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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, lipoxygenase, 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 syruping determination

Dough syruping 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. Microextention studies

Microextention 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.

2.8. High-Performance Size Exclusion Chromatography (HP-SEC) analysis of AX

A Waters Ultrahydrogel Linear 6–13 μm, 7.8 × 300-mm column and ultrahydrogel guard column (Waters, Milford, MA) were used to analyze. WE-AX. Samples were run using a Agilent 1200 series high-performance liquid chromatograph (Agilent Technologies, Wilmington, DE), equipped with an auto sampler. An Agilent refractive index detector and PC with ChemStation (HP ChemStation for LC Rev. A.04.01) were used for control and integration. All samples were analyzed at 40 °C with filtered deionized, distilled water as eluant. Flow rate was 0.3 mL/min and injection volume was 10 μL.

2.9. Carbohydrate analysis

The monosaccharide compositions of hydrolyzed aqueous extracts and the total monosaccharide composition of the lyophilized doughs were determined by Gas chromatography-Mass spectrometry (GC-MS) of alditol acetates. Ten to fifteen milligrams of lyophilized, ground doughs and extracted AXs were hydrolyzed at 110 °C for 1 h with 1 mL of 4.0 M TFA and for 2 h with 5.0 mL of 2.0 M TFA, respectively. Internal standard (inositol, 0.1%, 1.000 mL) and ammonia (25%, 1.0 mL) were added. After reduction with sodium borohydride and acetylation with acetic anhydride, alditol acetates were separated on a Supelco SP-2380 column (30 m–0.32 mm i.d., 0.2 ím film thickness) (Supelco, Bellefonte, PA) in an Agilent chromatograph (Agilent 6890 series, Wilmington, DE) equipped with an autosampler, a splitter injection port (split ratio 1:20), and a flame ionization detector. The carrier gas was He. Separation was at 225 °C, and injection and detection were at 270 °C. AXs were calculated as the sum of xylose and arabinose monosaccharides.

2.10. Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy analysis

^{1}H NMR analyses of AX samples were performed using a Varian Unity Inova 500 MHz NMR spectrometer (Varian Inc., Palo Alto, CA). Samples were first dissolved in 1 mL of deuterium oxide (D_2O), left for 2 h at room temperature, and lyophilized. Then, the samples were dissolved in 0.6 mL D_2O again and ^{1}H NMR spectra were obtained at 80 °C.

3. Results and discussion

3.1. Changes in dough syruping and dough stability

To examine the detailed structural changes of AXs during refrigerated storages, we used two flours with different psychochemical characteristics that exhibited different degree of dough syruping (DDS). The properties of these flours, Polar Bear and Qualitate, are given in Table 1. In order to rule out confounding factors from other ingredients, only water and flour were used in the preparation of the dough. Dough samples were stored at 6 °C, taken at specific storage times, and centrifuged to separate syrup. The separated syrup percentage was calculated from the difference in weight of the tubes before and after syrup removal and reported as a percentage of the initial dough weight. The DDS was 7.7% for Qualitate and 1.5% for Polar Bear at 1 day dough storage, but DDS increased to 20.5% for Qualitate and 13.4% for Polar Bear at day 16 (Fig. 1 bottom). At the end of 34 days of storage, DDS was 21.6% and 17.4% for Qualitate and Polar Bear, respectively. Sample Qualitate showed much higher DDS compare to Polar Bear in all the storage points.

