JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** *53,* 7623−7629 **7623**

Evidence for the Involvement of Arabinoxylan and Xylanases in Refrigerated Dough Syruping

CHRISTOPHE M. COURTIN,* WOUTER GYS, KURT GEBRUERS, AND JAN A. DELCOUR

Katholieke Universiteit Leuven, Laboratory of Food Chemistry, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium

The relationship between syruping in refrigerated doughs upon prolonged storage and different aspects of arabinoxylan (AX) hydrolysis was investigated using *Triticum aestivum* xylanase inhibitor (TAXI) and different xylanases in the dough formula. Dough characteristics were evaluated with strong emphasis on the AX population and its fate as a function of storage time. Selective reduction of part of the flour endogenous xylanase activity in dough by added TAXI reduced dough syruping after 12 and 20 days of storage by 50%, providing straightforward evidence for the involvement of xylanases and, thus, AX in the syruping phenomenon. Addition of xylanases with different inhibitor sensitivities [an inhibition-sensitive *Bacillus subtilis* xylanase (XBS_i) as well as a noninhibited mutant (XBS_{ni}) thereof] to dough confirmed the importance of xylanases in dough syruping, on one hand, and the power of wheat flour TAXI to constitute a significant barrier against xylanase-mediated dough syruping, on the other hand. Use of xylanases with different substrate selectivities [an *Aspergillus aculeatus* xylanase (XAA) versus XBS_{ni}] showed degradation of water-extractable AX (WE-AX) and solubilized AX to low molecular weight molecules rather than the conversion of water-unextractable AX (WU-AX) to high molecular weight water extractable components to be the main factor influencing dough syruping.

KEYWORDS: Arabinoxylan; xylanase; xylanase inhibitor; refrigerated dough; syruping

INTRODUCTION

The refrigerated dough industry offers a wide range of dough products, which ideally yield end products with the same texture, appearance, and taste as their fresh equivalents. This requires the use of correct flour, yeast or chemical leaveners, shortening, sugars, gums, and stabilizers.

One of the problems encountered with refrigerated doughs is syruping, i.e., the migration of a brownish liquid to the dough surface after prolonged dough storage (*1*−*4*). The main measures the industry takes today to prevent dough syruping are based on empirical grounds and consist of the right choice of flour together with addition of agents increasing the water-holding capacity of the dough (*2*). Syrup development is believed to originate from the natural constituents of the flour, namely the arabinoxylans (AX) and the endogenously present xylanases (*2*, *4*, *5*).

AX are cereal cell wall polysaccharides consisting of a backbone 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. Wheat flour contains approximately 1.5−2.5% AX of which 25−30% are water-extractable AX (WE-AX) (*6*) while the remainder are water-unextractable AX (WU-AX). While no major structural differences exist between the two different classes of AX in wheat endosperm flour (*7*−*9*), WU-AX and WE-AX differ in physicochemical (*6*, *10*, *11*) and functional properties (*12*).

Xylanases (EC 3.2.1.8), mainly classified in glycosyl hydrolyze families (GHF) 10 and 11 (*13*), impact the physicochemical properties of AX. They can solubilize WU-AX to form high molecular weight solubilized AX (HMW-S-AX) and can degrade HMW-WE-AX and HMW-S-AX to LMW-WE-AX and LMW-S-AX. Solubilization of WU-AX increases viscosity, while degradation of WE-AX and S-AX decreases it. Also, WU-AX solubilization induces loss of water-holding capacity.

Xylanases can, among others, differ in substrate specificity and substrate selectivity. The former implies that they differ in their relative capabilities to degrade the unsubstituted or arabinose-substituted parts of the xylan backbone (*14*). The latter indicates that they have a preference for either WE-AX degradation or WU-AX solubilization.

The most important property of AX in dough that has been hypothesized to influence refrigerated dough syruping is probably their water-holding capacity. Although it included no data on AX degradation or enzyme activity, a patent text mentioned that AX degradation by xylanases causes a loss in water-holding capacity of the dough and would be responsible for dough syruping (*2*). Another patent text showed that addition of

* Corresponding author. Tel: + 32 16 32 16 34. Fax: + 32 16 32 19 97. E-mail: christophe.courtin@biw.kuleuven.be.

10.1021/jf051161v CCC: $30.25
Published on Web 08/20/2005

xylanase inhibitor reduces dough syruping, indicating the importance of flour xylanases on dough syruping (*4*). It was further also demonstrated that reduction of xylanase and amylase enzyme activities in flour by debranning wheat prior to milling (*15*) reduces dough syruping (*16*), implying the importance of either one or both types of these enzymes in dough syruping.

