Effect of Baking on the Microstructure of Rye Cell Walls and Protein

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ABSTRACT

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Rye doughs and breads were baked from whole meals milled from two rye varieties, a Canadian Muskate and a Swedish Danko. A study was made of the effect of β-glucanase and xylanase incubation on the kernel cross-sections. β-Glucanase studies indicated that β-glucans are evenly distributed in the endosperm cell walls of the rye kernel. Xylanase treatment degraded only peripheral endosperm cell walls and had greater effect on the Muskate rye kernels. This finding suggests that wall structure varies in the different parts of the rye kernel; hence variety or growing conditions, or both, play an important role in the structural features of the plant. Examination of the microstructure of the doughs and breads indicated that proteins are of major importance in the structure of the rye dough just after mixing. The Muskate meal was milled finer, and protein was released from the cells to the dough matrix. The Muskate dough components were tightly stuck together. The Danko dough was less cohesive and more rigid than the Muskate dough just after mixing, evidently because of a higher content of big, unbroken particles in the Danko dough. As baking proceeds, endosperm walls are fragmented and, along with starch, their role in the formation of the continuous matrix increases. The Danko bread contained long, unbroken aleurone layers that, combined with a weak protein matrix, possibly decreased extensibility of the dough and distorted the gas cell structure. The Danko bread crumb was less elastic and more porous than the Muskate bread crumb.

In many countries, rye is chiefly used as an animal feed. In Finland, it is mainly used in rye bread, which is baked from whole meal containing bran and coarse endosperm particles. Gluten forms a more rigid and elastic network in a relatively neutral wheat dough than in an acidic rye dough (Pomeranz et al 1984). Because of the high pentosan content, rye proteins are not able to form a film (Drews and Seibel 1976). According to Holas et al (1992), rye protein neither forms the dough structure nor is the main water-absorbing matter in dough. The water-binding capacity of rye dough is largely dependent on the content of nonstarch polysaccharides, which are found primarily in the plant cell wall as both water-soluble and water-insoluble fractions

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(Girhammar and Nair 1992). Most of the nonstarch polysaccharides present in rye whole meal are polymers of xylose and arabinose, i.e., arabinoxylans (6.5–11.5%), and only 1.0–2.0% is (1→3),(1→4)-β-glucan (Saastamoinen et al 1989). Thus, compared with other cereals, rye is rich in arabinoxylans, while its (1→3),(1→4)-β-glucan content is lower than that of barley and oats (Saastamoinen et al 1989). Pentosans (mainly arabinoxylans and arabinogalactans) have been found to retain gas in the wheat dough (Hoseney 1984) and to retard wheat bread staling (Kim and D'Appolonia 1977, Jankiewicz and Michniewicz 1987).

The physical properties of rye dough depend mainly on water-binding substances (Pomeranz 1985b, Holas et al 1992). In normal rye dough, soluble pentosans form a sticky gel that plays an important role in keeping the parts of the dough together (Pomeranz 1985a, Holas et al 1992). Insoluble pentosans also have a high water-binding capacity and, in addition, a capacity to swell (Kulp 1968, Kim and D'Appolonia 1977, Pomeranz 1985a, Meuser and Suckow 1986). The viscous properties of rye doughs are extremely important for baking quality (Drews and Seibel 1976).

According to Bengtsson et al (1992), the main water-soluble arabinoxylan in rye contains at least two polymers or two fractions of a polymer. In one of these polymers (arabinoxylan I), a long 4-linked β-D-xylopyranosyl chain forms the backbone to which α-L-arabinofuranosyl side groups are attached at 0-3 in the manner presented in Fig. 1. In the other polysaccharide (arabinoxylan II), the xylose residues are substituted at 0-2 and 0-3. Water-soluble arabinoxylans of wheat also contain both mono- and disubstituted xylose residues (Izydorczyk and Biliaderis 1992a). The arabinose side chains cause the water solubility of arabinoxylans (Meuser and Suckow 1986). Much attention has been given to the isolation and characterization of water-soluble arabinoxylans, but the chemical structure of water-insoluble arabinoxylans has been less well characterized. It has been reported (Medcalf and Gilles 1968; Meuser and Suckow 1986) that, in wheat, the A-X ratio of the water-soluble and water-insoluble fraction is similar, but the molecular weight of arabinoxylans in the insoluble fraction is higher. According to Gruppen et al (1992), water-soluble and -insoluble arabinoxylans have largely the same chemical composition, but the arabinose substitution is different.

