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Baking Performance, Rheology, and Chemical Composition of Wheat Dough and Gluten Affected by Xylanase and Oxidative Enzymes

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ABSTRACT

Baking trials were performed with six wheat doughs prepared with xylanase, peroxidase, or glucose oxidase (GOX), and their combinations. Judging dough properties, baking performance, bread volume, and crumb structure, the dough containing xylanase plus peroxidase performed best. Flow relaxation measurements with doughs indicated that peroxidases introduced new transient linkages, whereas glucose oxidase also introduced cross-links permanent on long time scales. Rheological tests and chemical analysis revealed small differences between control and xylanase and/or peroxidase containing gluten and more pronounced differences for GOX containing gluten. No evidence was found that xylanase specifically removed arabinoxylans from gluten or that peroxidase catalyzed the formation of covalent bonds between arabinoxylans and gluten proteins.

Key Words: baking, wheat gluten, pentosan, peroxidase, xylanase, glucose oxidase

INTRODUCTION

ARABINOXYLANS ARE CELL WALL COMPONENTS OF WHEAT. They can be either water soluble or water insoluble and constitute 1% to 3% of the dry matter of wheat flour. The water-soluble pentosans can hold up to 10 times their weight in water, and their presence has been positively correlated with bread properties (Casier et al., 1973; Kim and D'Appolonia, 1977). To increase the amount of water-soluble pentosans, xylanases are added to bread dough (Sprössler, 1995). Insoluble arabinoxylans result in decreased bread volume (Krishnarau and Hoseney, 1994).

Xylanases can break glycosidic linkages in arabinoxylans in an endo- or exo- fashion, creating smaller fragments. Addition of xylanases improves the handling properties of dough, the ovenspring, and the bread volume. Furthermore, staling is retarded (Rouau et al., 1994; Sprössler, 1995; Poutanen, 1997). The soluble pentosans have several effects on dough. Because of their high water holding capacity, pentosans in dough influence the distribution of available water. It has been suggested (Weegels et al., 1990, van der Lugt et al., 1995) that xylanase removes arabinoxylans that interfere with the formation of a gluten network.

In case of extensive degradation or too much exo-activity, addition of xylanases, notably those of *Aspergillus niger* or *Trichoderma resei,* has adverse side effects and may result in sticky dough that is more difficult to handle (van Oort et al., 1995a). The addition of glucose oxidase or peroxidase to bread dough that was deliberately made sticky by addition of xylanase led to strengthening of the dough after 55 and 70 min proofing time. The shape and volume of rolls prepared with different peroxidases was (for most peroxidases) equal to or better than the results obtained with glucose oxidase (van Oort et al., 1995b; van Oort 1996).

Glucose oxidase (GOX) catalyzes the oxidation of glucose to gluconolactone by molecular oxygen that is converted to hydrogen peroxide. The H_2O_2 has been hypothesized to be responsible for the effect on the gluten network since the same effect was achieved by its addition or the addition of other enzymes that also produce H_2O_2 in situ, such as galactose oxidase (van der Lugt et al., 1995) or hexose oxidase (Poulsen and Bak Hølstrup, 1998).

Peroxidase can use a wide range of compounds, such as phenols, arylamines, halides, and thiols, as electron donors (Krylov and Dunford 1996; Obinger et al., 1996). In vitro the combination of peroxidase and peroxide catalyzes the gelation of arabinoxylans via the formation of diferulic acid linkages (Geissmann and Neukom, 1973; Schooneveld-Bergmans et al., 1998). In dough, such cross-linking was suggested to be responsible for the improvement of dough properties (van Oort, 1996). Furthermore, peroxidase has been suggested to cross-link arabinoxylans to side chains of amino acids (Neukom and Markwalder, 1978). Evidence for the occurrence of thiol-ferulic acid linkages in overmixed dough has been published (Jackson and Hoseney, 1986). So, peroxidase action in dough has been attributed to the formation of interchain bonds between arabinoxylans or by the coupling of arabinoxylans to gluten proteins. In the latter case, gluten properties and chemical composition would be expected to change.

We investigated the effects of different enzyme combinations on the handling and baking performance of dough. Our objective was to elucidate by rheological experiments and chemical analysis whether differences could be ascribed to the action of such enzymes on the gluten network.

