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Effect of Oxidative Enzymes on Bulk Rheological Properties of Wheat Flour Doughs

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ABSTRACT

The use of enzymes such as peroxidases or glucose oxidase instead of chemical oxidants is a very interesting option for improving breadmaking performance of doughs. In this study the effect of such enzymes on bulk rheological properties of dough was quantified and their influence on the polymer network in dough deduced.

Small deformation oscillation and relaxation tests (strain 0·001) are not suitable for discriminating between doughs prepared in the presence of the different enzymes. Flow relaxation tests at high deformation and long relaxation times showed a clear distinction between the effect of peroxidase and glucose oxidase. Peroxidase increases only the number or lifetime of transient bonds, whereas glucose oxidase additionally produced cross-links that were permanent on time scales up to 3 h. Peroxidase probably introduced a second, more transient structure (arabinoxylan network) through the gluten network, whereas glucose oxidase may also have strengthened the gluten network. A higher water addition could not compensate for the effect of peroxidase; on longer time scales the stress remained at a higher level. Similar results were obtained in large deformation biaxial and uniaxial extension tests. Peroxidases only increased stress levels. The addition of glucose oxidase resulted in a higher stress and more intense strain hardening. Only in biaxial extension was an influence of pH observed. An increase in stress level was accompanied by a decrease in fracture strain, making predictions of the effects on bread structure complicated.

Keywords: dough rheology, relaxation, peroxidase, glucose oxidase.

INTRODUCTION

Chemical oxidants are frequently added to flour to improve its breadmaking performance. Substituting these oxidants by enzymes such as peroxidases (POX) or glucose oxidase (GOX) is a very interesting option. These oxidative enzymes can also counteract the effect of xylanases (XYL), which have a positive effect on oven spring and loaf volume, but often result in sticky dough. In general POX gives a better dough handling, dough tolerance, crumb structure, loaf volume, and less staling. Thus oxidative enzymes like GOX and POX are already widely used in breadmaking[1,2].

It is thought that POX causes oxidative gelation of flour pentosans. As early as 1925 it was reported that hydrogen peroxide, a substrate for POX, increased the viscosity of wheat and flour suspensions and caused gelation of flour extracts[3]. The dough component affected by hydrogen peroxide was found to be water-soluble and was identified as water-soluble pentosan[4]. Gelation of water-soluble pentosans is

ABBREVIATIONS USED: POX = peroxidases; FPOX = wheat flour peroxidase; HPOX = horseradish peroxidase; LPOX = lacto peroxidase; SPOX = soy peroxidase; GOX = glucose oxidase; XYL = xylanase..

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irreversible and is affected by pentosan concentration, oxidant and its concentration, temperature and several soluble substances found in flour and bread doughs. Added bran extract liquefied the gel, presumably through enzymatic action.

Wheat flours contain 2–3% total pentosans, of which one-third to one half is water-soluble[5,6]. This water-soluble fraction is composed of arabinoxylan and arabinogalactan[6]. The oxidative gelation of pentosans by H_2O_2 is ascribed to ferulic acid residues, esterified to the arabinose in arabinoxylan[7–9]. One possible mechanism is through dimerization of ferulic acid residues on adjacent arabinoxylan chains[10]. Proteins also participate in gelation since the gel fraction contains about 25% protein and that is dissolved by proteolytic enzymes[4,10]. The proposed mechanism is by coupling of ferulic acid residues to tyrosine or cysteine residues on proteins[10,11]. H_2O_2 has no effect in the absence of POX[11].

The aim of this study was to quantify the effect of several POX's and GOX on the rheological properties of flour dough by small and large deformation tests and to deduce from these physical experiments their influence on the polymer network in dough. Effects of the enzymes on breadmaking properties and bread quality are published elsewhere[12].

MATERIALS AND METHODS

Materials

The flour used was a commercial biscuit -type mixture, named Kolibri, with 15·0% moisture and 10·5% protein, obtained from Meneba, The Netherlands. Its breadmaking quality is poor. NaCl (analytical grade), H_2O_2 (30% solution, stabilised, medical extra pure) and glucono-1,5-lactone were from Merck, Germany.

FPOX (0·13 peroxidase units/mg), LPOX (99 peroxidase units/mg) and XYL ex *Trichoderma* (Biobake CX 160, 351 xylanase units/mg) were obtained from Quest International, The Netherlands. SPOX was from Adumin, Israel (35 peroxidase units/mg). HPOX was from Boehringer, Germany (1000 peroxidase units/mg). GOX Oxygo L5 was from Genencor (5364 glucose oxidase units/ml). Peroxidase activities are determined on ABTS (2,2′-Azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid). Yeast was 'Koningsgist' from Gistbrocades, The Netherlands. The amylase was Biobake 5000 from Quest International. Deionised water was used in all experiments.