Our earlier work (*5*, *16*) suggested, but did not substantiate, that AX contribute to dough syruping. Hence, straightforward evidence for involvement of AX in dough syruping or lack thereof is needed. To contribute to insight in this area, we here use addition of TAXI (*Triticum aestivum* xylanase inhibitor) (*17*) and xylanases with different inhibitor sensitivities to establish the influence of xylanases (and thus AX) and TAXI, respectively, on dough syruping, while xylanases with different substrate selectivity were used to specifically analyze the contribution to dough syruping of WU-AX solubilization, on one hand, and WE-AX/S-AX degradation, on the other hand.

MATERIALS AND METHODS

Materials. *Aspergillus aculeatus* xylanase (XAA) [Shearzyme 500L, NCBI accession number AAE69552, (*18*)] was from Novozymes (Bagsvaerd, Denmark). *Bacillus subtilis* xylanase, which is inhibited by TAXI (XBS_i) [Grindamyl H640, XynA_BACSU, SWISS-PROT entry P18429, (*19*)], and *Bacillus subtilis* xylanase not inhibited by TAXI (XBS_{ni}) [obtained by site-directed mutagenesis and only differing in a few amino acids (D11F/R122D) from XBS_i] was from Danisco (Brabrand, Denmark). Whereas XBS_i and XBS_{ni} have a greater selectivity for WU-AX, XAA rather degrades WE-AX and S-AX and is insensitive to inhibition by TAXI (*17*).

Purified TAXI (*20*) was dissolved in 1.7 mL of sodium phosphate buffer (25 mM, pH 6.0). Its concentration (0.22 mg/mL) was determined spectrophotometrically at 280 nm using a specific extinction coefficient of 775 mAU/(mg/mL). The specific inhibition activity of TAXI against XBSi amounted to 3640 InhU/mg protein (*20*), where one InhU is defined as the amount of inhibitor per gram sample that inactivates 50% of a given activity ($E_{590} = 1.0$) of xylanase under the conditions described by Gebruers et al. (*21*).

Petrus, a wheat variety of moderate bread-making quality, was from Clovis Matton (Kerkhove, Belgium). After conditioning to 15.5% (24 h) and water addition (0.5%) 30 min prior to milling, flour (milling yield 70.6%) was produced with a Bühler MLU-202 laboratory mill (Uzwil, Switzerland) (*22*). It had moisture (*23*), ash [dm basis (*24*)], and protein contents [dm basis (*25*)] of 12.9, 0.59, and 11.8%, respectively.

Petrus flour contained 0.51% WE-AX and 1.80% WU-AX, as determined by gas−liquid chromatography (see below). Water absorption to reach 700 FU on a Brabender Farinograph "E" (Duisburg, Germany) was 57.9% (on 14% moisture basis) (*26*).

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

Xylanase Activity Determination. Apparent xylanase activity in Petrus flour was measured using the Xylazyme-AX method (Megazyme, Bray, Ireland). To that end, flour (1:5 w/v) was suspended in sodium phosphate buffer (25 mM, pH 6.0) and extracted for 1 h at 6 °C. After centrifugation [Beckmann (Fullerton, CA) J2−21 centrifuge, 3000 *g*, 10 min, 6 °C], the supernatant (1.0 mL) was preincubated at 40 °C for 10 min before adding an AZCL-AX tablet (Megazyme, Bray, Ireland). After incubation at 40 °C for 17 h, the reaction was stopped with 1.0% TRIS solution (10 mL). Following filtration, the E_{590} values (extinction at 590 nm) were measured against a control, prepared by incubating the solutions without the substrate tablet for 17 h at 40 °C and addition of the substrate tablet after adding 1.0% TRIS solution to the extract. Correction was made for nonenzymatic color release from the AZCL-AX tablets. 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.

Because of the presence of xylanase inhibitors in wheat, it is reasonable to expect that the xylanase activity, measured as above, is underestimated. Indeed, during aqueous extraction a variable proportion of xylanases is expected to bind to the inhibitors and is not measured in this or any other readily available assay. Thus, the measured xylanase activities must be regarded as apparent.

Xylanase activities of suitable diluted enzyme solutions (1.0 mL) were determined in the same way.

Dough Preparation. Dough was prepared at a Brabender Farinograph dough consistency of 700 FU using a dough formula containing 10.0 g of Petrus flour (14% moisture base), 0.18 g of salt, 5.69 mL of water containing sodium azide (0.06% w/v, Acros Organics, Geel, Belgium), and 100 μL sodium phosphate buffer (25 mM, pH 6.0) or the same amount of buffer containing xylanase or TAXI. In this way, seven different dough series were prepared: a control dough series, and dough series containing 80 InhU TAXI, 0.3 XU XBS_i or 0.3 XU XBS_{ni}, or 0.1 XU, 0.3 XU, and 0.6 XU XAA.

