

Effects of Bran Fermentation on Quality and Microstructure of High-Fiber Wheat Bread

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ABSTRACT

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We compared the effects of spontaneous fermentation of the bran fraction and fermentation with added yeast or added yeast and lactic acid bacteria (*Lactobacillus brevis*) on the quality of wheat bread supplemented with bran. Prefermentation of wheat bran with yeast or with yeast and lactic acid bacteria improved the loaf volume, crumb structure, and shelf life of bread supplemented with bran. The bread also had added flavor and good and homogenous crumb structure. Elasticity of the crumb was excellent. Spontaneous fermentation of the bran fraction did not have the same positive effects on bread quality. The microstructure of the breads

was characterized by light microscopy. The positive effect of fermentation of bran on bread quality was evident when comparing the well-developed protein network structure of the breads baked with fermented bran with the control bread. Prefermentation of the bran with yeast and lactic acid bacteria had the greatest effect on the structure of starch. The starch granules were more swollen and gelatinized in the breads made with prefermented bran. The pretreatments of the bran fraction had no detectable effect on the microstructure of the cell wall particles in the test breads.

Bran supplementation usually weakens the structure and baking quality of wheat dough and decreases bread volume and elasticity of the crumb. The effect has been attributed to the dilution of gluten, which would affect the gas-holding capacity of the dough (Pomeranz et al 1977; Gan et al 1992). The increased concentration of insoluble and soluble cell wall material may also have detrimental effects on dough structure. Bran particles can mechanically disrupt the structure of the gluten network and also force gas cells to expand in a particular dimension. The baking quality of bran is influenced also by its particle size (Lai et al 1989a,b; Moss 1989; Özboy and Köksel 1997). The soluble pentosans and β -glucan of the bran fraction affect dough development. As the specific volume of bread is one of the important characteristics determining acceptability, different bran pretreatments have been used to improve the volume of breads supplemented with bran. For example, washing the bran to remove harmful components, grinding the bran to obtain a smaller particle size, or using various heat treatments to inactivate enzymes have been successfully used to improve the quality of breads supplemented with bran (Lai et al 1989a,b; Rao and Rao 1991; Rasco et al 1991; Nelles et al 1998; de Kock et al 1999).

Sour dough fermentation is used to improve the texture, volume, and keeping quality of breads (Brümmer and Unbehend 1997). Lactic acid bacteria cause acidification of the dough, proteolysis of gluten, and moderate hydrolysis of starch. This modification of dough components may affect the physicochemical changes occurring during bread shelf life (Corsetti et al 1998). The flour type, temperature, water content, and type and amount of added yeast and bacteria all affect acidification of the dough and bread quality (Brümmer and Unbehend 1997; Rouzaud and Martínez-Anaya 1997). Flour type has been the main factor in the acidification of sour doughs, with most acid produced in sour doughs made from whole meal flour (Salovaara and Valjakka 1987; Hansen and Hansen 1994).

The aim of the present study was to improve the quality of wheat bread supplemented with bran by prefermentation of the bran fraction. We compared the effects of spontaneous fermentation and fermentation with yeast or with yeast and lactic acid bacteria on the quality and microstructure of bread supplemented with 20% wheat bran.

MATERIALS AND METHODS

Commercial white wheat flour and wheat bran were used for baking (Melia Ltd., Raisio, Finland). The chemical composition of the

materials is given in Table I. The dry matter (DM) content of the wheat flour and bran were determined by oven drying at 130°C for 1 hr. Protein content was determined by the Kjeldahl method (Kjeltec Auto 1030 Analyzer, Tecator AB, Sweden) and the starch content by the Megazyme colorimetric method. Total dietary fiber and insoluble and soluble fiber were determined by the enzymatic-gravimetric method of Asp et al (1983), total pentosan by the colorimetric method of Douglas (Douglas 1981; Rouau and Surget 1994) and β -glucan enzymatically by the Megazyme method (McCleary and Codd 1991). The commercial coarse bran (particle size 53% > 1,000 μ m, 80% > 750 μ m) was further ground to obtain finer particle size (6% > 750 μ m, 47% > 355 μ m, 78% > 132 μ m). The starter culture of *Lactobacillus brevis* L62 was obtained from Chr. Hansen, Denmark. Instant active dry yeast (Fermipan red, Gist-brocades, Delft, The Netherlands) was used for bran pretreatment and baking.