MATERIALS & METHODS

Materials

The flour was the commercial untreated cookie type flour Kolibri with 15.0% moisture and 10.5% protein, obtained from Meneba Meel B.V. (Rotterdam, The Netherlands). Inherently a cookie type flour has poor bread making quality. Yeast was "Koningsgist" from Gist-brocades (Delft, The Netherlands). Glucose oxidase (GOX, Oxygo L5, 5364 U/ml) was from Genencor, (Helsinki, Finland). Fungal amylase (Biobake 5000, 175 FFA/mg), xylanase ex *Trichoderma* (Biobake CX160) (XYL, 351 U/mg), and soy peroxidase (POX, 35 U/mg) were from Quest International BV (Naarden, The Netherlands). Horseradish peroxidase (1000 U/mg) was from Boehringer Mannheim (Germany).

Baking trials

For baking trials, doughs were prepared (Table 1) with a 1000-fold lower amount of GOX. The dough was mixed in a Kemper spiral mixer at 300 slow turns (2 min) and 800 fast turns (3 min), resulting in a dough temperature of 26 °C. For crispy rolls, after 15 min of first proofing, the dough was divided into 55-g pieces, using a Record Automat type 3-10 TLG, from Kemper. The dough pieces were shaped using an Frilado type 2 shaper (Fritz Laureck). After 50 or 65 min proofing, they were baked in a Winkler Wachtel

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4-Deck Oven type AE 416/38 for 19 min at 220 °C. White tin breads were baked using the same process conditions, but the dough was divided after 5 min of first proofing and baked after 55 or 70 min final proofing.

The doughs were scored manually (rheological techniques cannot be applied to yeasted doughs) two minutes after mixing for dryness, stiffness, elasticity, extensibility, and coherency on a 10-point scale. Scoring was done blindly by 3 skilled bakers, each having more than 5 years experience, per set of 3 doughs, including a reference dough. Dryness was determined by touching the dough; 1 point was extremely sticky, and 10 was very dry. Stiffness (firmness) was the resistance of the dough when applying manual pressure; 1 was very soft, and 10 was very stiff. Elasticity was the capability of the dough to regain its original shape after being extended; 1 was nonelastic (deformation not recoverable), and 10 was very elastic, (deformation totally recoverable). Extensibility was the deformation to fracture when extended manually with a speed of about 5 cm/s; 1 was not extensible, and 10 was very extensible. Coherency was measured as the sheet forming capacity; 1 was non-coherent, and 10 was very coherent.

The fermentation stability of the crispy rolls was scored manually after the standard proofing time of 50 min and at 65 min proofing time (15 min over-proofing). The baked crispy rolls were scored by experienced test bakers on a 10-point scale for roll shape and crumb structure; 1 was a very coarse structure, and 10 was small cells and very regular structure. Product volume was measured using the rape seed displacement method. Tin loaves were judged for stability, ovenspring, crumb structure, and softness.

Dough and gluten preparation

Flow relaxation measurements were performed with flour doughs composed of Kolibri flour, water (60% flour weight basis), NaCl (2% flour weight basis), and either xylanase (75 ppm flour basis) or horse radish peroxidase (25 ppm flour basis), plus 10 ppm H_2O_2 or GOX (20 µL/10g flour). Doughs without enzymes, or containing combinations of xylanase with either peroxidase or glucose oxidase were also used. No yeast or ascorbic acid was added. All doughs were mixed for 4 min in a Mixograph (National Mfg. Co., USA).

For gluten isolation, doughs were prepared (Table 1), in a Kemper spiral mixer, at 300 slow turns and 800 fast turns, resulting in a dough temperature of 26 °C. Dough was rested for 30 or 45 min at 26 °C. From this dough, gluten was prepared by hand washing a portion of the dough with 2% NaCl solution for 15 min. Part of the fresh gluten prepared after 30 min of dough development was used for rheological measurements. The remainder of this gluten and the gluten prepared after 45 min of dough development were frozen immediately in liquid nitrogen, freeze dried, and ground prior to further analysis.

Rheological measurements

Flow relaxation measurements were done in a Bohlin VOR strain-controlled rheometer with parallel plates (dia 30 mm), covered with emery paper to prevent slip. The gap between the plates was set to 3 mm. After mixing, the flour dough was loaded between the plates. To prevent drying of the test piece, the rim of the sample was coated with grease, water drops were placed around the geometry, and the whole geometry was covered. Immediately after loading, a shear rate of 0.000665 s^{-1} was applied during 1500 s, resulting in a final deformation of 1. The applied shear rate was the lowest possible and was in the same order of magnitude as the deformation rates during fermentation of dough (Bloksma, 1990). After reaching the final deformation of 1, the stress was recorded during 3 h. Experiments were done in duplicate.