Fluorescence microscopy is a useful tool for studying the microstructure of cereals when these have been marked with specific fluorochromes (Fulcher 1982). It has been used to observe the specific compounds in barley (Fulcher et al 1977), for revealing the effect of processing on oats (Yiu 1986, Autio et al 1992) and rapeseed (Yiu et al 1983), and for several other applications (Fulcher et al 1989). Our aim in the present work was to compare the microstructures of Muskate and Danko rye kernels and their corresponding doughs and breads and to study the location of arabinoxylans, β-glucans, and protein.

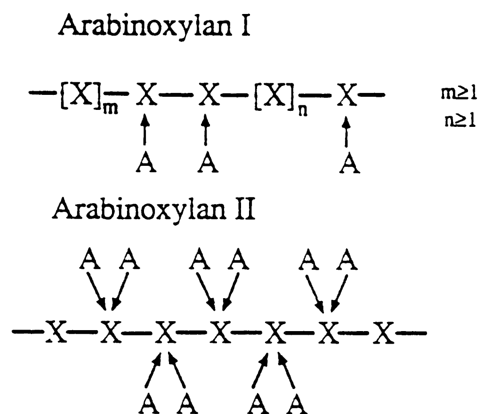


Fig. 1. Structure of arabinoxylan I and II in rye grain. A = arabinose, X = xylose. (Reprinted, with permission, from Bengtsson et al 1992)

MATERIALS AND METHODS

Rye Samples and Chemical Analysis

Two different rye varieties, a Canadian Muskate and a Swedish Danko, were examined. Before the chemical analysis, samples were ground in a cyclotec 1093 sample mill (Tecator, Höganäs, Sweden) to pass a 0.5-mm screen. All analysis were made in duplicate and are reported on a dry matter basis.

Starch was determined enzymatically (Åman and Hesselman 1984), and crude protein (N × 6.25) was analyzed by a standard method (AOAC 1984). Nonstarch polysaccharide residues were determined as described by Theander and Westerlund (1986). Mixed-linked (1→3),(1→4)-β-glucans were analyzed according to Åman and Graham (1987).

Water-soluble nonstarch polysaccharides were extracted from rye meal (400 mg) with sodium acetate buffer at 95°C during starch hydrolysis. Polymers were isolated by ethanol precipitation, and nonstarch polysaccharides were analyzed by sugar analysis (Bengtsson et al 1992).

Baking Quality of Rye Meals

Milling. Grains were milled in a Bauermeister Universal Mill (Hamburg, Germany) fitted with a 1.5-mm sieve. Particle size distribution of 100 g of milled grains was determined by sieving in a laboratory sieve (Buhler Miag DLKP-2040, Milan, Italy) fitted with different screens and separating into three fractions: <180, 180–355, and >355 μm.

Baking. Doughs consisted of milled rye (675 g), water (575 and 540 g, Muskate and Danko, respectively), salt (12 g), yeast (20 g), lactic acid (1.9 ml), and acetic acid (0.13 ml) (pH 5.1). The amount of water was adjusted according to the water absorption of the rye variety (85 and 80%, Muskate and Danko, respectively).

The doughs were mixed in a fork mixer (John Holmström, Stockholm, Sweden) for 6 min. After floor time (45 min at 36°C), dough pieces (500 g) were molded by hand and proofed (90 min at 36°C). The breads (pH 5.1) were baked at 175°C for 55 min. The crumb characteristics were determined by an experienced baker.