Bran Fermentation

The optimum conditions for fermentation were determined by preliminary experiments. Bran (100 g) was mixed with 350 g of water and the dry microorganisms (1.25 g of yeast, 0.162 g of *L. brevis* L62) in a large beaker; the beaker was then covered with aluminum foil and incubated in temperature cabinet. After the treatment, the pH and total titratable acidity (TTA) were measured (Standard-Methoden für Getreide, Mehl und Brot 1978). The total titratable acidity was expressed as milliliters of 0.1M NaOH/10 g of bran suspension. Bran without any pretreatment was added to the control bread. The conditions for the fermentations were 1) 1.25% yeast, 4 hr, 28°C; 2) 1.25% yeast + *L. brevis* L62, 16 hr, 25°C; 3) spontaneous fermentation (no added microbes) 4 hr, 28°C; and 4) spontaneous fermentation 16 hr, 25°C.

Baking

The recipe for the control bread (g) used in this study was wheat flour (500), yeast (7.5), salt (7.5), sugar (7.5), fat (7.5), emulgator Panodan (2.4) and water (345). The optimal water addition was

TABLE I
Chemical Composition of the Raw Materials (dwb)

Component	Wheat Flour	Wheat Bran	80% Flour + 20% Bran
Protein (N \times 6.25)	13.2	13.0	13.2
Starch	78.8	12.0	65.4
Total dietary fiber	2.7	52.0	12.6
Soluble dietary fiber	1.2	1.9	1.3
Total pentosan	1.9	29.0	7.3
Soluble pentosan	0.6	0.4	0.6
β -Glucan	0.3	1.2	0.5

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69% measured by a farinograph. The recipe for the bread (g) with added bran was wheat flour (400), wheat bran (100), yeast (7.5), salt (7.5), sugar (7.5), fat (7.5), emulgator Panodan (2.4), and water (400). Test baking determined the optimal water addition at 80. The doughs were mixed with a Diosna spiral mixer for 8 min. After a floor time of 20 min at 28°C and 76% rh, the dough was divided into 400 g loaves. The loaves were proofed in pans (55 min at 35°C, 76% rh) and baked at 200°C for 25 min. Bread volume was determined by rapeseed displacement. The test breads were 1) control, baked without bran; 2) control, bran without pretreatment 20%; 3) prefermented wheat bran 20%, fermentation 1; 4) prefermented wheat bran 20%, fermentation 2; 5) prefermented wheat bran 20%, fermentation 3; and 6) prefermented wheat bran 20%, fermentation 4.

Crumb Firmness and Elasticity Measurements

Crumb firmness was measured on days 0, 1, and 3 to assess the potential shelf life of the breads. Bread crumb firmness during storage was determined as maximum compression force (40% compression) (Approved Method 74-09, AACC 2000) using texture profile analysis (TPA) (TA-XT2 Texture Analyzer, Stable Micro Systems, Godalming, England).

Statistical Analysis

The results are means of the analyses of four replicate breads. The test bakings were repeated twice. One-way variance analysis was performed to evaluate the statistical significance of the results.

TABLE II
Effect of Fermentation on the pH and Total Titratable Acids (TTA) of Bran-Water Slurries

	Fermentation			
	1	2	3	4
Yeast	yes	yes	no	no
<i>Lactobacillus brevis</i>	no	yes	no	no
Fermentation time, hr	4	16	4	16
Temperature, °C	28	25	28	25
Initial pH	6.6	6.6	6.6	6.6
Final pH	6.3	5.8	6.6	6.5
Initial TTA	3.3	3.3	3.3	3.3
Final TTA	5.9	8.7	3.3	4.4

Microscopy

Microstructure of the breads was studied in four to six pieces taken from two loaves originating from separate test bakings. Pieces of bread crumb (0.5 cm) were taken from the middle of the loaf, embedded in 1% agar, fixed in 1% glutaraldehyde in 0.1M phosphate buffer, pH 7.0, dehydrated with ethanol and embedded in hydroxyethyl methylacrylate as recommended by the manufacturer (Historesin, Leica, Heidelberg, Germany). Sections were cut 4 µm thick in a rotary microtome (HM 355, Leica) using a steel knife. The sections were transferred onto glass slides and stained with Fuchsin Acid and Calcofluor White or Light Green and Lugol's iodine solution (Fulcher and Wong 1980; Wood et al 1983; Parkkonen et al 1994).