Sinusoidal oscillation tests were performed with freshly prepared gluten, using the same Bohlin VOR rheometer with the same geometry. Frequency-sweeps were performed at a strain of 0.0167 and at frequencies from 0.007 to 0.5 and back down. Gluten was loaded between the plates immediately after isolation. To prevent drying of the test piece the rim of the gluten was coated with grease, and a wetted tissue was placed around the geometry. The test was started after 16 min. Results were means of measurements on 3 gluten samples.

Biaxial extension tests were performed with fresh gluten by compression of a cylindrical test piece of gluten between two parallel Teflon plates with radius R = 10 mm, lubricated with paraffin oil (110 mPa s) (Van Vliet et al., 1992). A 6-g gluten piece was placed in a lubricated Teflon cylinder with dia 19 mm and covered with a lubricated solid Teflon plunger. After 45 min, the sample was compressed in a Lloyd instruments material testing machine (LR5K) equipped with a 50-N load cell, at room temperature (27 °C). At least 18 test pieces were compressed at 3 speeds v (5, 12, and 60 mm/min) to a final height of 1 mm. The deformation and force F were recorded. The point at which a force of 0.01 N was reached was taken as the starting point of compression. Hencky strain $_H$ and strain rate $\dot{}$ were calculated as $_H = -\ln(h_t/h_0)$ and $\dot{} = d\ _H/dt = dh/(hdt) = v/h_t$, respectively; biaxial strain and biaxial extensional rate as $_B = \ _H/2$ and $\dot{}_B = \dot{}_H/2 = v/2h_t$, respectively; h_0 is the initial height of the test piece and h_t the height at time t. Stress was calculated as $\sigma = F_t/(\pi R^2)$ (Van Vliet et al., 1992; Van Vliet, 1998).

Analytical techniques

The protein content was determined by a semi micro Kjeldahl method and calculated from the nitrogen content (N ∞ 5.75) (Gruppen et al., 1989). Starch was determined enzymatically with a test kit (Boehringer Mannheim). Prior to the determination of neutral sugars, gluten was extracted with DMSO/H_2O/ethanol (4:16:80 v/v) to remove (galacto)lipids (Gruppen et al., 1989). The neutral sugars in the gluten were determined as alditol acetates, using inositol as internal standard (Englyst and Cummings, 1984). Hydrolysis was performed with 1 M H_2SO_4 for 3 h at 100 °C, so cellulosic glucose was not determined (Englyst et al., 1982).

Alditol acetates were separated on a 2 m ∞ 2 mm i.d. glass column (packed with Chrom WAW 80 to 100 mesh coated with 3% OV275) in a Carlo Erba Fractovap 2300 GC operated at 200 °C and equipped with a F.I.D. detector set at 270 °C. Non-starch glucose was calculated from the difference in glucose found by the alditol acetate method and by the starch determination.

Phenolic acid content was determined as described by Gruppen et al. (1989). The samples were analyzed by HPLC with a reversed phase Lichrosorb 10 RP 18 column (Merck; 250 mm ∞ 4.6 mm), at room temperature, at a flow rate of 1 mL/min. Column elution conditions were modified: elution started with 95% 0.01 M acetic acid pH 5.0 and 5% methanol for 5 min. The methanol concentration increased to 50% in 20 min, to 90% in 5 min, and subsequently to 95% in 5 min. After 10 min at 95% methanol, the initial conditions were established over a period of 1 min, followed by 14 min washing. The eluent was monitored from 200 to 360 nm with a Spectra System UV3000 rapid scanning UV/VIS detector (Thermo Separation Products). Phenolic acid concentrations were calculated with respect to p-hydroxybenzoate as internal standard and corrected for the response factors of coumaric and ferulic acid at 280 nm.

Statistical analysis

All determinations were performed in duplicate. Reproducibility was ≥ 2%. Data were analyzed using the Statistical Analysis System (SAS Institute Inc., Cary, N.C., U.S.A.). Significantly different means were separated, using least significant difference at $P \leq 0.05$.

