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Refrigerated Dough Syruping in Relation to the Arabinoxylan Population

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Refrigerated doughs develop syruping upon prolonged storage. To assess the role of arabinoxylans (AX), in this phenomenon, the evolution of the AX population and syruping in refrigerated doughs during storage were studied. When doughs were kept at 6 °C for up to 34 days of storage, dough syruping increased from 0% (fresh dough) to 22% of dough weight, reaching a plateau after 16 days of storage. High-performance size exclusion chromatography and gas−liquid chromatography showed hydrolysis of water-unextractable AX in the refrigerated dough, resulting in increased levels of solubilized AX in the first 2 days of storage. Longer storage resulted in further degradation of solubilized and water-extractable AX. Increased syruping was accompanied by a decrease in farinograph dough consistency. The results support the hypothesis that loss of water-holding capacity due to degradation of AX by endogenous xylanases is responsible for dough syruping.

KEYWORDS: Arabinoxylan; endoxylanase; refrigerated dough; syruping

INTRODUCTION

Some of the unique advantages of refrigerated doughs are their convenience of use and extended storage possibilities. However, the potential development of a syrup at the wheat dough surface after prolonged storage of refrigerated doughs, which affects processing and organoleptic properties, is an important concern. A possible cause is loss of water-holding capacity in the dough, which, in view of the high water-holding capacity of arabinoxylans (AX), may be related to degradation of AX during refrigerated storage.

Within the AX, which are cereal cell wall materials and which consist of β-1,4-linked D-xylopyranosyl units substituted with α-L-arabinofuranosyl units at the C(O)-2 and/or C(O)-3 position, two different classes can be distinguished: water-extractable AX (WE-AX) and water-unextractable AX (WU-AX). WE-AX, approximately one-third (∼0.5% of flour weight) of the total AX fraction in wheat flour (∼1.5−2.0%), are loosely bound to the cell wall material (*1*). Chemical bonds such as between AX-linked ferulic acids (*2*) and physical interactions (inclusion of AX in other cell wall constituents) determine the water unextractability of the other two-thirds of AX. The physicochemical properties of AX, such as their viscosity-forming potential (*3*, *4*), water-binding capacity (*5*−*7*), solubility (*8*), and gel-forming capacity (*9*−*11*), differ for the two classes and are closely related to the fine structure of AX (*12*).

To our knowledge, no reports on refrigerated dough syruping can be found in the traditional peer-reviewed scientific publications. The patent literature suggests that the degradation of AX influences dough syruping (*13*, *14*). Atwell (*13*) reduced syrup development by the addition of commercial birchwood and oat spelt xylans to the refrigerated dough recipe. According to the inventors, the added xylan is a competitive substrate for endoxylanases. In other words, the endoxylanases endogenously present will preferentially degrade the added xylan rather than native AX in wheat flour, which reduces syruping. A further interesting observation was made by Poulsen and Sørensen (*14*), who added wheat endogenous endoxylanase inhibitor (*15*) to dough. Addition of sufficient endoxylanase inhibitor to inhibit endogenous endoxylanases reduced dough syruping drastically. Both experiments indicate that the degradation of AX by endoxylanases from wheat flour is a key factor in dough syruping. However, neither Atwell (*13*) nor Poulsen and Sørensen (*14*) investigated the fate of the AX population during refrigerated dough storage.

Earlier limited work of Beldman et al. (*7*) showed that the addition of AX-degrading enzymes and, in particular, (1,4)-β-D-endoxylanases to a mixture of wheat flour and 0.05 M acetate buffer in a 2:3 ratio increased the amount of liquid loss after 30 min of incubation at 37 °C and centrifugation of the mixture. Although the experimental conditions in the Beldman test system differ strongly from those found in refrigerated dough systems, the experiments clearly showed that degradation of AX can cause the loss of a considerable amount of liquid from a flour/water mixture.

The objective of the present work was therefore to study the water extractability and the apparent molecular weight distribution of the AX population in relation to dough properties. To this end, doughs were made and kept at 6 °C for up to 34 days. After different storage periods, doughs were analyzed: dough syruping and consistency were measured, and the evolution of the AX population was studied. We here report on the outcome of this work.

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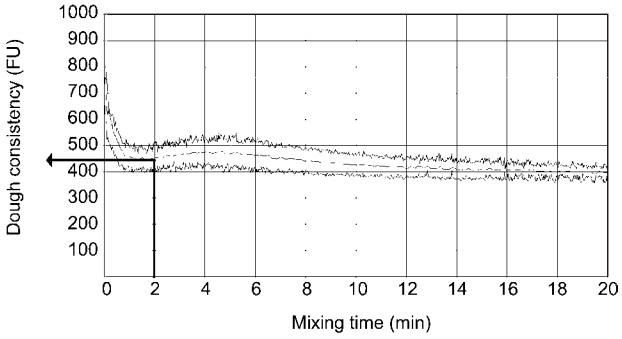

Figure 1. Typical farinogram of a dough after refrigerated storage. Dough consistency (farinograph units, FU) is defined as the resistance measured 2 min after mixing has started. In this particular farinogram, dough consistency was 455 FU.