Fuchsin Acid and Calcofluor White. Protein was stained with aqueous 0.1% (w/v) Fuchsin Acid for 1 min (Gurr, BDH Ltd, Poole, England), and β-glucan was stained with aqueous 0.01% (w/v) Calcofluor White for 1 min (Fluorescent brightener 28, Aldrich, Germany). In fluorescent light (excitation 330–385 nm, fluorescence >420 nm), intact cell walls stained with Calcofluor appear blue and proteins stained with Fuchsin Acid appear red. Starch is unstained and appears black.

Light Green and iodine staining. Protein was stained with aqueous 0.1% (w/v) Light Green for 1 min (Gurr, BDH Ltd, Poole, England) and starch with 1:10 diluted Lugol's iodine solution (I₂ 0.33%, w/v, and KI 0.67%, w/v). Light Green stains protein green. Iodine stains the amylose component of starch blue and amylopectin brown.

The samples were examined with an Olympus BX-50 microscope (Tokyo, Japan). Micrographs were obtained using a SensiCam PCO CCD camera (Hamamatsu Photonics K.K., Hamamatsu-City, Japan) and the AnalySIS 3.0 image analysis program (Soft Imaging System, Münster, Germany).

RESULTS AND DISCUSSION

Baking

The wheat bran used in the test bakings had a low starch content (12.0% dry matter, Table I). Total dietary fiber content of the bran was very high (52.0%), while the soluble dietary fiber content was 1.9%. Pentosan content of the bran was 29.0%. A 20% substitution of bran for flour decreased starch content of the dough by 17% and almost quadrupled the amount of total pentosan. Even though the bran-flour protein content did not change, the gluten concen-

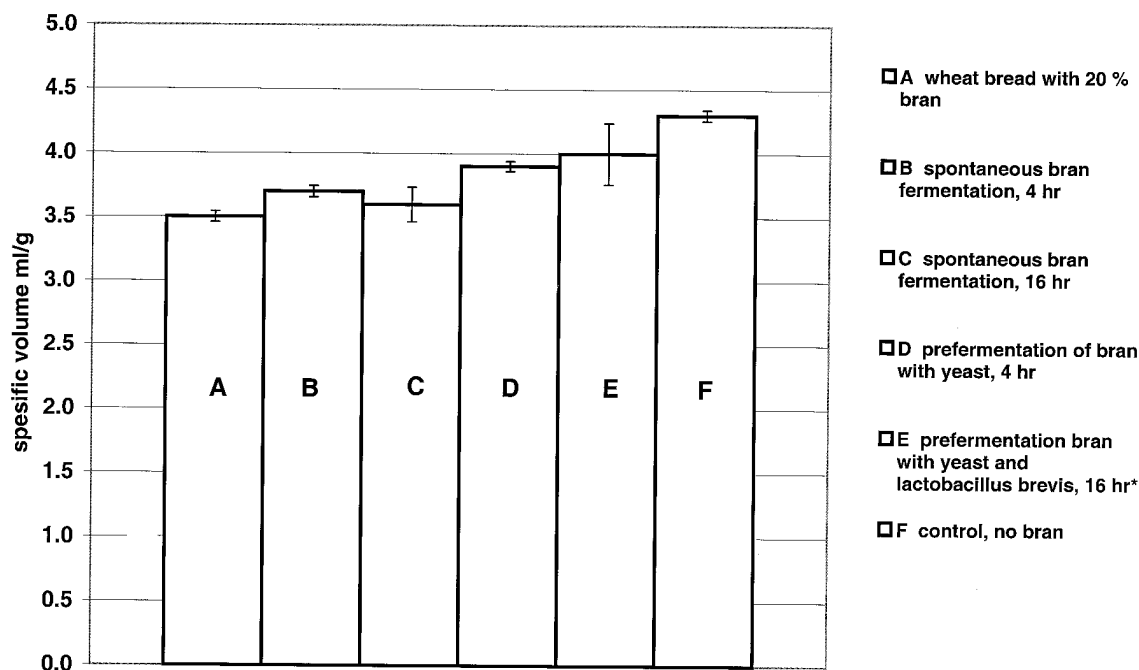


Fig. 1. Effect of bran fermentation on loaf volume of breads supplemented with 20% bran.

tration decreased by 20% because the bran does not contain gluten. The wheat bread baked with 20% wheat bran substituted for flour contained 10% total dietary fiber (10 g/100 g of fresh bread).