Rheological measurements. The rheological measurements were made using a Bohlin (VOR, Lund, Sweden) rheometer with parallel-plate geometry in a high-temperature cell. Doughs without yeast were prepared in the same way as described above, using 80% absorption. The rye dough sample was slowly compressed by the upper plate until the gap between the plates (25 mm diameter) was 1.5 mm, and the expelled dough was carefully trimmed off with a razor blade. The sample was allowed to rest for 2 min. An O-ring was used in the lower plate, and silicon oil was applied around the plate edges to prevent the sample from drying. The temperatures of the dough and the gas were measured by two thermocouples. The measurements, performed at 36°C, were made just after dough mixing and after incubation of the dough at 36°C for 70 min. The frequency was 5 Hz and the strain 3.0×10^{-4}. At this strain, the samples were within the linear region. The strain dependence was studied from 3×10^{-5} to 1×10^{-3}. All data reported are the means of at least two (generally more) replicate tests.

The crumb firmness during storage was determined as maximum compression force (20% compression with a 21-mm diameter plunger, compression rate 2 cm/min) measured by an Instron (High Wycombe, England) Universal Testing Apparatus, model TM-MA 0636. The maximum force needed for compression was calculated from the curves, and the results were expressed as the means of eight measurements from four bread slices (thickness 3 cm).

Microscopy

Plastic sections. Bread samples were taken from the center of the loaf. Small samples of dough (just after mixing) and bread (~16 mm^3) and halves of rye kernels were fixed in 5% glutaraldehyde in 0.1*M* phosphate buffer (pH 7.0) (Fulcher 1982), dehydrated with ethanol, and embedded in Historesin (Reichert-Jung,

Heidelberg, Germany) embedding medium, as recommended by the manufacturer. Sections (5 μm) were cut with a Reichert-Jung (Heidelberg, Germany) microtome.

Enzyme preparations. The endo-β-xylanase and endo-β-glucanase used were isolated and purified from *Trichoderma reesei* by the Biotechnical Laboratory of VTT. Endo-β-xylanase (E.C. 3.2.1.8.), pI 9.0, was purified as described by Tenkanen et al (1992), and endo-β-glucanase (E.C. 3.2.1.4.) was endoglucanase II (Saloheimo et al 1988) purified as described (J. Pere, M. Siika-Aho, J. Buchert, and L. Viikari, *unpublished data*). Xylanase activity was assayed by using 1% birchwood xylan (Roth 7500, Karlsruhe, Germany) as substrate, as described al (1992). β-Glucanase activity was assayed in a similar way by using 1% barley β-glucan (Biocon Biochemicals, Cork, Ireland) as substrate (Zurbriggen et al 1990). Enzyme activity was expressed as katals (1 kat = 1 mol sec^{-1}). Xylanase showed no activity toward amylose or β-glucan. In the case of the β-glucanase, no activity was detected toward amylose or arabinoxylan.

Enzyme treatment. The plastic sections of rye kernel were incubated with these enzyme preparations (300 nkat/ml) in a humid chamber at 37°C for 24 hr at pH 4.5. The reference sections were incubated without enzyme. The sections were rinsed with distilled water and dried.

Staining. For the fluorescence microscopic examinations, the sections were stained with specific fluorochromes (Fulcher and Wong 1980). The kernel sections were stained with 0.01% Calcofluor White M2R New. The dough and bread sections were stained with 0.1% acid fuchsin and 0.01% Calcofluor White M2R New. The sections were rinsed with distilled water and dried. Calcofluor stained the cell walls blue. Acid fuchsin stained the aleurone protein red and the endosperm protein orange or light brown. Starch was unstained and appeared black.

Microscopic examination. The samples were examined and photographed with an Olympus (Tokyo, Japan) Vanox-T microscope. The stained sections were examined with a filter set BH2-DMV with maximum transmission at 405/>455 nm. Photomicrographs were obtained using Kodak Gold 400 film.

