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Effects of Laccase and Xylanase on the Chemical and Rheological Properties of Oat and Wheat Doughs

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The effects of *Trametes hirsuta* laccase and Pentopan Mono BG xylanase and their combination on oat, wheat, and mixed oat−wheat doughs and the corresponding breads were investigated. Laccase treatment decreased the content of water-extractable arabinoxylan (WEAX) in oat dough due to oxidative cross-linking of feruloylated arabinoxylans. Laccase treatment also increased the proportion of water-soluble polysaccharides (WSNSP) apparently due to the β-glucanase side activity present in the laccase preparation. As a result of the laccase treatment, the firmness of fresh oat bread was increased. Xylanase treatment doubled the content of WEAX in oat dough and slightly increased the amount of WSNSP. Increased stiffness of the dough and firmness of the fresh bread were detected, probably because of the increased WEAX content, which decreased the amount of water available for β-glucan. The combination of laccase and xylanase produced slight hydrolysis of β-glucan by the β-glucanase side activity of laccase and enhanced the availability of AX for xylanase with concomitant reduction of the amount and molar mass of WSNSP. Subsequently, the volume of oat bread was increased. Laccase treatment tightened wheat dough, probably due to cross-linking of WEAX to higher molecular weight. In oat−wheat dough, laccase slightly increased the proportion of WSNSP between medium to low molecular weight and increased the specific volume of the bread. Xylanase increased the contents of WEAX and WSNSP between medium to low molecular weight in oat−wheat dough, which increased the softness of the dough, as well as the specific volume and softness of the bread. The results thus indicate that a combination of laccase and xylanase was beneficial for the textures of both oat and oat−wheat breads.

KEYWORDS: Oat; wheat; dough; bread; laccase; xylanase; arabinoxylan; ferulic acid; cross-linking

INTRODUCTION

Whole grain oats contain high amounts of valuable nutrients such as soluble fibers, proteins, unsaturated fatty acids, vitamins, minerals, and phytochemicals. The dietary fiber complex with its antioxidants and other phytochemicals is assumed to be protective toward cardiovascular disease and some types of cancer (*1–3*). The main water-soluble polysaccharide of oat, β-glucan, is reported to decrease blood cholesterol and intestinal absorption of glucose (*4, 5*).

Traditionally, oat bread contains up to 20% oat of the weight of wheat flour. In Germany, the bread can be labeled as "oat bread" if it contains 20 g oat/100 g wheat flour (*6*). Oat endosperm and the aleurone layer contain a high amount of globulins and a relatively low quantity of prolamins. The lack of gluten proteins as well as the high content of β-glucan lead easily to tight, moist, and gummy bread. However, the addition of at least 50% whole grain oat flour of the weight of wheat flour in bread is needed to attain 0.75 g β-glucan/serving. This dose of β-glucan permits the use of a health claim that oat-soluble fiber can help reduce blood cholesterol (*7*) (United Kingdom) or the risk of coronary hearth disease (*8*) (United States). Good quality oat−wheat bread, with 51% whole grain oat of flour weight and 0.8 g β-glucan content/serving, was developed by optimization of gluten, water, and processing conditions (*9*).

Cell walls of cereal grains contain arabinoxylan (AX) and (1→3),(1→4)-β-D-glucan as major constituents. AX can be further divided into water-unextractable (WUAX) and water-extractable arabinoxylan (WEAX). The reported AX and WEAX contents of whole grain oat flour range between 2.0 and 4.5% of d.w. and 0.2 and 0.4% of d.w., respectively (*10–12*). The total AX content of endosperm wheat flour varies between 1.4 and 2.9%, and the content of WEAX varies between 0.26 and

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0.75% of d.w (*13, 14*). Ferulic acid (FA) is the main phenolic compound of oat and wheat, and it is mainly linked to AX by ester linkage and dehydrodiferulate esters contributing to cross-links between cell wall polysaccharides in soluble and insoluble dietary fiber (*15, 16*). The total monomeric FA content of whole grain oat varies between 10 and 276 μg/g (*17–19*), and endosperm wheat flour varies between 15 and 136 μg/g (*15, 19, 20*). According to Adom and Liu (*21*), 98% of FA present in whole grain oat was in bound form, 2% was soluble conjugate, and 0.4% was free. In wheat flour, the corresponding values were 98, 1, and 0.1%, respectively.

Enzymes are potential tools to improve the bread properties. Traditionally, hydrolytic enzymes are used in baking, but nowadays, cross-linking enzymes such as laccases and peroxidases are also gaining a lot of attention. Xylanases (endoxylanases EC 3.2.1.8) cleave the xylan backbone of WUAX, subsequently increasing the level of WEAX. Concomitantly, xylanases also reduce the molecular weight of the WEAX (*22*). The solubilization of WUAX, and possibly the subsequent water redistribution from WUAX to gluten, improves the bread-baking quality (*22*). Laccase (benzene-diol:oxygen reductase, EC 1.10.3.2) is a multicopper enzyme catalyzing the oxidation of a variety of organic substrates with concomitant reduction of molecular oxygen to water. The primary oxidation products are generally reactive radicals that can undergo nonenzymatic reactions, such as cross-linking of monomers, degradation of polymers, and ring cleavage of aromatics (*23, 24*). Laccase can oxidize FA into a phenoxy radical that reacts nonenzymatically to produce di- and triFA. As a result, cross-links are formed between feruloylated AXs (*25, 26*). The thiols of cysteine and glutathione reduce the phenoxy radicals, formed by laccase, back to the original FA with concomitant oxidation into disulfides (*25, 27*). Also, heteroconjugate formation between tyrosine and tyrosine-containing peptides or proteins and feruloylated AX has been reported by laccase (*28*) and peroxidase (*29–31*).

Laccase and xylanase alone, or in combination, can improve the gluten index and thus the gluten quality (*32*). The effect of laccase on wheat bread quality has been studied by Si, Primo-Martin et al., and Selinheimo et al. (*33–35*). According to them, the addition of laccase increased the volume of the white wheat breads by 4−9%. The fungal laccase at activity levels of 5−50 nkat/g flour has been reported to decrease the extensibility and increase the resistance to stretching of wheat dough (*36*). At high activity levels (50 nkat/g flour), resistance to stretching decreased as a function of dough resting time, suggesting depolymerization of AX (*36*). Laccase increased wheat dough consistency in comparison with the control and decreased AX extractability by increasing the oxidative gelation of AX through dimerization of feruloyl esters (*27*). With added FA, the SH oxidation increased by 47%, when compared with control dough. However, the effect of laccase on SH oxidation has been measured only with added FA (*27*).

Gluten, which was extracted from xylanase-treated dough, showed improved rheological characteristics, probably by interfering in the protein−AX interaction (*32*) or releasing those AXs that are entrapped in the gluten network (*37, 38*). The combination of laccase and xylanase increased the specific volume of wheat breads by 10−11% when compared to breads with xylanase or laccase alone (*34*), while Si (*33*) and Selinheimo et al. (*35*) reported the same specific volumes for wheat breads with a combination of laccase and xylanase as for breads with laccase or xylanase alone.

The aim of this study was to investigate the possibilities to improve the structure of breads with high β-glucan contents by using laccase, xylanase, and a mixture of the two. Means to improve the structure of health-promoting breads are highly needed and appreciated by the baking industry. AXs, proteins, FAs, and rheological properties were characterized for oat, wheat, and oat−wheat doughs. The biochemical and rheological features were related together to better understand the effects of these enzymes on the bread-making quality of oat and oat−wheat breads.

Table 1. Quality Attributes of Flours

attribute	whole oat flour	endosperm wheat flour	oat−wheat flour
moisture (% db)	9.1	12.4	10.7
protein (% db) tot.[a]	18.2	13.2	
-insoluble proteins (mg/ml)	3.4	3.6	3.4
-soluble proteins (mg/ml)	3.1	3.1	3.2
ash (% db)	2.4	0.7	
wet gluten (%)[a]		30.9	
farinograph			
absorption (14% moisture)	86.9	59.8	75.6
sieves (μm) (%)			
630	21		
475	12		
355	8		
250	7		
132	50		
<95	0.5		

[a] Nx5.7 for wheat, Nx6.25 for oat, and Nx5.98 for oat−wheat.

MATERIALS AND METHODS

Raw Materials. Whole grain oat flour (Helsinki Mills Ltd., Järvenpää, Finland) and/or commercial endosperm wheat flour (Raisio Group plc, Raisio, Finland) were used in all experiments. Oat grains were steamed before milling to inactivate lipase (a usual practice in commercial oat flours). Chemical analyses of the flours were made in duplicate (**Table 1**). Moisture, ash, protein, and wet gluten contents as well as the falling number of the flours were determined according to standard methods 44-15A, 08-01, 46-11A, 8-12A, and 56-81B, respectively (*39*). The sieve analysis of oat flour was carried out by sieving two 100 g portions of the flour for 10 min.