RESULTS & DISCUSSION

Dough and loaf properties

We selected the dough composition (Table 1) using Kolibri

flour with a protein content of 10.5 % instead of 11% to 12 %, because the effects of enzyme addition would be more pronounced in the resulting dough. The doughs were manually scored for dough properties 2 min after completion of mixing (Table 2). Addition of XYL in combination with POX or GOX improved the handling properties, as did POX alone. Extreme values for different dough properties cause problems when doughs are processed mechanically. The results for GOX in combination with XYL confirmed those published by van der Lugt et al. (1995). Clear differences in dough properties 2 min after mixing, indicated enzyme action during the initial stages of the bread making process.

We also scored the fermentation stability of the crispy roll dough against overproofing and the shape of the rolls (Table 2). The shape of those prepared with XYL-POX and XYL-GOX was judged best for they had no flat bottom, and a good split combined with a large volume (Fig. 1).

Addition of XYL to dough increased loaf volume and improved crumb structure of the baked product, but made the dough more sticky and less firm. GOX and POX alone did not have such an effect, whereas the combinations XYL-POX and XYL-GOX gave the same increase in volume as XYL. This effect has been ascribed to redistribution of water from the hemicellulose to gluten, which would render the gluten more extensible (Maat et al., 1992).

White tin loaves were judged for crumb structure (data not shown). Addition of XYL or POX improved crumb structure whereas GOX had a negative effect. The combination XYL-POX gave a finer, more regular crumb structure. The XYL-GOX combination baked unevenly and resulted in a coarse crumb structure. Results confirmed the observations reported for combinations of oxidative enzymes and xylanase by van Oort (1996).

From an application point, the combination of *Trichoderma* xylanase with soy peroxidase gave the best handling properties for dough, the best crumb structure in white tin loaves and the largest roll volume. Replacement of soy peroxidase with horseradish peroxidase had the same effect on handling properties and baking performance (van Oort et al., 1995b).

Rheological measurements

To compare blank dough with those containing combinations of xylanase, horseradish peroxidase, or glucose oxidase, we performed flow relaxation experiments. The doughs were composed of flour, water, salt, and enzymes as indicated. In flow relaxation experiments, horseradish peroxidase increased the peak stress with about 40% and glucose oxidase with about 155% (Fig. 2). During 3 h relaxation, the stress in the dough with horseradish peroxidase decreased to the same stress as that of the blank (20 Pa). This indicated that POX action did not result in additional permanent bonds

Table 1—Composition of doughs. (Flour was "as is")

Compound	Quantity (g)	Control	XYL	POX	XYL-POX	GOX	XYL-GOX
Flour	2000	+	+	+	+	+	+
Water	1180	+	+	+	+	+	+
Yeast	80	+	+	+	+	+	+
Salt	40	+	+	+	+	+	+
Ascorbic acid	0.04	+	+	+	+	+	+
Amylase	0.1	+	+	+	+	+	+
Xylanase	210,000 U	–	+	–	+	–	+
Soy Peroxidase	154 U	–	–	+	+	–	–
Glucose oxidase	21,500 U	–	–	–	–	+	+

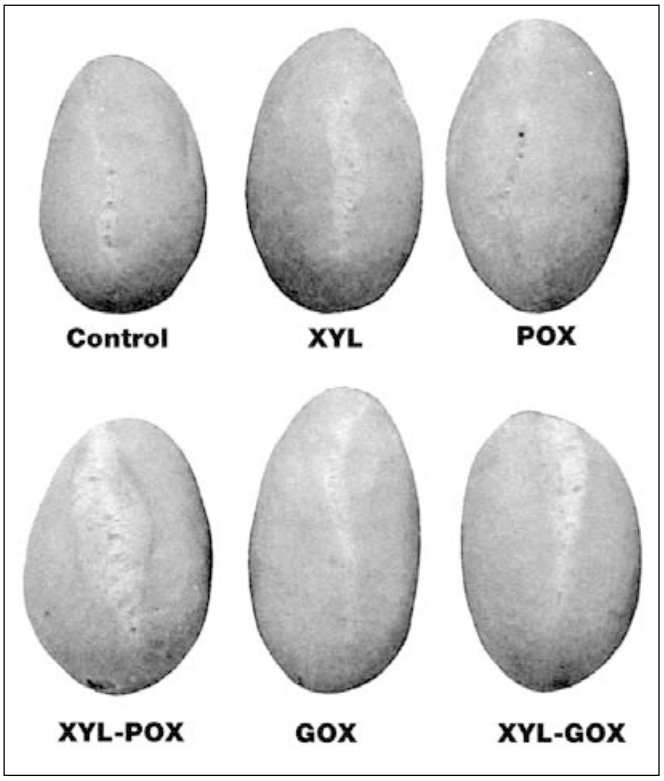

Fig. 1—Baking performance of doughs for the preparation of crispy rolls.