RESULTS AND DISCUSSION

Rye Kernel

The rye varieties were chosen on the basis of their different chemical compositions (Table I). Microscopic examination was made of approximately 100 sections of each variety, cut from many different grains. The cell walls were stained with Calcofluor (Figs. 2–9), which has a high affinity for (1→3),(1→4)-β-glucan (Fulcher et al 1989). (1→3),(1→4)-β-Glucan content was 2.1% in both varieties (Table I). No difference was detected in the thickness of the Calcofluor-stained cell walls of the two varieties. The Calcofluor-induced fluorescence of the walls was similar in all parts of the endosperm, indicating that (1→3),(1→4)-β-glucan is not confined to the walls of cells in certain regions (Figs. 3 and 5).

After β-glucanase treatment, the fluorescence of the endosperm walls disappeared almost totally from both varieties (Figs. 2 and 4). Under intense illumination, Calcofluor binds nonspecifically to storage proteins (Fulcher et al 1989), and its blue emission is sometimes difficult to differentiate from the blue cell walls, as in Fig. 4.

TABLE I
Chemical Compositions of Rye Samples (% of dry matter)

Component	Danko	Muskate
Starch	66.6	64.4
Protein	9.5	11.9
Xylans[a]	8.0	6.8
Water-soluble arabinoxylans[b]	2.1	1.9
(1→3), (1→4)-β-Glucans	2.1	2.1

[a]Calculated as the sum of arabinose, xylose, and uronic acid residues of nonstarch polysaccharides.
[b]Calculated as the sum of arabinose and xylose.

Compared with the reference sections (Figs. 7 and 9), in both varieties, the fluorescence intensity of the endosperm cell walls was weaker after the xylanase treatment (Figs. 6 and 8). According to Wood et al (1983), the Calcofluor used for cell wall staining induces fluorescence in (1→3),(1→4)-β-D-glucan and does not stain arabinoxylan or interact with it in solution. The endoxylanase that was used hydrolyses arabinoxylans from inside the polymer that, together with β-glucan, forms the cell walls. Evidently, when one component, arabinoxylan, is enzymatically split off from the wall, the wall structure is loosened, thereby decreasing the affinity for Calcofluor of the other wall component, β-glucan.

Aleurone walls fluoresced after both β-glucanase and xylanase treatments (Figs. 2, 4, 6, and 8). This may be the result of the limited accessibility of the enzymes to their substrates in the thick aleurone walls. On the other hand, aleurone walls contain some cellulose, which fluoresces in the presence of Calcofluor.

Fluorescence was weakest in the peripheral endosperm wall in the xylanase-treated section (Figs. 6 and 8). Possibly, arabinosyl substitution of the arabinoxylan polymer decreases from the inner endosperm toward the outer endosperm, as has been shown for wheat kernel (Izydorczyk and Biliaderis 1992b), and, possibly, cell wall hydrolysis by xylanase increases in the same direction. In the Danko variety, xylanase incubation affected only the cell walls of the very outer layers of the endosperm (Fig. 6), whereas in the Muskate variety, it had an effect also deeper in the endosperm (Fig. 8). This difference must be related to the different structure or arabinoxylan content of the cell walls of the two varieties.

The Danko rye contained more arabinoxylan (arabinose + xylose), 8.0%, than the Muskate rye, 6.8% (Table I). The Danko rye also contained more water-soluble arabinoxylan than the Muskate rye (2.1 vs. 1.9%) (Table I). The pentosan content of rye increases in wet growing conditions (Saastamoinen et al 1989). Drews and Seibel (1976) report that the water-soluble pentosan content is higher in rye cultivated in the more humid growing conditions in Europe than in rye cultivated in Canada. The effect of environmental factors on the other soluble components is not significant (Drews and Seibel 1976).