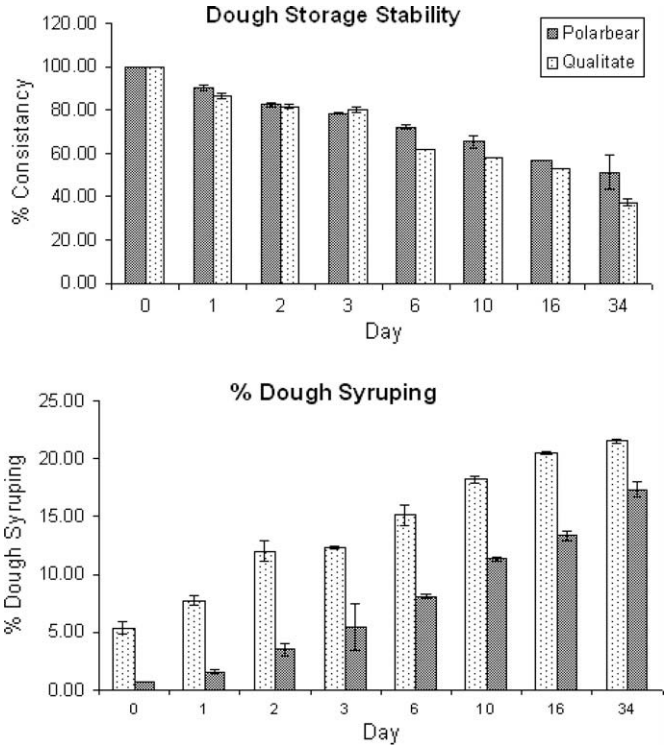

Fig. 1. (Top) Dough consistency changes as percentage of the decrease in initial dough consistency (measured after 2 min of mixing in the farinograph) of refrigerated dough, stored for 0, 1, 2, 3, 6, 10, 16, and 34 days, (Bottom) Dough syruping (percent dough weight) of refrigerated dough, stored for 0, 1, 2, 3, 6, 10, 16, 24, and 34 days.

Consistency was determined by using a farinograph and calculated as a percentage of the decrease in initial dough consistency. Dough consistency diminished quickly during the first 6 days of refrigerated storage. For Qualitate, consistency was 61.7% of the initial value, whereas Polar Bear had 72.3% of the initial value at day 6. Consistency diminished slowly after day 16 for sample Polar Bear. On the other hand, Qualitate sample's dough consistency had a significant decrease even for longer periods of storage, which was in correspondence with the trends measured in syruping. After the 34 days of storage, Qualitate was able to retain 37.2% of its initial consistency while Polar Bear kept 51.1% of its initial consistency. Thus, dough prepared with Qualitate flour had overall low consistency and higher DDS.

3.2. Analysis of refrigerated dough samples by microextention test

Changes in extensibility and resistance to extension, measured by a texture analyzer as described by Kieffer et al. (1998), were detected in doughs prepared with both flours during refrigerated storage. In these tests, maximum measured force stands for "maximum resistance (R_{max})" while the corresponding distance stands for "Extensibility", which are the rheological parameters illustrated in Fig. 2. The extension tests showed that R_{max} and extensibility changed dramatically during the refrigeration storage. They were inversely correlated to each other. R_{max} was increasing in the early stages of storage and decreasing a little after 10 days of storage for both flours (Fig. 2, top). From day 0 to 6, R_{max} increased by 31%, and at the end of the storage at day 34, R_{max} was 9% lower than the value on day 0 for sample Qualitate. Sample Polar Bear exhibited lower R_{max} values for every storage point. It reached maximum value at day 3 (32% of the initial value), and reached the number that is 46% lower than the initial value. In contrast, extensibility was

