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IMPROVEMENT OF RHEOLOGICAL PROPERTIES OF FLOUR DOUGHS WITH (PER)OXIDASES

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Abstract

The effect of hydrogen peroxide, peroxidases and glucose oxidase on the rheological properties of flour dough has been studied by small deformation oscillation tests and biaxial and uniaxial extension tests. (Per)oxidases caused the dough to become slightly more elastic. All additions caused stress in biaxial and uniaxial deformation to increase. Only glucose oxidase increased strain hardening. In all cases a higher stress level was accompanied by a lower fracture strain. Calculations showed that the combined effect of higher stress and strain hardening improves the stability against disproportionation to a large extent. However, the accompanying smaller fracture strain may induce earlier coalescence of the gas cells.

Résumé

L'influence de peroxyde d'hydrogène, des peroxydases et de glucose oxydase sur les propriétés rhéologiques de la pâte de farine a été déterminée en faisant des essais d'oscillations sur une petite déformation d'une part, et des essais d'extension biaxial et uniaxial d'autre part. Les (per)oxydases ont légèrement entrainé l'élasticité de la pâte de farine. Toutes les additions ont augmenté les valeurs de tension. Seul le glucose-oxydase a augmenté le renfort causé par la déformation (strain hardening). Dans tous les cas, une plus grande tension était suivie d'une plus petite déformation à fracture. Les calculs ont montré que l'effet combiné de l'augmentation de tension et le renfort causé par la déformation améliore largement la stabilité de la pâte contre la disproportion des cellules de gaz. Toutefois une plus petite déformation à fracture accompagnante peut avoir une coalescence plus tôt pour résultat.

Introduction

As early as 1925 it was reported that hydrogen peroxide increased the viscosity of wheat and flour suspensions and caused gelation of flour extracts (1). The substance affected by peroxide was identified as the water-soluble pentosans (2) composed of arabinoxylan and arabinogalactan (3). The oxidative gelation of pentosans by H_2O_2 is ascribed to the ferulic acid residues, esterified to the arabinose units in arabinoxylan (3,4,5,6). As possible mechanisms of oxidative gelation are proposed dimerization of ferulic acid residues on different arabinoxylan chains and coupling of ferulic acid residues to cysteine or tyrosine residues on protein (7,8). H_2O_2 had no effect in the absence of peroxidase (7).
In the past chemical oxidants were added to dough to improve its breadmaking performance. In order to substitute these oxidants by naturally occuring oxidants it is very interesting to study the effect of enzymes as peroxidases or glucose oxidase on the dough behaviour.
To quantify the effect of (per)oxidases on the rheological properties of dough, small deformation oscillation tests and biaxial and uniaxial extension tests were performed. Elasticity, deformation at fracture, stress level, strain hardening and strain rate thinning behaviour were determined.

The results from biaxial extension were used to estimate the effect of stress level and strain hardening on disproportionation (Ostwald ripening) of the gas cells in dough. During disproportionation the dough around the larger (growing) gas cell is extended biaxially, which gives rise to a bulk stress σ opposing further growth (13). The Laplace pressure (ΔP) in the smaller (shrinking) gas cell increases. For stopping disproportionation the increase of the bulk stress in the dough around the larger gas cell (σ_l) has to be larger than the increase in Laplace pressure in the smaller gas cell (ΔP_s), taking changes in σ_s and ΔP_l into account (9). So:

$$\frac{d\sigma_l}{dR_l} + \frac{d\Delta P_l}{dR_l} > \frac{d\sigma_s}{dR_s} + \frac{d\Delta P_s}{dR_s} \qquad [1]$$

R is the radius of the gas cell. The subscripts s and l denote the smaller and larger gas cell respectively. Starting from this formula, the next criterium for stability against Ostwald ripening was derived:

$$\frac{\mathrm{d}\ln\sigma(R)}{\mathrm{d}\varepsilon} > \frac{2\gamma / R_\mathrm{s}}{\sigma(R_\mathrm{l})}\left[\frac{R_\mathrm{l}}{R_\mathrm{s}} + \frac{R_\mathrm{s}}{R_\mathrm{l}}\right] \qquad [2]$$