MATERIALS AND METHODS

Flour and Reagents. Meunier, a wheat variety of good bread-making quality, was obtained from Clovis Matton (Kerkhove, Belgium). After conditioning to 15.5% for 24 h and final water addition (0.5%) prior to milling (30 min), flour (milling yield 76.4%) was produced with a Bühler MLU-202 laboratory mill (Uzwill, Switzerland) (*16*). The flour had a moisture content of 13.3% (*17*), an ash content of 0.68% [dry matter (dm) basis] (*18*), and a protein content [determined with the Dumas method, an adaptation of the AOAC Official Method (*19*) to an automated Dumas protein analysis system (EAS varioMax N/CN, Elt, Gouda, The Netherlands)] of 10.2% (dm basis). Rheological properties of the flour were determined using a Brabender farinograph E (Duisburg, Germany) (*20*). Water absorption to reach 700 farinograph units (FU) was 53.1% (on a 14% moisture basis).

All chemicals and reagents were purchased from Sigma-Aldrich (Bornem, Belgium) and were of analytical grade unless specified otherwise.

Sample Preparation. Dough was prepared at a Brabender farinograph dough consistency of 700 FU using a lean dough formula consisting of 10.0 g of flour (14% moisture base), 0.18 g of salt, and 5.31 mL of water containing sodium azide (0.06% w/v, Acros Organics, Geel, Belgium). Dough was mixed in a 10-g pin mixer (National Manufacturing, Lincoln, NE) for 3.5 min, sheeted, molded, and stored in a small, polyethylene jar for 0 h (analysis immediately after mixing), 8 h, and 1, 2, 3, 6, 10, 16, 24, and 34 days at 6 °C. The jar (27.0 mm diameter, 52.6 mm height, Nalgene, Rochester, NY) was closed with a snap cap.

For each point in time, seven identical doughs were made: three were used to measure dough syruping, two were used for dough farinograms, and two were frozen, lyophilized, ground, and stored until further analysis.

Quantification of Dough Syruping. Dough syruping was measured as the volume of liquid released by the dough after centrifugation, in line with the principle of the method of Atwell (*13*). The stored dough was divided in four pieces of ~3.0− 4.0 g. Accurately weighed dough pieces were centrifuged in a centrifuge tube of 30 mL (height = 92 mm, diameter = 25.5 mm) in a Beckman (Fullerton, CA) J2-21 centrifuge (JA 20 rotor, 22000*g*, 20 °C, 30 min). After centrifugation, the tubes were placed upside down to allow the liquid to run out. After 2 h, the liquid adhering to the sides of the centrifuge tube was removed with a cotton ball. The syrup was quantified as the difference in weight between the tubes before and after syrup removal and expressed as percentage of initial dough weight. Dough analyses were carried out in triplicate, such that the amount of dough syrup was determined as the average of 12 measurements.

Dough Farinograms. Farinograms of stored doughs were recorded with a Brabender farinograph (S-10 mixing bowl, 10 g, dynamometer speed = 63 min^{-1}). In contrast to AACC Method 54-21 (*20*), where flour is mixed with water to form a dough of desired consistency, we examined the farinogram of a previously fully developed dough after storage. An example of such an obtained dough farinogram is shown in **Figure 1**. Although dough consistency is normally measured at the highest point reached after dough development in the farinograph mixing bowl, doing so did not allow dough consistency for a fully developed and refrigerated dough to be measured in a reproducible way. On the basis of preliminary experiments we measured dough consistency 2 min after the start of Farinograph mixing. This allowed for equilibration of the refrigerated dough temperature (6 °C) to that of the mixing bowl (30 °C).

Extraction of Flour and Dough Samples. Water extraction of doughs was performed as described by Courtin et al. (*21*). Hence, lyophilized and ground dough samples (5.0 g) were accurately weighed in a centrifuge tube of 250 mL. Deionized water (100 mL, 4 °C) was added, and the centrifuge tubes were shaken (30 min, 4 °C). After centrifugation (10000*g*, 15 min, 4 °C), the supernatant was transferred to a flask and immediately frozen in liquid nitrogen. The residue in the centrifuge tube was washed with water (50 mL, 4 °C) and centrifuged again as above. The supernatant was added to the flask and frozen. After lyophilization, boiling water (150 mL) was poured into the flask, which was then kept in a bath with boiling water (30 min) to inactivate endogenous enzymes. Following cooling and freezing (liquid nitrogen), its contents were lyophilized again. The lyophilized material was dispersed in water (50 mL) and centrifuged (10000*g*, 15 min, 4 °C) to remove heat-coagulated protein material. The supernatant was frozen until further analysis.

Soluble Protein Content. The protein content of aqueous extracts of doughs lyophilized after storage was determined with the Dumas method, an adaptation of the AOAC Official Method (*19*) to an automated Dumas protein analysis system (EAS varioMax N/CN, Elt, Gouda, The Netherlands).

Carbohydrate Analysis. *With Hydrolysis.* The monosaccharide compositions of hydrolyzed aqueous extracts and the total monosaccharide composition of the lyophilized doughs were determined by gas−liquid chromatography (GLC) of alditol acetates (*22*). Supernatants (2.5 mL) and lyophilized, and ground doughs (~0.1 g, accurately weighed) were hydrolyzed at 110 °C for 1 h with 2.5 mL of 4.0 M TFA and for 2 h with 5.0 mL of 2.0 M TFA, respectively. Internal standard (allose, 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 μm 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. AX were calculated as the sum of xylose and arabinose monosaccharides. The variation coefficient, defined as the ratio of the standard deviation and the mean of the obtained results, was <10%.