The bran possessed a good buffering capacity, the pretreatments had only a slight effect on the pH of the bran slurries (Table II). The bran fraction of the grain is especially rich in phytin, a reserve compound of phosphate and mineral ions. The high content of minerals, such as calcium and phosphorus, in whole meal flour contributes to the high buffering capacity of the sour doughs (Spicher and Stephan 1982). The bran fermented by lactic acid bacteria and yeast was the most acidic (pH 5.8, TTA 8.7), whereas spontaneous fermentation of the bran for 16 hr had a much smaller effect, pH 6.5, and TTA 4.4. Wheat sour doughs usually have values of pH 3.5 to 4.5 and TTA values from 8 to 22, depending on the ash content of the flour and the starter culture used (Salovaara and Valjakka 1987; Hansen and Hansen 1994). Higher ash content of the flour gives more acidic pH and higher TTA values. In comparison to the pH and TTA values presented in literature, lactic acid bacteria-yeast fermented bran was not very acidic (pH 5.8, TTA 8.7). Addition of baker's yeast to the sour dough slows the souring. Bran contains a much higher number of microbes than flour. Therefore, adding starter cultures to the bran instead of spontaneous souring is advisable for the production of bread with an even and repeatable quality.

The test breads were evaluated by a bakery panel of three experienced bakers (data not shown). Short fermentation with yeast and long fermentation with yeast and lactic acid bacteria improved the taste and mouthfeel of bread supplemented with wheat bran. The bread had added flavor and good and homogenous crumb structure. Elasticity of the crumb was excellent. Spontaneous fermentation of the bran did not improve the taste and mouthfeel of the test bread.

Baking experiments showed that wheat bran at a substitution level of 20% decreased loaf volume by 19% in comparison to white bread (Fig. 1). Bran supplementation has both mechanical and chemical effects on the structure of the dough. Wheat bran is more detrimental to loaf volume than would be expected from simple dilution of gluten protein (Pomeranz et al 1977; Lai et al 1989b; Gan et al 1992). Bran particles cause physical disruption of the foam structure of the dough. Different methods have been used to improve the volume of breads supplemented with bran. Fine grind-

ing and presoaking of wheat bran had a beneficial effect on loaf volume (Lai et al 1989a,b). Hydration of bran before addition to the dough increased loaf volume and improved bread quality in wheat bread containing 12% bran (Nelles et al 1998). Nelles et al (1998) proposed several possible mechanisms for the observed improvement: improved hydration of all brown flour components, lipoxigenase activation, and a washing out of free reduced glutathione. Chemical or enzymatic modification of wheat bran were used to enhance the functional properties of bran (Rasco et al 1991). Optimal mixing time increased and loaf volume and crumb grain decreased for yeast-raised breads (sponge and dough) containing the treated ingredients in comparison to white wheat bread at the same flour substitution level (10–20% w/w). Cell-wall-degrading enzymes have also been used to improve the quality of bran-enriched wheat bread (Laurikainen et al 1998).

Bran treated by short fermentation with yeast and long fermentation with yeast and lactic acid bacteria improved the specific bread volume over untreated bran by 10–15% (Fig. 1). For long fermentation with yeast and lactic acid bacteria, the difference was statistically significant ($P > 0.05$). The 4-hr spontaneous fermentation of the bran slightly improved specific volume, whereas the 16-hr spontaneous fermentation had no effect on bread volume.

Four-hour fermentation with yeast and 4-hr spontaneous fermentation of the bran slowed the crumb firming slightly during three days of storage in comparison to white bread (Fig. 2). The bread baked with bran treated by 4-hr fermentation with yeast was slightly more firm than the white bread after storage for three days, but the firming rate (the change in firmness from day 0 to day 3) was much smaller. The 16-hr fermentation with yeast and lactic acid bacteria improved the crumb softness and keeping qualities most. On the day of baking, all breads supplemented with bran were firmer than the white wheat bread, but after storage for only one day, the crumb firmness of the white wheat bread was the same as that of the bread baked with fermented bran. After storage for three days, the bread baked with bran fermented with yeast and lactic acid bacteria was softer (20%) than the white wheat bread. After the 16-hr fermentation with yeast and lactic acid bacteria, the improvement was statistically significant ($P > 0.05$). The 16-hr spontaneous fermentation increased the crumb staling rate.