The Glucose/Fructose UV-test (Boehringer, Mannheim) was used to determine glucose in the flour.

Dough preparation

Water addition was 60% on a flour basis (as is) for all doughs. The water contained 2% NaCl, on flour basis, and the enzyme. Enzymes were added to a concentration found to be optimal in baking experiments (M. van Oort, Quest International, pers. comm.). The concentration of FPOX, HPOX, LPOX, SPOX, GOX and XYL was 130, 25 000, 9900, 525, 10 730 and 26 300 U/kg on a flour basis, respectively. In baking tests H_2O_2 is produced by the yeast[13] and no H_2O_2 has to be added as a substrate for POX (R. Orsel; Quest International, pers. comm.). However in the absence of yeast there was no effect of the POX on dough rheology. Therefore, 20 μL of diluted H_2O_2 (10 ppm H_2O_2 on flour basis) was added in experiments with POX; 20 μL water for the blank and GOX.

Water and flour temperatures were 20 °C. All doughs were prepared in a 10 g mixograph (National Mfg. Co., Lincoln, Nebraska, USA). The average peak time, 4 min, was taken as a standard mixing time. After mixing the temperature and pH of the dough were determined. Rheological measurements were started after a resting period of 45 min, except for the (flow) relaxation measurements. Dough with NaCl as the only additive was the designated control dough. Glucono-1,5-lactone was used to lower the pH of the dough in the same time course as with the added yeast. To estimate the optimal amount of glucono-1,5-lactone, doughs were mixed with 4% yeast and 100 ppm amylase and compared to doughs mixed with different amounts of glucono-1,5-lactone. Additions were made after 0·5 min mixing. The optimal glucono-1,5-lactone concentration was 23·5 mg for all doughs except for GOX (13 mg). The final pH values were about 5·4.

Mixograms

Mixograms recorded during 10 min mixing, were analysed for time to peak, peak height, and slope of the ascending and descending portions of the curve at the peak were determined according to AACC Method 54–40A.

Sinusoidal oscillation, relaxation and flow relaxation tests

Sinusoidal oscillation, relaxation and flow relaxation tests were performed with a Bohlin VOR constant shear rheometer equipped with a plate–plate assembly (diameter 30 mm), covered with emery paper to prevent slip. The gap between the plates was set to 3 mm and the temperature at 30 °C. Doughs were loaded between the plates immediately after mixing. To prevent drying out of the test piece, the rim of the dough was coated with grease, water drops were placed around the edge of the piece and the whole was covered. Disappearance of the water drops indicated that the sample was drying out.

Frequency-sweeps were performed at a strain of 0·001. Results reported are the mean of measurements on three different doughs.

In relaxation experiments after a resting time of 6 h in the geometry a deformation of 0·0013 was applied in 1 s and the stress was measured during 3 h relaxation.

For flow relaxation studies three methods were used. In the first method there was no resting period and the sample was sheared immediately during 1500 s at a shear rate of 0·000665 s^{-1}. This shear rate was the lowest possible and is of the same order of magnitude as the deformation rates during fermentation of dough[14]. As no resting period is included, there could still be some influence of sample loading on the results. Therefore, in the second method a resting period of 3 h in the apparatus was included and the sample was deformed at 0·0208 s^{-1} during 50 s. The higher shear rate, compared to the first method, demonstrates more clearly the existence of fast relaxing bonds. A third method was used to illustrate an effect of the rheolgical history of the sample on the results. In this method the resting time was 1450 s after which the sample was deformed at 0·0208 s^{-1} during 50 s. In all methods a final strain of 1 was applied. The force was measured during 3 h. Both relaxation and flow relaxation tests were performed at least in duplicate.

Biaxial extension tests

Biaxial extension tests were made by compressing a cylindrical test piece between two parallel Teflon plates with radius $R = 10$ mm, lubricated with paraffin oil (110 mPa · s)[15]. A dough piece, 7 · 0 g, was placed in a lubricated 19 mm diam. Teflon cylinder. The sample was covered with a lubricated solid Teflon plunger that just fitted in the cylinder. After 45 min the sample was allowed to slide out of the cylinder onto the lower plate. A few drops of paraffin oil was placed on top of the sample, which was compressed in a Zwick material testing machine equipped with a 50 N load cell, at 30 ± 1 °C. At least 18 test pieces were compressed at three different speeds v (5, 12 and 60 mm/min) to a final height of 1 mm. The deformation and force F were recorded. The starting point of compression was chosen as the point at which a force of 0·01 N was reached. The initial sample height h_0 was calculated as the final height plus the total displacement of the plunger starting from the force of 0·01 N. Hencky strain and relative deformation rate were calculated as $\varepsilon_H = -\ln(h_t/h_0)$ and $\dot{\varepsilon} = d\varepsilon_H/dt = dh/(h_t dt) = v/h_t$, respectively; biaxial strain and biaxial strain rate as $\varepsilon_B = \varepsilon_H/2$ and $\dot{\varepsilon}_B = \dot{\varepsilon}_H/2 = v/2h_t$, respectively; h_t is the height of the test piece at time t. Stress was calculated as $\sigma = F_t/(\pi R^2)$[15,16].