Enzymes and Enzyme Activity Measurements. Laccase was produced by a white-rot fungus *Trametes hirsuta* and partially purified by anion exchange chromatography. The activity of laccase was measured with ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] as the substrate (*40*). One nkat corresponded to the oxidation of 1 nmol of ABTS/s at 25 °C and pH 4.3. Xylanase was a commercial product (Pentopan Mono BG, Novozymes A/S, Bagsverd, Denmark). The activity of xylanase was determined using birch glucuronoxylan as the substrate (*41*). The β-glucanase activity was measured with a method described by ref *42* and modified by the method of ref *43*. The endoglucanase (*44*), α-amylase (*45*), protease (Protazyme AK tablets, Megazyme International Ireland Ltd.), and glucoseoxidase (modified method of ref *46*) activities were also determined from the enzymes. Laccase preparation had slight protease and β-glucanase side activities, and xylanase preparation had a slight endoglucanase side activity (**Table 2**).

Preparation of Doughs for Chemical and Rheological Analysis. The amount of water added to the doughs was determined according to the water absorption of the farinograph at the consistency of 500 BU (**Table 1**). The flour contents of the doughs were 47.3 g for oat, 49 g for wheat, or 24.5 g for oat and 23.5 g for wheat flour. Laccase (14 nkat/g flour) was added to the water just before mixing with flours, and xylanase (46 nkat/g flour) was added prior to mixing of oat, wheat, and a combination of these flours (51% oat and 49% wheat). The mixing times were optimized with a plastograph (Plasti-Corder PL2100, Brabender, Duisburg, Germany). A 50 g farinograph bowl and *z*-blades were tempered to 26 °C, and the doughs were mixed until the time to peak the torque of duplicate doughs was reached. After mixing (oat doughs for 6 min, wheat doughs for 4 min, and oat−wheat doughs for

Table 2. Enzyme Activities (nkat/mL) of Laccase and Xylanase Preparates Used in This Work

enzyme	laccase	protease	xylanase	endoglucanase	β-glucanase	glucoseoxidase	α-amylase
laccase	7800	25	0	0	2.3	ND[a]	0
pentopan xylanase	ND	0	132600	52	0	0	0

[a] Not determined.

5 min), the doughs for chemical analysis were incubated at 30 °C (Panimatic PSF10, Souppes-Sur-Loin, France) for 60 min. The doughs (10 g) were then frozen immediately in liquid nitrogen and freeze-dried. The dried doughs were ground in a laboratory mill (Prolab #62130) for 1 min.

Rheological properties of doughs were measured for duplicate doughs by uniaxial extension measurements using Kieffer dough extensibility rig fitted onto a TA.XT2 Texture Analyzer (Stable Micro Systems Ltd., Godalming, United Kingdom) and equipped with a 5 kg load cell. Kieffer tests were performed according to Kieffer et al. (*47*) with a few modifications concerning resting times and temperatures. The doughs were mixed with a farinograph (Brabender, Duisburg, Germany) at 25 °C using the same mixing times as optimized with the plastograph. After mixing, the doughs were divided in to four pieces of 18 g, rounded in an extensigraph (Brabender, Duisburg, Germany), and molded to bars by hand to fit to the Kieffer extension rig. The dough bars with the extension rigs were kept at 80% relative humidity and 30 °C for 20 and 60 min to allow stress relaxation before measurements.

Analysis of AX and WEAX Contents and Molecular Weight Distributions. Aqueous extracts of nonstarch polysaccharides (NSP) of flour and dough samples were prepared according to Rouau and Surget (*48*) with the following modifications. Flour and dough samples were extracted with deionized water (1 g/4 mL) at 4 °C with a magnetic stirrer for 15 min. After centrifugation (12000*g*, 15 min, 4 °C), the supernatant was boiled at 100 °C for 10 min to inactivate the enzymes and cooled down in a cold water bath for 30 min. After centrifugation (10 min, 3500 rpm, Beckman TJ-6), the supernatant was frozen until further analysis. The carbohydrate contents of flour, dough, and aqueous extracts thereof were estimated by gas−liquid chromatography following the procedure of ref *49*. Alditol acetates, obtained after sulfuric acid hydrolysis (3N, 100 °C, 2 h), reduction with sodium borohydride, and acetylation with acetic anhydride of the samples, were injected on a DB-225 capillary column (J6W Scientific, Folsom, CA), using inositol as the internal standard. The AX content was then defined as 0.88 × the sum of the monosaccharides xylose and arabinose. Because the galactose content of oat was higher (0.3 ± 0.02) than the content of arabinose and the structure or content of arabinogalactan in oat has not been studied, no correction of the arabinose present in arabinogalactan was made.

The apparent MW distribution of the aqueous extract components of flour and dough samples was studied by size-exclusion chromatography (SE-HPLC) with a procedure described by ref *50* using a Waters (Millipore Co., Milford, MA) Ultrahydrogel 1000, 10 μm, column (7.8 mm × 300 mm), with a pullulan limit exclusion 10^6 Da, and a Waters 410 differential refractometer was used for detection.

Analysis of β-Glucan and Phenolic Compounds. β-Glucan contents of samples were analyzed by method 32-23 (*39*). The weight-average molecular weights (M_w) of β-glucan of oat dough with and without laccase ($n = 7$) were analyzed after stirring 1 g of the sample overnight with a magnetic stirrer in 100 mL of 0.1 M NaOH containing 0.1% $NaBH_4$. The samples were analyzed by HPLC-SEC with Calcofluor staining by using right-angle laser light scattering for detection (*51*).

The contents of total phenolic compounds were analyzed after alkaline hydrolysis by RP-HPLC with diode array detection with modifications (*52*). The 20 mg sample was first hydrolyzed with 1.1 mL of 2 M NaOH at room temperature for 16 h. Before extraction with ethyl acetate (3 × 2 mL), the sample was acidified with 0.7 mL of 5 M HCl. The combined ethyl acetate extract was evaporated into dryness, and the residue was dissolved into 50% MeOH prior to RP-HPLC analysis on an Agilent Hypersil BDS-C18 column (4.6 mm × 150 mm, 5 μm). The free phenolic compounds were analyzed correspondingly but using water instead of NaOH and 50 μL of 5 M HCl for acidification. The linear gradient was from 5 to 95% MeOH in 1% acetic acid at a flow rate of 0.8 mL/min. Quantitation was based on calibration curves of reference substances at 324 nm. A 8-5′-coupled diferulate (benzofuran) was synthetized for use as a standard on DFA (dehydrodimers of FA) analysis.

Table 3. Recipes of the Oat and Oat−Wheat Bread

	oat		oat−wheat	
bread ingredients	g	% of flour weight	g	% of flour weight
whole grain oat flour	1287.0	100	585.0	51.0
endosperm wheat flour			562.5	49.0
gluten (Raisio Group plc)			175.0	15.2
water	1243.0	96.6	1050.0	91.5
sugar	39.2	3.0	35.0	3.0
salt	25.2	2.0	22.0	2.0
margarine	39.2	3.0	35.0	3.0
yeast	39.2	3.0	35.0	3.0
Panodan A2020	7.7	0.6		
Cekol 50 000	9.6	0.8		
total	2690.1		2500.0	

Protein Analyses. The contents of sodium dodecyl sulfate (SDS)-insoluble and SDS-soluble proteins of the flours were analyzed by Kjeldahl method 46-12 (*39*) (**Table 1**). Extraction of protein and the molecular size distribution of SDS-soluble and -insoluble proteins in doughs was analyzed by SE-HPLC (*53*) with modifications as described by ref *54*. The samples were centrifuged at a speed of 39191*g* (Beckman Avanti). Once corrected for their different solid-to-solvent ratios during extraction, areas of soluble and insoluble proteins (in arbitrary units) were added, and the sum was assumed to correspond to the total protein content of the sample. The SH content of flours and doughs was determined using Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid or DTNB) as described by ref *55* except that centrifugation was performed at a speed of 20800*g* instead of 15000*g*.

Fractionation of Oat Flour Proteins and SDS-PAGE Analysis. SDS polyacrylamide gel electrophoresis (PAGE) analysis of oat protein fractions incubated with and without laccase was performed. Oat protein fractions were extracted from whole grain oat flour (Helsinki Mills Ltd., Finland), which was defatted by extracting the flour with acetone (40 g flour/L) for 30 min with a magnetic stirrer. Albumin and globulin were isolated with the sequential extraction described by ref *56* and prolamin with the method of ref *57*. Albumin was freeze-dried, and globulin and prolamin were frozen at −20 °C. The protein contents of fractions were analyzed by Lowry method (DC protein assay, Bio-Rad, CA). Albumin and prolamin (3 mg/mL) were suspended and treated in 50 mM Na-acetate buffer, pH 5.0. A 0.2 M concentration of NaCl was added to globulin buffers. Laccase (100 and 500 nkat/g protein) was added to the protein suspensions and incubated at 30 °C for 16 h. A reference sample without enzyme addition was prepared from each fraction. After incubation, the reaction was stopped by adding 1 volume (1:1) of SDS-PAGE sample buffer with 1% (w/v) DTT (Sigma, Germany) and boiling for 2 min. The prolamin sample was boiled for 5 min. A 12% Tris-HCl gel (Bio-Rad, Hercules, CA) was used. The molecular mass range of the standard (Bio-Rad, Hercules, CA) was between 21.5 and 113 kDa.