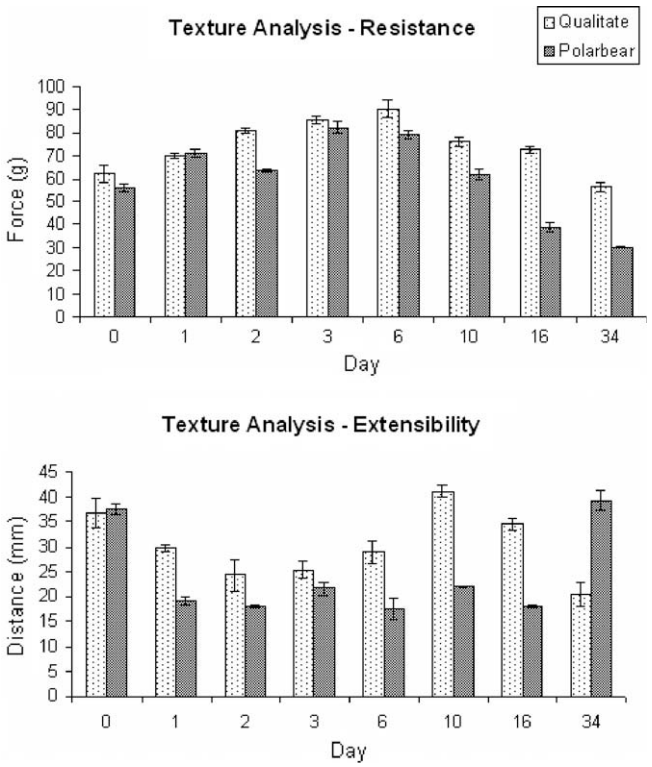

Fig. 2. Analysis of refrigerated dough samples stored for 0, 1, 2, 3, 6, 10, 16, 24, and 34 days with TA-XT2*i* texture analyzer. (Top) Maximum force in grams required to tear dough strips, (Bottom) Maximum distance in millimetres dough strips stretched.

decreasing during storage and then increasing a little after day 6 for both samples. For sample Qualitate, from day 0 to 6, extensibility decreased by 21%. On day 34, extensibility was only 35% of the initial value. It has been reported that the performance in these tests is largely determined by protein quality and flours of high protein content show high resistance and low extensibility (Tronsmo, Magnus, Faergestad, & Schofield, 2003). These empirical tests showed that more resistance to extension and lower extensibility in doughs made with Qualitate flour were associated with more syruping.

Changes in R_{max} and extensibility did not follow a regular trend, which was observed in DDS values. The possible explanation for this observation could be the changes in the ratio of WE-AX to WU-AX during storage due to xylanase activity in dough samples. Therefore, the water distribution among the macromolecules could be changing during the refrigerated storages, which affects the rheological behavior of dough samples.

3.3. Structural characterization of AXs by GC-MS, HPSEC and ^{1}H NMR

Degree of substitution refers to the number of arabinose moieties on the xylose backbone, and it can be quantified as the A/X ratio. The degree of substitution and distribution of side chains are important factors on the physicochemical properties of AX. Based on our results obtained from GC-MS, the total AX composition of both samples was similar (about 3.3%), whereas A/X was 0.74 for doughs prepared with Polar Bear flour and 0.79 for those prepared with Qualitate (Table 1). Percentages of water extractable solids (WES) and water unextractable solids (WUS), and the AX composition and A/X ratio in WES and WUS were also measured to determine the structural basis for AX solubility in the samples (Table 2).

The WES and WUS were separated by centrifugation, freeze-dried, and measured. The percentage of WES increased with storage time: from 14.6% for Qualitate and 12.9% for Polar Bear at day 1 to 16.7% for Qualitate and 15.6% for Polar Bear at day 34 (Table 2). Chemical analysis of WES with GC-MS showed that it contained AXs, which shows different structural details in each storage point. A/X ratio in WES from Polar Bear was 0.80 at day 0, and it decreased to 0.69 at day 34. A/X in WUS from the same sample had the opposite trend in which the ratio increased from 0.61 to 0.77 at day 0 and 34, respectively. Qualitate flour, which has higher DDS, yielded a similar trend in regards to storage time. However, the A/X ratio in the water extractable fraction was 0.88 for day 0, showing that Qualitate flour has a higher arabinose substitution in the WE-AX compared to Polar Bear. The effect of storage time on doughs prepared with Qualitate flour was different from that on doughs made with Polar Bear flour, as the A/X ratio in the WUS decreased from 0.69 to 0.66. However, at the end of 34 days of storage, this value has reached to 0.74, which is 16% lower than initial value. These findings may be a result of endoxylanase activity (higher in the Qualitate sample), which hydrolyzes the AX at non-substituted regions.

The other important observation was that the percentage of WES was positively correlated to DDS. For example, Polar Bear exhibited a relatively lower level of DDS and lower percentage of WES formation during refrigerated storage. Importantly, xylanase activity was one of the main differences between the flours.

