

Effect of Germination and Water Content on the Microstructure and Rheological Properties of Two Rye Doughs

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ABSTRACT

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Two rye cultivars, Marder and Motto, with falling numbers 314 and 309, respectively, were germinated *in vitro*. Relative to the native grains, germination induced minor local changes in the microstructure of cell walls and proteins in the kernels. Kernels of germinated and native grains were milled, and doughs were prepared from the flours, with water content and incubation time varied according to experimental design. The viscoelastic properties of the doughs were measured just after mixing and after various incubation times. The area of blue fluorescence, a measure of intact cell walls, was quantified by computer-assisted image analysis in thin sections of rye dough after mixing and incubation, and the starch

structure was studied under the microscope after iodine staining. The water content of the doughs was explained well by the rheological behavior. Doughs made from flours of germinated grains were always softer than doughs made from flours of native grains, and Marder doughs were always more rigid than Motto doughs. The higher the water content, and the longer the incubation time, the greater the rheological changes during incubation. Microstructural studies showed that germination and incubation caused changes in the cell wall structures of dough that might explain the softening of the doughs.

Rheology plays an important role in almost every step of the rye breadmaking process. Finnish rye breads are baked from whole meal rye, and the dough rheology is highly dependent on the particle size and structural state of the cell walls (Autio et al 1996; Koskinen et al 1997; Parkkonen et al, *in press*). We have shown that very large particles originating from aleurone and endosperm layers are dispersed in the protein-starch matrix immediately after mixing (Parkkonen et al 1994). As the baking proceeds, the cell walls fragment and become part of the continuous phase. The rheological properties of dough change during the process, and the extent of the changes depends on, among other things, the enzyme activities of the raw material. The increase of α -amylase activity due to sprouting has been well documented and is one reason for the rheological changes of doughs. We have also demonstrated that sprouting can have great effect on the structure of cell walls (Koskinen et al 1997).

Resistance to preharvest sprouting is considered an important property of a cultivar, since low falling number due to sprouting is a major cause of poor baking quality. Hybrid breeding, developed in Germany (Geiger 1982), has become an interesting alternative to population breeding, and the hybrid cultivars grown in the Nordic countries, Amando and Marder, exhibit good sprouting resistance (Gunnarsson 1995).

Arabinoxylans present in rye endosperm cell walls have excellent water-holding capacity. Estimates suggest that the water uptake of pentosans is ≈ 15 g water/g (db) (Bushuk 1966). Water-binding not only depends on the content of arabinoxylans, but also on their structure and on the activity of endogenous enzymes. The addition of cell-wall degrading enzymes usually results in a decrease in the water-binding of cell walls (Maat et al 1992, Autio et al 1996), an increase in the fragmentation of cell walls, and a redistribution of the water. In wheat doughs, water is transferred from cell walls to gluten and in rye doughs from cell walls to the starch-protein matrix.

The aims of the present work were to study the effects of falling number and water content on the rheological properties of rye doughs by using a statistical experimental design, and to determine whether the rheological differences could be explained through reference to dough microstructure. A population cultivar, Motto, and a hybrid cultivar, Marder, were compared.

MATERIALS AND METHODS

Rye Samples

Two rye cultivars, Motto and Marder (both harvested in 1995), were examined. The dry matter content of the flour was determined by oven drying at 130°C for 1 hr. The total arabinoxylan content was determined by the spectrophotometric method, and the soluble arabinoxylan content was determined by the same method after a 45-min aqueous extraction at room temperature (Douglas 1981). Falling number was measured by a standard method (ICC 1995).

Germination

Rye samples were steeped at 14°C to 27% moisture content (mc) and germinated at 14°C (20 hr Motto and 19 hr for Marder). The kilning temperature was 30°C, and the samples were dried to 14% mc.

Enzyme Activities

α -Amylase activity was assayed by using an α -amylase assay procedure kit (Ceralpha, CER 6/93, Megazyme Ltd., Sydney, Australia). The sample, 2 g/20 mL of buffer (50 mM sodium malate, 50 mM sodium chloride, 2 mM calcium chloride, 3 mM sodium azide), was extracted at room temperature at pH 5.2 for 30 min (McCleary and Sheehan 1987). Xylanase activity was assayed by a modification of the method of Bailey et al (1992) with incubation of 2 hr at 40°C for the enzyme reaction. Before the assay, the extracted samples were diluted (1+2 vol) with 50 mM sodium citrate buffer to decrease the high background caused by the solubilized sugars in the sample. Three to five parallel assays were performed for each sample.

Swelling Curve

In the swelling test (Drews 1971), the viscosity of a rye flour-buffer suspension (120 g of flour in 410 mL of buffer (10 mM sodium phosphate-citric acid) (pH 5.0) was measured in a viscosograph (Brabender OHG, Duisburg, Germany) with a 500-cmg measuring cartridge.