Uniaxial extension tests

Uniaxial extension tests were performed at 30 ± 1 °C with a Kieffer extensibility rig[17] (micro-extensograph) fitted on a Zwick material testing machine equipped with a 50 N load cell. The dough was formed into a roll, put on the lower plate of the Teflon form of the extensibility rig and compressed with the top plate. The plates were lubricated with paraffin oil. After 45 min at room temperature the dough was pushed out of the form using a stick, and using tweezers, the sample was placed on the lower plate of the extensibility rig. About 18 samples were tested at 12, 60 and 300 mm/min. The deformation and force F_m were recorded. The starting point for all experiments was taken as the position y_0 of the hook at 2 mm above the top of the lower plate.

Hencky strain and relative deformation rate were calculated as $\varepsilon_H = \ln[\sqrt{(9^2 + (y_t + y_0)^2)}/\sqrt{(9^2 + y_0^2)}]$ and $\dot{\varepsilon} = 4(y_t + y_0)v/l_t^2$, respectively. Stress was calculated as $\sigma = F_d/(V/l_t)$. In these equations y_t is the displacement of the hook from the point y_0 (2 mm) at which the actual extension starts, 9 is half the size of the gap in the lower plate through which the hook passes, F_d is the force actually exerted on the dough string, V is the volume of the extended dough and $l_t = 2\sqrt{(9^2 + (y_t + y_0)^2)}$ is the length of the sample at time t.

From the biaxial and uniaxial extension tests the strain rate thinning ($\partial \ln \sigma / \partial \ln \dot{\varepsilon}_B$) and strain hardening ($\partial \ln \sigma / \partial \varepsilon_B$) behaviour of the dough were calculated[18,19].

RESULTS AND DISCUSSION

Temperature and pH

The temperature of the doughs after mixing averaged 28 °C. The pH of all doughs, except GOX dough, averaged 5·93 directly after mixing. The pH did not change in time during the 45 min resting at 30 or 50 °C. In the presence of GOX the pH decreased to 5·81 and 5·73 directly after mixing and after 45 min, respectively. GOX produces gluconо-1,5-lactone, which slowly hydrolyses, releasing protons.

Mixograms

GOX increased the peak time and by adding 10 ppm H_2O_2 the developing slope was increased (Table I). Strictly speaking, the dough with 10 ppm H_2O_2 should be regarded as the blank in relation to the doughs with POX. Taking this into account, there were no other significant differences in mixogram characteristics, but the peak time tended to be higher when 10 ppm H_2O_2 was added and to increase further when HPOX or SPOX was added. Furthermore, doughs with 10 ppm H_2O_2 and POX tended to be more tolerant to overmixing (lower weakening slope). By using a standard mixing time of 4 min, all doughs were mixed up to their peak time (within 95% confidence interval), except GOX dough, which was undermixed.

Sinusoidal oscillation tests

Frequency-sweeps were performed at a strain of 0·001, which is the upper limit of the linear region. At this strain G' was found not to decrease after starting an oscillation at 1 Hz, whereas at a strain of 0·003 G' decreased from $2{\cdot}3 \cdot 10^4$ to $2{\cdot}0 \cdot 10^4$ Pa during the first 300 s. In the oscillation tests $\log(G')$ of all doughs, except for GOX dough, increased from 3·6 at 0·007 Hz to 4·3 at 10 Hz linearly as a function of log oscillation frequency. The value of $\tan\delta$ was between 0·37 and 0·43. Only GOX caused an increase of G' by a factor 1·2 and a slight decrease in $\tan\delta$ (results not shown). This is in accordance with observations of e.g. Vemulapalli *et al.*[20]. In this experiment GOX doughs were also mixed up to their peak according to mixograph tests (5·5 min, Table I). This increased G' by 10%. No effect of POX was observed by this method. This test is unable to discriminate between flour doughs that behave differently in baking trials, as observed earlier[19].