Baking Procedures. The effects of enzymes on the specific volume and firmness of the oat (100% whole grain oat flour) and oat−wheat bread (51% whole grain oat flour and 49% wheat flour) were studied by baking breads with straight baking methods in duplicate. The recipes of the breads are in **Table 3**. Laccase (14 nkat/g flour) was added to the water just before mixing with flours, and xylanase (46 nkat/g flour) was added prior to mixing of wheat, oat, and a combination of these flours (51% oat and 49% wheat). Wheat flour was blended thoroughly with the sugar and salt and placed in a spiral mixer (Diosna SP 12 F,

Table 4. AX and β-Glucan Contents of Wheat and Oat Doughs and Flours[a]

sample	enzyme	AX total (% of d.w.)	WEAX (% of d.w.)	ara/xyl (in WEAX)[b]	β-glucan (% of d.w.)
whole grain oat flour	0	2.6 ± 0.16 a	0.13 ± 0.00 a	1.88 ab	5.33 ± 0.12 a
oat dough	0	2.6 ± 0.25 a	0.22 ± 0.03 b	1.68 b	5.21 ± 0.62 a
oat dough	lac		0.19 ± 0.01 a	1.92 a	5.11 ± 0.15 a
oat dough	xyl		0.49 ± 0.04 c	0.97 c	5.31 ± 0.59 a
oat dough	lac + xyl		0.44 ± 0.02 c	0.93 c	5.80 ± 0.15 a
endosperm wheat flour	0	1.9 ± 0.08 a	0.65 ± 0.15 a	1.11 a	0.19 ± 0.08 a
wheat dough	0	1.8 ± 0.07 a	0.70 ± 0.02 a	1.01 ab	0.21 ± 0.02 a
wheat dough	lac		0.67 ± 0.04 a	1.00 b	0.23 ± 0.02 a
wheat dough	xyl		1.42 ± 0.10 b	0.76 c	0.25 ± 0.05 a
wheat dough	lac + xyl		1.41 ± 0.05 b	0.78 c	0.34 ± 0.11 a
oat−wheat dough	0		0.42 ± 0.03 a	1.14 a	2.91 ± 0.14 a
oat−wheat dough	lac		0.39 ± 0.01 a	1.18 a	2.84 ± 0.35 a
oat−wheat dough	xyl		0.84 ± 0.03 b	0.82 b	2.93 ± 0.03 a
oat−wheat dough	lac + xyl		0.83 ± 0.01 b	0.81 b	2.89 ± 0.06 a

[a] Mean values ± standard deviations. Mean values ($n = 4$) followed by a different letter within the same column and flour type are significantly different ($P < 0.05$). [b] Without correction of arabinose in arabinogalactan. Mean values followed by a different letter within the same cereal and column are significantly different ($P < 0.05$).

Dierks and Söhne GmbH, Osnabrück, Germany). The yeast was suspended in water (24 °C) and added to the mixture with the tempered shortening.

The oat dough was mixed with low speed (100 rpm) for 2.5 min and with fast speed (200 rpm) for 5 min. Intermediate proof was performed at room temperature (22 °C) for 20 min, and the dough was handled further as the oat−wheat dough, except that proofing conditions were 65 min at 39 °C and the baking temperature was 220 °C. The oat−wheat dough was mixed with fast speed (200 rpm) for 2.5 min, and the oat flour with the rest of the water was added to the dough and mixed at a low speed (100 rpm) for 6 min. After intermediate proofing at 28 °C and 80% relative humidity for 12.5 min, the dough was divided into six 400 g pieces, rounded, and molded to tempered pans that were sprayed with pan grease and proofed at 80% relative humidity and 35 °C for 65 min. The breads were baked at 195 °C for 30 min and with 5 s of steam in the beginning.

After baking, the loaves were cooled for 2 h before they were weighed. The loaf volume was determined by the rapeseed displacement method (*58*). The crumb hardness was measured at 2 and 72 h after baking by the TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, United Kingdom) using the Texture Profile Analysis (TPA) test. Six 25 mm thick slices were used for the analysis; two slices were taken from each of the three different breads. The crust of the slices was removed so that only textural parameters from the crumb were measured. The slices were compressed by 10 mm (40%) with a speed of 1.7 mm/s. The results are presented as an average of six replicated loaves baked on two different days.

Statistical Analysis. One-way analysis of variance and Tukey's test were performed to study the possible differences between samples using the statistical program SPSS 14.0.1 for Windows (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Chemical Properties of Oat and Wheat Flours and Doughs. The AX content of steamed whole grain oat flour was 2.6% of d.w., and the WEAX content was 0.13% of d.w. (**Table 4**). The AX and WEAX contents of wheat endosperm flour (1.9 and 0.65% of d.w., respectively) were similar to those previously reported (*14, 36*). Only 5% of oat flour AX was water-soluble, while that of wheat flour was 34%. The content of WEAX was thus lower in oat than in wheat. Mixing and incubation of the dough increased the relative amount of WEAX in oat by 41% and in wheat by 7% when compared to the WEAX content of the corresponding flours. This increase is expected to be caused by hydrolysis of AX by wheat flour-associated endoxylanases or by disaggregation of AX chains weakly bound in endosperm cell walls by a temperature increase or mechanical work input (*59–62*). Solubilization of WUAX is reported to correlate significantly with endogenous xylanase activity during the resting phase of the dough but not during the mixing phase (*61*). This was supposed to indicate that solubilization of WUAX during the short mixing phase was caused by mechanical forces, while that during the resting phase was caused by endogenous xylanases (*61*).

The MW distribution of WSNSP of flours and doughs is presented in **Figure 1A−D**. The changes in MW for oat were due to changes in both AX and (1→3,1→4)-β-D-glucan populations. The peak MW of WSNSP of oat flour increased and shifted from 300000−500000 to 500000−800000 in dough, indicating the increase of the size and amount of WEAX and water-soluble β-glucan during mixing and incubation. The significant increase in WEAX content from oat flour to incubated dough (**Table 4**) was in accordance with these results. Because of the low (1→3,1→4)-β-glucan content in wheat flour, variations in HPSEC profiles can be attributed to AX. Preparation and incubation of wheat dough increased the proportion of WEAX with MW < 20000 (**Figure 1C**, black line), when compared to the MW curve of wheat flour (**Figure 1A**, red line) and suggesting a slight increase in WEAX contents as well. The total β-glucan contents (about half of it is water-soluble) of oat, wheat, and oat−wheat doughs were 5.2, 0.2, and 2.9% of d.w., respectively (**Table 4**).

The free and total monomeric FA contents of whole grain oat flour were 9 and 250 μg/g of d.w., and the corresponding contents of endosperm wheat flour were 1 and 97 μg/g of d.w., respectively (**Figure 2A**,**B**), indicating that most FAs in both oat and wheat are bound to AXs. The values obtained correspond to those previously reported (*15, 17–19*), with the exception of free FA in our oat flour, which was higher than those reported by Sosulski et al. (*15*) (2.4 μg/g of debranned oat flour) or Zielinski et al. (*17*) (1.5 μg/g of whole grain oat). Hydrothermal processing, as steaming of the grain, may liberate phenolic acids and their derivatives from the wall cells (*17*). The amount of total FA was 3-fold higher in whole grain oat dough than in endosperm wheat dough (**Figure 2A**).

Mixing and incubation did not significantly affect the content of SH groups in oat dough (0.53 μmol SH/g flour)—so, in dough, 92% of the initial flour SH remained (**Table 5**). The behavior of oat proteins during baking is poorly studied, but it is known that 70−85% of oat proteins consist of globulins, which do not form a protein network comparable to gluten, which in turn are the main proteins (70−80%) of wheat. Also, heat treatment (kilning and steaming of oat before milling to inactivate lipase) may affect the behavior of oat proteins and endogenous oxidases present in oat grain. The amount of SH groups in wheat dough