Table 2—Evaluation of manually scored dough properties 2 min after mixing and baking performance of the different doughs at 50 min proofing time or 15 min overproofing time (65 min)

Dough properties		Control	XYL	POX	XYL-POX	GOX	XYL-GOX
Dryness		5	4	7	7	7	7
Stiffness		6	4	8	7	9	8
Elasticity		4	5	6	6	7	6
Extensibility		3	4	2	4	2	3
Coherency		5	6	7	7	4	5
Properties of fermenting dough and baked rolls	**Proofing time**						
Fermentation stability	50	6	7	7	8	7	8
	65	4	6	6	7	6	7
Specific volume [%]	50	100	112	102	114	100	110
	65	100	116	104	120	104	117
Shape of roll	50	5	6	6	7	6	7
	65	4	5	5	6	5	6
Crumb structure	50	5	7	6	7	4	5
	65	3	6	5	7	4	5

in the dough. However, the differences in stress relaxation at time scales up to 3000 s indicated the increased occurrence of transient interactions. Such transient interactions, however, may be important for bread making as they would be effective on time scales relevant to the process. During proofing and baking, dough is extended at a strain rate of about 10^{-3} s^{-1} (Bloksma, 1990). The increased occurrence of entanglements may be explained by peroxidase catalyzed cross-linking of the arabinoxylans to high molecular weight aggregates that are intertwined with each other and with the gluten network via non-covalent interactions.

After 3 h, the stress in the dough prepared with glucose oxidase was still 30 Pa higher than in the control. Although transient interactions had apparently been introduced, additional permanent cross-links were formed. Due to glucose oxidase action, the network extending throughout the whole dough had been strengthened, probably because of the formation of more covalent bonds. This was in agreement with the view that glucose oxidase strengthens the gluten network via the action of hydrogen peroxide (van der Lugt et al., 1995, Poulsen and Bak Hølstrup, 1998).

Addition of XYL lowered the peak stress in all cases, but the effect in the GOX dough was strongest. Interestingly, in the presence of POX, the final stress for dough with and without XYL attained the same value. In the other doughs, differences became smaller in time but persisted up to 10,000 s. At 45 °C, the effect of the enzymes was similar, but stress levels were lower. In other words, POX could counteract the effects of xylanase at temperatures and on time scales relevant for the bread making process.

We performed sinusoidal oscillation tests with the six glutens to obtain information on the changes in viscoelastic properties of the gluten. Viscous behavior is reflected by the loss modulus (G″) and elastic behavior by the elastic or storage modulus (G′). Tan δ reflects the ratio G″/G′ of these moduli. A lower tan δ indicates a more elastic behavior of the material.

As can be seen (Fig. 3), XYL tended to reduce G′, but this effect could be counteracted by addition of GOX. POX gluten had a lower G′ than the control. XYL gave a somewhat higher tan δ, as compared to the control gluten. Autio et al. (1996) observed a similar effect on G′ and tan δ for xylanase in rye dough. GOX and XYL-GOX gave the lowest tan δ, i.e., behaved the most elastic, whereas POX and XYL-POX had a slightly lower tan δ than the control gluten. Application of small deformations indicated that the GOX-treated gluten behaved differently.

Similar results were obtained when large deformations were applied in biaxial extension tests. We calculated results on the basis of a constant biaxial strain rate of 0.01 s^{-1}. The stress was determined as a function of the biaxial strain at a constant rate (Fig. 4), so the slope of the curve was the strain hardening value, $\leq \ln\sigma/\leq_{B}$ (Van Vliet et al., 1992; Van Vliet, 1998). The values, indicating the increase in resistance towards extension with increasing strain, are indicated in the legend. The addition of XYL resulted in a lower stress in comparison to the control gluten. All other enzymes and combinations increased stress. These results, obtained with gluten isolated after 30 min dough development, and the strain hardening values of gluten were in agreement with the manual assessment of the doughs (performed after 2 min). XYL resulted in a soft, elastic dough, whereas GOX and XYL-GOX resulted in a firm dough, with the others intermediate.