For simplicity the stress around the smaller gas cell and the change in surface tension with radius was neglected in formula 2. The surface tension γ was assumed to be 40 $\mathrm{mNm^{-1}}$ (15). The relative change in stress with biaxial extension $\mathrm{d}\ln\sigma(R)/\mathrm{d}\varepsilon$ was taken equal to the strain hardening at constant strain rate. If Ostwald ripening between two gas cells has stopped, the strain rate of the dough around both is zero.
Allthough the performed calculations on only two gas cells are very simple compared to the real situation with initially about 10000 gas cells per $\mathrm{mm^3}$ (16), the combined effect of stress and strain hardening on Ostwald ripening can be demonstrated very clearly.

Materials and Methods

The flour used, was a commercial mixture Kolibri with 15.0% moisture and 10.5% protein, obtained from Meneba. It has a poor bread making quality. NaCl (analytical grade) and H_2O_2 (30% solution, stabilized, medical extra pure) were from Merck, Germany. Wheat flour (0.13 U/mg) and lacto (99 U/mg) peroxidase were obtained from Quest International, The Netherlands. Soya peroxidase was from Adumin, Israel (35 U/mg). Horse radish peroxidase was from Boehringer, Germany (1000 U/mg). Glucose oxidase Oxygo L5, was from Genencor (5364 U/ml). Activities are on ABTS. The water used was deionised.

Water addition was 60% on flour basis for all doughs. The water contained 2% NaCl, on flour basis, and the enzyme. De concentration of wheat flour, horse radish, lacto and soya peroxidase were 1000, 25, 100 and 15 ppm on flour basis, respectively. The concentration glucose oxidase was 20 µl/10 g flour. Allthough in baking tests no H_2O_2 has to be added as a substrate for peroxidases (R. Orsel; Quest International, personal communication), no effect of peroxidases was found in experiments on doughs without yeast. Therefore, 20 µl diluted H_2O_2 (10 ppm H_2O_2 on flour basis) was added in case of peroxidases; 20 µl water for the blanc and glucose oxidase. All doughs were mixed for 4 minutes in a mixograph (National Mfg. Co., Lincoln). Rheological measurements were started after a resting period of 45 minutes.

Sinusoidal oscillation tests were performed with a Bohlin VOR constant shear rheometer equipped with a plate-plate geometry (diameter 30 mm), covered with emery paper to prevent slip. The gap between the plates was set to 3 mm. Frequency-sweeps were performed at a strain of 0.001. 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, waterdrops were put around the geometry and the whole was covered. Results are the mean of measurements on three different doughs.

Biaxial extension tests were performed by compression of a cylindrical test piece between two parallel plates with radius R = 10 mm, lubricated with paraffin oil (110 mPa·s) (10). A dough piece of 7.0 g was put in a lubricated teflon cylinder with a diameter of 19 mm and covered with a lubricated solid teflon plunjer. After 45 minutes the sample was compressed in a Zwick material testing machine equipped with a 50 N load cell, at 30 ± 1°C. 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 point at which a force of 0.01 N was reached was taken as the starting point of compression. 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; relative biaxial strain and relative biaxial extensional rate as $\varepsilon_B = \varepsilon_H/2$ and $\dot{\varepsilon}_B = \dot{\varepsilon}_H/2 = v/2h_t$ respectively; h_o is the initial height of the test piece and h_t the height at time t. Stress was calculated as $\sigma = F_t/(\pi R^2)$ (10, 11).