Additionally, arabinofuranisadase, which hydrolyzes the arabinose from the AX backbone, is active during refrigerated storage, and when we analyzed the WES with GC-MS without hydrolysis (this method only shows the free sugars), free arabinose was 33.98 μg/mg for day 0, 41.87 μg/mg for day 6 and 42.75 μg/mg for day 16 for the Polar Bear sample (Table 3). On the other hand,

Table 2
Analysis of water extractable solids (WES) and water unextractable solids (WUS) from whole-lyophilized-ground refrigerated dough.

Storage (Days)	WES[a] (%)		WUS[b] (%)		A[c]/X[d] ratio WES		A/X ratio WUS	
	Polar Bear	Qualitate	Polar Bear	Qualitate	Polar Bear	Qualitate	Polar Bear	Qualitate
0	12.3	12.5	87.7	87.5	0.80	0.88	0.61	0.69
1	12.9	14.6	87.1	85.4	0.76	0.83	0.64	0.72
2	11.6	14.5	88.4	85.5	0.79	0.84	0.70	0.68
3	13.9	16.0	86.1	84.0	0.77	0.79	0.71	0.65
6	13.9	15.5	86.1	84.6	0.77	0.84	0.73	0.66
10	13.5	15.6	86.5	84.4	0.71	0.82	0.74	0.70
16	14.3	15.8	85.8	84.2	0.72	0.79	0.76	0.66
34	15.6	16.7	84.4	83.3	0.69	0.74	0.77	0.73

[a] Water extractable solids.
[b] Water unextractable solids.
[c] Arabinos.
[d] Xylose.

Table 3
Monosaccharide composition analysis of water extractable solids from whole-lyophilized-ground refrigerated dough. All the numbers are presented as μg/mg in the table.

Sugar[a]	0 Day	1 Day	2 Day	3 Day	6 Day	10 Day	16 Day	34 Day
Ara								
Polar Bear	33.98	33.21	31.92	39.95	41.87	36.26	42.75	35.79
Qualitate	47.87	39.47	38.40	37.86	48.66	36.04	37.06	34.72
Xyl								
Polar Bear	42.51	54.06	40.17	51.76	53.87	51.18	59.12	51.80
Qualitate	43.67	47.56	45.97	47.89	57.61	43.97	46.78	46.41
Man								
Polar Bear	11.85	11.56	14.77	14.52	14.76	13.21	14.47	14.29
Qualitate	10.23	11.28	11.16	11.38	12.00	10.16	9.90	9.77
Gal								
Polar Bear	26.43	19.36	17.76	24.32	24.32	24.08	24.36	23.27
Qualitate	20.21	26.25	25.97	31.48	31.01	25.72	26.00	31.37
Glc								
Polar Bear	222.59	237.28	249.26	289.74	309.40	286.40	315.34	306.14
Qualitate	283.50	307.11	335.08	319.22	393.48	329.40	330.05	382.12
Free ara								
Polar Bear	12.42	13.63	11.55	11.19	14.09	13.08	14.26	15.28
Qualitate	13.60	9.14	10.84	11.98	13.62	12.96	12.71	15.17
Free xyl								
Polar Bear	ND	ND	ND	ND	ND	ND	1.18	2.29
Qualitate	ND	ND	ND	ND	ND	0.71	1.10	2.38
Free man								
Polar Bear	58.11	56.63	55.53	49.17	52.81	52.74	46.97	48.52
Qualitate	46.16	39.02	42.24	32.90	37.77	38.66	36.93	35.38
Free gal								
Polar Bear	3.13	3.07	2.83	2.72	2.72	3.22	3.13	3.40
Qualitate	ND	1.85	2.25	2.04	2.28	2.46	3.24	3.76
Free glc								
Polar Bear	86.16	84.33	81.24	77.11	81.99	89.86	83.08	90.78
Qualitate	57.97	64.45	65.58	58.82	69.83	76.00	76.36	86.03

[a] Ara, arabinose; xyl, xylose; man, mannose; gal, galactose; glc, glucose.