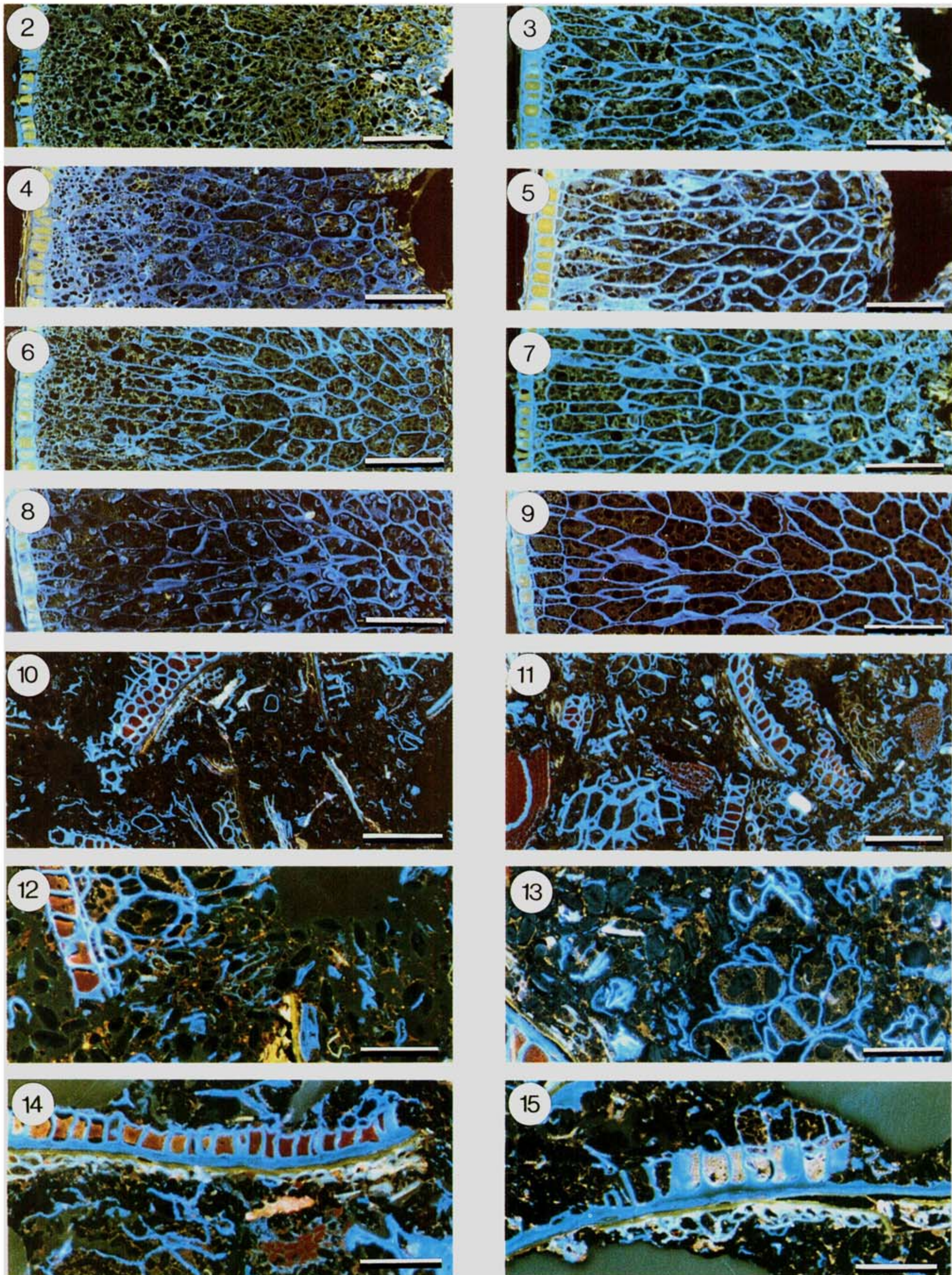
The differences in the microstructure and chemical composition of the two varieties are probably dependent on both the dissimilarities of the varieties and the growing conditions of the plant.