Relaxation tests

In relaxation experiments the modulus G varied from 5500 to 6500 Pa when the strain of 0·0013 was reached. G subsequently decreased to 200–350 Pa in 3 h. The relaxation time is defined as the time in which G decreases to $1/e$ ($= 0{\cdot}37$) times G at time zero. In these experiments the relaxation time was on an average 73 s. No significant effects of H_2O_2 and POX's were detected. GOX also had no effect (results not shown). From these tests and the sinusoidal oscillation tests it was concluded that the effects of oxidative enzymes on breadmaking properties were not due to a change in the small deformation properties of dough. Small deformation properties of bread dough are determined to a large extent by the starch granules[21]. Thus, any effect of added enzymes on the components other than starch may have been not noticeable in this dough, which was otherwise of the same composition.

Table I Averages and least significant difference (95% level) of characteristics of mixograms recorded during 10 min mixing. MU = mixogram units (arbitrary scale)

	n	Time to peak (min)	Peak height (MU)	Developing slope (MU/min)	Weakening slope (MU/min)
Control	3	3·60	460	34	11
10 ppm H_2O_2	3	4·03	487	52	6
Flour POX, 130 U	3	4·13	502	44	5
Horseradish POX, 25 000 U	3	4·39	477	41	5
Lacto POX 9900 U	3	3·94	491	47	4
Soya POX, 525 U	3	4·40	499	47	5
GOX, 10 730 U	6	5·31	495	33	10
Least significant difference		0·43	38	11	6

Flow relaxation tests: effect of enzymes

Flow relaxation measurements are relaxation measurements at strains far outside the linear region. Dough was sheared to a strain γ of 1 at two quite different shear rates, $\dot{\gamma}$ and resting times in the apparatus before shearing. The measurements were very reproducible (Fig. 1, FPOX and LPOX not measured). With SPOX the initial stress σ_i was 182 and 276 Pa for 1500 s at $0{\cdot}000665\,s^{-1}$ after no resting time, and 50 s at $0{\cdot}0208\,s^{-1}$ after 3 h resting time, respectively. In SPOX dough σ decreased in a manner similar to that in H_2O_2 and POX doughs (not shown). Immediately after γ had been applied, in both methods the initial stress, σ_i, was higher for all additions but to different extents. GOX had by far the largest effect. The addition of H_2O_2 was disadvantageous, as it had an effect on its own.

After 3 h H_2O_2 and POX doughs had relaxed to the same final stress σ_f (20 Pa) as the control dough. Thus due to the action of POX the number of relatively short-lived cross-links ('entanglements') was increased. On the time scale of these experiments no permanent cross-links were formed. However, the increased number of entanglements may well be important for breadmaking as on relevant time scales (1000 up to 3000 s) σ is still higher in comparison with the blank. After 3 h σ_f of dough with GOX was still 30 Pa higher than all other doughs. Although also non-permanent cross-links were introduced, as in SPOX and HPOX doughs, additional cross-links were formed, which were permanent on the time scale of the experiment. The time after which σ had decreased to $1/e$ (37%) of the initial σ, was not or only slightly increased by POX's, but by a factor 2–3 by GOX (Fig. 2).

From these measurements it may be concluded that POX's only cross-linked pentosans. The resulting increase in their molecular weight may be responsible for more non-permanent cross-links between the pentosans and between the pentosans and the protein network. This may be interpreted as an introduction of a second, more transient structure independent of the gluten network. GOX may have had the same effect, as released H_2O_2 could serve as a substrate for the native FPOX. However, in addition, it may also have strengthened the network extending throughout the whole dough and thus may also affect gluten. The difference in action between the POX's and GOX was verified by chemical analysis. Addition of POX hardly affected the chemical properties of gluten, whereas GOX did[12]. Vemulapalli and Hoseney[22] observed a reduction in the SH content of the SDS-soluble protein on addition of glucose oxidase, although its amount and relative viscosity did not change.

At 45 °C the enzymes had similar effects although stress levels were 25% lower than at 30 °C (only