Uniaxial extension tests were performed at 30 ± 1°C with a Kieffer extensibility rig (12), also called a micro-extensograph, fitted on a Zwick material testing machine equipped with a 50 N load cell. The dough was made into a roll, put on the lower plate of the teflon form which belongs with the extensibility rig and compressed with the top plate. The plates were lubricated with paraffin oil. After 45 min. the dough was pushed out of the form and put on the lower plate of the extensibility rig. 18 samples were tested at 12, 60 and 300 mm/min. The lowest speed was a minimum from an experimental point of view, in order to avoid excessive sagging and drying out of the sample. The position of the hook at 2 mm above the top of the lower plate (y_0) was taken as the starting point of the actual extension (ε_H zero) for al experiments. Hencky strain and relative strain rate were calculated as $\varepsilon_H = \ln(l_t/l_0) = \ln\left(\sqrt{9^2 + (y_t + y_0)^2} \;/\; \sqrt{9^2 + y_0^2}\right)$ and $\dot{\varepsilon} = d\varepsilon_H/dt = dl/l_t dt = 4(y_t+y_0)v/l_t^2$, respectively; l and y are the length of the sample and the displacement of the hook, respectively, l_0 and y_0 at ε_H zero and l_t and y_t at time t starting from ε_H zero, v is the hook speed and 9 is half the width (mm) of the gap in the lower plate over which the dough extends. The force acting on the dough (F_d) and the resulting stress were calculated from the measured (vertical) force (F_v) and the volume of the dough piece (V) according to $F_d = F_v l_t/(4(y_t+y_0))$ and $\sigma = F_d/(V/l_t)$. The volume of the dough piece that was extended was about 375 mm^3.

From the uniaxial and biaxial extension tests the strain hardening $\partial \ln\sigma/\partial\varepsilon$ behaviour of the dough was calculated (13,14).

Results and discussion

Experiments were done with different concentrations H_2O_2 (0-500 ppm) in order to estimate its effect and to find an optimum concentration for studying an additional effect of peroxidases. In biaxial extension tests, stress and strain hardening increased with increasing concentration H_2O_2 (results not shown). The additional effect became smaller with increasing concentration H_2O_2. Similar results were obtained from uniaxial extension and dynamic experiments. A concentration of 10 ppm H_2O_2 was taken for the experiments with peroxidases.

In small deformation oscillation tests the elasticity modulus was slightly higher and the loss angle slightly lower when a peroxidase was added (results not shown). The effect of glucose oxidase was larger than that of peroxidases. The differences, however, were very small.
In biaxial extension wheat flour, soya and horse radish peroxidase increased the stress above the effect of H_2O_2, horse radish peroxidase being highest (figure 1A). Lacto peroxidase did not show an additional increase in stress above that of H_2O_2 alone. Glucose oxidase gave the highest stress values. The strain hardening of the doughs, which is equal to the slope of the curves in figure 1A, was only increased by using glucose oxidase. Effects in uniaxial extension were similar, except for lacto peroxidase having a negative effect compared to H_2O_2 alone (figure 1B). The deformation at which fracture of the dough occured was much smaller if the stress level was higher, meaning a much shorter dough.
The results show that through the action of (per)oxidases more bonds are formed resulting in a more extensive or stiffer macromolecular network.