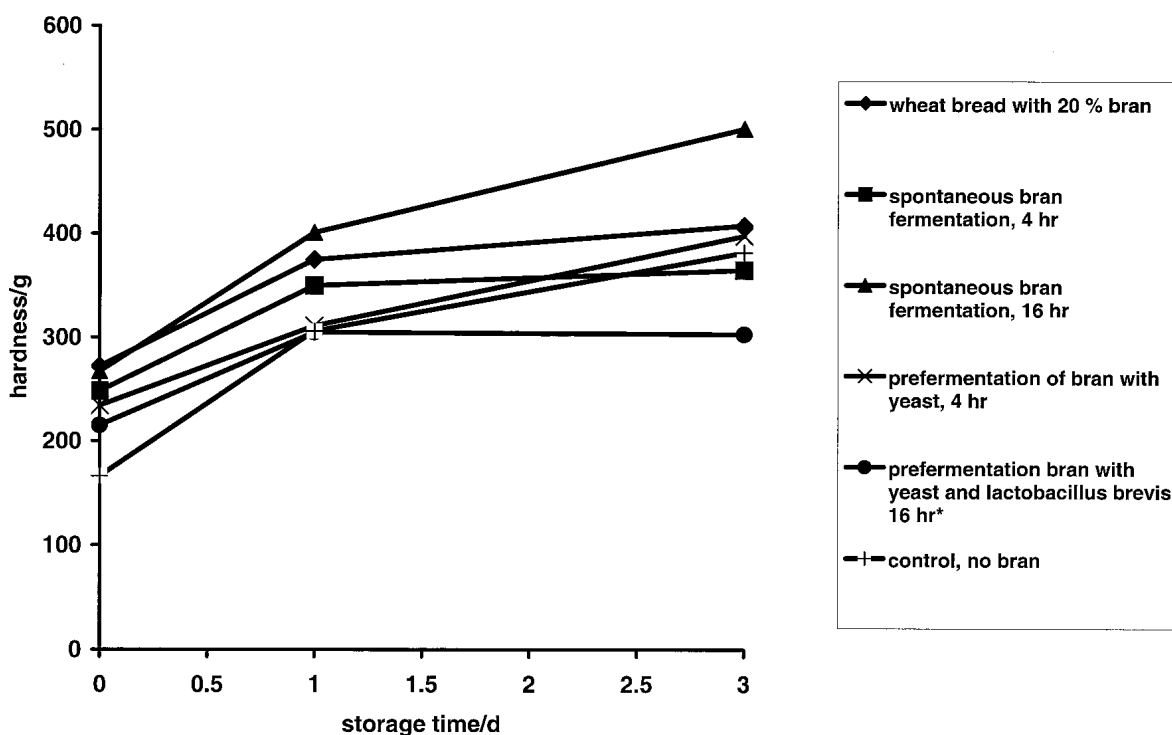


Fig. 2. Effect of bran fermentation on crumb firmness of breads supplemented with 20% bran.