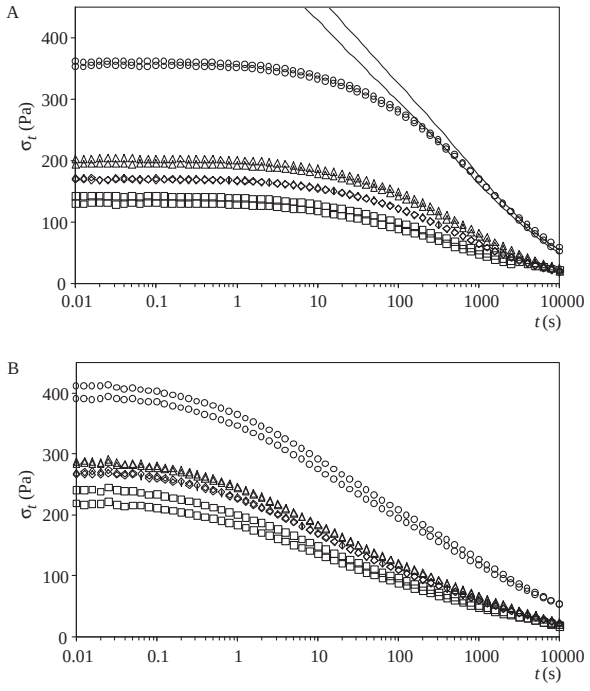

Figure 1 Shear stress σ_t as a function of time t after cessation of flow in a flow relaxation experiment at 30 °C and pH 5·9. No resting time, shearing 1500 s at $0{\cdot}000665\,s^{-1}$ and 1450 s resting time, shearing 50 s at $0{\cdot}0208\,s^{-1}$ (solid lines, only measured for GOX) (A) and 3 h resting time, shearing 50 s at $0{\cdot}0208\,s^{-1}$ (B). Control (□), H_2O_2 (◇), HPOX (△) and GOX (○).

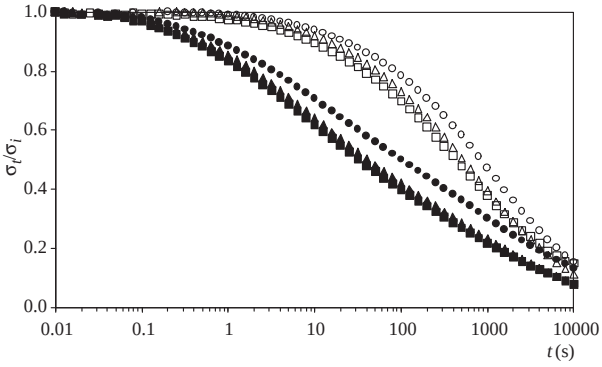

Figure 2 Shear stress over initial shear stress σ_t/σ_i as a function of time t after cessation of flow in a flow relaxation experiment at 30 °C and pH 5·9. Open symbols: no resting time, shearing 1500 s at $0{\cdot}000665\,s^{-1}$; Closed symbols: 3 h resting time, shearing 50 s at $0{\cdot}0208\,s^{-1}$. Control (□), HPOX (△) and GOX (○).

measured after shearing for 1500 s at 0·000665 s^{-1} and no resting time, results not shown). The measurements were less reproducible than at 30 °C.

The effect of GOX was more pronounced than observed by Wikström and Eliasson[23]. Apart from the differences in flour and enzyme, this may be due to the higher $\dot{\gamma}$ (0·0367 s^{-1}) and the smaller final γ (0·367) they applied. A higher $\dot{\gamma}$ leads to a higher σ_i and, in consequence, the differences in σ caused by changes in the gluten network are less pronounced. In addition, at small γ the influence of the starch granules on σ will be larger. Moreover, if we had followed stress relaxation for only 1200 s the difference in relaxation behaviour between POX's and GOX would not have been so clear. Thus the high strain and the long relaxation times were essential to show a clear distinction between the effects of POX and GOX. Effects that are important for the processes proceedings over time scales used during baking.

At low $\dot{\gamma}$ nearly all relatively fast relaxing bonds will already relax during the shearing procedure. After shearing at a high $\dot{\gamma}$ these bonds will relax as soon as the strain is applied. From Figure 1 this effect of shearing history on the relaxation behaviour is clearly visible. The higher $\dot{\gamma}$ resulted in a higher initial stress σ_i of the relaxed doughs (Fig. 1B). It is likely that this is a result of the long resting period before shearing, within seconds σ in the relaxed doughs decreased below the stress in doughs to which the strain was applied in 1500 s without prior resting. A resting time of 3 h implies that the strain is applied to a relaxed dough. To illustrate that the differences were caused by shearing history effects a measurement on GOX dough was performed to which γ was applied for 50 s ($\dot{\gamma} = 0{\cdot}0208\ s^{-1}$) after a resting time of 1450 s. So γ was also 1 after 1500 s, but with different shearing history. Figure 1A (solid lines) shows that indeed in this dough σ_i was also much higher because of the higher $\dot{\gamma}$, but finally the curves coincided with those for no resting time and 1500 s at 0·000665 s^{-1}.

Flow relaxation tests: Effect of water dosage

Controlling the stress levels in dough by varying the amount of added water is common practice. Doughs without additions and doughs with HPOX and GOX were used to check if the effect of oxidative enzymes on stress level and relaxation behaviour could also be achieved by simply changing the amount of water added. When 3% more water was added, this reduced σ_i with 40 to 50 Pa for the control dough and HPOX dough and with 120 Pa in the case of GOX (Fig. 3). This is about 2/3 of σ_i with normal 60% water addition. The value of σ_i in HPOX dough dropped to the same level as the control dough with the normal 60% water addition.