Without Hydrolysis. Omitting hydrolysis in the above-described GLC procedure allowed measurement of the level of free monosaccharides in the aqueous extracts. Supernatants (2.5 mL) were mixed with 4.0 M TFA (2.5 mL), 1.0 mL of ammonia (25%), and 1.000 mL of internal standard solution (allose, 0.1%). Reduction was with sodium borohydride and acetylation with acetic anhydride. The remainder of the procedure was carried out as described above.

High-Performance Size Exclusion Chromatography (HPSEC). Apparent molecular weight distributions of AX were studied by HPSEC on a Shodex SB-804 HQ column (Showa Denko K.K., Tokyo, Japan). Supernatants (50 μL) were eluted with 0.3% NaCl (0.5 mL/min) at 30 °C. The unit (pump unit 325, Kontron, Milan, Italy) was equipped with autoinjection, and monitoring was with a refractive index detector (VSD Optilab, Berlin, Germany). Glucose and pullulan standards (Shodex standard P-82, Showa Denko K K.) with molecular weights of 788, 404, 212, 112, 47.3, 22.8, 11.8, and 5.9 kDa were used as molecular weight markers.

Viscosity Measurements. Viscosity of dough extracts was measured using an Ostwald type viscometer. Flow times of supernatants (5.0 mL) were measured at 30 °C. Results are expressed as relative viscosity (η_{rel}), that is, they were divided by the flow time of deionized water under the experimental conditions. Specific viscosity (η_{sp}) was calculated as $\eta_{sp} = \eta_{rel} - 1$.

Characterization of Proteins by Sodium Dodecyl Sulfate—Polyacrylamide Gel Electrophoresis (SDS-PAGE). Meunier flour and

Table 1. Monosaccharide Composition after Hydrolysis (Percent Dough Dry Matter) and Protein Content (Percent Dough Dry Matter) of Aqueous Extracts of Doughs Lyophilized after Storage for 0 and 8 h and 1, 2, 3, 6, 10, 16, 24, and 34 Days

storage	ara	xyl	gal	gal_{cor}[a]	glc	AG[b]	AG_{cor}[c]	AX[d]	AX_{cor}[e]	A/X[f]	A/X_{cor}[g]	protein[h]
0 h	0.45	0.65	0.20	0.20	3.26	0.30	0.30	0.84	0.84	0.48	0.48	1.45
8 h	0.45	0.67	0.17	nd[i]	4.38	0.26	nd	0.88	nd	0.49	nd	1.43
1 day	0.58	0.89	0.20	nd	5.50	0.31	nd	1.17	nd	0.49	nd	1.38
2 days	0.60	0.94	0.19	nd	5.84	0.29	nd	1.24	nd	0.50	nd	1.44
3 days	0.68	1.07	0.21	0.19	6.45	0.32	0.29	1.41	1.42	0.50	0.51	1.52
6 days	0.66	1.06	0.20	0.17	6.72	0.30	0.26	1.40	1.41	0.49	0.51	1.59
10 days	0.72	1.05	0.22	nd	7.15	0.33	nd	1.43	nd	0.54	nd	1.71
16 days	0.73	0.95	0.24	0.20	7.51	0.36	0.31	1.34	1.36	0.60	0.62	1.88
24 days	0.73	0.89	0.25	0.20	7.86	0.38	0.31	1.27	1.30	0.63	0.66	2.03
34 days	0.75	0.88	0.27	0.21	8.26	0.41	0.32	1.26	1.31	0.63	0.69	2.45

[a] $gal_{cor} = gal_{with\ hydrolysis} - gal_{without\ hydrolysis}$, i.e., the galactose content coming from AG, i.e., corrected for galactose most probably originating from galactolipids. [b] % AG = (gal + 0.7 gal) × 0.89, where 0.7 gal = arabinose content originating from AG with ara/gal = 0.7. [c] AG_{cor} = (gal_{cor} + 0.7 gal_{cor}) × 0.89. [d] % AX = [(ara − 0.7 gal) + xyl] × 0.88, where 0.7 gal = arabinose content originating from AG with ara/gal = 0.7. [e] % AX_{cor} = [(ara − 0.7 gal_{cor}) + xyl] × 0.88. [f] A/X = (ara − 0.7 gal)/xyl. [g] A/X_{cor} = (ara − 0.7 gal_{cor})/xyl. [h] Protein content of aqueous extracts. [i] Not determined.

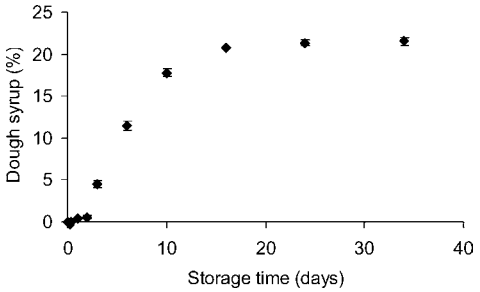

Figure 2. Dough syruping (percent dough weight) of refrigerated dough, stored for 0 and 8 h and 1, 2, 3, 6, 10, 16, 24, and 34 days.