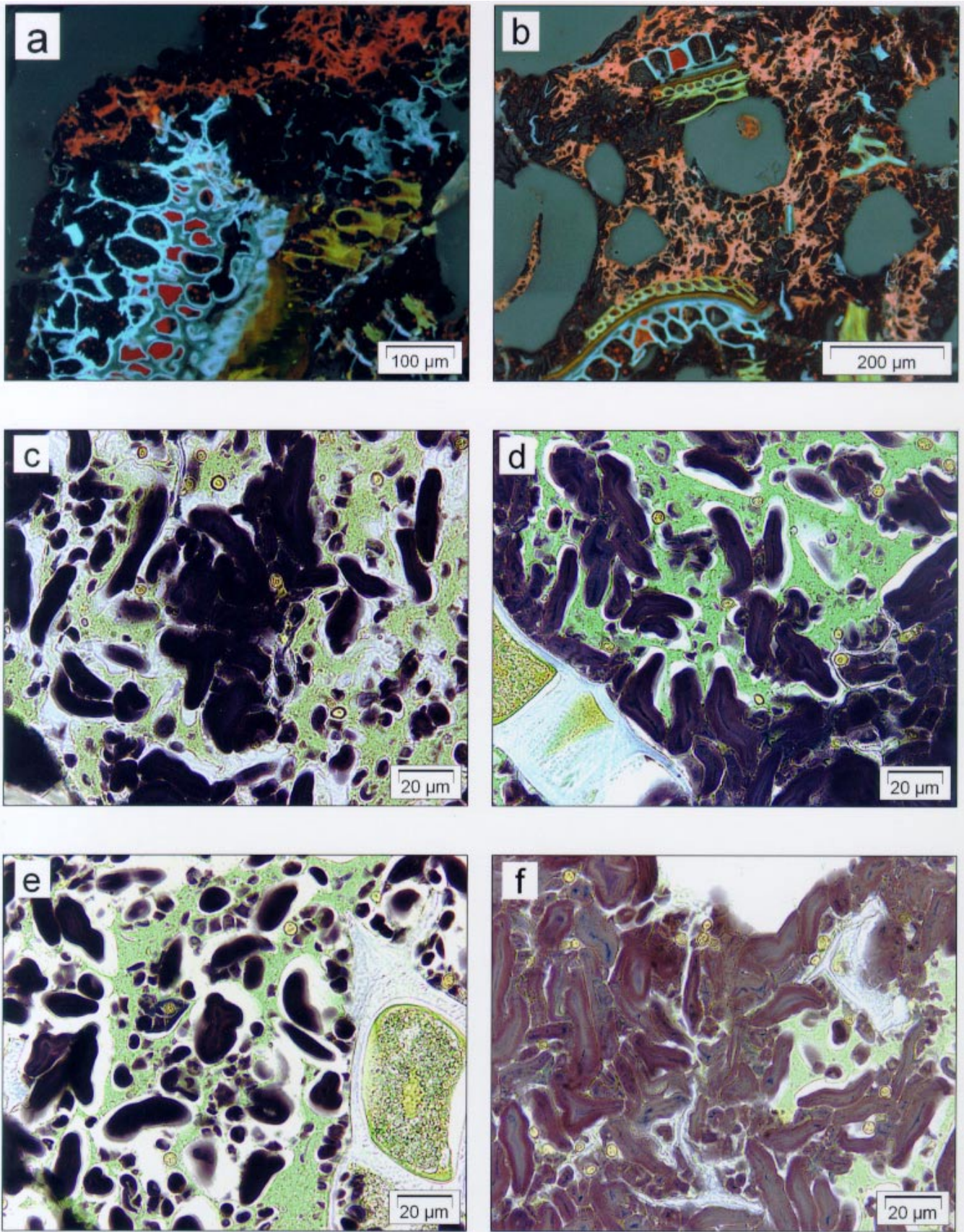


Fig. 3. Effect of bran fermentation on the microstructure of breads supplemented with 20% bran. Micrographs of test breads with Calcofluor-stained cell walls. Bread baked with bran treated with spontaneous fermentation for a) 4 hr and b) 16 hr. Epifluorescence micrographs taken from embedded bread sections stained with Acid Fuchsin and Calcofluor. Endosperm cell walls appear blue, protein red, and starch black; the outer lignified pericarp layers appear yellowish green. Micrographs of test breads with iodine-stained starch. Bread baked with bran treated with spontaneous fermentation for c) 4 hr and d) 16 hr; e) 4 hr fermentation with baker's yeast; f) 16 hr fermentation with lactic acid bacteria and baker's yeast. Iodine stains amylose component of starch blue and amylopectin brown, Light Green stains protein green.