In the control doughs with 60% and 63% water addition, the double logarithmic curves were shifted, i.e. the relaxation proceeded in the same manner, independent of the water addition. The same applies to GOX doughs. In the case of HPOX, however, relaxation at 63% water was slower than at 60% water. The stress level in both HPOX doughs finally coincided. Thus over longer time scales, that are more relevant for breadmaking, the effect of more water on $\sigma(t)$ seemed to be smaller in the case of added POX than for the control. In other words, when using HPOX 3% more water can be added (compared with the control) with little alteration of the rheological properties over time scales relevant for breadmaking. With 3% less water in the control dough σ_i did not increase to the level of the HPOX dough (Fig. 3).

The fact that the relaxation behaviour of the control dough and of GOX dough was independent of a water addition at 60% or 63%, suggests that water had nearly the same effect on various kinds of physical bonds in the doughs. Apparently the effect of a higher water addition in this range is simply a dilution of physical interactions resulting in a lower stress but equal relaxation behaviour. This was unexpected as dough is a visco-elastic material containing many different compounds and physical interactions, which would be expected to affected differently by water. In this aspect POX dough behaved differently in this range of water addition.

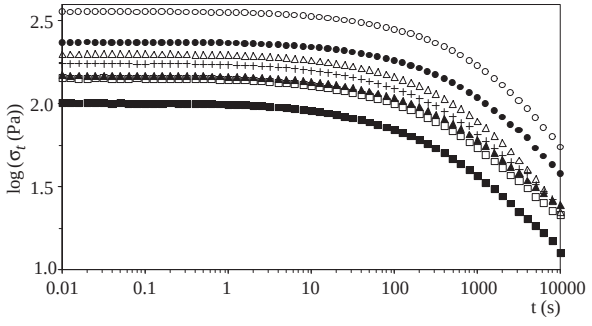

Figure 3 Influence of water dosage on shear stress σ_t as a function of time t after cessation of flow in flow relaxation of doughs at 30 °C (no resting time, shearing 1500 s at 0·000665 s^{-1}). Water dosages were: standard 60% (open symbols), 63% (closed symbols), 57% (+). Control (□, +), HPOX (△) and GOX (○).

Biaxial extension tests

The forces measured in biaxial extension tests at different biaxial strain rates $\dot{\varepsilon}_B$ were expressed graphically as $\ln(\sigma)$ at $\dot{\varepsilon}_B$ 0·01 s^{-1} versus biaxial strain ε_B according to the procedure described by Kokelaar *et al.*[19]. Addition of H_2O_2 resulted in a higher σ (Fig. 4). POX addition resulted in an additional increase, although the extent varied with the origin of POX. GOX addition resulted in the largest increase. For this experiment GOX doughs were also kneaded to the peak in the mixograph (5·5 min, Table I). This was found to give a still higher σ.

The experiments were also performed at an elevated temperature (50 °C), but well below the gelatinization temperature of starch. At 50 °C the effect of H_2O_2 addition on σ was of the same magnitude as its effect at 30 °C, but the effect of POX's and GOX on σ at 50 °C was less pronounced. At 50 °C LPOX addition resulted in an even lower σ as compared to H_2O_2 dough. At 50 °C σ in all doughs was lower than at 30 °C. Kokelaar *et al.*[19], on the contrary, observed a higher σ at 55 °C in comparison with 20 °C for a flour dough of Spring (a mixture of North American wheat cultivars with reasonable baking quality). They observed the opposite with gluten dough mixed from gluten prepared from the same mixture.

The slope of the curves in Figure 4 gives the strain hardening $y = \partial \ln \sigma / \partial \varepsilon_B$ of the dough[18]. It is clear that only GOX gave a stronger strain hardening and that kneading to the peak time according to the mixogram further increased the value (Table II). The slope of curves of $\ln(\sigma)$ at ε_B 0·5 *vs.* $\ln \dot{\varepsilon}_B$ was taken as the strain rate thinning behaviour $z = (\partial \ln \sigma / \partial \ln \dot{\varepsilon}_B)$[18]. For the GOX dough its value tended to be somewhat smaller than for the other doughs. Van Vliet *et al.*[24] showed that when $y + 2z > 2$ dough films between gas cells are stable against rupture during baking. So the higher these values the stronger the so-called 'repairing' mechanism in dough films against local thinning and the lesser coalescence of gas cells will occur. All values are clearly below 2 as expected for biscuit flour (Table II). Only GOX slightly improved this stability against rupture.