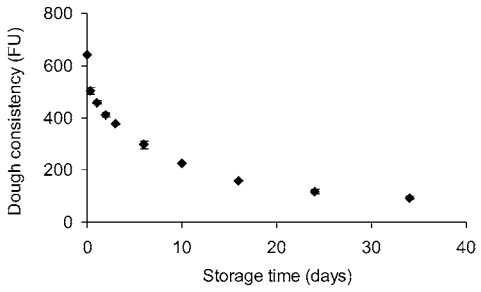

Figure 3. Dough consistency (measured after 2 min of mixing in the farinograph, farinograph units, FU) of refrigerated dough, stored for 0 and 8 h and 1, 2, 3, 6, 10, 16, 24, and 34 days.

lyophilized dough samples were extracted with deionized water (30 min) at room temperature. After centrifugation (10000*g*, 15 min, 20 °C), the pellet was lyophilized and gently ground with a pestle and mortar. The material (2.0 mg) was then examined by SDS-PAGE (*23*), as described by Bleukx et al. (*24*). It was thus suspended in sample buffer (0.25 mL) containing 17.7 mM tris(hydroxymethyl)aminomethane−HCl (Tris-HCl, pH 6.8), 1.0% (w/v) SDS, 20.0% (w/v) sucrose, 5.0% (v/v) β-mercaptoethanol, and 0.01% (w/v) bromophenol blue. Samples were shaken overnight (6 °C), equilibrated to room temperature, and clarified by centrifugation (11000*g*, 3 min). Samples (30 μL) were subjected to SDS-PAGE in a SE 600 series gel electrophoresis unit (Hoefer Pharmacia Biotech Inc., San Francisco, CA). The slab gel (14.0 × 16.0 × 1.5 cm) consisted of a stacking gel (3.88% T, 1.33% C) and a separating gel (17.57% T, 0.455% C). Separation was at 18 °C and 30 mA. Molecular weight marker proteins were phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa). Staining (3 h) was with 0.025% Coomassie Brilliant Blue R250 in 40% methanol containing 7.0% acetic acid. Destaining was with 5.0% methanol containing 7.0% acetic acid until the background was clear. Under the conditions of the assay, mainly gluten proteins (gliadins and glutenin subunits) are visualized.

RESULTS

Dough Syruping. Although very limited, dough syruping was already measurable after 1 day of storage (**Figure 2**). Small differences in weight before and after centrifugation could be detected (0.4 and 0.6% for doughs stored for 1 and 2 days, respectively). Under such conditions, all liquid adhered to the side of the centrifuge tube and was removed with a cotton ball. After only 3 days of storage, dough syrup ran out easily, which facilitated measurements. Dough syruping increased from 4.5% after 3 days of storage to 20.8% after 16 days, when a plateau was reached. When doughs were stored for an extra period of 18 days, dough syruping increased only slightly by 0.7% to a final level of 21.5%.

Dough Farinograms. A few hours after the dough was mixed, a decrease in farinograph consistency was already measurable (**Figure 3**). Dough consistency diminished from 640 FU immediately after mixing to 505 FU for doughs stored for 8 h. After 6 days, farinograph consistency was reduced by >50% and continued to decrease with increasing storage times, although to a lesser extent. Dough consistency after storage for 34 days amounted to 94 FU, which was ∼15% of the original value.

Table 2. Free Monosaccharide Composition[a] (Percent Dough Dry Matter) of Aqueous Extracts of Doughs Lyophilized after Storage for 0, 3, 6, 16, 24, and 34 Days

storage	ara	xyl	gal	glc	% $AX_{polymer}$[b]	$A/X_{polymer}$[c]
0 days	0.01	0.00	0.00	0.09	0.84	0.47
3 days	0.06	0.01	0.02	0.61	1.36	0.46
6 days	0.10	0.02	0.03	0.92	1.30	0.42
16 days	0.24	0.08	0.04	1.60	1.09	0.41
24 days	0.30	0.12	0.05	1.87	0.94	0.38
34 days	0.37	0.17	0.06	2.17	0.83	0.33

[a] Results are obtained with GLC performed without hydrolysis during preparation of the sample. [b] % $AX_{polymer}$ = [($ara_{with\ hydrolysis}$ − $ara_{without\ hydrolysis}$ − 0.7 gal_{cor}) + ($xyl_{with\ hydrolysis}$ − $xyl_{without\ hydrolysis}$)] × 0.88. [c] $A/X_{polymer}$ = [($ara_{with\ hydrolysis}$ − $ara_{without\ hydrolysis}$ − 0.7 $gal_{cor\ with\ hydrolysis}$)]/($xyl_{with\ hydrolysis}$ − $xyl_{without\ hydrolysis}$).

Carbohydrate Analysis. Monosaccharide contents of hydrolyzed aqueous extracts from doughs lyophilized after storage are given in **Table 1**. The free monosaccharide content, obtained with GLC of unhydrolyzed aqueous extracts, is given in **Table 2**. The percentage of (polymeric) AX was calculated as the sum of monomeric arabinose and xylose, multiplied by a factor 0.88 for the release of water. A correction was made for arabinoses

originating from arabinogalactans (AG). In wheat flour, the arabinose-to-galactose ratio (A/G) of the latter is fairly constant and amounts to 0.7 (*22*). Hence, % AX = [(% arabinose − 0.7% galactose) + % xylose] × 0.88. The same approach was used in the calculation of the level of AG and the arabinose-to-xylose ratio (A/X). Additionally, for each of the above-mentioned parameters (i.e., AX, AG, and A/X) a corrected value is listed. These values were calculated with corrected galactose contents (gal_{cor}). This was necessary because the free (monomeric) galactose content in the aqueous extracts of doughs increased with increasing storage time (**Table 2**). This increase in free galactose content can probably be ascribed to degradation of galactolipids. A corrected value for galactoses originating from AG is therefore % $galactose_{cor}$ = (% $galactose_{with\ hydrolysis}$ − % $galactose_{without\ hydrolysis}$).