Experimental Design

Factors considered important were cultivar, water content, and incubation time. The software used for planning experiments and for evaluation of experimental data was Modde for Windows (Umetri, AB, Umea, Sweden). The fractional factorial design for native and germinated samples is presented in Table I.

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Preparation of Doughs

Doughs consisted of milled rye (175 g), water, and salt (1.5% on flour basis). The amount of water was varied to give three different dough yields: 175, 180, and 185. The doughs were mixed for 3 min in a household mixer (Kenwood Chef Excel, New Lake, Great Britain) fitted with a K-beater. Dough pieces were incubated at 36°C and 80% rh for different lengths of time.

Rheological Measurements of Doughs

The rheological measurements were made with a rheometer (StressTech, Reologica, Lund, Sweden) with parallel-plate geometry. Two replicate doughs were prepared as described above and three pieces from each dough were tested. The dough sample was slowly compressed by the upper plate until the gap between the plates (25 mm dia) was 2.0 mm, and the expelled dough was carefully trimmed off with a razor blade. The sample was allowed to rest to reach a 0 normal force. Silicon oil was applied around the plate edges to prevent the sample from drying. The measurements were made at 36°C just after dough mixing. The frequency was 1 Hz and the strain was 4×10^{-4} .

Microscopy and Image Analysis

Six small pieces of dough were taken from the center of each dough immediately after mixing and after 60, 90, or 120 min of incubation (two doughs were prepared from each rye cultivar). Dough pieces and halves of kernels were fixed in 5% (v/v) glutaraldehyde in 0.1M phosphate buffer (pH 7) (Fulcher 1982), dehydrated with ethanol, and embedded in Histo-resin medium (Jung, Heidelberg, Germany), as recommended by the manufacturer. Sections (5 µm) were cut with a microtome (Leica Jung RM2055, Nussloch, Germany). Two sections (5 µm) were cut randomly from each dough piece. For the fluorescence microscopic examinations, the sections were stained with specific fluorochromes (Fulcher and Wong 1980, Fulcher et al 1989). The

dough sections were counterstained with 0.1% (w/v) green (Fast Green) and intact cell walls were stained with 0.01% (w/v) blue (Calcofluor White M2R New). After each staining, the sections were rinsed with distilled water and dried. Altogether, 24 sections (50 fields/section), with a total area of 480 mm², were examined with a microscope (Vanox-T, Olympus, Tokyo, Japan) using filter set BH2-DMV with maximum transmission at 405 ≥ 455 nm, with a chilled color video camera (CCD, C5310-11, Hamamatsu Photonics K.K., Tokyo, Japan) and analyzed with a computer-assisted image analyzer (CUE-3, version 4.6p, Olympus, Tokyo, Japan). The area of blue fluorescence relative to the total area analyzed was calculated by applying the volume fraction operation according to the method of Parkkonen et al (*in press*). Thresholding values were adjusted according to the fluorescence of the blue cell walls. For bright-field microscopy, the sections were stained with 0.1% (w/v) Light Green and iodine.

The kernel sections for photography were stained with 0.1% (w/v) red (Acid Fuchsin) and 0.01% (w/v) blue (Calcofluor White M2R New). After each staining, the sections were rinsed with distilled water and dried. The intact cell walls were stained blue. The aleurone protein was stained red and the endosperm protein was stained orange or light brown. Starch was unstained and appeared black. Photomicrographs were obtained with the microscope using Kodak Gold 400 film. Photomicrographs of grain cross sections were prepared from several photographs of a single grain section to reveal the highly localized differences between the cultivars. Several grains from each sample were examined, and a representative grain was chosen for presentation in the figures.

RESULTS AND DISCUSSION

Differences in the Grain Properties

Marder, representing a German hybrid cultivar, had slightly higher pentosan content than Motto, a Swedish population cultivar (Table II).

The falling number of the rye samples is shown in Table II. Germination of Motto and Marder resulted in a decrease of the falling number. It was difficult to keep conditions constant during germination, and falling numbers differed for the two batches of germinated grain.

The enzyme activities were studied in more detail for batch 1. Germination increased the activity of both α-amylase and endo-β-xylanase (Table II).

Micrographs of the longitudinal sections revealed smaller cells and thinner cell walls for Motto (Fig.1, plate 1) than for Marder (plate 2). Relative to the native grains of Motto (plates 3 and 4), germination induced only local changes in the microstructure of grains: fading of the blue fluorescence of cell walls in local parts of subaleurone (plate 5), disappearance of the blue fluorescence near the endosperm cavity (plate 6), and change in the microstructure of the protein matrix in some grains of Motto (plates 3 and 5). The small B-type starch granules were not visible because the germination caused protein to spread out over them.