As POX had no influence on the parameters y and z, a positive effect of POX on baking behaviour may be a result of the higher stress. A higher stress in the beginning of proofing is advantageous for a good bread crumb structure as it slows down Ostwald ripening. In later stages of proofing, when the dough

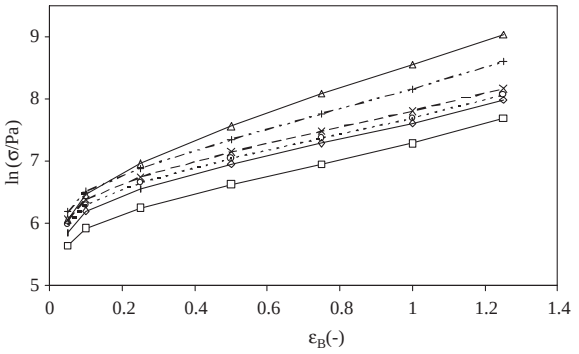

Figure 4 Stress σ as a function of biaxial strain ε_B at a constant biaxial strain rate of 0·01 s^{-1} at 30 ° C and pH 5·9. Control (□), H_2O_2 (◇), HPOX (×), SPOX (○), GOX mixed for 4 min (+) and GOX mixed for 5·5 min (△). FPOX and LPOX were similar to SPOX and H_2O_2, respectively.

Table II Strain hardening (y) and strain rate thinning (z) in biaxial extension and the values for $y + 2z$. pH was 5·9

	y		z		$y+2z$
	30 °C	50 °C	30 °C	50 °C	50 °C
Control	1·41	1·09	0·28	0·29	1·68
10 ppm H_2O_2	1·37	1·12	0·28	0·23	1·58
Flour POX, 130 U	1·41	1·28	0·26	0·22	1·72
Horseradish POX, 25 000 U	1·35	1·19	0·23	0·23	1·66
Lacto POX, 9900 U	1·38	1·06	0·24	0·27	1·59
Soya POX, 525 U	1·36	1·14	0·25	0·25	1·63
GOX, 10 730 U	1·66	1·41	0·20	0·22	1·84
GOX, 10 730 U, 5·5 min mixing	1·95	—	0·21	—	—

around the gas cells becomes more extended, strain hardening will make an increasing contribution to this. Both higher stress level and stronger strain hardening will then also favour equal growth of gas cells. In theory a finer crumb structure should therefore result when using GOX[24]. However, the extensibility and the stability against local thinning of dough films will also play an important role in this. The latter improves through increased y and z, but the extensibility decreases drastically (see below).

Effect of pH and addition of other components in biaxial extension tests

Doughs were also prepared in the presence of glucono-1,5-lactone to represent the changes in pH during fermentation with yeast. The pH was 5·4 at the time the tests were performed. For all doughs at pH 5·4, higher stress levels were observed than at

pH 5·9. The increase was by a factor 1·3 for the control and for the doughs with H_2O_2, FPOX and GOX and by a factor 1·1 to 1·2 for the other POX's. Minimal changes in strain hardening and strain rate thinning were observed. The significance of these observations for baking practice is not clear. Test baking indicated that all additions affected dough properties within 2 min of mixing[12] by then the decrease in pH is only about 0·1 units.

The effect of GOX at 30 °C was not dependent on addition of glucose up to 100 ppm. The effect of GOX was comparable to the effect of 100 ppm H_2O_2, a reaction product of GOX, equivalent to a conversion of 530 ppm glucose. As the glucose content of the flour was 3000 ppm, oxygen was presumably the limiting factor. In practice, in the presence of yeast, oxygen will be depleted earlier than in the present experiments. Thus, any effect of GOX has to be attained during mixing and in the very early stages of fermentation.

When XYL was added the stress was 0·1 unit on a $\ln(\sigma)$ scale lower in all doughs, but strain hardening was not affected. In glutens containing no added enzymes other than XYL, the effect on stress was similar, but the strain hardening was slightly less[12]. In the present research (S)POX and XYL did not show a synergistic effect on stress and strain hardening of dough, whereas a slightly synergistic effect was observed in biaxial extension tests of gluten[12].

As in baking practice no H_2O_2 is needed to allow the action of POX's, biaxial extension tests were also performed to check whether a trace of H_2O_2 could initiate a chain reaction involving POX and if iodide and manganese ions affect this. Iodide is present in the salt used for breadmaking and POX can oxidise iodide ions to reactive molecules, which can act as intermediates in oxidative reactions[25]. Oxidation of glutathione by HPOX was reported to be extremely slow unless Mn^{2+} and certain phenols were added[26]. Thus, manganese addition might promote the oxidation reaction of POX. Iodide was added as KI to a final amount of 1·2 ppm, equal to the amount added with 2% commercial bread salt. $MnCl_2$ was added to a final concentration of 5 ppm Mn^{2+}, which is equal to the difference in concentration found in bread and flour[27]. Addition of 0·001 ppm H_2O_2, with or without HPOX, did not affect the stress and strain hardening. Furthermore, iodide and manganese ions, alone or in combination, did not have any influence on the measured parameters. The reaction of POX in dough without the addition of H_2O_2 is not explained by these factors. Presumably POX uses H_2O_2 produced by baker's yeast[13].