The percentage of soluble arabinose increased continuously during storage, whereas that of soluble xylose increased during the first 3 days of storage, remained constant for a few days, but even diminished when doughs were stored for up to 16 days or more (**Table 1**). This was reflected in the polysaccharide composition of the dough: an increase in the level of AX in aqueous extracts of doughs was seen during the first 10 days of storage. The concentration of soluble AX, consisting of solubilized AX (S-AX) and WE-AX, increased from 0.84% immediately after mixing to 1.41% after 3 days of storage. This indicated a solubilization of WU-AX by dough endoxylanases, which was confirmed by HPSEC analysis. During the next 7 days, AX content in aqueous extracts remained at a constant level. The percentage of soluble AX decreased in doughs stored for >10 days.

To explain the opposite trends in arabinose and xylose levels, we hypothesized that arabinofuranosidase action may have been responsible for the degradation of AX. Ergo, such action may have resulted in AX with lowered A/X, more linear and therefore less soluble (*8*), or even xylan. This may then explain the observed decreasing xylose content in aqueous extracts. To test this hypothesis, carbohydrate analysis was performed such that only monosaccharides present in dough samples were measured; that is, GLC was carried out without hydrolysis of AX (**Table 2**). The amounts of both free arabinose and xylose in aqueous extract increased during storage of refrigerated doughs. Immediately after mixing and after 34 days of storage, free arabinose content equaled 0.01 and 0.37%, respectively, whereas free xylose contents were 0.00 and 0.17%, respectively. The increase in free xylose was probably due to wheat flour endogenous xylosidase activity, present in the dough.

The percentage of monosaccharides originating from AX polymer in aqueous extracts was calculated as the difference in percent monosaccharides analyzed with GLC with and without the hydrolysis step and was corrected for arabinoses originating from AG. Therefore, % $AX_{polymer}$ = 0.88 × [(%$arabinose_{with\ hydrolysis}$ − % $arabinose_{without\ hydrolysis}$ − 0.7% $galactose_{cor}$) + (%$xylose_{with\ hydrolysis}$ − % $xylose_{without\ hydrolysis}$)].

After 0, 3, 6, 16, 24, and 34 days of storage, 0.84, 1.36, 1.30, 1.09, 0.94, and 0.83% AX polymer was found, respectively. Again, a decrease was measured after 3 days of storage. When the A/X ratio of AX polymer in aqueous extracts was calculated, a decrease from 0.47 immediately after mixing to 0.46, 0.42, 0.41, 0.38, and 0.33 after 3, 6, 16, 24, and 34 days of storage was seen, respectively.

Moreover, AX polymers in the residue, obtained after centrifugation of aqueous extracts and calculated by difference between total monosaccharide content and monosaccharide content in aqueous extracts, had A/X ratios varying from 0.62 immediately after mixing to 0.25 after 34 days of mixing. These low A/X ratios confirmed the hypothesis that AX polymers had become insoluble after prolonged storage: during the first days of storage, 60% of flour WU-AX had been solubilized (total AX content of Meunier flour is 1.93%; WE-AX content totals 0.69%). Due to arabinofuranosidase action, WU-AX, WE-AX, and S-AX had become less soluble. Even more, AX solubilized after 3 days of storage had lost arabinose side chains and had become more susceptible to aggregation with other low A/X ratio AX polymers. As a consequence, the content of AX in dough extracts had decreased.

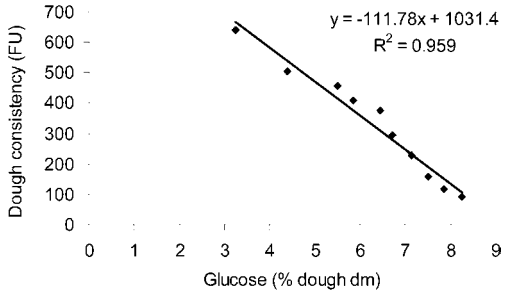

Figure 4. Dough consistency (measured after 2 min of mixing in the farinograph, farinograph units, FU) of refrigerated dough, stored for 0 and 8 h and 1, 2, 3, 6, 10, 16, 24, and 34 days plotted against the glucose content (percent dough dry matter) of aqueous extracts of the refrigerated dough.

In contrast to AX, glucose and protein contents in aqueous extracts of doughs increased continuously with extended dough storage. Glucose increased from 3.26% of dough weight to 8.26%, whereas the protein content increased from 1.45 to 2.45%. It is of note that a linear correlation was found between the increase in glucose content in aqueous extracts of doughs and the decrease in farinograph consistency of a dough (**Figure 4**). No correlation was found with the protein content.

HPSEC. Figure 5 illustrates the formation of S-AX with HPSEC apparent molecular weights between 50 and 800 kDa during the first 2 days of storage. An increase in AX with lower apparent molecular weight was noticed as well. The formation of high molecular weight (HMW) S-AX was, in all probability, caused by solubilization of WU-AX by endogenous endoxylanases, whereas the increase in LMW-AX content was probably caused by hydrolysis of HMW-S-AX and HMW-WE-AX by endogenous endoxylanases as well. During the next days, less solubilization and more degradation of S-AX and WE-AX were noticed (**Figure 5**). After 34 days of storage, almost all HMW-S-AX and HMW-WE-AX were degraded and only AX with low apparent molecular weight was present in the dough.