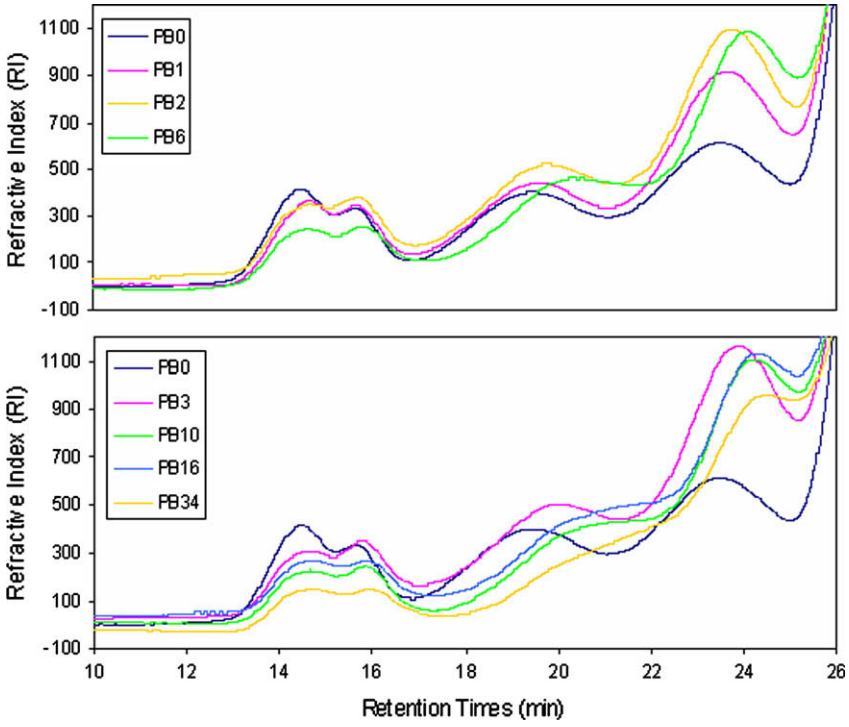

Fig. 3. Analysis of water extractable arabinoxylans (WE-AXs) from refrigerated dough by High-Performance Size Exclusion Chromatography (HP-SEC) (PB: Polar Bear. Numbers in the boxes represents each storage point as days.). Peaks were labeled as described in Table 4. Dextrans were run under the same condition with AXs and retention times were labeled in chromatogram at the bottom.

Qualitate sample showed very complex trend for the free arabinose formation during storage. Free arabinose was 47.87 μg/mg at day 0 and it decreased to 34.72 μg/mg at day 34.

We have also observed changes in the formation of free xylose, mannose, galactose and glucose, which indicate hydrolyzation of starch and NSP in the dough system during refrigerated storage, which needs further investigation.

The molecular weights of the WE-AXs in stored doughs were also determined at 0, 1, 2, 3, 6, 10, 16 and 34 days. As shown in Fig. 3 four distinct peaks (I, II, III, IV) of different molecular weights (compared to Dextran standards; arrows) were detected in each chromatogram, and peak integration and retention times were determined for each sample (Table 4). This provides significant information about the solubilization and hydrolysis of AX during refrigerated storage for the two flours. For Polar Bear flour, a decrease in the peak area values of the high molecular weight fraction (HMW) of AX was observed. Also, the retention time of the low molecular weight (LMW) fraction of the AXs changed slightly. The effect of storage time on doughs prepared with the Qualitate flour was more complex (data not shown). An increase in the HMW fraction of the AX was observed for day 1, which is probably due to an increase in solubility of the HMW AX from the WU-AX fraction. However, these AXs were subsequently subjected to hydrolysis by day 6 (decrease in the peak area). This could be one reason why doughs made with Qualitate flour have a higher DDS; i.e., the results show that WU-AX, which has a high water holding capacity, is being solubilized during refrigerated storage. Therefore, even though hydrolysis of WE-AX was detected in the Polar Bear sample, the effect on DDS was lower than that produced by solubilization of WU-AX, which occurred only in the Qualitate sample.

Table 4
Retention time and peak area for the water extractable arabinoxylans from sample Polar Bear (PB) and Qualitate (QL) using High-Performance Size Exclusion Chromatography (HP-SEC).