Fermentation of wheat bran with yeast or with yeast and lactic acid bacteria improved the loaf volume, crumb structure, and shelf life of bread supplemented with bran. Even though we used bran instead of flour for the fermentations, the results are similar to the effects of sour dough on bread quality presented in the literature. Endogenous enzymes of flour, especially amylases and proteases, as well as the enzymes produced by yeast and *L. brevis* during fermentation seem to have a positive effect on the dough properties and the structure of the bread. Acids produced during fermentation lower the pH level of the dough, thereby affecting the enzyme activity and gluten characteristics. The pH optima of carbohydrate-degrading enzymes such as amylase, pentosanase, or cellulase vary widely (3.6–5.6) depending on wheat cultivar and germination status (Fox and Mulvihill 1982). Proteinases and peptidases in flour are active at pH 4–9, depending on the substrate. The rapid drop in pH level in sour dough can cause reduced amyolytic activity, whereas the slower drop in the pH level in spontaneously fermented dough permits further starch degradation (Wehrle and Arendt 1998). Modifications in starch during fermentation alter the gelatinization characteristics of the starch granules (Siljeström et al 1988; Eynard et al 1995). In normal practice, pH 5.1–5.4 is generally accepted to produce a good crumb structure in white bread (Pylar 1988).

Effects of Bran Pretreatment on Bread Microstructure

Bran-supplemented bread had large bran particles consisting mainly of the pericarp and aleurone cells (Fig. 3a,b). The micrographs show the structure of the breads baked with bran treated by spontaneous fermentation for 4 hr (Fig. 3a) and 16 hr (Fig. 3b). The samples had been stained by cell-wall staining. The β -glucan component of the cell walls stained blue and the aleurone cell walls rich in β -glucan and cell wall fragments originating from the starchy endosperm show blue fluorescence. The lignified cell walls of the pericarp appear yellowish green and protein has been stained red. The disrupting effect of the large bran particles on the structure of the dough can clearly be seen in the micrographs. The bran particles appear very large in comparison to the other structures of the bread (e.g., the protein network, starch granules, and gas cells) even though the bran used in the baking experiments was finely ground. The different bran fermentations had no detectable effects on bran particles or cell wall fragments. There were notable differences between the samples in the formation of gluten-starch matrix. In the breads baked with untreated bran and bran treated by spontaneous fermentation for 4 hr, the gluten network was coarser, and starch and protein appeared more in large separate areas than in the breads in which bran had been fermented for 16 hr (Fig. 3a,b).

The different bran fermentations affected the starch microstructure in the breads (Fig. 3c–f) (iodine stains pure amylose blue and amylopectin brown with varying intensity). The short fermentations (4 hr, Fig. 3c and e) affected starch structure less than the longer fermentations (16 hr, Fig. 3d and f). In the bread baked with bran treated by spontaneous fermentation for 4 hr (Fig. 3c), the starch granules appear only slightly swollen. They were stained dark brown-violet, indicating that amylose had not leached out of the starch granules. In bread baked with bran treated by fermentation with yeast for 4 hr (Fig. 3e), the starch granules also appeared only slightly swollen. The protein matrix (green) was clearly separated from the large starch granules, indicating that adhesion between gluten and starch had been reduced in the process. In the bread baked with bran treated by spontaneous fermentation for 16 hr (Fig. 3d), the starch granules appeared more swollen than in the previous samples. The granules also stained more brown and the equatorial groove of the large granules stained blue in the bread baked with bran treated by spontaneous fermentation for 16 hr (Fig. 3d) in comparison to the breads made with bran treated for spontaneous (Fig. 3c) or yeast fermentation for 4 hr (Fig. 3e), indicating phase separation of amylose and amylopectin in the starch granules. The 16-hr incubation of the bran with added lactic acid bacteria and baker's yeast

had the greatest effect on the structure of the starch granules. The starch granules were more swollen in the breads made with bran treated by fermentation with added lactic acid bacteria and baker's yeast for 16 hr (Fig. 3f) than in the other samples. The granules were stained more brown and the equatorial groove of the large granules stained blue, indicating phase separation of amylose and amylopectin in the starch granules.

Phase separation of amylose and amylopectin can take place both in swollen granular systems and in molecular dispersions (Kalichevsky and Ring 1987; Langton and Hermansson 1989; Hermansson et al 1995; Hermansson and Svegmärk 1996). Wheat starch amylopectin is less soluble than amylose, and phase separation takes place due to solubilization and diffusion of amylose out of the swollen granules (Hermansson et al 1995). The most important diffusion path of water is through the equatorial groove and the solution inside the granule can easily be squeezed out by shear or external pressure through that path as well (Langton and Hermansson 1989). In the oven, when the temperature rises, the starch granules swell and gelatinize becoming quite flexible. As the gas cells expand, the flexible starch granules within the cell wall are elongated (Pylar 1988). Phase separation of amylose and amylopectin has been studied by light microscopy using iodine in wheat starch suspension (Langton and Hermansson 1989; Hermansson et al 1995; Hermansson and Svegmärk 1996) and bread (Hug-Iten et al 1999; Salmenkallio-Marttila et al 2000).