Uniaxial extension tests

In uniaxial extension, stress levels were not increased when POX's were added and LPOX even had a negative effect on stress compared to H_2O_2 alone (Fig. 5). Again GOX caused a much higher stress. Strain hardening values were calculated using the data between the Hencky strain 0·1 and 1·0, although the lines were curved (Fig. 5B). As with biaxial extension, only GOX gave a stronger strain hardening. Figure 5A clearly shows that for

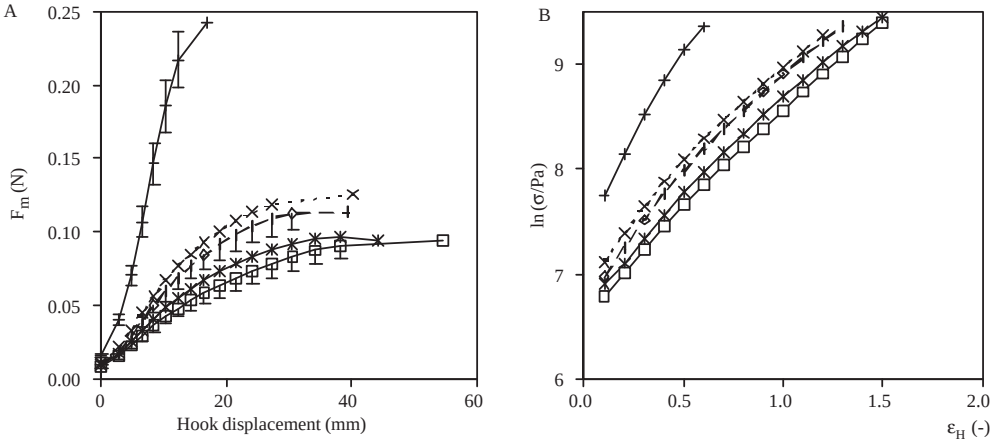

Figure 5 Measured force F_m as a function of hook displacement at 12 mm/min (A) and stress σ as a function of strain ε_H at a constant strain rate of 0·01 s^{-1} (B) in uniaxial extension at 30 ° C and pH 5·9. Control (□), 10 ppm H_2O_2 (◇), horseradish (×) and lacto (*) POX, GOX (+). FPOX and SPOX were similar to 10 ppm H_2O_2. Error bars give 90% confidence interval of the average at that specific displacement.

all additions the deformation at which the dough fractured was much smaller if the stress level was higher, and would lead to a much shorter dough. This will counteract the positive effect of higher stress and stronger strain hardening on bread crumb structure, as mentioned with the biaxial extension tests, as it may induce earlier coalescence of the gas cells, as was seen in the baking trials[12]. For GOX the decrease in fracture strain was extreme. This means that depending on the conditions, the negative effect of GOX could over-ride its positive effects, resulting in lower breadmaking quality. No differences were observed between doughs at pH 5·4 and 5·9.

Addition of XYL did not affect the force and fracture strain measured at 60 mm/min hook speed.

POX's may catalyse the oxidation of ascorbic acid to dehydroascorbic acid[28]. Dehydroascorbic acid, in turn, may oxidise glutathione, thereby preventing it from depolymerizing gluten and, as a consequence, soften the dough. It was reasoned that POX would elevate the effect of added ascorbic acid used in practice. In uniaxial extension addition of 5 ppm ascorbic acid in combination with 10 ppm H_2O_2 had the same effect as 10 ppm H_2O_2 alone. It was expected that the combination of 5 ppm ascorbic acid, 10 ppm H_2O_2 and LPOX would result in a larger effect. Surprisingly the stress was reduced to the level of the control dough. When using 50 ppm ascorbic acid the stress was higher than with 10 ppm H_2O_2 alone, but again dropped when POX was also added. The cause of this phenomenon is not clear, but can probably be attributed to the complicated redox system in dough.

CONCLUSIONS

The effects of oxidative enzymes on breadmaking properties of flour doughs are not due to alterations in small deformation properties. In large deformation tests POX increased stress levels only, whereas GOX increased stress levels and strain hardening. In POX doughs the number of non-permanent cross-links was increased, whereas GOX introduced both more non-permanent cross-links and more cross-links that survived over time scales up to 3 h. The effect of POX could not simply be achieved by the addition of less water. An increase in the stress level was accompanied by a decrease in the fracture strain. All enzymes tested had positive and negative effects, complicating any prediction of the overall effect on bread structure.

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