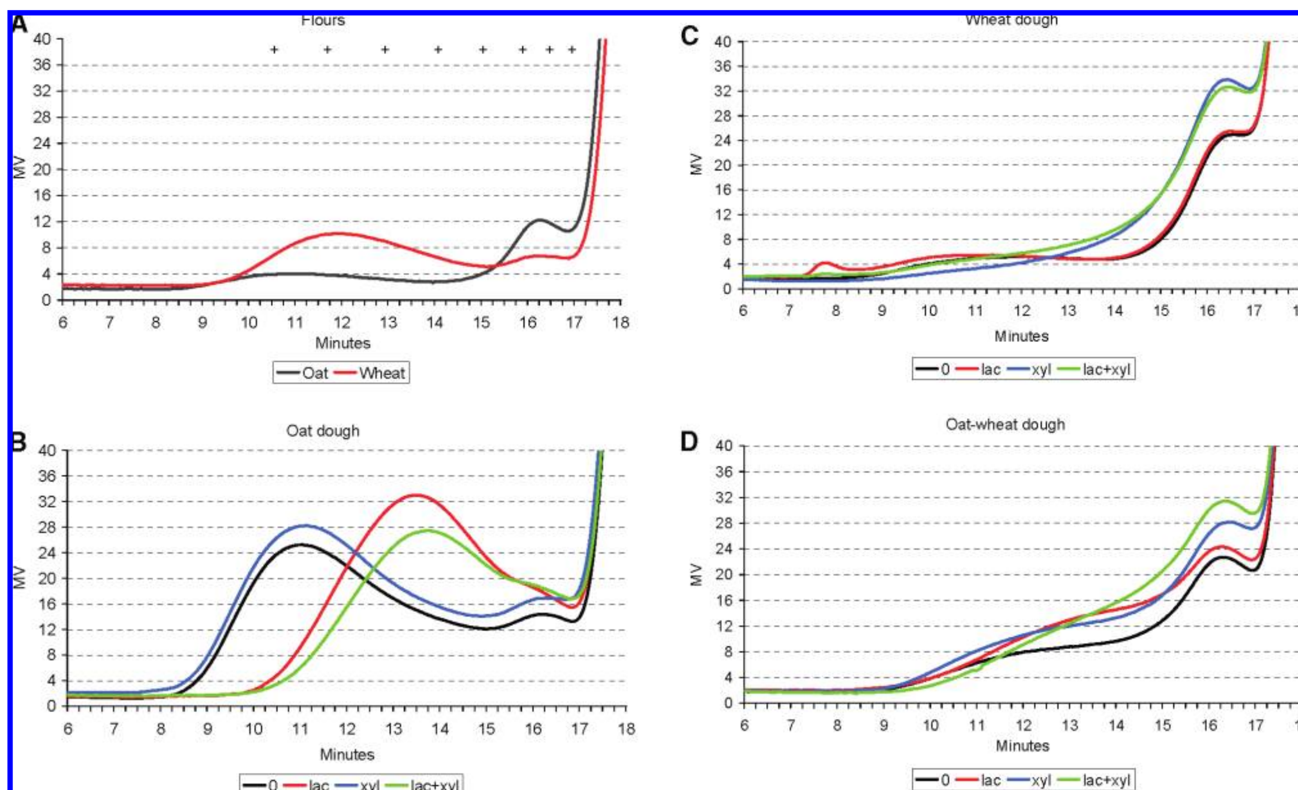

Figure 1. Size-exclusion HPLC elution profiles of water-soluble nonstarch polysaccharides (WSNSP) of flours (**A**), oat doughs with or without enzymes (**B**), wheat doughs with or without enzymes (**C**), and oat−wheat doughs with or without enzymes (**D**).

was only 14% of the original content of SH groups in flour (0.77 μmol/g) (**Table 5**), indicating a strong thiol-oxidizing mechanism during dough mixing and incubation, probably due to ascorbic acid present in commercial wheat flour. The amount of SH groups in different flours was not significantly different. The SH groups in the mixture of oat−wheat flour decreased to the same level as in wheat when mixed with the dough. It may be hypothesized that the ascorbic acid and/or endogenous oxidative enzymes present in wheat flour could also oxidize the SH groups present in oat flour during mixing and incubation.

Effects of Enzymes on the Chemical Properties of Doughs. The impact of laccase (dosage 14 nkat/g flour) and xylanase (dosage 46 nkat/g flour) treatments on the chemical compositions of doughs was investigated first. A significant decrease in the amount of WEAX (14%) and increase in the ratio of ara/xyl in WEAX (12%) was detected in oat dough treated with laccase (**Table 4**). This was apparently due to cross-linking of WEAX and concomitant decrease in the water extractability of the AX to WUAX. Laccase treatment also significantly decreased FA contents (**Figure 2A**,**B**). The size of WSNSP decreased (from MW 400000−800000 to 100000−200000), and the amount of WSNSP slightly increased when compared to control dough in **Figure 1B**. Because the β-glucan content of oat dough was higher than the AX content, it may be postulated that more β-glucan than WEAX was present in the WSNSP of oat dough. Because laccase decreased the amount of WEAX, the higher peak observed in **Figure 1B** by laccase is probably caused by β-glucan. A minor side activity of β-glucanase in laccase preparation (2.3 nkat/mL, corresponding 0.08 nkat/g β-glucan in oat dough) may be the reason for a small but significant ($p < 0.01$) decrease of the M_w of total β-glucans of oat dough from 1 million of untreated oat dough to 0.9 million of laccase-treated oat dough (data not shown). The β-glucanase activity could have changed mainly the molecular weight distribution of easily water-soluble β-glucans and thus affected the decrease in the molecular size and slight increase in the amount of water-soluble β-glucans observed in **Figure 1B**. Even though xylanase treatment increased the WEAX content of oat dough by 45%, it increased only slightly the amount of WSNSP (**Figure 1B**). This may indicate that WSNSP consisted mostly of β-glucan, which was not affected by xylanase. When laccase and xylanase were added together, the WSNSP content decreased more than with laccase alone. By degrading AX from insoluble cell walls to WEAX, xylanase may have enhanced the accessibility of β-glucanase from laccase preparation to β-glucan molecules, resulting in higher amounts and smaller molecular sizes of water-soluble β-glucans.

In the case of wheat doughs, laccase did not significantly affect the WEAX content of wheat doughs analyzed after 60 min of incubation (**Table 4**). According to Labat et al. (*27*), laccase caused an increase in WEAX content (from 26 to 28% of total AX) of wheat dough at the peak time of the mixing, when maximum consistency of the dough was reached, but a decrease to 23.5% after 30 min of mixing. The higher dosage of laccase (30 nkat) and longer mixing time used by Labat et al. (*27*) may have favored the formation of a higher proportion of cross-linked AX and reduced WEAX content, while in our case, a lower dosage of laccase (14 nkat/g flour) was not sufficient to oxidize WEAX completely. However, an increased amount of high molecular size WEAX (MW > 800000) of wheat dough was obtained as can be seen from the peak that eluted between 7 and 8 min (**Figure 1C**). This could be cross-linked WEAX formed by laccase. No changes in 8-5′-dehydrodiferulic acid (DFA) (benzofuran) content was detected (results not shown), but phenoxy radicals may have dimerized to other DFAs instead with different structures, such as 8-5′-DFA (decarboxylated), 8-8′, and 8-O-4′-DFA, which were not detected by the method used in this study. Xylanase treatment increased the amount of WEAX significantly in wheat dough

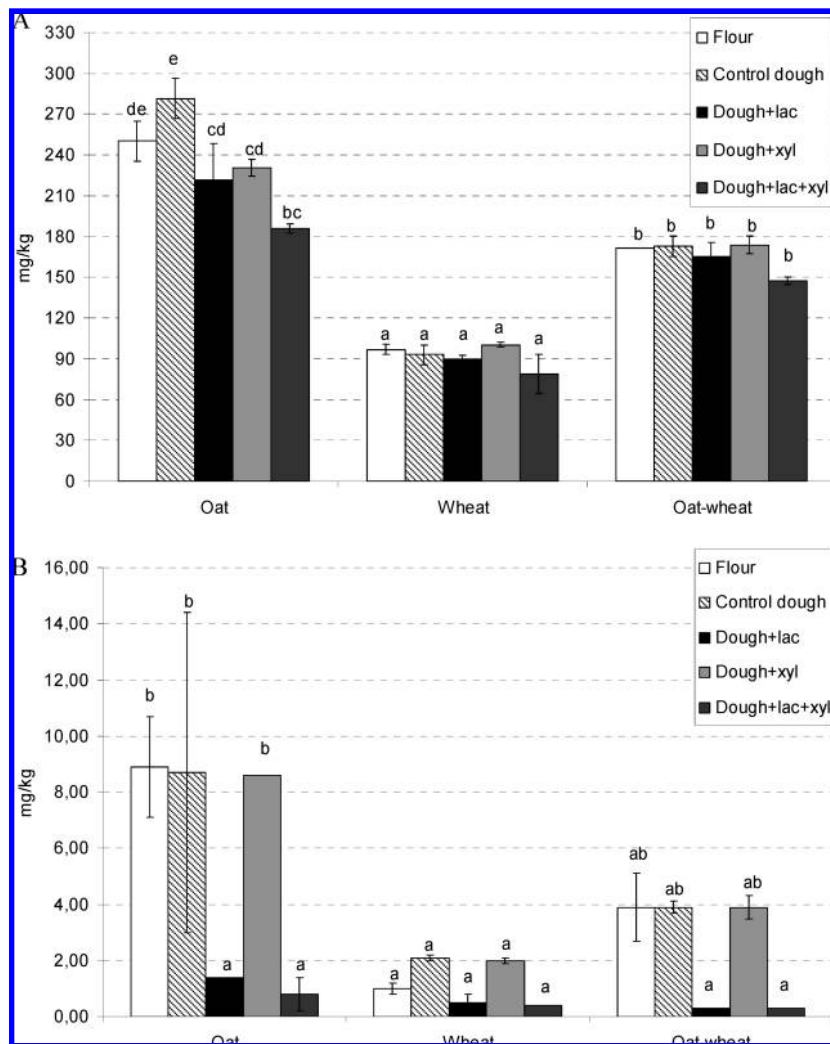

Figure 2. Total (**A**) and free (**B**) FA contents of doughs and flours.

Table 5. Alteration of Sulfhydryl (SH) Contents (DNTB Method) and Size Distribution of Protein Polymers by Enzymes in Doughs as Assessed by Ratio of Peak Areas of SDS-Insoluble Proteins/Total Proteins (%) (SE-HPLC)[a]

		dough			
	flour	0	lac	xyl	lac + xyl
oat					
μmol SH/g db	0.53 b	0.49 b	0.22 a	0.43 b	0.17 a
% SH content of dough/flour		92	42	81	32
insoluble proteins/total proteins, % db	9.4 a	11.1 a	9.6 a	9.8 a	10.4 a
wheat					
μmol SH/g db	0.77 b	0.11 a	0.10 a	0.16 a	0.17 a
% SH content of dough/flour		14	13	21	22
insoluble proteins/total proteins, % db	14.1 c	6.4 b	4.7 a	6.3 b	4.2 a
oat−wheat					
μmol SH/g db	0.77 b	0.11 a	0.09 a	0.09 a	0.16 a
% SH content of dough/flour		14	11	11	20
insoluble proteins/total proteins, % db	6.4 b	7.3 d	6.5 bc	6.1 a	6.7 c

[a] Mean values ± standard deviations. Mean values (n = 4) followed by a common letter within the same line are not significantly different ($P < 0.01$).