Stress and strain hardening are important factors for bread crumb structure. Stress is most important at the beginning of proofing, while strain hardening is important at the end. Higher stress favors good crumb structure, and strain hardening is an important factor for dough film stability. The higher the strain hardening the better the stability against rupture of dough films between gas cells (van Vliet, 1998). The coarse crumb structure of the baked product can be related to dough fracture. During fermentation, the gas cells increase in volume, and the liquid dough films between the gas cells are extended. When the extension becomes too large, the films fracture and the gas cells coalesce and lower the gas holding capacity of dough. So at a certain extension, determined by the increase in dough volume during fermentation, XYL-GOX dough may have reached the fracture strain. The biaxial extension tests show that the dough prepared with peroxidase and xylanase was more extensible because of synergistic effects of the enzymes on gluten stress and strain hardening. The increased extensibility and the stronger strain hardening may explain the finer crumb structure of the XYL-POX loaves. Although GOX had a positive effect on strain hardening, the effect on crumb structure

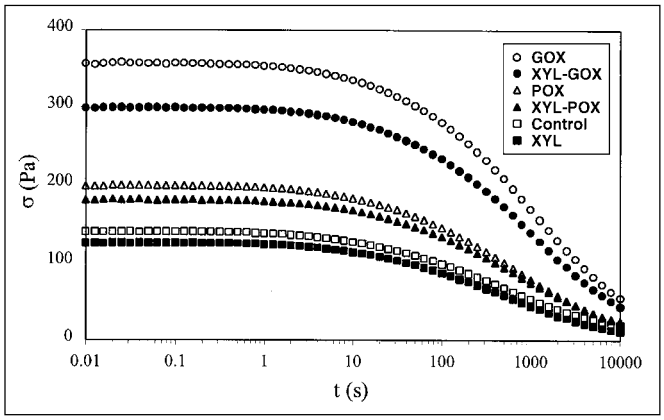

Fig. 2—Shear stress σ as a function of time *t* after cessation of shear in flow relaxation measurements for flour doughs. Shear was applied during 1500 s at a shear rate of 0.000665 s^{-1}.

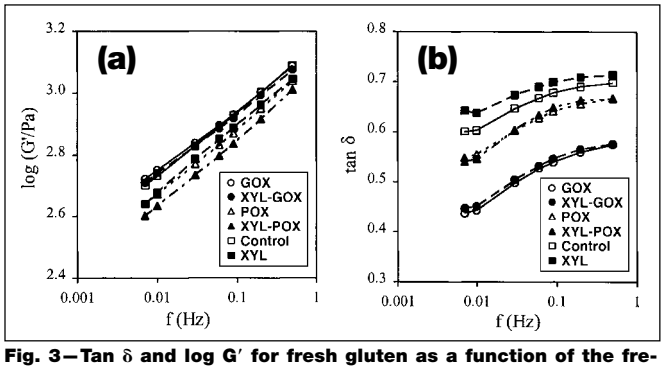

Fig. 3—Tan δ and log G′ for fresh gluten as a function of the frequency.

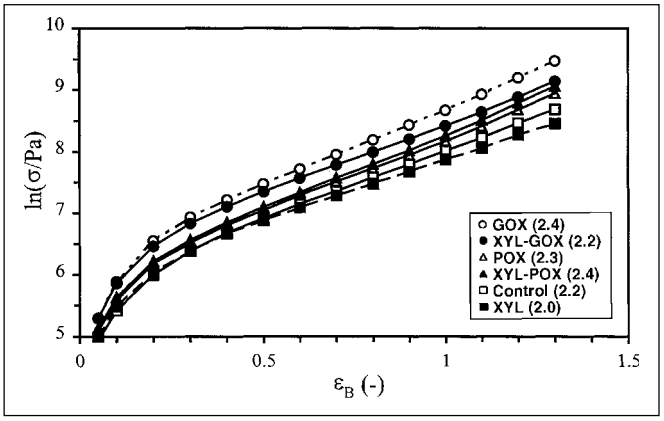

Fig. 4—Stress σ of fresh gluten as a function of the biaxial strain in biaxial extension, calculated on the basis of a biaxial strain rate of 0.01 s^{-1}. The strain hardening value is indicated in brackets.