Viscosity. In accordance with the results obtained with GLC and HPSEC, the specific viscosity of aqueous extracts from lyophilized doughs increased during the first 2 days of storage **(Figure 6)**. This increase could be ascribed to the solubilization of AX with relatively high molecular weight. In the period between 3 and 10 days of storage, degradation of S-AX and WE-AX was responsible for a decrease in viscosity. However, the extracts remained more viscous than the reference extract (i.e., the aqueous solution of the dough immediately after mixing). Further storage led to degradation of S-AX and WE-AX and subsequently to removal of AX with high apparent molecular weight in the extracts and formation of small AX fragments. As a result, the viscosity of the extracts decreased continuously.

Characterization of Digested Proteins by SDS-PAGE. As seen in **Figure 7**, small changes in protein quantity during the

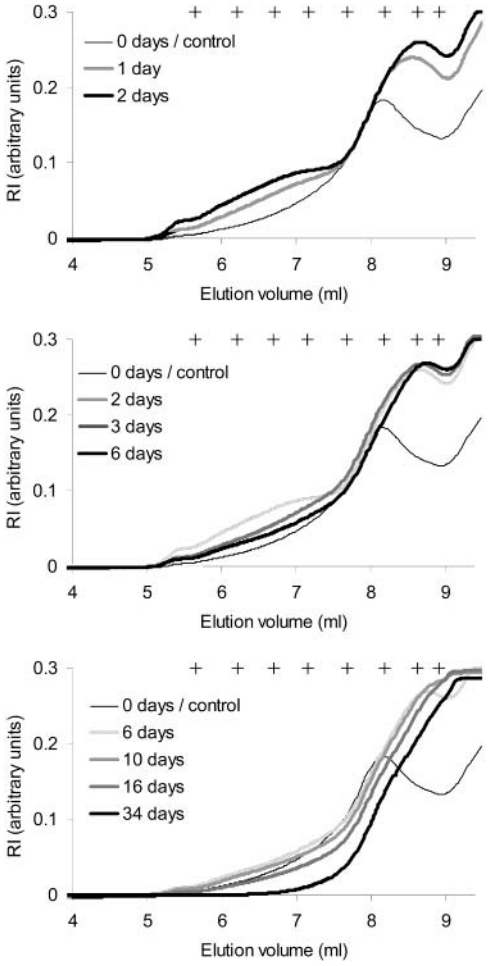

Figure 5. HPSEC molecular weight profiles of AX in aqueous extracts of dough lyophilized after storage for 0, 1, 2, 3, 6, 10, 16, and 34 days. Molecular weight markers from left to right are 788, 404, 212, 112, 47.3, 22.8, 11.8, and 5.9 kDa. The column was a Shodex SB-804 HQ.

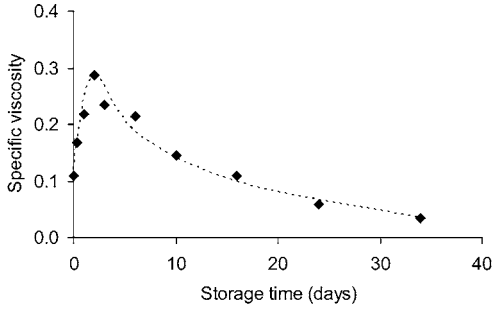

Figure 6. Specific viscosity of aqueous extracts of doughs lyophilized after storage for 0 and 8 h and 1, 2, 3, 6, 10, 16, 24, and 34 days.

storage of refrigerated doughs occurred. The levels of HMW fragments, with MW ranging from 90 to 120 kDa, slightly decreased when dough was stored for 6 or 16 days (lanes 3 and 4, respectively) compared to a dough immediately after mixing (lane 2). The decrease in HMW fragments was more pronounced, even though still small, in a dough after storage for 34 days (lane 5). Small differences in low molecular weight (LMW) fragments were seen as protein bands around 40 kDa became more visible. Around 15 kDa, the formation of a new protein band could be noticed.

The same observations could be made in the study of the HPSEC profile of SDS-soluble proteins of lyophilized doughs. A small decrease in HMW glutenin quantity and a small increase in small protein fractions was seen (results not shown).

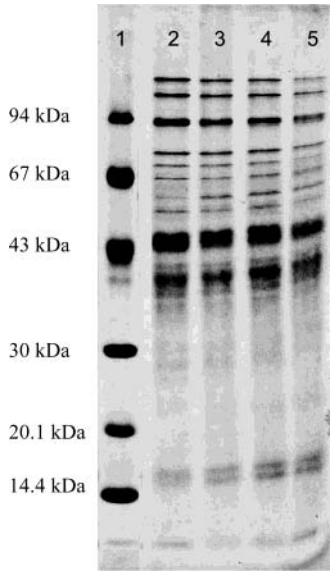

Figure 7. SDS-PAGE patterns of digested gluten polymers from lyophilized dough pellets as a function of storage time. Doughs were stored for 0 days (immediately after mixing) and 6, 16, and 34 days (lanes 2–5). Lane 1 contains molecular weight markers.

Although small changes in gluten subunits can have significant effects on dough rheology, we assume, on the basis of the above and the results presented by Atwell (*13*) and Poulsen and Sørensen (*14*), that if gluten interferes in the development of dough syrup, it does so to a much smaller extent than AX.