Dough was mixed in a 10-g pin mixer (National Mfg., Lincoln, NE) for 3.5 min, sheeted, molded, and stored in small, polyethylene jars (27.0 mm diameter, 52.6 mm height, Nalgene, Rochester, NY) for 0 h (analysis immediately after mixing), 1, 3, 5, 8, 12, 16, 20, 30, and 40 days at 6 °C except for TAXI-enriched doughs, which were stored for 0 h and 12, 20, and 40 days. The jars were closed with a snap cap. For each series and each point in time, four identical doughs were made: two doughs were used to measure dough syruping and two were frozen in liquid nitrogen, 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 (*2*) and described in Gys et al. (*5*). To that end, the two stored doughs, prepared for measurement of dough syruping, were each divided in four pieces of approximately 3.0−4.0 g. The accurately weighed dough pieces were centrifuged in a centrifuge tube of 30 mL (height, 92 mm; diameter, 25.5 mm) in a Beckmann J2-21 centrifuge (JA 20 rotor, 22 000*g*, 20 °C, 30 min). After centrifugation, the tubes were inverted to allow liquid to drain. After 2 h, any liquid adhering to the sides of the centrifuge tube was removed with a cotton bud. 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. As outlined above, for each time point, dough analyses were made on two separate doughs; thus, each value for dough syrup recorded was the average of eight measurements.

The coefficient of variation, defined as the ratio of the standard deviation and the mean of the obtained results, was lower than 10%.

Extraction of Dough Samples. Water extracts of doughs were prepared as described by Courtin et al. (*12*). Lyophilized and ground dough samples (5.0 g), two for each series and each point in time, 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 (10 000*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 (10 000*g*, 15 min, 4 °C) to remove heat-coagulated protein material. The supernatant was frozen until further analysis.

Carbohydrate Analysis. The monosaccharide compositions of aqueous extracts and total monosaccharide composition of the lyophilized and ground dough samples after hydrolysis were determined by gas−liquid chromatography (GLC) of alditol acetates (*27*). Supernatants (2.5 mL) and flours (approximately 0.1 g, accurately weighed) were hydrolyzed at 110 °C for 1 h with 2.5 mL of 4.0 M trifluoroacetic acid (TFA) and for 120 min 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 acid anhydride (*27*), 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 autosampler, splitter injection

port (split ratio 1:20), and flame ionization detector. The carrier gas was He. Separation was at 225 °C, and injection and detection were at 270 °C.

The monosaccharide compositions of aqueous extracts were expressed as a percentage of dough dry matter (% dough dm), and the total monosaccharide composition of the flours was expressed as % flour dm. Apparent solubilization of total xylose was calculated as % solubilized xylose = [% $\text{xylose}_{(\text{dough extract})}$ − % $\text{xylose}_{(\text{flour extract})}$]/[% $\text{xylose}_{(\text{flour})}$ − % $\text{xylose}_{(\text{flour extract})}$]. Apparent solubilization of total arabinose (i.e. arabinose coupled to the xylan backbone) was calculated in the same way. Samples, two for each series and each point in time, were analyzed in duplicate. The coefficient of variation, defined as the ratio of the standard deviation over the mean of the values, was lower than 5%.

High-Performance Size-Exclusion Chromatography. Apparent MW distributions of dough aqueous extracts were studied by high-performance size-exclusion chromatography (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 MW of 788, 404, 212, 112, 47.3, 22.8, 11.8, and 5.9 k were used as MW 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. The relative viscosity (η_{rel}) was the flow times of supernatant divided by that of deionized water under the same conditions. Results are expressed as specific viscosity (η_{sp}), which was calculated as $\eta_{sp} = \eta_{rel} - 1$.

RESULTS

Dough Series. The wheat flour contained 0.03 XU/g flour, which implied that the total apparent endogenously present xylanase activity in the control dough (10 g) amounted to 0.3 XU. We added the same amount of enzyme, i.e., 0.3 XU of XBS_i and XBS_{ni}, to the different dough series. In addition, XAA was added in increasing amounts (0.1, 0.3, and 0.6 XU). Finally, a dough series containing TAXI was prepared by adding 100 μL of the TAXI solution, i.e., 80 InhU of TAXI.

Dough Syruping. Figure 1 shows the dough syruping as a function of storage time for all dough series. Dough syruping in the control dough series manifested itself at day 8 (1.3% dough syrup) and reached a maximum of 18.8% after 40 days of storage, similar to earlier observations (*5*).

Addition of TAXI almost halved dough syruping after 12 and 20 days of storage. This is remarkable, since not all xylanases in the dough are inhibited by TAXI (see below). This result provides straightforward evidence that TAXI-sensitive xylanases have a major impact on dough syruping. It equally shows that AX are a main player in dough syruping.