Table 3—Gluten composition in percent w/w after 30 min dough development. Values are averages of 2 independent determinations

	Control	XYL	POX	XYL-POX	GOX	XYL-GOX
Starch	9.1	5.9[a]	5.7[a]	4.2[b]	4.1[b]	2.7
Protein	80	84[c]	84[c]	87[c]	86[c]	86[c]
NSP	1.9	1.3	1.6	1.3	2.7	1.4
ferulic acid (μg/mg)	0.080[d]	0.074[d]	0.060[ef]	0.071[de]	0.048[fg]	0.046[g]

[a-g] Means with same letter are not significantly different (P > 0.05).
[h] Non-starch, non-cellulose sugar content. NSP was calculated as the difference between total amounts of sugars and starch. Statistical errors are the sums of the errors in rha, ara, xyl, man, gal, glc, and starch, so differences were not significant.

Table 4—Non-starch non-cellulose sugar composition of gluten after 30 min dough development[a]

	control	XYL	POX	XYL-POX	GOX	XYL-GOX
Rha	0.04	0.024[a]	0.022[a]	0.019[ab]	0.019[ab]	0.013[b]
Ara	0.301[c]	0.248[d]	0.226[de]	0.221[e]	0.294[c]	0.226[de]
Xy	0.253[f]	0.210	0.189[g]	0.177[g]	0.354	0.238[f]
Man	0.085	0.067	0.057[h]	0.059[h]	0.049	0.041
Gal	0.212[ij]	0.192[ijkl]	0.183[ijkl]	0.195[ijl]	0.177[jkl]	0.164[kl]
Glc	1.056	0.600[mn]	0.969[mn]	0.670[no]	1.839[mn]	0.682[o]
ara +xy[q]	0.43	0.35	0.31	0.29	0.54	0.37
ara/xy ratio[r]	0.71	0.66	0.64	0.62	0.55	0.56
fer/xy[r]	0.022	0.024	0.022	0.027	0.009	0.013

[a-o]Means with the same letter are not significantly different (P>0.05).
[p]Values are weight percent with respect to gluten and are averages of 2 independent determinations. Rha, rhamnose; ara, arabinose; xy, xylose; man, mannose; gal, galactose; glc, glucose; fer, ferulic acid.
[q] For calculation of the amount of arabinose in arabinogalactan, a molar ratio of 0.7 was used (Fincher and Stone, 1974). We assumed that all remaining arabinose was incorporated in arabinoxylan.
[r]Expressed as molar ratio.

was negative. Apparently, the higher strain hardening could not compensate for lower extensibility.

Neither the large nor the small deformation tests provided evidence that xylanase removed covalently bound polysaccharides from the gluten, leading to a better aggregation of the gluten proteins (as suggested by van der Lugt et al., 1995). In that case xylanase should have caused a higher stress and stronger strain hardening, except if it also caused the gluten network to become much less homogeneous (which is less likely).

Analysis of chemical composition

Baking trials and rheological measurements showed differences in dough and gluten properties caused by addition of XYL, POX, or GOX. It has been suggested that each of these enzymes may affect gluten properties, so we compared the chemical composition of gluten isolated from the different doughs after 30 and 45 min of development.

The dry weight of gluten isolated after 45 min of dough development ranged from 53 mg/g dough for GOX gluten to 58 mg/g dough for the control gluten. These differences were attributed to variations in washing efficiency, since trends found for this series of gluten differed from those for another series (data not shown).

The starch, protein, non-starch non-cellulose polysaccharide and ferulic acid contents were determined (Table 3) on the gluten isolated after 30 min of dough development. The starch and protein content of the gluten isolated from the control dough were different ($P < 0.05$) from the other doughs. Also in gluten prepared after 45 min of dough development (data not shown), the starch content was higher than in the other gluten fractions. Kokelaar (1994) provided evidence that this was due to a less efficient aggregation of the gluten.

The polysaccharide content varied between the glutens. Saulnier et al. (1997) have noted a positive correlation between arabinoxylan and starch content in gluten and attributed this to less efficient washing. However, samples with a similar starch content varied in amounts of non-starch, non-cellulose polysaccharides.