DISCUSSION

Although of clear importance for the refrigerated dough industry, no peer-reviewed papers describing the phenomenon of dough syruping were found. Two previous experiments, found in the patent literature (*13*, *14*), suggest that AX are involved in the development of dough syrup. More particularly, dough syruping can be diminished by adding xylan (as competitive substrate for degradation of AX by endoxylanases) or by adding an endoxylanase inhibitor. The inventors state that the formation of dough syrup can possibly be explained on the basis of the capacity of AX to hold a large amount of water in dough. When degraded by endoxylanases, AX lose their water-holding capacity. This in turn leads to free liquid inside the dough and is responsible for syruping.

It was our goal to contribute to the state of the art by investigating the AX population during refrigerated dough storage.

The quantitative results show three different phases in dough syruping: in a first period, no syrup development is observed (the first 2 days); in a second phase—from 3 days to 16 days of storage—dough syrup increased; and in a last period, the maximum level of syruping is reached. We will discuss each period separately.

First Phase: No Apparent Syrup Development. Although there was a large decrease in farinograph dough consistency from 0 to 2 days of storage, no syrup development was measured. During these 2 days, WU-AX were solubilized due to endogenous endoxylanases present in wheat flour. This led to an increase in the level of HMW-S-AX (measured with GLC and HPSEC) and could be a reason for the decrease in farinograph dough consistency. Dough syrup could not be measured, probably because the water-holding capacities of HMW-S-AX and HMW-WE-AX in dough were high enough to counteract

the loss of water-holding capacity by degradation of WU-AX. Indeed, the water-holding capacity of WE-AX is lower than that of WU-AX, but WE-AX can still hold a large amount of water in dough (*5*, *6*). Moreover, the formation of HMW-S-AX increased the viscosity of aqueous dough extracts significantly, which can be a potential additional cause for a delay in dough syruping.

It is clear that the speed of this process determines the start of apparent dough syrup development. The more slowly WU-AX are solubilized and HMW-S-AX and HMW-WE-AX are degraded, the longer syrup development will be retarded. This is most probably the reason for the fact that no syrup development was seen when endoxylanase inhibitors were added to refrigerated doughs (*14*).

Second Phase: Start of Apparent Syrup Development. Dough syruping started after 3 days of storage and increased continuously up to 16 days of storage. During this period, both solubilization and degradation of S-AX and WE-AX took place, with the emphasis on degradation of AX. Free arabinoses were released from the xylan backbone of AX, probably due to arabinofuranosidases present in the dough. This resulted in two actions: a decreased solubility of AX and increased degradation of AX by endoxylanases.

AX solubility decreased because unsubstituted regions in the xylan backbone facilitate coagulation of two or more AX polymers (*8*), which decreased the levels of HMW-S-AX and HMW-WE-AX and the viscosity of aqueous extracts. It was probably also the reason for a lowered water-holding capacity in the dough. Both effects probably stimulated dough syruping.

A second action that took place due to arabinofuranosidases was that unsubstituted regions in AX were more easily attacked by most endoxylanases. Because of that, degradation of HMW-S-AX and HMW-WE-AX increased and the water-holding capacity of the dough decreased. As a result, syrup developed.

Third Phase: Maximum Syrup Development. In a third phase, dough syruping reached its maximum. In a period of 18 days, from 16 to 34 days of storage, syrup increased only from 20.8 to 21.5%. However, carbohydrate analysis with GLC and HPSEC showed that degradation of AX to small fragments had continued and that AX polymers had continued to lose solubility. That no further syrup development had occurred is probably due to the fact that the water-holding capacity of AX was already at its lowest level at 16 days of storage, even if not all AX polymers had been fully degraded. Further degradation of AX had, consequently, no effect, neither on the water-holding capacity nor on dough syruping.

The loss in farinograph dough consistency also diminished, probably because a maximum decrease had been reached. Dough consistency after 34 days of storage was ~15% of the consistency immediately after mixing.

It is, however, of note that a linear correlation was found between the increase in glucose in aqueous extracts of doughs and the decrease in farinograph consistency. A possible explanation is that the release of glucose was caused by degradation of damaged starch with, consequently, loss of water-holding capacity (with the knowledge that damaged starch is also responsible for a part of the water-holding capacity of the dough). Although this loss seems to have affected farinograph consistency, no apparent correlation was found between the glucose content in aqueous extracts of doughs and dough syruping. Further research is necessary.

Because no correlation was found between the protein content in aqueous extracts of refrigerated doughs, neither with dough syruping nor with dough farinograph consistency, and taking into account the results obtained with the addition of xylan (*13*) and xylanase inhibitors (*14*), we assume that the role of the proteins is much less important than the role of AX and glucose.

Concerning the HPSEC and viscosity measurements, more or less the same results—but on a much shorter time scale—were obtained in bread-making experiments (*21*). Adding *Bacillus subtilis* endoxylanase to flour resulted in increased viscosity of bread samples when low dosages were used but decreased viscosity when the enzyme dosage was raised 10-fold. Adding *Aspergillus aculeatus* endoxylanase—which preferentially degrades WE-AX—resulted in an immediate decrease of viscosity, even at low enzyme dosage.

On this basis, it is tempting to speculate that the enzymic degradation of AX during storage is caused by xylanase action that preferentially solubilizes WU-AX.

In summary, when analyzing dough during refrigerated storage, we found solubilization and degradation of AX by endogenous endoxylanases to go hand in hand with dough syruping. HPSEC profiles in combination with syrup measurements clearly showed that AX degradation (partially accelerated due to arabinofuranosidase action) accompanied the development of dough syrup. The loss of water-holding capacity and the decrease in viscosity, caused by degradation of AX, probably lie at the base of the formation of dough syrup. Loss of dough consistency was probably influenced by the same process as well. Therefore, the use of endoxylanase inhibitors can be an effective tool in the refrigerated dough industry.