Analysis of the impact of xylanases with different inhibitor sensitivities also showed remarkable results. XBS_{ni} already led to 9.6% of dough syrup after only 3 days of storage and 17.2% syrup after 5 days of storage. Since the difference between this dough series and the control series (where no syrup development was found after 5 days of storage) could be solely ascribed to addition of XBS_{ni} and, thus, to AX solubilization and degradation, these results again show the importance of the AX hydrolyzing activity in dough syruping. After 40 days of storage, more than 25% dough syrup was found.

When XBS_i was added to the dough, the onset in dough syruping was slightly retarded. These results showed the impact of both xylanase as well as endogenous TAXI on dough syruping (see also below).

Comparison of syruping in doughs containing 0.3 XU of the TAXI-insensitive XAA or XBS_{ni} showed that doughs with

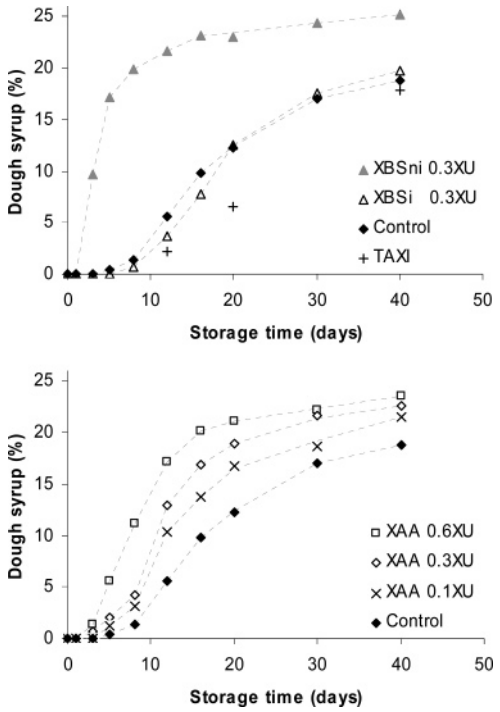

Figure 1. Dough syruping (% dough weight) as a function of storage time in refrigerated control dough and doughs with TAXI, XBS_{ni}, XBS_i, and different amounts of XAA added. The coefficient of variation for each of the data points is lower than 10%.

XBS_{ni} released dough syrup faster and to a larger extent than did doughs with XAA.

Analysis of the level of dough syrup caused by increasing dosages of XAA showed that the time to onset decreased and the speed of dough syruping increased with increasing dosages. Furthermore, the level of dough syrup at day 40 for all three dosages was higher than in the control dough series and amounted to 21.5%, 22.6%, and 23.6% for 0.1, 0.3, and 0.6 XU XAA, respectively.

Carbohydrate Analysis. The apparent degrees of solubilization of xylose and arabinose in the different doughs (**Figure 2**) showed almost equal trends, i.e., increased solubilization during the first 20 days, after which a plateau was reached. The latter contrasts with results by Gys et al. (*5*, *16*), where a decrease in the level of apparent solubilized xylose after longer storage was found, due to dearabinosylation and loss of solubility.

Addition of TAXI to a dough had no marked impact on apparent arabinose or xylose solubilization. Since addition of TAXI drastically reduced syruping, and since AX solubilization and degradation were shown to go hand in hand, these results seemed somewhat surprising. However, it is of note that the GLC analysis provides no information on the distribution of HMW and LMW AX fragments in the population.

Addition of 0.3 XU XBS_{ni} led to a remarkable 63.1% solubilization of AX by the end of mixing. A maximum solubilization of 93.4% was reached after 5 days of storage. At the same dosage, XBS_i increased xylose solubilization from 7.3% immediately after mixing up to 71% after 40 days of storage (**Figure 2**). This again showed the importance of xylanases in dough syruping and the impact of endogenously present TAXI on the activity of AX degrading enzymes. Under the present experimental conditions, uninhibited xylanase (XBS_{ni}) immediately solubilized and degraded AX chains, while