The data (Table 3) suggested only minor differences between the glutens. A clearer picture was obtained when changes in the amounts of individual sugars were analyzed. The sugar composition of the control gluten was (for most sugars) significantly different from the other glutens. This higher NSP content may be related to less efficient removal of starch and NSP as suggested by Saulnier et al. (1997). The other doughs in most cases, did not differ with respect to rha, gal, and glc, but differences in xy and ara were significant in most cases.

Arabinoxylans are the substrates for xylanases, so their content in the gluten was expected to decrease due to enzyme action. In the gluten isolated from doughs prepared with XYL, POX, XYL-POX, or XYL-GOX, the amount of xylose and arabinose had decreased as expected, but not for GOX gluten. Similar results were obtained with gluten isolated after 45 min dough development (data not shown). Arabinose could originate from either arabinoxylans or arabinogalactan-proteins, which can be extracted with cold water (Fincher and Stone, 1974). However, extraction with NaCl retained them in the gluten fraction. We used a molar ratio of ara/gal of 0.7 (Fincher and Stone, 1974) to calculate the amount of arabinogalactan, assuming that all galactose originated from arabinogalactan-protein. The remainder of arabinose was assumed to originate from arabinoxylans. The resulting amounts of arabinoxylan (Table 4) ara+xy and the ara/xy ratio were rather low, indicative of low substituted less soluble arabinoxylans. Xylanase addition resulted in a lower ara+xy content in their gluten as compared to the control. Gluten from POX and XYL-POX dough contained the same amount of ara+xy. This was also the case in gluten isolated after 45 min of dough development (data not shown). When the amount of ara+xy was expressed as percentage of all sugars minus glucose, in all cases arabinoxylans constituted 50 % of the amount of sugars minus glucose, except for the GOX gluten where this was 60 %. Neither rheological nor chemical data provided evidence to support the hypothesis (van der Lugt et al., 1995) that xylanase removed specifically arabinoxylans from gluten, rendering aggregation more efficient.

Using the data (Table 3), the ferulic acid units per backbone unit could be calculated. POX alone reduced the amount of ferulic acid, but in combination with xylanase there was hardly any effect on the amount whereas the ratio fer/xy had increased. In the presence of GOX, with or without XYL, not only the absolute ferulic acid content but also the fer/xy ratio was lower.

The data (Table 3 and 4) indicated that addition of POX to dough affected the properties of gluten less than the GOX or XYL-GOX combination. Rheological properties confirmed this. The effect of POX on the gluten could not be ascribed to changes in arabinoxylan content, as gluten derived from POX containing doughs contained less arabinoxylans than the control. Neither less efficient removal of large arabinoxylan aggregates from gluten during washing nor cross-linking of arabinoxylans to gluten protein appeared to apply. The changes in gluten properties might be explained by POX converting ascorbic acid to dehydroascorbic acid, which could oxidize gluten via a thiol/disulphide interchange reaction. Since POX hardly affected the gluten, but yielded dough with good handling properties and a good baking performance, it must act on other fractions of the dough. Another study confirmed that POX changed the properties of water unextractable solids by cross-linking arabinoxylans present in this fraction (Hilhorst et al., 1999). Resulting products may form a second, entangled network throughout the dough, as suggested by rheological data.

Chemical analysis confirmed rheological data that gluten derived from GOX and XYL-GOX dough differed from other glutens. GOX addition led to less efficient removal of cellulose, glucans, and arabinoxylans from gluten. The presence of these components could, in combination with the formation of additional covalent bonds in gluten be responsible for the changed properties of the gluten. The GOX and XYL-GOX doughs differed in baking

performance, but their gluten were rather similar in chemical and rheological properties. We concluded that the properties of the gluten per se did not suffice to provide a good quality dough and a good baking performance. Properties of other fractions of the dough were also relevant for the XYL-GOX treated dough.

CONCLUSION

THE RESULTS PRESENTED HERE SHOW THAT AN INTEGRATED APproach, combining baking trials, rheological experiments, and chemical analysis could give answers to complicated questions that cannot be answered by any of these approaches alone. Baking trials showed that the use of peroxidase in combination with xylanase improved the handling properties of the doughs and the final baked product, justifying their commercial use. Although their mechanism of action is not well understood, we concluded that these enzymes had only a marginal effect on gluten properties and were likely to exert their action on other fractions.

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