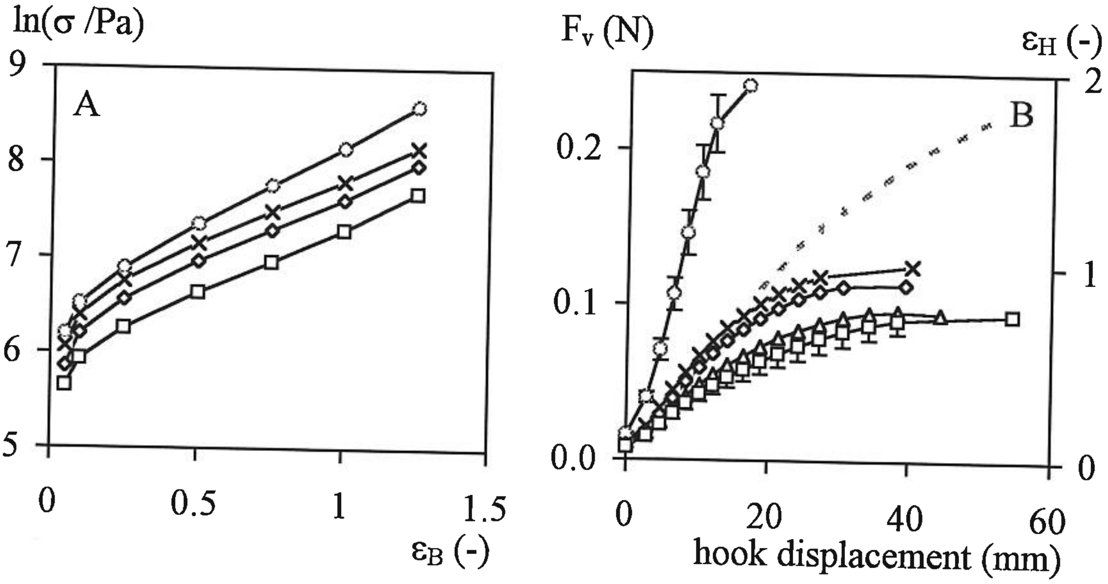

Figure 1 A: Stress in biaxial extension, calculated on the basis of a biaxial deformation rate of 0.01 s^{-1}. B: The measured (vertical) force in uniaxial extension at 12 mm/min and the strain (dotted line) as a function of the hook displacement. Error bars give 90% confidence interval of the mean, calculated at the specific deformation. No additives (□), H_2O_2 (◊), horse radish (×) and lacto (△) peroxidase, glucose oxidase (o).

To estimate the effect of the (per)oxidases on disproportionation of gas cells, formula 2 was rewritten so that only $\sigma(R_l)$ was at the left-hand side of the "greater than" sign and all other terms on the right-hand side.

$$\sigma(R_l) > \frac{2\gamma / R_s}{\mathrm{d}\ln\sigma(R)/\mathrm{d}\varepsilon}\left[\frac{R_l}{R_s} + \frac{R_s}{R_l}\right] \qquad [3]$$

The latter will be called the criterium value; it represents the stress in the biaxially extended dough around the larger gas cell required for stability. For the calculation of the criterium value for Ostwald ripening according to equation 3, a fixed value for R_s was taken. Then for various R_l, which should be larger than R_s, the criterium value could be calculated. The so calculated criterium values are plotted as a function of R_l for different values of R_s in figure 2A (dotted lines). The criterium value increases with R_l as a result of the increasing difference in Laplace pressure between the gas cells.

As the larger gas cell grows the dough around it will be extended biaxially, building up a stress which opposes or even inhibits further growth. This