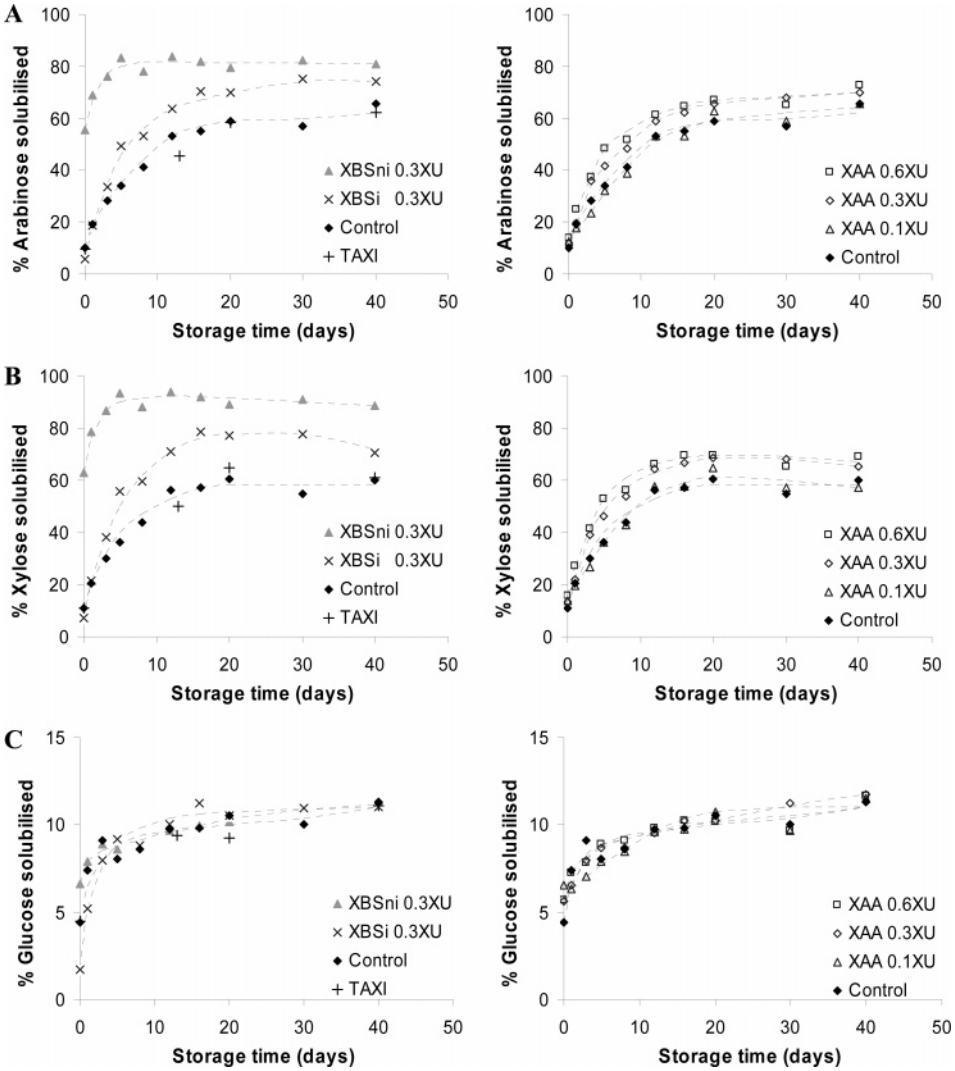

Figure 2. Apparent solubilization of total (**A**) arabinose, (**B**) xylose, and (**C**) glucose as a function of storage time in refrigerated control dough and doughs with TAXI, 0.3 XU XBS_{ni}, 0.3 XU XBS_i, and different amounts of XAA (0.1, 0.3, and 0.6 XU) added. The coefficient of variation for each of the data points is lower than 5%.

addition of TAXI-sensitive xylanase (XBS_i) resulted in a smaller shift in AX population than in the control dough (see below).

Increased concentrations of XAA in dough led to increased WU-AX solubilization. Whereas in the control dough maximum xylose solubilization after 40 days was 60%, addition of 0.6 XU XAA increased this level up to 69%. The smallest concentration of XAA (0.1 XU), however, did not markedly impact AX solubilization.

That 0.6 XU XAA and 0.3 XU XBS_i led to different dough syruping profiles while the levels of arabinose and xylose solubilization were more or the less the same shows that not only the level of S-AX in dough is responsible for dough syruping but also the distribution of HMW and LMW AX fragments in the population (see below).

No major differences in glucose solubilization between the different dough series were observed (**Figure 2**). Glucose solubilization increased from approximately 4.5% immediately after mixing to approximately 11.5% after 40 days of storage.

High-Performance Size-Exclusion Chromatography. When analyzing the HPSEC profiles of the aqueous extracts of the doughs of the different series (profiles not shown), it was clear that in the control dough series, during the first 5 days of storage, the level of the extractable AX fraction with a MW exceeding 400 k increased. This is most likely due to solubilization of WU-AX to HMW-S-AX. Further storage showed decreased HMW-S-AX and HMW-WE-AX levels, while the profile in the low MW region (LMW-S-AX and LMW-WE-AX) continuously shifted to lower MW, due to degradation of S-AX and WE-AX by flour xylanases.

Addition of TAXI led to more or less the same profiles, but the degradation of HMW-S-AX and HMW-WE-AX was slower.

Added XBS_{ni} solubilized a large level of WU-AX during mixing (in analogy with the GLC results). Storage of these doughs led to a continuous decrease in the levels of the high MW AX and a concomitant increase in the levels of their low MW counterparts.