Rye Meal, Dough, and Bread

Since the rye dough was baked from whole meal, the structure of the dough was very complex. The micrographs shown are representative of a number of samples. Irrespective of the variety, the aleurone layers were resistant to breakage during dough mixing, whereas part of framework of endosperm walls disintegrated (Figs. 10 and 11). Both dough samples contained unbroken bran, aleurone, and endosperm particles with size varying from under 10 μm up to 600 μm in diameter. In the Muskate dough, the components were stuck tightly together and the structure was very cohesive (Fig. 11). The Danko rye meal did not form such a cohesive dough; the fragile dough structure can be seen in Figs. 10 and 12.

Over 25% of the particles of the Danko rye meal were more than 355 μm in diameter, but only 10% of those of the Muskate meal were (Table II). About half (57%) of the particles of the Danko meal and 76% of the Muskate meal were less than 180 μm in diameter. The fact that the values of G′ obtained for wheat doughs (Lindahl 1990, Weibert 1992) have been substantially lower than for rye doughs suggests that both the rigidity and the size distribution of particles in rye doughs make a major contribution to the magnitude of G′. The Danko dough had higher G′ (57 ± 3 kPa, Table III) than the Muskate dough (46 ± 2 kPa), evidently because of the higher content of big, unbroken particles in the Danko dough.

During incubation at 36°C, the Muskate dough exhibited a smaller decrease of G′ (5 kPa) than the Danko dough (11 kPa) (Table III). The decrease of G′ is related to the softening and degradation of cell walls and to changes in the water distribution.



Figs. 2-15. Photomicrographs of Calcofluor-stained kernels and acid fuchsin-calcofluor-stained dough and bread samples from two rye varieties. Reference samples were incubated without enzyme. Bar = 250 μm, except where noted. **2,** Danko rye kernel after β-glucanase incubation. **3,** Reference sample for Fig. 2. **4,** Muskate rye kernel after β-glucanase incubation. **5,** Reference sample for Fig. 4. **6,** Danko rye kernel after xylanase incubation. **7,** Reference sample for Fig. 6. **8,** Muskate rye kernel after xylanase incubation. **9,** Reference sample for Fig. 8. **10,** Danko rye dough. Acid fuchsin and Calcofluor staining. **11,** Muskate rye dough. Acid fuchsin and Calcofluor staining. **12,** Danko rye dough. Acid fuchsin and Calcofluor staining, bar = 100 μm. **13,** Muskate rye dough. Acid fuchsin and Calcofluor staining, bar = 100 μm. **14,** Danko rye bread. Acid fuchsin and Calcofluor staining, bar = 100 μm. **15,** Muskate rye bread. Acid fuchsin and Calcofluor staining, bar = 100 μm.

The increase of dough volume during fermentation was greater for the Muskate dough (370 cm^3) than for the Danko dough (320 cm^3) (Autio et al 1993). The softer Muskate dough expanded more during fermentation.

In the Muskate dough, starch granules, bran, and endosperm particles were dispersed in a protein matrix (Fig. 13). Orange-stained protein was distributed all around the starch granules, endosperm, and bran particles. Because of the weak matrix structure and lack of cohesion in the Danko dough, the dough structure collapsed during sample preparation (Fig. 12). Unstained plastic was the dominating factor between the cereal components in the Danko dough section, and orange protein could be detected only in patches in the matrix. The probable reason for the loose protein-starch matrix is the lower protein content (9.5%) and higher content of large particles in the Danko meal than in the Muskate meal (11.9% protein). Microscopic examination with brightfield (*not shown*) revealed a more intense staining of the protein matrix of the Muskate dough than of the Danko dough. According to Holas et al (1992), both starch and pentosans form highly concentrated gels when the amount of water present is limited, as in rye dough. Our studies showed that proteins, too, play an important role in the dough stage of rye bread baking. These observations were made just after dough mixing. In the formation of the continuous matrix, the importance of protein decreased as the baking proceeded, and the role of the other water-binding components, like starch and cell wall fragments, increased.