The bread crumb is composed mainly of a bicontinuous system of gluten and gelatinized starch (Eliasson and Larsson 1993). Owing to the limited amount of water present in bread dough used in our study, the starch granules were only partially gelatinized and swollen in the baking process. Moreover, only some amylose molecules leach out of the granules. Both amylose and amylopectin are present in the swollen granules. The degree of separation of amylose and amylopectin is expected to be lower at lower water concentrations. The interrelationship between amylose and amylopectin is not completely understood, although the amylose fraction is assumed to exist in the granule as an entity that is largely separated from the amylopectin fraction. Thus, amylose is able to leach out of the granule. Concentrated wheat starch suspensions had an amylose-rich zone in the granule center after heating to 75°C (Langton and Hermansson 1989). The authors postulated that there are openings in the equatorial groove allowing for transport of amylose out of and into the central zone without diffusion through the granular structure. The passage through the equatorial groove could facilitate transport of amylose from the interior zone out of the granule and vice versa. An unhomogenous structure of the starch network in bread, consisting of swollen and interconnected starch granules and leached starch, has been reported for wheat (Hug-Iten 1999) and rye breads (Autio et al 1996).

Differences in starch granule structure in baked products reflect differences in the availability of water in the various products (Derby et al 1975). Starch gelatinization progresses further in bread in which more water is used. Differences in the degree of starch gelatinization as affected by moisture content will also affect bread volume. The differences noted between the samples, the degree of gelatinization and swelling of the starch granules, the extent to which amylose has leached from the starch granules, and the degree of adhesion between the gluten and starch are all dependent on the water content of the dough and distribution of water between the various dough components. The starch granules were more swollen and gelatinized in the breads made with sour bran than in the other samples. This is probably due to the enzymatic activities of the added lactic acid bacteria and yeast. Modification of starch by amyolytic enzymes during fermentation alters the gelatinization characteristics of the starch granules. The state of starch and its interactions with other bread constituents, particularly proteins, was suggested to cause differences in amylolysis in breads prepared by conventional or acid fermentation (Siljeström et al 1988; Eynard et al 1995). The hydrolysis of cell wall material can also affect the microstructure

of starch granules. Cell-wall-degradation has caused more water to be available for the gelatinization of starch (Autio et al 1996). As the dough contains a high concentration of cell wall components with high water absorption capacity (e.g., pentosans can absorb $\approx 10\times$ their weight of water), the enzymes of flour and microorganisms hydrolyzing the cell wall material can cause more water to be available for the gelatinization of starch.

During sour dough fermentation, the enzymes of yeast and lactic acid bacteria modify the dough components. The sour dough process affects binding of starch and gluten, recrystallization of gelatinized starch granules, retrogradation of amylose leaking out of the starch granules, and the rigidity of the starch matrix. Use of fermented bran affects the pH level of the dough supplemented with it. Enzyme activities and the charge of gluten proteins are affected by pH, thus altering the dough strength. Proteolytic activity of lactic acid bacteria used in the sour dough process modifies the gluten network, affecting the physical properties of gluten and the texture of bread (Armero and Collar 1997; Corsetti et al 1998; Wehrle et al 1999). In our experiments, fermentation of the bran fraction clearly affected the properties of the gluten matrix of the bread. The difference was especially clear when comparing the microstructure of the breads after storage. The improved structure of the gluten network probably also improves the keeping quality of bread.

CONCLUSIONS

Fermentation of wheat bran with yeast or with yeast and lactic acid bacteria improved loaf volume, crumb structure and shelf life of bread supplemented with 20% bran. The bread had added flavor and good and homogenous crumb structure. The observed differences in the microstructure of the test breads, the degree of gelatinization and swelling of the starch granules, the extent to which amylose leached from the starch granules and the degree of adhesion between the gluten and starch, are all dependent on the water content of the dough and the distribution of water between the various dough components. By optimizing the baking process it is possible to produce good consumer quality wheat bread containing up to 10% dietary fiber.

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