(**Table 4**) as has been reported also by Rouau et al. (*59*) and Courtain et al. (*63*). Degradation of WEAX and WUAX resulted in a higher proportion of smaller WEAX with MW up to 200000 in wheat dough (eluted after 13 min) (**Figure 1C**). This was also observed when laccase and xylanase were added together to the wheat dough as well as a small peak of high molecular size WEAX at MW 800000 (**Figure 1C**).

As expected, the WEAX contents of oat−wheat doughs with laccase, xylanase, and a combination of the enzymes were lower than in wheat but higher than in oat doughs. The molecular size distribution of WEAX of mixed oat−wheat doughs (**Figure 1D**) also reminded us more of the elution profiles of wheat doughs than oat doughs, because the content of WEAX originating from wheat was higher than that of oat in this dough. Laccase increased the content of WSNSP with molecular size between 600000 and 10000 when compared to the control (**Figure 1D**). Because the WEAX content did not change significantly (**Table 4**), the observed increase in **Figure 1D** was possibly caused by degradation of insoluble β-glucan of oat to water-soluble by β-glucanase side activity of laccase preparation. In contrast to wheat dough, the formation of high molecular weight WEAX was not detected by laccase in this dough. It is likely that high content of other WSNSP than wheat WEAX in oat−wheat dough hindered the cross-linking of wheat WEAX. When laccase and xylanase were added together to this dough, degradation of WUAX of wheat and oat to the doubled content of WEAX enabled more β-glucan to be degraded by the side activity of β-glucanase in laccase preparation. The combined effects of these enzymes resulted in the highest amount of WSNSP with molecular weight ≤400000.

Laccase treatment decreased the amount of total and free FA of oat dough by 21 and 84%, respectively (**Figure 2A**,**B**),

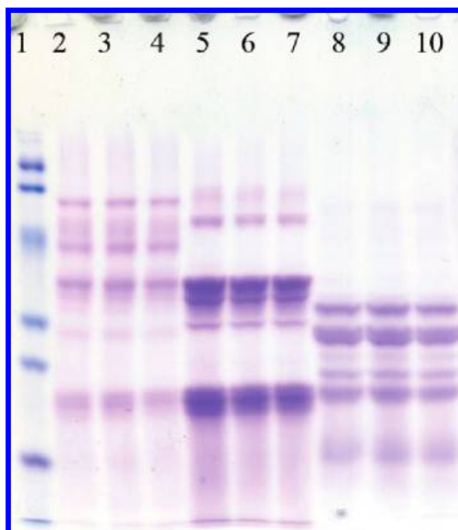

Figure 3. Reduced SDS-PAGE profile of laccase-treated oat protein fractions: protein markers from the top to the bottom: 113, 92, 52.9, 35.4, 29, and 21.5 kDa (lane 1); oat albumin (lane 2); oat albumin with 100 and 500 nkat laccase/g protein, respectively (lanes 3 and 4); oat globulin (lane 5); oat globulin with 100 and 500 nkat laccase/g protein (lanes 6 and 7); oat prolamin (lane 8); and oat prolamin with 100 and 500 nkat laccase/g protein (lanes 9 and 10).

indicating the formation of FA dimerization products that were not detectable by the assay. Surprisingly, xylanase decreased the total FA content of oat dough by 18%, without a concomitant increase in free FA (**Figure 2B**) or 8-5′-DFA benzofuran form (results not shown). The reason for this remains to be studied, but one explanation could be nonenzymatic oxidation reactions occurring to the WEAX. Because of the 2-fold WEAX content and concomitant increased FA anount, the phenolics could have reacted nonenzymatically during mixing and incubation of the dough. The content of total FA in oat dough with a mixture of the enzymes lowered by 34%, which was significantly ($p < 0.05$) more than by laccase or xylanase alone (**Figure 2A**), while only laccase decreased the content of free FA significantly (**Figure 2B**). Laccase or xylanase did not affect significantly the amount of detectable FA in wheat and oat−wheat dough, indicating that only a limited amount of DFA cross-linking was formed.

In oat dough, the amount of SDS-insoluble proteins/total proteins was not affected by laccase (**Table 5**). The amount of middle-size SDS-soluble proteins (630−65 kDa) increased, and small-size SDS-soluble proteins (>65 kDa) decreased as a result of laccase treatment in oat dough (HPSEC, results of the fractions not shown). Laccase decreased 55% of the SH groups (and increased middle-size soluble proteins) present in oat dough. It is likely that oat dough contained a sufficient amount of free FA for laccase to form phenoxy radicals, which in turn have led to disulfide bonds between proteins and low M_r thiols, as proposed in wheat dough with added laccase and FA (*27*). These disulfide bonds were not formed in control dough. No novel subunits of proteins could be detected on reduced SDS-PAGE (**Figure 3**), indicating that no polymerization between larger proteins occurred in laccase-treated oat flour. Xylanase did not affect the thiol content of the dough, so the reduced thiol content of oat dough with combination of enzymes was caused by laccase (**Table 5**).

Laccase alone and together with xylanase significantly decreased the relative amount of SDS-insoluble proteins/total proteins (mainly glutenin macropolymers in wheat) of wheat doughs (**Table 5**). The decrease in the ratio of insoluble proteins of total proteins was 27% for laccase and 34% for combination of enzymes. This result is in agreement with observations of Labat et al. (*27, 64*) and could be due to the acceleration of thiol/disulfide interchanges during mixing, leading to an earlier depolymerization of the glutenin macropolymer during mixing than in control wheat dough. Laccase treatment did not affect the SH groups present in wheat dough (**Table 5**). When wheat flour and water are mixed together, the formation of a gluten network starts after hydration of gluten proteins. They start to unfold and form long gluten strands, which form interchain disulfide bonds by oxidation of their thiol groups; thus, only 13% of the free SH groups are left in control wheat and oat−wheat doughs when compared to the original content of free SH groups in wheat flour (**Table 5**). The formation of gluten network is affected by mixing shear stress, addition of oxidants, and endogenous oxidases naturally present in wheat flour. The reaction of phenoxy radicals resulting from laccase activity is quite limited because of the lack of mobile phenolic acids in endosperm wheat flour. Thus, there may be less possibilities for phenoxy radicals to oxidize the remaining protein thiol groups into disulfides. According to Figueroa-Espinoza et al. (*25*) and Labat et al. (*27*), laccase did not affect SH oxidation of wheat dough without added FA. With added FA, the SH oxidation increased 47%, when compared with control dough (*27*). This was probably due to production of mobile thiol radicals through a displacement reaction from phenoxyl radicals to SH groups. Thiol radicals could have partially blocked the reformation of protein interchain disulfide bonds in favor of disulfide between proteins and low M_r thiols. Therefore, the depolymerization of glutenin polymers associated with mixing was not compensated by reformation of disulfide bonds between protein chains. This mechanism proposed by ref *27* could also explain why the amount of SDS-insoluble proteins/total proteins in laccase-treated wheat dough was reduced in our study.

As in wheat dough, laccase alone and in combination with xylanase decreased the ratio of SDS-insoluble proteins/total proteins also in oat−wheat dough by 11 and 8%. Unexpectedly, xylanase also decreased the content of SDS-insoluble proteins/total proteins of oat−wheat dough by 16%. Either xylanase degraded WUAX entrapped in the protein network or the degradation of WUAX in dough increased the accessibility of solvent to SDS-soluble proteins.

Effect of Enzymes on the Rheological Properties of the Doughs. Prolonged incubation (from 20 to 60 min) significantly decreased ($p < 0.05$) the resistance to stretching (R_{max}) of oat, wheat, and oat−wheat doughs without enzyme addition or with laccase (**Figure 4A−C**). Oat dough has very poor viscoelastic properties as can be seen from small changes in resistance to extension and its very low extensibility, and thus, the effects of enzymes on dough rheology remained very low (**Figure 4A**). Control dough and dough with laccase softened similarly during incubation as observed in wheat dough but without an increase in extensibility. Because endogenous enzymes are inactivated in flour milled of kiln-dried oat, the softening in this case may be mainly due to hydration of dough components during incubation, as can be seen from a slight increase in the WEAX content of dough when compared to WEAX content of flour (**Table 4**). Xylanase increased the resistance to extension after 60 min of incubation. Because xylanase doubled the WEAX content with slightly increased content of WSNSP, it may have increased the viscosity and tightened the dough. When xylanase and laccase were added together to the oat dough, it was softer after 20 min of incubation than other oat doughs, but after 60 min, it was only softer than oat dough with xylanase alone. By