Solubilization of WU-AX by XBS_{ni} was, in other words, very fast, while degradation of AX also slightly started during mixing, but was more pronounced after 5 days of storage and more.

XBS_i caused continuous solubilization of WU-AX to HMW-S-AX during the first 12 days of storage. Meanwhile, also the medium molecular weight S-AX (MMW-S-AX) and MMW-WE-AX (peak MW between 50 k and 200 k) and LMW-S-AX/LMW-WE-AX (peak MW around 20 k) fractions were enriched. Probably both solubilization and degradation took place. In each case, the processes of solubilization and degradation proceeded much slower than in the doughs where XBS_{ni} was added.

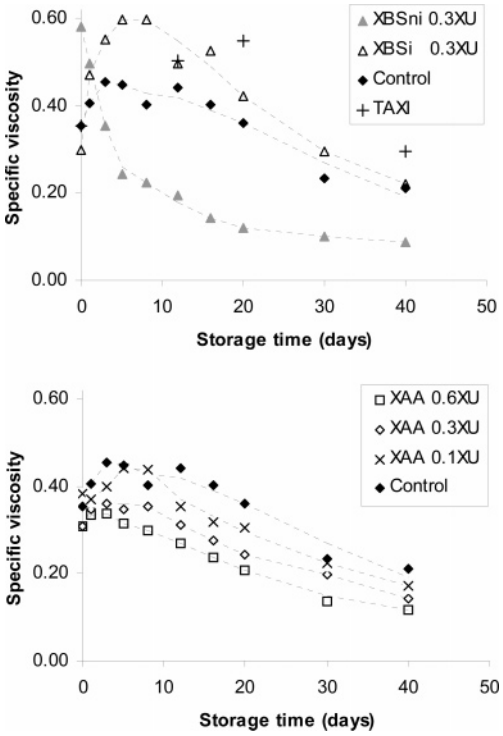

Figure 3. Specific viscosities of aqueous extracts as a function of storage time in refrigerated control dough and doughs with TAXI, XBS_{ni}, XBS_i, and different amounts of XAA added.

In contrast to XBS_{ni} and TAXI, XAA only mildly affected WU-AX solubilization. As could be expected, XAA primarily degraded WE-AX and S-AX to lower MW. This increase in LMW-S-AX/LMW-WE-AX was more pronounced in doughs with 0.3 and 0.6 XU XAA.

Viscosity Measurements. Figure 3 shows the specific viscosities of aqueous extracts of the control and TAXI- and xylanase-treated doughs. The control series showed an increase in viscosities during the first 3 days of storage, which was due to the formation of HMW-S-AX. Further storage led to a decrease in viscosity, caused by degradation of HMW-S-AX/HMW-WE-AX.

The addition of TAXI increased specific viscosities of the dough aqueous extracts up to 20 days of storage. This indicated either that the WE-AX/S-AX are of higher MW (higher viscosity) after 20 days of storage or that the WE-AX/S-AX concentration in the TAXI supplemented dough aqueous extracts is higher than in the control series. Since the arabinose and xylose solubilization in the TAXI-supplemented doughs was comparable to that in the control doughs, the higher viscosity probably results from the presence of larger MW AX molecules. Only after 20 days was a decrease noticed. The specific viscosity after 40 days of storage was still 0.3, which was the highest value of all dough series.

In XBS_{ni}-treated doughs, aqueous extracts reached maximum specific viscosity immediately after mixing (0.58), which is in agreement with the GLC and HPSEC results. Further storage of these doughs led to a continuous decrease in the specific viscosities of the dough extracts.

In contrast to what was observed with the inhibition-insensitive XBS_{ni}, XBS_i increased the specific viscosity of the dough aqueous extracts up to a value of 0.6 after 5 days of storage, which again showed the solubilizing effect of XBS_i on WU-AX. After 40 days of storage, the specific viscosity was equal to that of the control.

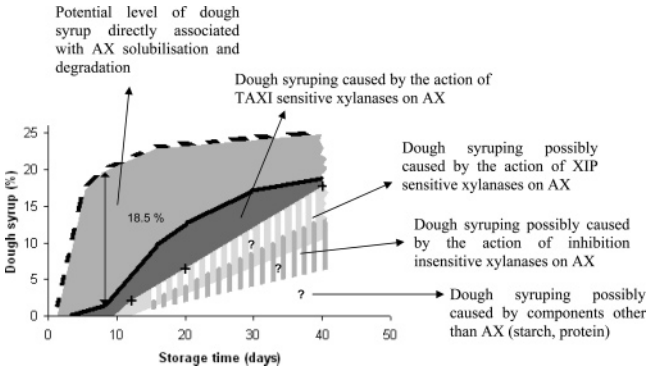

Figure 4. Dough syruping (% dough weight) as a function of storage time in refrigerated control dough (—) and doughs with TAXI (+++) and XBS_{ni} (---) added. The figure shows that AX is clearly involved in the dough syrup phenomenon.