TABLE I
Experimental Design

Grains	Dough Yield	Incubation Time (min)	Batch No.
Native			
Motto	175	120	
Marder	175	60	
Motto	185	60	
Marder	185	120	
Motto	180	90	
Marder	180	90	
Germinated			
Motto	175	60	1
Marder	175	60	2
Motto	185	120	1
Marder	175	120	1
Marder	185	120	2
Motto	185	60	2
Marder	185	60	1
Motto	175	120	2

TABLE II
Grain Properties of Two Ryes

	Pentosans		Falling Number	Enzyme Activities	
	Total Arabinoxylans (% of dry matter)	Soluble Arabinoxylans (% of dry matter)		α-Amylase (U/g)	Xylanase (nkat/g)
Motto					
Native	8.2	1.6	309	0.17	1
Germinated, batch 1	...		124	0.51	5
Germinated, batch 2	...		163
Marder					
Native	8.8	1.7	314	0.20	1
Germinated, batch 1	...		112	0.40	5
Germinated, batch 2	...		160

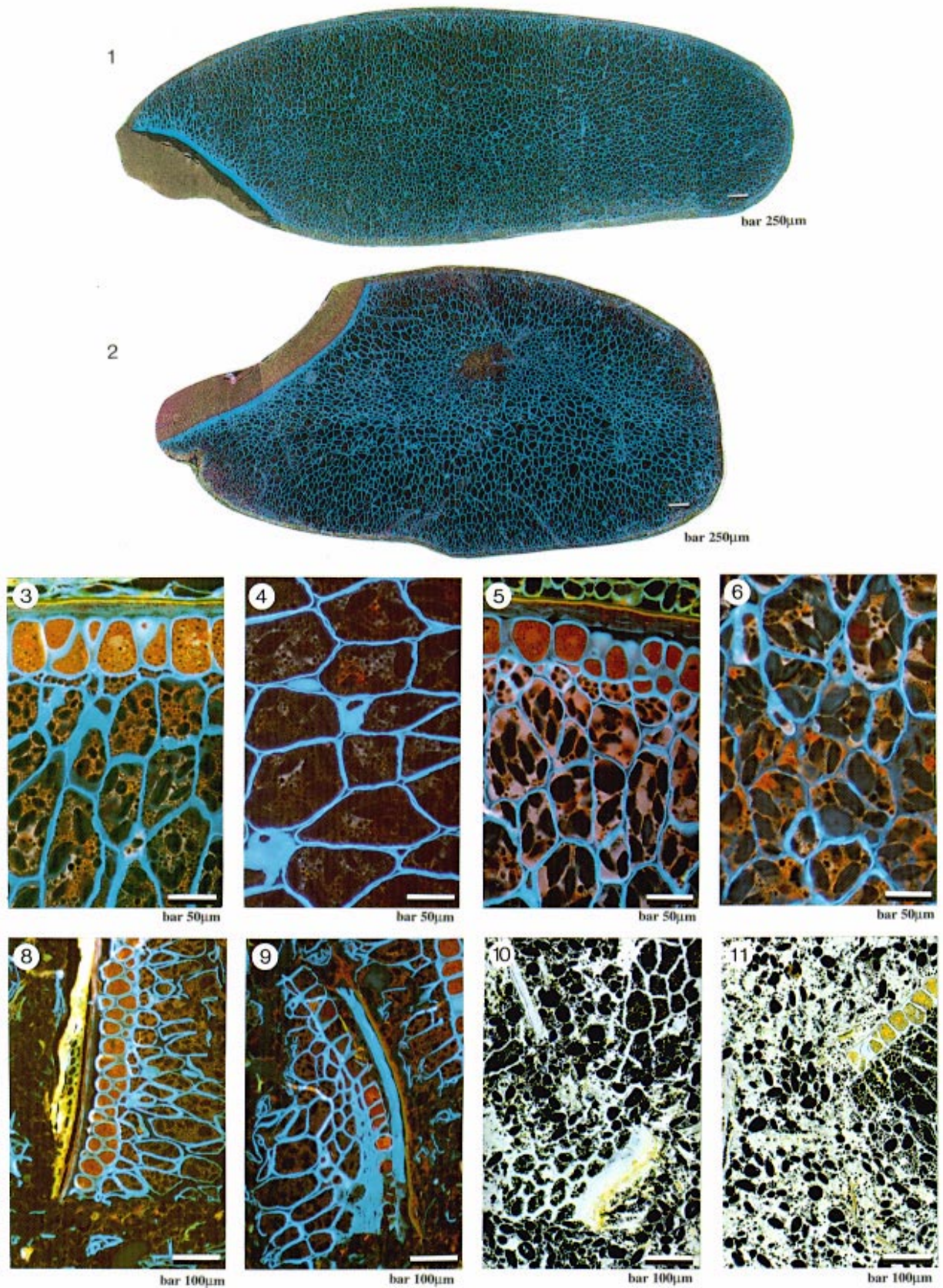


Fig. 1. 1, Longitudinal section of native Motto kernel (cell walls appear blue). 2, Longitudinal section of native Marder kernel. 3, Vertical section of native Motto kernel showing the aleurone layer and outer starchy endosperm. 4, Vertical section of native Motto kernel showing the inner starchy endosperm. 5, Vertical section of germinated Motto kernel showing the aleurone layer and the outer starchy endosperm. 6, Vertical section of germinated Motto kernel showing the inner starchy endosperm. 8, Cross section of Motto dough (made from germinated grains, batch 2) with 75 water content (area of blue fluorescence was lowest in this sample; cell walls appear blue and the appear proteins red). 9, Cross section of Motto dough (made from germinated grains, batch 1) with 85 water content (area of blue fluorescence in the dough section was highest; staining as in 8). 10, Cross section of Motto dough (made from germinated grains) with 75 water content (starch granules appear black). 11, Cross section of Motto dough with 85 water content (made from germinated grains; the starch granules appear black).

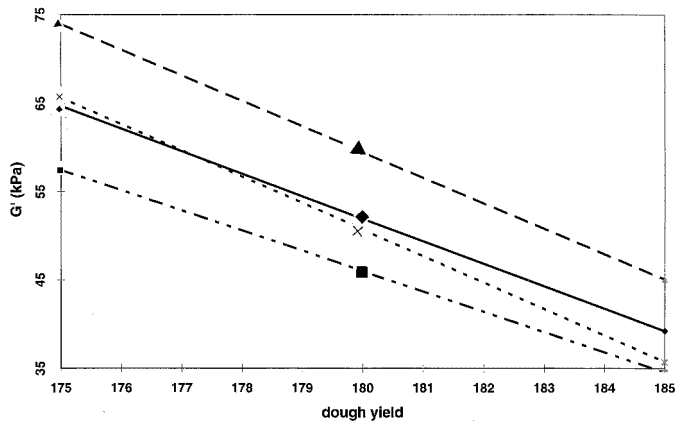


Fig. 2. Effect of water content on the storage modulus (G') of dough after mixing (Motto \blacklozenge , Marder \blacktriangle) and incubation (Motto \blacksquare , Marder \times).

Swelling Tests of Flours

Marder had higher viscosity at all temperatures studied (Table III). Germination also caused higher onset viscosity. Higher viscosity is related to higher solubility and swelling of the cell walls. In an earlier study, we showed that addition of β -glucanase causes (50 nkat/g) an increase, and xylanase causes a decrease in the onset viscosity but no decrease during the holding period (Autio et al 1996). Addition of a mixture of cell-wall degrading enzymes caused the onset viscosity to decrease significantly, relative to control samples, and caused the viscosity to decrease during the holding period.