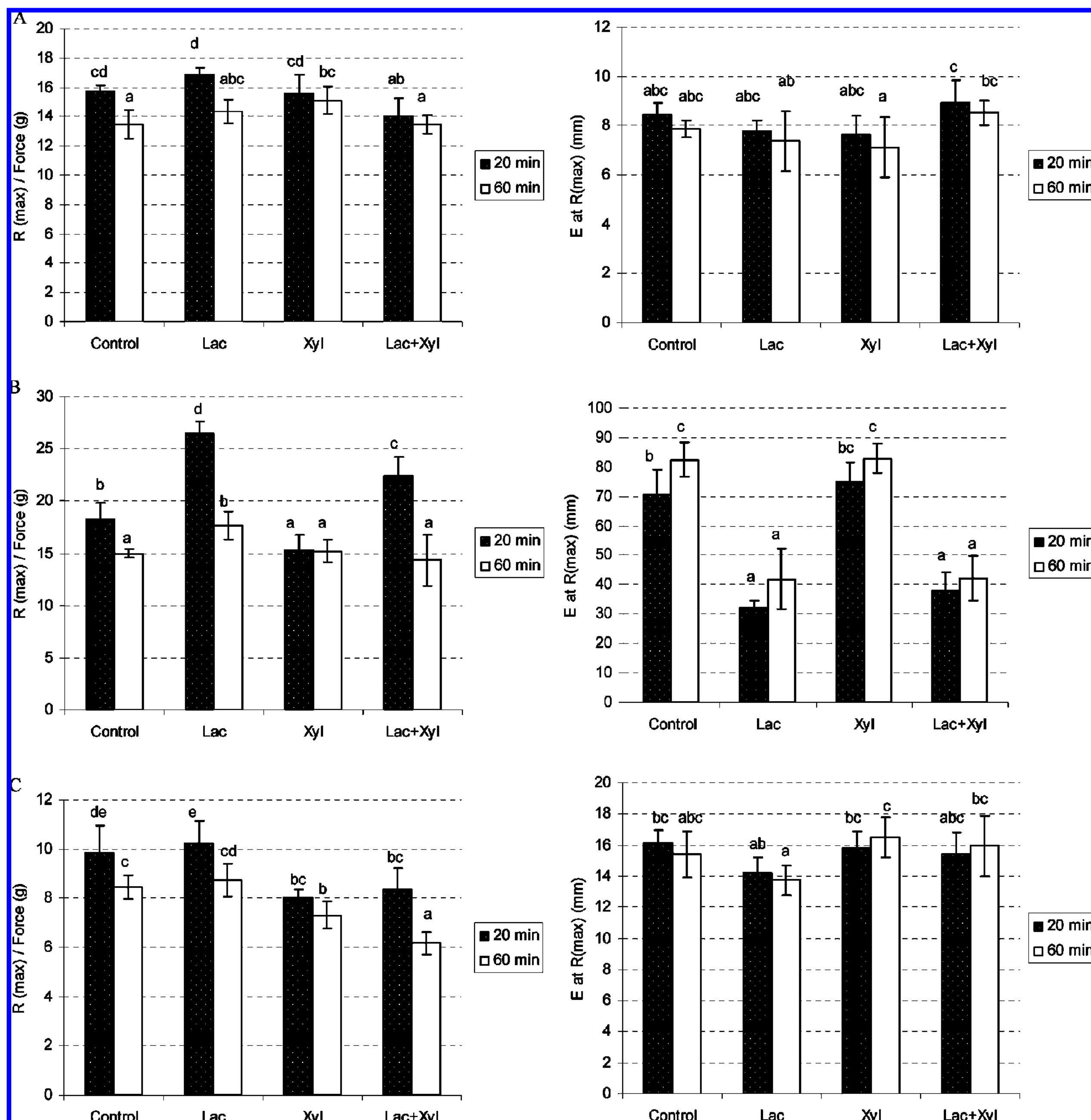

Figure 4. Effects of enzymes on rheological properties of oat (**A**), wheat (**B**), and oat−wheat (**C**) doughs.

degradation of WUAX, xylanase enabled more β-glucan to be degraded from the cell walls by the small β-glucanase side activity present in laccase preparation, leaving the dough softer and more extensible than xylanase-treated dough.

Besides hydration and relaxation of macromolecular network of dough after mixing and during incubation, also the endogenous enzymes of wheat, for example, xylanases, start to solubilize AX during resting (*61*), which could cause softening of the dough. Wheat dough with added xylanase had lower resistance to stretching already after 20 min of incubation, which did not change after 60 min of incubation (**Figure 4B**). This could indicate the higher activity of added xylanase already at the mixing stage of the dough preparation, while endogenous xylanases hydrolyze AX mainly during incubation of the wheat dough (*61*). Laccase increased and xylanase decreased significantly the resistance to stretching of wheat dough, when compared to control dough after 20 min of incubation (**Figure 4B**). After 60 min of incubation, only the wheat dough with laccase had higher resistance to stretching than other doughs. When laccase and xylanase were added together to the wheat dough, the role of laccase seemed to dominate the rheological properties, because the extensibility of the dough was reduced. Because laccase decreased the content of SDS-insoluble proteins/total proteins and no effect on free SH groups could be detected in wheat doughs, the cross-linking of WEAX to a small amount of WUAX and high molecular weight WEAX could be the explanation for rheological changes observed. High molecular weight WEAX formed in laccase-treated dough has a higher viscosity and could have tightened the structure of the dough and thus increased the resistance to extension and reduced the extensibility (E) of the dough. Prolonged incubation (from 20 to 60 min) increased significantly ($p < 0.05$) the extensibility of the control wheat dough, while with enzymes, the increase in extensibility from 20 to 60 min was more moderate and not significant (**Figure 4B**). Laccase has been shown to greatly increase the maximum resistance and decrease the extensibility

of wheat dough at an activity level of 5−50 nkat/g flour (*35, 36*), but the laccase-treated wheat dough softened during incubation, while with control dough, the effect of incubation time on softening of the control dough was very small (*36*). Possible reasons for the observed differences in control doughs may be different activities of endogenous xylanases, as different incubation temperatures (21 instead of our 30 °C) and incubation times (45 instead of 60 min) were used. Laccase increased the resistance to extension significantly after 20 and 60 min of incubation, when compared to control dough. The reduction in resistance to extension between 20 and 60 min incubation times was more pronounced in laccase-treated dough than in control dough. It can be postulated that laccase cross-links WEAX and forms high molecular weight WEAX and a small amount of WEAX-derived WUAX. This increases the resistance to extension in the beginning. During incubation, endogenous xylanases also depolymerize part of these large WEAX polymers. This could cause a larger drop in resistance to extension of laccase-treated dough from 20 to 60 min than depolymerization of smaller WEAX polymers in control dough during 20−60 min. Xylanase did not affect the resistance to extension or extensibility of wheat dough. Similar results have been previously reported by Selinheimo et al. (*35*) with a dosage of 50 nkat/g flour of the same xylanase, while higher dosages softened the dough significantly. Also, Primo-Martin et al. (*32*) detected no significant effects of laccase or xylanase or their combination on the resistance to extension or extensibility of wheat dough, when compared to control after 60 min of incubation. This is in accordance with our results with no effects of xylanase on the rheological properties of the wheat dough, but different effects of doughs with laccase and combination of laccase and xylanase might be explained by different laccase and flours used in this study. The influence of laccase on wheat dough rheological properties seemed to be predominant when dosages of 46 nkat xylanase/g wheat flour and 14 nkat laccase/g wheat flour were used.

The resistance to extension of control oat−wheat dough after 20 min of incubation was lower than in oat or wheat dough. The softening of the dough was also more pronounced in complete farinogram curves of oat−wheat dough. After 20 min of mixing, the drop in consistency from 500 BU was larger for oat−wheat dough than for oat or wheat doughs (results not shown). Besides competition of water between gluten and fiber, also endogenous enzymes of wheat may affect starch and NSP of oat and contribute to the weakening of the dough during 20 min of incubation. The resistance to extension (R_{max}) decreased significantly ($p < 0.05$) during incubation from 20 to 60 min, except in dough with xylanase (**Figure 4C**). Laccase did not affect the resistance to extension in oat−wheat dough, when compared to the control dough. No such changes in molecular size distribution as in wheat dough by laccase were recognized, and the amount of WEAX was smaller than in wheat dough. Xylanase increased the softness of the dough also after 60 min of incubation when compared to the control, while in wheat dough, the effect was significant only after 20 min of incubation. Besides doubled WEAX content, this can be explained by smaller endogenous xylanase activity of the oat−wheat control dough than in wheat dough. A combination of the enzymes in oat−wheat dough resulted in the softest dough. Xylanase catalyzed hydrolysis of WUAX in wheat and oat and resulted in a doubled amount of WEAX; thus, more β-glucan was degraded from insoluble cell walls by the side activity of β-glucanase in laccase preparation. The extensibility of the oat−wheat dough was not affected by either of the enzymes used.

Table 6. Effects of Laccase and Xylanase on Specific Volume and Instrumental Hardness of the Oat Breads ($n = 6$) and Oat−Wheat Breads ($n = 12$)[a]

Oat Bread

	specific volume	hardness (kg)	
	cm^3/g	2 h	48 h
control	1.70 b	1.04 a	1.54 ab
laccase 14 nkat/g flour	1.65 ab	1.43 c	1.83 b
xylanase 46 nkat/g flour	1.65 ab	1.42 c	1.85 b
laccase + xylanase	1.78 c	0.91 a	1.39 a

Oat−Wheat Bread

	specific volume	hardness (kg)	
	cm^3/g	2 h	72 h
control	3.6 ± 0.07 a	0.14 ± 0.017 b	0.28 ± 0.036 bc
laccase 14 nkat/g flour	3.7 ± 0.11 b	0.12 ± 0.016 ab	0.31 ± 0.029 c
xylanase 46 nkat/g flour	3.7 ± 0.06 b	0.11 ± 0.014 a	0.24 ± 0.023 a
laccase + xylanase	4.0 ± 0.06 c	0.12 ± 0.010 a	0.26 ± 0.028 ab

[a] Mean values followed by a common letter within the same column and bread type are not significantly different ($P < 0.05$).