Increasing concentrations of XAA consistently resulted in specific viscosities which were lower than those measured for the control, due to increased degradation of WE-AX/S-AX.

DISCUSSION

Previous research illustrated that AX solubilization and degradation occur simultaneously with dough syruping (*5*). Furthermore, removal of part of the xylanases and amylases in the flour (*15*) reduces AX solubilization and degradation and slows down dough syruping (*16*). However, no causal relationship between AX modification and dough syruping has been established.

We here were able to verify or deny the involvement of AX degradation in the formation of refrigerated dough syrup and to gain insight in AX-based factors affecting this process.

TAXI. Inhibition of wheat flour borne xylanases by added TAXI was clearly evidenced by viscosity measurements. Compared to the control, solubilization of WU-AX and degradation of HMW-S-AX and HMW-WE-AX was slowed. The impact on dough syruping was clear: after 12 and 20 days of storage, dough syrup was reduced by 50%, providing straightforward evidence for the hypothesis that xylanases, and thus AX, are a very important factor influencing dough syruping.

The here observed reduction in dough syruping upon addition of TAXI was achieved despite only partial inhibition of the xylanase population in the dough. Indeed, as TAXI does not inhibit GHF 10 xylanases and as all xylanases from cereals and plants identified so far belong to GHF 10 (*28*), implying that all wheat endogenous xylanases are most probably GHF 10 xylanases, only part of the xylanase population in the dough [i.e. the microbial GHF 11 xylanases that "contaminate" the flour endogenous xylanases (*15*)] is inhibited by TAXI (**Figure 4**).

It is most remarkable that endogenous TAXI is so powerful in reducing the contribution to syruping of TAXI-sensitive xylanase and, at the same time, that addition of 22 μg (80 InhU) of TAXI to 10 g of flour, which is approximately 5% of the total estimated amount of TAXI present in the dough, can reduce dough syruping. This probably indicates that not all endogenous TAXI present in the flour and dough is able to bind the xylanases present in the flour. A possible explanation for this observation is that TAXI or xylanases in the dough system lack sufficient mobility to interact with each other. The small amount of predissolved TAXI in part of the mixing water might not lack this mobility and cause inhibition of xylanases that cause syruping.

Furthermore, since TAXI is only able to inhibit the bacterial and fungal GHF 11 xylanases present in the dough, and since xylanase inhibiting protein (XIP) (*29*) is able to additionally inhibit fungal GHF 10 xylanases, addition of the latter xylanase inhibitor on top of TAXI may result in the inhibition of a broader spectrum of xylanases, thus further reducing the level of dough syruping (**Figure 4**).

Xylanases. The large difference in dough syruping between XBS_{ni}-supplemented doughs and the control dough series (a difference of 9.6% after 3 days of storage, of 17.2% after 5 days of storage, of 18.5% after 8 days of storage) can solely be ascribed to the addition of the AX-degrading enzyme and, thus, to changes in the AX population, again indicating the involvement of AX in dough syruping. It furthermore shows that, under the experimental conditions, a potential of at least 18.5% liquid loss is specifically associated with AX solubilization and degradation. **Figure 4** illustrates the involvement of AX in refrigerated dough syruping and clearly indicates the potential of AX to hold large levels of water.

In addition, the present experiments show that solubilization of WU-AX into HMW-S-AX alone has no significant impact on dough syruping. This is clearly demonstrated by two points of dough series XBS_{ni}: after mixing and after 1 day of storage, solubilization reached 63.1% and 78.3%, respectively, without causing dough syruping. In other words, solubilization of WU-AX into HMW-S-AX is not directly responsible for the loss of the water-holding capacity of the dough. The fact that XBS_{ni} causes a rapid increase in dough syruping after 3 days of storage and more is, on the contrary, caused by the rapid degradation of the formed HMW-S-AX and the native HMW-WE-AX. Indeed, HPSEC results showed degradation into MMW-S-AX/MMW-WE-AX already during the first day of storage. Increased storage time led to further degradation of HMW-S-AX/HMW-WE-AX and MMW-S-AX/MMW-WE-AX to LMW-S-AX/LMW-WE-AX and to dough syruping. Despite the specificity of the xylanase toward WU-AX, its high activity also resulted in rapid degradation of AX in solution.

This leads us to conclude that the degradation of HMW-WE-AX/HMW-S-AX to LMW-S-AX/LMW-WE-AX rather than the level of S-AX determines the onset of dough syruping.