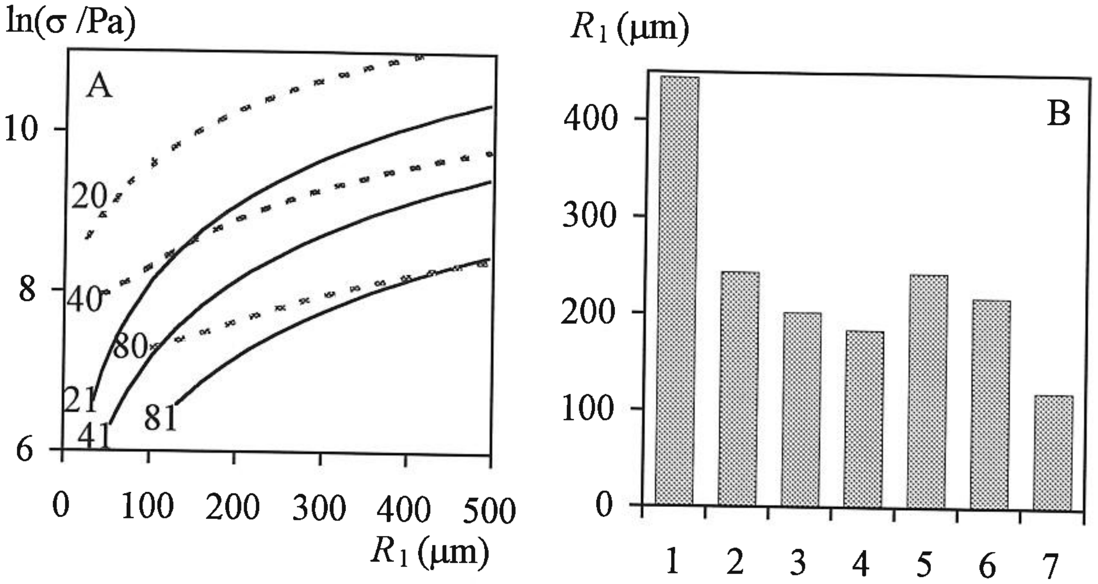

Figure 2 A: Comparison of the actual stress $\sigma(R_{\mathrm{l}})$ reached by biaxial extension of the dough around a larger gas cell (solid lines), with the criterium stress for stability against Ostwald ripening (dotted lines) for dough without additions. The actual radius of the smaller gas cell and the initial radius of the larger gas cell is indicated near the curves.

B: The radius of the larger gas cell R_{l} at which the criterium is met, calculated for a fixed radius of the smaller gas cell of 80 μm and an initial radius of the larger gas cell of 81 μm. Numbers 1 to 7: no additives, H_2O_2, wheat flour, horse radish, lacto and soya peroxidase and glucose oxidase, respectively.

stress can be calculated from the various curves in figure 1A, knowing the relative biaxial deformation from an assumed initial radius $R_{\mathrm{l},0}$ and the actual radius R_{l}. This stress is represented in figure 2A for different initial radii $R_{\mathrm{l},0}$ by the solid lines. The criterium is met when $\sigma(R_{\mathrm{l}})$ (solid line) crosses the criterium line (dotted line). At that point $\sigma(R_{\mathrm{l}})$ is high enough to compensate for the driving force for Ostwald ripening, the difference in Laplace pressure. The stress $\sigma(R_{\mathrm{l}})$ should only be compared with the criterium value when $R_{\mathrm{l},0}$ was higher than R_{s} (the values at the start of the various curves in figure 2A), otherwise Ostwald ripening proceeds in the reverse direction.
From figure 2A can be concluded that in this dough (no additions) all small gas cells will disappear. A gas cell of 81 μm initial radius has to grow to about 445 μm to build up a stress high enough to compensate for the difference in Laplace pressure. It is not usefull to extend the scale above 500

μm, because the amount of gas present in the dough and the time available for Ostwald ripening are limiting factors. The time required for gas diffusion scales with the distance squared.

Results for other doughs are shown in figure 2B. Due to the higher σ in the doughs with peroxidases, the larger gas cells have to grow less to meet the criterium. The combined effect of higher stress and stronger strain hardening in the dough with glucose oxidase makes that in this dough stability against Ostwald ripening is obtained even sooner.

The calculations show the relevance of various factors, but more factors than taken into account here play a role, which may affect the absolute values in a real dough.

It can be shown that if the gas cells are stabilized against Ostwald ripening they will also grow equally due to CO_2 production in the liquid dough phase (in preparation). If Ostwald ripening is stopped soon after mixing, many very small gas cells will grow, resulting in a very fine crumb structure. However, figure 1B indicates that if the stress level was higher the deformation at which fracture of the dough occured was smaller. This was the case for all additions to different extent. If deformation at fracture becomes too small, fracture of dough films will occur before the end of oven rise and again a very coarse crumb structure will be obtained. So for a good bread structure there will be an optimum in the action of the added enzymes. It may well be that horse radish peroxidase which results in less stabilization against Ostwald ripening than glucose oxidase, gives therefore a finer and more regular crumb structure.

Conclusions

Addition of H_2O_2 with or without a peroxidase resulted in slightly more elastic dough and higher stress upon extension, biaxial as well as uniaxial. Glucose oxidase resulted in a more elastic dough. In uniaxial and biaxial extension this enzyme caused a higher stress as well as a stronger strain hardening. Calculations showed that H_2O_2, peroxidases and glucose oxidase through their effect on dough properties very much improved the stability of the gas cells against disproportionation. However, all additions resulted in a lower fracture strain, glucose oxidase most. For obtaining a fine and regular crumb structure, there will be an optimum between stabilization against disproportionation, equal growth and fracture strain. Moreover, more detailed calculations on Ostwald ripening have to be done before more definitive conclusions can be drawn about the absolute values of resulting gas cell size.

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