Effect of Enzymes on Bread Characteristics. In 100% oat bread, laccase or xylanase increased the firmness of fresh oat breads without affecting the specific volume of bread, while the combination of these enzymes increased the specific volume significantly (**Table 6**). The softness of these breads remained at the same level as in control bread after 2 days of storage. Photographs of the oat-wheat breads are presented in Figure 5. Laccase, xylanase, and especially their combination increased the specific volume of oat−wheat bread significantly (**Table 6**). Similarly, an increase in the volume of wheat bread by laccase, xylanase, and their combination has been reported (*33–35*). Fresh oat−wheat bread with xylanase and with combination of enzymes was significantly softer than control bread. After 3 days of storage, only the bread with xylanase remained significantly softer than control bread. Xylanase and a combination of xylanase and laccase were also most effective in retaining the softness of wheat bread after 3 days of storage (*34, 35*).

By combining the enzyme-induced changes in molecular structures to the rheological properties of doughs and to the quality attributes of oat and oat−wheat breads, it can be concluded that laccase decreased the contents of WEAX and total and free monomeric FA in oat doughs. The increased content of WSNSP with smaller molecular weight was detected as well, which was proposed to be water-soluble β-glucan and WEAX. A slight β-glucanase side activity in laccase preparation could have hydrolyzed part of the insoluble β-glucan to water-soluble and thus increased the proportion of WSNSP with smaller molecular size. This in turn could have enhanced the possibilities of laccase to form phenoxy radicals and formation of cross-links between FA moieties of WEAX molecules resulting in WUAX. Laccase also decreased the content of SH groups and shifted the molecular size of SDS-soluble proteins toward a larger size, without concomitant effects on the subunits of oat protein fractions, as shown by reduced SDS-PAGE. Laccase did not affect the rheological properties of the oat dough, probably because its high β-glucan content and lack of

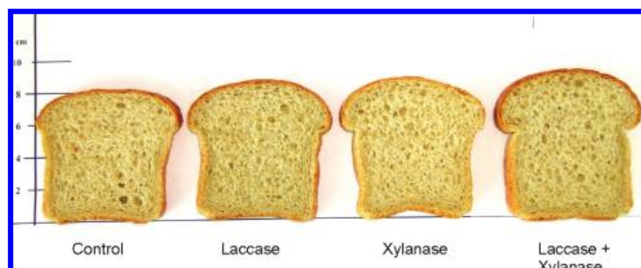

Figure 5. Photographs of the oat−wheat breads.

gluten. It is suggested that the formation of WUAX by laccase increased middle-size SDS-soluble proteins by formation of disulfide bonds between proteins and/or low M_r thiols increased the firmness of fresh oat bread. Xylanase doubled the content of WEAX in oat dough and slightly increased the amount of WSNSP. This increased the resistance to extension of the dough and firmness of the fresh bread, probably because WEAX decreased the amount of water available for β-glucan. When laccase and xylanase were added together, the slight degradation of AX by xylanase enhanced the degradation of β-glucan by laccase. The synergistic effect of the enzymes reduced the amount and size of WSNSP, allowing more water to the gluten phase of the dough, which was assumed to be the main reason for increased volume of this oat bread.

In wheat dough, laccase increased slightly the proportion of high molecular weight WEAX and decreased the ratio of SDS-insoluble proteins/total proteins, resulting in increased resistance to stretching and decreased extensibility of the dough. The cross-linking of WEAX to high molecular weight was proposed to produce tighter dough due to increased water absorption of WEAX.

In oat−wheat dough, laccase slightly increased the amount of WSNSP with molecular size between 600000 and 10000 when compared to the control. The increased content of water-soluble β-glucan in the WSNSP extract by β-glucanase side activity of laccase preparation may have increased the WSNSP and thus the specific volume of the bread. Xylanase increased the contents of WEAX and WSNSP of oat−wheat doughs, which increased the softness of the dough, and thus increased also the specific volume and softness of the bread. When laccase and xylanase were added together to this dough, degradation of WUAX of wheat and oat to double the content of WEAX enabled more β-glucan to be degraded by the side activity of β-glucanase in laccase preparation. The combined effects of these enzymes resulted in the highest amount of WSNSP with molecular weight $\leq$400000, which was assumed to be the main reason for softest dough and highest specific volume of oat−wheat bread.

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LITERATURE CITED

(1) Thompson, L. U. Antioxidants and hormone-mediated health benefits of whole grains. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 473–497.
(2) Jacobs, D. R., Jr.; Marquart, L.; Slavin, J.; Kushi, L. H. Whole-grain intake and cancer: an expanded review and meta-analysis. *Nutr. Cancer* **1998**, *30*, 85–96.
(3) Slavin, J.; Marquart, L.; Jacobs, D. R., Jr. Consumption of whole-grain foods and decreased risk of cancer: Proposed mechanisms. *CFW* **2000**, *45*, 54–58.
(4) Wood, P. J. Physicochemical characteristics and physiological properties of oat (1→3) (1→4)-β-D-glucan. In *Oat Bran*; Wood, P. Ed.; AACC Inc.: St. Paul, MN, 1993; pp 83−112.
(5) Mälkki, Y. Physical properties of dietary fiber as keys to physiological functions. *CFW* **2001**, *46*, 196–199.
(6) Brümmer, J.-M.; Morgenstern, G.; Neumann, H. Herstellung von Hafer-, Gerste., Mais-, Reis-, Hirse- und Buchweizenbrot. *Getreide, Mehl Brot* **1988**, *5*, 153–158.
(7) JHCI. Generic Health Claim for Oats and Blood Cholesterol; Joint Health Claims Initiative: Leatherhead, U.K. 2004.
(8) FDA. Food and Drug Administration Food Labeling: Health claims; Oats and coronary heart disease. *Fed. Regist.* **1997**, *62*, 3584−3601.
(9) Flander, L.; Salmenkallio-Marttila, M.; Suortti, T.; Autio, K. Optimization of ingredients and baking process for improved wholemeal oat bread quality. *LWT* **2007**, *40*, 860–870.
(10) Bhatty, R. S. Total and extractable β-glucan contents of oats and their relationship to viscosity. *J. Cereal Sci.* **1992**, *15*, 185–-192.
(11) Frölich, W.; Nyman, M. Minerals, phytate and dietary fibre in different fractions of oat-grain. *J. Cereal Sci.* **1988**, *7*, 73–82.
(12) Westerlund, E.; Andersson, R.; Åman, P. Isolation and chemical characterization of water-soluble mixed-linked β-glucans and arabinoxylans in oat milling fractions. *Carbohydr. Polym.* **1993**, *20*, 115–123.
(13) Michniewicz, J.; Biliaderis, C. G.; Bushuk, W. Water-insoluble pentosans of wheat: composition and some physical properties. *Cereal Chem.* **1990**, *67*, 434–439.
(14) Ordaz-Ortiz, J. J.; Saulnier, L. Structural variability of arabinoxylans from wheat flour. Comparison of water-extractable and xylanase-extractable arabinoxylans. *J. Cereal Sci.* **2005**, *42*, 119–125.
(15) Sosulski, F.; Krzysztof, K.; Hogge, L. Free, esterified, and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flours. *J. Agric. Food Chem.* **1982**, *30*, 337–340.
(16) Bunzel, M.; Ralph, J.; Marita, J. M.; Hatfield, R. D.; Steinhart, H. Diferulates as structural components in soluble and insoluble cereal dietary fibre. *J. Sci. Food Agric.* **2001**, *81*, 653–660.
(17) Zielinski, H.; Kozlowska, H.; Lewczuk, B. Bioactive compounds in cereal grains before and after hydrothermal processing. *Innovation Food Sci. Emerg. Technol.* **2001**, *2*, 159–169.
(18) Guth, H.; Grosch, W. Quantitative analysis of caffeic and ferulic acids in oatmeal—Comparison of a conventional method with a stable isotope dilution assay. *Z. Lebensm.-Unters. Forsch.* **1994**, *199*, 195–197.
(19) Mattila, P.; Pihlava, J.-M.; Hellström, J. Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. *J. Agric. Food Chem.* **2005**, *53*, 8290–8295.
(20) Peyron, S.; Surget, A.; Mabille, F.; Autran, J. C.; Rouau, X.; Abecassis, J. Evaluation of tissue dissociation of durum wheat grain (*Triticum durum* Desf.) generated by the milling process. *J. Cereal Sci.* **2002**, *36*, 199–208.
(21) Adom, K. K.; Liu, R. H. Antioxidant activity of grains. *J. Agric. Food Chem.* **2002**, *50*, 6182–6187.
(22) Courtin, C. M.; Delcour, J. A. Arabinoxylans and endoxylanases in wheat flour bread-making. *J. Cereal Sci.* **2002**, *35*, 225–243.
(23) Claus, H. Laccases: Structure reactions, distribution. *Micron* **2004**, *35*, 93–96.
(24) Flurkey, W. H. Laccase. In *Handbook of Food Enzymology*; Whitaker, J. R., Voragen, A. G. J., Wong, D. W. S. Eds.; Marcel Dekker, Inc.: New York, NY, 2003; pp 525−537.
(25) Figueroa-Espinoza, M.-C.; Morel, M.-H.; Rouau, X. Effect of lysine, tyrosine, cysteine, and glutathione on the oxidative cross-linking of feruloylated arabinoxylans by a fungal laccase. *J. Agric. Food Chem.* **1998**, *46*, 2583–2589.
(26) Carvajal-Millan, E.; Guigliarelli, B.; Belle, V.; Rouau, X.; Micard, V. Storage stability of laccase induced arabinoxylan gels. *Carbohydr. Polym.* **2005**, *59*, 181–188.