That XBS_i did not increase dough syruping, but even reduced it during early storage, is probably due to the formation of HMW-S-AX and MMW-S-AX. The reduction of dough syruping during the first days of storage, compared to the control, indicates that HMW-S-AX have higher water-holding capacity than WU-AX. This is consistent with literature where, on one hand, similar water-holding capacities were described for WE-AX and WU-AX (*30*), while, on the other hand, the water-holding capacity of alkali-solubilized AX was found to be twice that of WE-AX (*10*) and thus, consequently, WU-AX. In this case, the higher water-holding capacity of HMW-S-AX was probably high enough to delay dough syruping. The high water-holding capacity of HMW-S-AX and HMW-WE-AX was probably only lost after degradation of the polysaccharide chain into LMW-S-AX/LMW-WE-AX. **Figure 5** schematically shows the impact of AX solubilization and degradation on dough syruping.

The difference in dough syrup profiles caused by the addition of XBS_i and XBS_{ni} again showed the importance of TAXI in flour. It is clear that the inhibitors present in flour can inhibit xylanases that are added to the dough formula, at least when they are inhibitor-sensitive.

Addition of XAA speeded up the onset and increased the level of dough syruping. Higher dosages of XAA increased AX solubilization and degradation and led to more syrup development. Solubilization of WU-AX by XAA did not slow syruping, as solubilization went hand in hand with degradation of S-AX/WE-AX to low MW fragments. Indeed, no increase in HMW-S-AX could be found when studying the HPSEC profiles.

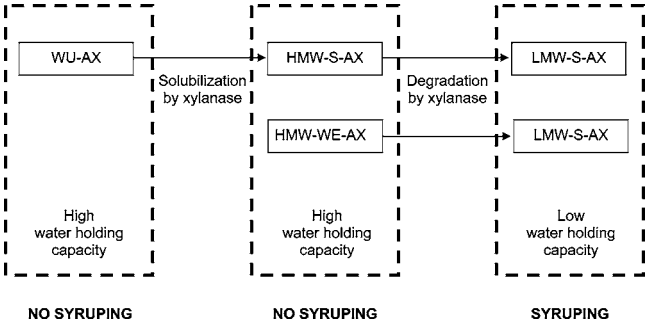

Figure 5. Schematic overview of the impact of AX solubilization and degradation on dough syruping.

In conclusion, this paper investigated the relationship between syruping in refrigerated doughs upon prolonged storage and different aspects of AX hydrolysis using TAXI and different xylanases in the dough formula. Selective elimination of part of the flour endogenous xylanase activity in dough by addition of TAXI decreased dough syruping by 50% compared to the control, providing straightforward evidence for the involvement of xylanases and thus AX in the syruping phenomenon.

Addition of xylanases with different inhibitor sensitivities to dough confirmed the importance of xylanases and AX in dough syruping, on one hand, and the power of wheat flour TAXI to constitute a significant barrier against xylanase mediated dough syruping, on the other hand.

Use of xylanases with different substrate selectivities clearly showed that not conversion of WU-AX to high MW water extractable components but degradation of WE-AX and solubilized AX to low MW molecules was the main factor influencing dough syruping.

From a practical point of view, it is of importance to analyze the flour to be used for production of refrigerated dough for both xylanase and xylanase inhibitor activity. Flours with as limited xylanase activity as possible should be chosen to prevent syruping. Xylanase inhibitors, be they endogenous or exogenous, can reduce syruping. It can be postulated that, since TAXI is not able to inhibit GHF 10 xylanases, addition of a cocktail of TAXI and XIP, which have different characteristics, could inhibit a larger and more varied range of xylanases, which in turn could reduce dough syruping to a larger extent.

ABBREVIATIONS USED

AX, arabinoxylan(s); dm, dry matter; FU, Farinograph units; GHF, glycosyl hydrolyze family; GLC, gas−liquid chromatography; HMW, high molecular weight; HPSEC, high erformance size-exclusion chromatography; LMW, low molecular weight; MMW, medium molecular weight, S-AX, solubilized arabinoxylan(s); TAXI, *Triticum aestivum* xylanase inhibitor; WE-AX, water-extractable arabinoxylan(s); WU-AX, water-unextractable arabinoxylan(s); XAA, *Aspergillus aculeatus* xylanase; XBS, *Bacillus subtilis* xylanase; XIP, xylanase inhibiting protein.

ACKNOWLEDGMENT

Helpful discussions with Jens Frisbaek Sørensen are gratefully acknowledged.

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Received for review May 19, 2005. Revised manuscript received July 14, 2005. Accepted July 18, 2005. Financial support was provided by Danisco (Brabrand, Denmark). This study was also carried out in the framework of research project GOA/03/10 financed by the Research Fund K.U.Leuven.

JF051161V