ABBREVIATIONS USED

AG, arabinogalactan(s); AX, arabinoxylan(s); A/X, arabinose-to-xylose ratio; dm, dry matter; FU, farinograph units; GLC, gas−liquid chromatography; HMW, high molecular weight; HPSEC, high-performance size exclusion chromatography; S-AX, solubilized arabinoxylan(s); TFA, trifluoroacetic acid; WE-AX, water-extractable arabinoxylan(s); WU-AX, water-unextractable arabinoxylan(s).

LITERATURE CITED

(1) Mares, D. J.; Stone, B. A. Studies on wheat endosperm. I. Chemical composition and ultrastructure of the cell walls. *Aust. J. Biol. Sci.* **1973**, *26*, 793−812.

(2) Iiyama, K.; Lam, T. B. T.; Stone, B. A. Covalent cross-links in the cell wall. *Plant Physiol.* **1994**, *104*, 315−320.

(3) Udy, D. C. The intrinsic viscosities of the water soluble components of wheat flour. *Cereal Chem.* **1956**, *33*, 67−74.

(4) Udy, D. C. Interactions between proteins and polysaccharides of wheat flour. *Cereal Chem.* **1957**, *34*, 37−46.

(5) Jelaca, S. I.; Hlynca, I. Water binding capacity of wheat flour crude pentosans and their relation to mixing characteristics of dough. *Cereal Chem.* **1971**, *48*, 211−222.

(6) Kim, S. K.; D'Appolonia, B. L. Bread staling studies. III. Effect of pentosans on dough, bread and bread staling rate. *Cereal Chem.* **1977**, *54*, 225−229.

(7) Beldman, G.; Osuga, D.; Whitaker, J. R. Arabinoxylan degrading enzymes of wheat flour. Effects on the quality of doughs and breads. In *Proceedings of the 2nd International Conference on the Impact of Food Research on New Product Development*, 2nd ed.; Ali, R., Barlow, P. J., Whitaker, J. R., Eds.; University of Karachi: Karachi, Pakistan, 1995; pp 173−188.

(8) Andrewartha, K. A.; Philips, D. R.; Stone, B. A. Solution properties of wheat-flour arabinoxylans and enzymically modified arabinoxylans. *Carbohydr. Res.* **1979**, *77*, 191−204.

(9) Izydorczyk, M. S.; Biliaderis, C. G.; Bushuk, W. Oxidative gelation studies of water-soluble pentosans from wheat. *J. Cereal Sci.* **1990**, *11*, 153−169.

(10) Geissmann, T.; Neukom, H. On the composition of the water-soluble wheat flour pentosans and their oxidative gelation. *Food Sci. Technol.-Leb.* **1973**, *6*, 54−62.
(11) Figueroa-Espinoza, M. C.; Rouau, X. Oxidative cross-linking of pentosans by a fungal laccase and horseradish peroxidase: mechanism of linkage between feruloylated arabinoxylans. *Cereal Chem.* **1998**, *75*, 259−265.
(12) Izydorczyk, M. S.; Biliaderis, C. G. Effect of molecular size on physical properties of wheat arabinoxylan. *J. Agric. Food Chem.* **1992**, *40*, 561−568.
(13) Atwell, W. A. Method for reducing syruping in refrigerated doughs. U.S. Patent 5,792,499, 1998.
(14) Poulsen, C. H.; Sørensen, J. F. Process of forming a refrigerated dough. Patent Appl. WO 01/52657, 2001.
(15) Gebruers, K.; Debyser, W.; Goesaert, H.; Proost, P.; Van Damme, J.; Delcour, J. A. *Triticum aestivum* L. endoxylanase inhibitor (TAXI) consists of two inhibitors, TAXI I and TAXI II, with different specificities. *Biochem. J.* **2001**, *353*, 239−244.
(16) American Association of Cereal Chemists. AACC Method 26-31. *Approved Methods of the AACC*, 10th ed.; AACC: St. Paul, MN, 2000.
(17) American Association of Cereal Chemists. AACC Method 44-15A. *Approved Methods of the AACC*, 10th ed.; AACC: St. Paul, MN, 2000.
(18) American Association of Cereal Chemists. AACC Method 08-12. *Approved Methods of the AACC*, 10th ed.; AACC: St. Paul, MN, 2000.
(19) Association of Official Analytical Chemists. AOAC Method 990.03. *Official Methods of Analysis*, 16th ed.; AOAC: Washington, DC, 1995.
(20) American Association of Cereal Chemists. AACC Method 54-21. *Approved Methods of the AACC*, 10th ed.; AACC: St. Paul, MN, 2000.
(21) Courtin, C. M.; Gelders, G. G.; Delcour, J. A. Use of two endoxylanases with different substrate selectivity for understanding arabinoxylan functionality in wheat flour breadmaking. *Cereal Chem.* **2001**, *78*, 564−571.
(22) Loosveld, A.-M. A.; Grobet, P. J.; Delcour, J. A. Contents and structural features of water-extractable arabinogalactan in wheat flour fractions. *J. Agric. Food Chem.* **1997**, *45*, 1998−2002.
(23) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.
(24) Bleukx, W.; Roels, S. P.; Delcour, J. A. On the presence and activities of proteolytic enzymes in vital wheat gluten. *J. Cereal Sci.* **1997**, *26*, 183−193.

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