Peak number	Retention time (min)		Peak area (%)	
	Polar Bear	Qualitate	Polar Bear	Qualitate
Day 0				
I	14.47	15.00	15.70	8.56
II	15.47	15.68	9.33	4.95
III	19.48	19.77	29.43	13.10
IV	23.41	24.08	45.54	73.33
Day 1				
I	14.68	15.05	10.74	12.13
II	15.61	16.34	9.58	9.06
III	19.64	20.05	28.17	29.05
IV	23.60	24.53	51.50	49.77
Day 2				
I	14.67	15.78	8.93	7.49
II	15.71	16.36	9.56	9.21
III	19.82	20.05	29.37	29.16
IV	23.74	25.20	52.13	44.26
Day 3				
I	14.70	15.69	8.13	13.84
II	15.70	16.42	8.98	18.72
III	20.09	20.28	27.58	56.80
IV	23.92	25.12	55.30	10.63
Day 6				
I	14.63	14.72	8.43	3.45
II	15.84	17.70	7.80	5.29
III	20.33	20.86	29.87	47.81
IV	24.08	25.16	53.90	43.46
Day 10				
I	14.67	15.37	7.63	10.45
II	15.85	16.54	7.50	11.76
III	22.08	21.98	29.19	53.54
IV	24.24	25.03	55.69	24.26
Day 16				
I	14.79	14.78	7.36	18.67
II	15.88	16.62	7.19	30.84
III	22.25	23.14	31.88	13.91
IV	24.31	25.02	53.57	36.58
Day 34				
I	14.75	14.76	7.92	12.01
II	15.99	16.67	7.23	10.55
III	22.62	19.81	32.68	17.77
IV	24.47	24.96	52.17	59.67

Structural changes and enzyme mechanisms for xylanases were monitored using ^{1}H NMR spectroscopy (Fig. 4). The analyses were performed on purified AX from the WES of dough made with Polar Bear flour. The resonances at δ5.80–5.60 and 5.00–5.20 ppm arise from the anomeric protons of arabinoses and xyloses, respectively. Arabinose units linked to the C-(O)-3 positions of xylose residues show resonances at δ5.75-5.82 ppm and those linked to the C-(O)-2 resonate at 5.65 ppm. In addition resonances at δ5.07, δ5.05 and δ5.02 ppm correspond to disubstituted, monosubstituted and unsubstituted xyloses, respectively (Hoffmann, Kamerling, & Vliegenthart, 1992). The spectra for the AX from day 0 show all the xylose residues either disubstituted or monosubstituted; xylose without arabinose substitution was not detected. However, unsubstituted xyloses were detected at day 16, which shows that arabinofructofuranases hydrolyzed some of the arabinoses from the AX backbone. It also appears that this enzyme preferentially hydrolyzes the arabinose residues at the C-(O)-3 positions.

4. Conclusions

Structural changes of AXs in relation to changes in dough properties in refrigerated dough samples were investigated in this study. To better understand the mechanism behind the syrup formation in refrigerated dough, AXs (WE-AX and WU-AX) at different storage times (0, 1, 2, 3, 6, 10, 16 and 34 days) were structurally characterized. HP-SEC was used to determine molecular weight changes and GC-MS for substitution patterns. ^{1}H-NMR was used to study xylanase enzyme action, to determine which arabinoses in the backbone was hydrolyzed first (C-2 bonded vs C-3), and other related structural changes. From this we were able to monitor structural changes in AX at the basic level in refrigerated dough systems. We observed that the molecular weight and arabinose substitution pattern change during storage and are correlated to DDS. Results obtained from HP-SEC and NMR showed that total AX percentage in the WES from Polar Bear, a flour that provided dough with lower DDS, was 6.7% for day 0, and it increased to 8.4% at day 6 (a 20% increase), and the A/X ratio decreased from 0.80 at day 0 to 0.77 for day 6. Total AX percentage in the WUS decreased from 1.88% to 1.49% during 6 days of storage, whereas the A/X ratio changed from 0.61 at day 0 to 0.73 at day 6, indicating that WU-AX with less arabinose substitution preferentially becomes soluble during refrigerated storage. The ratio of unsubstituted xylose in water extractable AXs increased during storage. These results showed that changes in AX chemistry are correlated to the refrigerated dough quality.