The endosperm cell walls were highly fragmented in baked breads (*not shown*), and the number of particles over 250 μm in diameter decreased dramatically. The bread baked from Danko rye meal consisted of very long, unbroken aleurone layers (Fig. 14). In the bread baked from the Muskate rye meal, the aleurone layers were more broken, and the protein inside the cells was released to the matrix (Fig. 15). The Muskate meal was finer than the Danko meal, which probably was an important reason for the aleurone cell wall fragmentation of the Muskate dough during floor time, proofing, or the early oven stage.

The crumb structure of the Danko bread was slightly more porous and less elastic to the thumb than that of the Muskate bread. The crumb of the fresh Danko bread was softer than that of the fresh Muskate bread. The Danko bread contained more soluble arabinoxylans than the Muskate bread (Autio et al 1993). The crumb softness was indicated by the maximum force needed for 20% compression, which was 4.8 N for the Danko bread and 5.7 N for the Muskate bread. In studies of whole-meal wheat bread, Gan et al (1992) found that nonendosperm components like brans cause serious distortion of gas cells and probably contribute to the resultant crumb morphology. Even though unbroken aleurone and bran particles may provide a coherent and continuous dough or bread structure (Pomeranz 1985a), in the Danko rye bread the unbroken aleurone layers, combined with the weak protein matrix, possibly decreased the extensibility of the dough and affected the gas cell structure. Big, unbroken particles may have contributed to the gas cell formation, but their sizes and effects were not systematically studied.

Since the amount of water is limited during baking, it is highly probable that the degree of cell wall hydrolysis not only depends on structural differences between the varieties but also on the different hydration levels of the cell walls. The effects of naturally occuring cell wall hydrolases on rye dough rheology and structure is difficult to judge because whole-meal rye baking is so complex. By adding β-glucanase and xylanase to rye dough, enzymes can be used as tools in studying their role in rye baking; this is discussed in more detail by Autio et al (submitted).

TABLE II
Particle Size Distribution of the Danko and Muskate Rye Meals

	Particle Size, %		
Variety	**< 180 μm**	**180–355 μm**	**> 355 μm**
Danko	57	17	26
Muskate	76	14	10

TABLE III
Storage Modulus (G′) for Rye Doughs

	G′ (kPa) at 36°C		
Dough	**After Mixing**	**After Incubation[a]**	**Δ**
Danko	57 ± 3	46 ± 2	11
Muskate	46 ± 2	41 ± 1	5

[a] At 36°C for 70 min.

CONCLUSIONS

After incubation of the rye cross-sections with β-glucanase and staining with Calcofluor, the previously uniform fluorescence of the endosperm cell walls disappeared in both rye varieties. This indicates that β-glucans are evenly distributed in all the endosperm cell walls of the rye kernel. After xylanase incubation and subsequent staining, the cell walls in the peripheral layers of the kernels showed reduced fluorescence in both varieties. Because of the different cell wall structures, however, the reduction of the fluorescence was more complete in the Muskate rye.

The rye meal consisted of starch, cell wall fragments, bran, and aleurone particles incorporated by protein and gumlike material to form a dough. Compared to the Muskate dough, the amount of large endosperm and bran particles was greater in the Danko dough, which was the main reason for the higher G′ after mixing and bigger decrease of G′ during incubation at 36°C. Proteins certainly play an important role in the rye dough structure just after mixing, since degradation of cell walls and hence contribution of pentosans to the continuous matrix is limited. The structure of the Muskate dough was far more compact than that of the Danko dough because of the denser protein matrix and finer meal. In the Danko rye dough, the aleurone layers were highly resistant to baking and for the most part stayed intact. The Muskate nonendosperm particles were more broken in the bread, and contents of the aleurone cells were dispersed in the bread matrix.

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