(27) Labat, E.; Morel, M.-H.; Rouau, X. Effects of laccase and ferulic acid on wheat flour doughs. *Cereal Chem.* **2000**, *77*, 823–828.
(28) Mattinen, M.-L.; Kruus, K.; Buchert, J.; Nielsen, J. H.; Andersen, H. J.; Steffensen, C. L. Laccase-catalyzed polymerization of tyrosine-containing peptides. *FEBS J.* **2005**, *272*, 3640–3650.
(29) Oudgenoeg, G.; Hilhorst, R.; Piersma, S. R.; Boeriu, C. G.; Gruppen, H.; Hessing, M.; Voragen, A. G. J.; Laane, C. Peroxidase-mediated cross-linking of a tyrosine-containing peptide with ferulic acid. *J. Agric. Food Chem.* **2001**, *49*, 2503–2510.
(30) Oudgenoeg, G.; Dirksen, E.; Ingemann, S.; Hilhorst, R.; Gruppen, H.; Boeriu, C. G.; Piersma, S. R.; van Berkel, W. J. H.; Laane, C.; Voragen, A. G. J. Horseradish peroxidase-catalyzed oligomerization of ferulic acid on a template of a tyrosine-containing tripeptide. *J. Biol. Chem.* **2002**, *277*, 21332–21340.
(31) Boeriu, C. G.; Oudgenoeg, G.; Spekking, W. T. J.; Berendsen, L. B. J. M.; Vancon, L.; Boumans, H.; Gruppen, H.; van Berkel, W. J. H.; Laane, C.; Voragen, A. G. J. Horseradish peroxidase-catalyzed cross-linking of feruloylated arabinoxylans with β-casein. *J. Agric. Food Chem.* **2004**, *52*, 6633–6639.
(32) Primo-Martin, C.; Valera, R.; Martinez-Anaya, M. A. Effect of pentosanase and oxidases on the characteristics of doughs and the glutenin macropolymer (GMP). *J. Agric. Food Chem.* **2003**, *51*, 4673–4679.
(33) Si, J. Q. Use of laccase in baking. International Patent Application PCT/DK94/00232, 1994.
(34) Primo-Martin, C.; Martinez-Anaya, M. A. Influence of pentosanase and oxidases on water-extractable pentosans during a straight bread making process. *J. Food Sci.* **2003**, *68*, 31–39.
(35) Selinheimo, E.; Autio, K.; Kruus, K.; Buchert, J. Elucidating the mechanism of laccase and tyrosinase in wheat bread making. *J. Agric. Food Chem.* **2007**, *55*, 6357–6365.
(36) Selinheimo, E.; Kruus, K.; Buchert, J.; Hopia, A.; Autio, K. Effects of laccase, xylanase and their combination on the rheological properties of wheat doughs. *J. Cereal Sci.* **2006**, *43*, 152–159.
(37) Roels, S. P.; Grobet, P. J.; Delcour, J. A. Distribution of carbohydrates in gluten fractions isolated from European wheats (*Triticum aestivum* L.) in a batter system. *J. Agric. Food Chem.* **1998**, *46*, 1334–1343.
(38) Saulnier, L.; Andersson, R.; Åman, P. A study of the polysaccharide components in gluten. *J. Cereal Sci.* **1997**, *25*, 121–127.
(39) American Association of Cereal Chemists. *Approved Methods of the AACC*, 10th ed.; The Association: St. Paul, MN, 2003.
(40) Niku-Paavola, M.-L.; Karhunen, E.; Salola, P.; Raunio, V. Ligninolytic enzymes of the white-rot-fungus *Phlebia radiate*. *Biochem. J.* **1988**, *254*, 877–884.
(41) Bailey, M. J.; Biely, P.; Poutanen, K. Interlaboratory testing methods for assay of xylanase activity. *J. Biotechnol.* **1992**, *23*, 257–270.
(42) Zurbriggen, B.; Bailey, M. J.; Penttilä, M. E.; Poutanen, K.; Linko, M. Pilot scale production of a heterologous *Trichoderma reesei* cellulase in *Saccharomyces cerevisiae*. *J. Biotechnol.* **1990**, *13*, 267–278.
(43) Institute of Brewing Analysis Committee. *Recommended Method of Analysis*; London, 1982.
(44) IUPAC, International Union of Pure and Applied Chemistry. Measurement of cellulase activities. *Pure Appl. Chem.* **1987**, *59*, 257−268.
(45) Fisher, E. H.; Stein, E. A. Alfa-amylase from human saliva. *Biochem. Prep.* **1961**, *8*, 27–33.
(46) Werner, W.; Rey, H.-G.; Wielinger, H. Über die eigenschaften eines neuen chromogens für die blutzuckerbestimmung nach der GOD/GOP-methode. *Z. Anal. Chem.* **1970**, *252*, 224–228.
(47) Kieffer, R.; Wieser, H.; Henderson, M.; Graveland, A. Correlations of the breadmaking performance of wheat flour with rheological measurements on a micro-scale. *J. Cereal Sci.* **1998**, *27*, 53–60.
(48) Rouau, X.; Surget, A. A rapid semi-automated method for the determination of total and water-extractable pentosans in wheat flours. *Carbohydr. Polym.* **1994**, *24*, 123–132.
(49) Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113*, 291–299.
(50) Carvajal-Millan, E.; Landillon, V.; Morel, M.-H.; Rouau, X.; Doublier, J.-L.; Micard, V. Arabinoxylan gels: impact of feruloylation degree on their structure and properties. *Biomacromolecules* **2005**, *6*, 309–317.
(51) Suortti, T. Size-exclusion chromatographic determination of betaglucan with post-column reaction detection. *J. Chromatogr.* **1993**, *632*, 105–110.
(52) Bartolomé, B.; Gómez-Gordovés, C. Barley spent grain: release of hydroxycinnamic acids (ferulic and *p*-coumaric acids) by commercial enzyme preparations. *J. Sci. Food Agric.* **1999**, *79*, 435–439.
(53) Dachkevitch, T.; Autran, J. C. Prediction of baking quality of bread wheats in breeding programs by size exclusion high performance liquid chromatography. *Cereal Chem.* **1989**, *66*, 448–456.
(54) Morel, M.-H.; Dehlon, P.; Autran, J. C.; Leygue, J. P.; Bar-L'Helgouac'h, C. Effects of temperature, sonication time, and power settings on size distribution and extractability of total wheat flour proteins as determined by size-exclusion high-performance liquid chromatography. *Cereal Chem.* **2000**, *77*, 685–691.
(55) Morel, M.-H.; Bonicel, J.; Micard, V.; Guilbert, S. Protein insolubilization and thiol oxidation in sulfite-treated wheat gluten films during aging at various temperatures and relative humidities. *J. Agric. Food Chem.* **2000**, *48*, 186–192.
(56) Mikola, M.; Jones, B. L. Characterization of oat endoproteinases that hydrolyze oat globulins. *Cereal Chem.* **2000**, *77*, 572–577.
(57) Mikola, M.; Brinck, O.; Jones, B. L. Characterization of oat endoproteinases that hydrolyze oat avenins. *Cereal Chem.* **2001**, *78*, 55–58.
(58) Siebert, G. Determination of volume in bakery products. *Bäcker Konditor* **1971**, *4*, 103–104.
(59) Rouau, X.; El-Hayek, M.-L.; Moreau, D. Effect of an enzyme preparation containing pentosanases on the bread making quality of flours in relation to changes in pentosan properties. *J. Cereal Sci.* **1994**, *19*, 259–272.
(60) Cleemput, G.; Booij, C.; Hessing, M.; Gruppen, H.; Delcour, J. A. Solubilisation and changes in molecular weight distribution of arabinoxylan and protein in wheat flours during bread-making, and the effects of endogenous arabinoxylan hydrolysing enzymes. *J. Cereal Sci.* **1997**, *26*, 55–66.
(61) Dornez, E.; Gebruers, K.; Cuyvers, S.; Delcour, J. A.; Courtin, C. M. Impact of wheat flour-associated endoxylanases on arabinoxylan in dough after mixing and resting. *J. Agric. Food Chem.* **2007**, *55*, 7149–7155.
(62) Trogh, I.; Courtin, C. M.; Andersson, A. A. M.; Åman, P.; Sörensen, J. F.; Delcour, J. A. The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and (1→3,1→4)-β-D-glucan levels. *J. Cereal Sci.* **2004**, *40*, 257–267.
(63) 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.
(64) Labat, E.; Morel, M.-H.; Rouau, X. Effect of laccase and manganese peroxidase on wheat gluten and pentosans during mixing. *Food Hydrocolloids* **2001**, *15*, 47–52.

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