AX contains FA, which is esterified to some arabinose residues at O-5 (Fausch, Kundig, & Neukom, 1963). FA is the key component involved in oxidative gelation of AXs, a cross-linking reaction believed to be unique to the WE-AX fraction (Biliaderis, Izydorczyk, & Rattan, 1995;Hoseney & Faubion, 1981). In addition to covalent cross-links (di-FA, tri-FA), the involvement of physical interactions between AX chains may contribute to the arabinoxylan gelation and gel properties. Therefore, AX structural characteristics such as FA content and location, molecular weight and xylan backbone substitution (A/X ratio) are related to the WE-AX gel properties, and this impacts characteristics, such as taste and

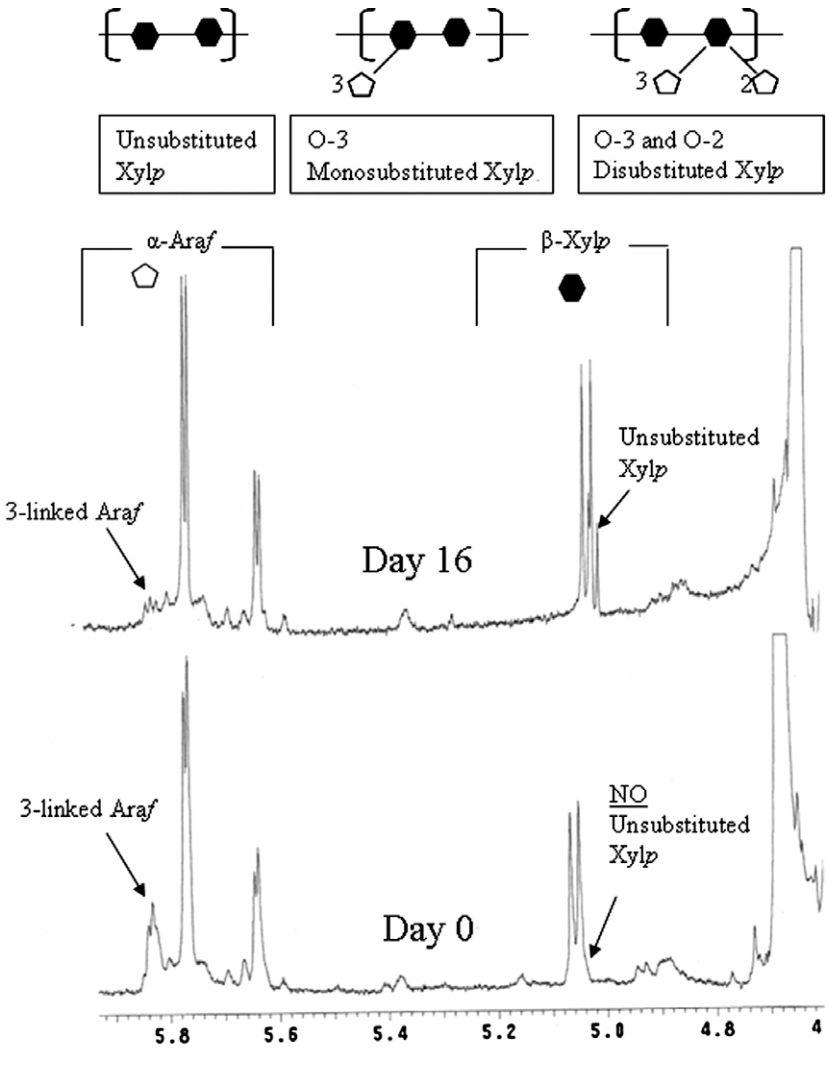

Fig. 4. Analysis of water extractable arabinoxylans from refrigerated dough (prepared using sample Polar Bear) by ^{1}H NMR.

odor, high water absorption capacity (up to 100 g of water/g of dry polymer), and absence of pH or electrolyte susceptibility (Carvajal-Millan, Guilbert, Doublier, & Micard, 2006; Izydorczyk & Biliaderis, 1995). Thus, total and free ferulic acid contents are important factors in dough syruping since they impact AX-AX and AX-protein interactions, and gel formation, which needs further investigation.

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