Rheological Measurements

The effect of water content on the storage modulus (G') of dough after mixing and incubation is presented in Fig. 2 for doughs made from native and germinated grains. The effect is shown as linear models fitted to the experimental data. Although falling numbers differed for the two germination batches, there was little effect on the rheological properties. The higher the water content, the lower the G' . Effect of water content was more pronounced for Marder than for Motto doughs.

Marder doughs were more rigid than Motto doughs. Moreover, doughs made from flours of germinated grains were always softer than doughs made from flours of native grains.

Microstructure and Image Analysis of Doughs

The area of visible cell walls were quantified in thin sections of rye dough. In native grains, the area after mixing varied between 11.6 and 13.6% depending on the dough yield, incubation time and cultivar (Table IV). After incubation the area was smaller and was reduced to between 9.8 and 11.3%. The germination-induced microstructural changes of cell walls in doughs were very much dependent on the germination batch. In batch 1, the area of cell walls in doughs after mixing varied between 13.5 and 16.9%, and after incubation it varied between 12.4 and 14.0%. The larger values for the germinated than for the native grains suggest that germination induces swelling of cell walls. In batch 2, the area of blue fluorescence after mixing varied from 7.4 to 11.6%, and after incubation it varied from 5.8 to 9.9%. The smaller values suggest that germination causes fading of cell walls. Thus, the effects in the two batches were opposite. Microstructural examination of dough sections showed germination caused two types of structural changes in the cell walls: 1) swelling and 2) fading of the blue fluorescence of cell walls. Samples with smallest (Fig. 1 plate 8) and highest area of blue fluorescence were chosen for microstructural examination. Figure 1 plate 9 very clearly demonstrates a swelling in the upper and lower cell walls of the aleurone layer resulted in the separation of fibers and cells from each other. Fig-

TABLE III
Viscosity Values of a Swelling Test for the Samples

Sample	Viscosity, BU		
	A ^a	B ^b	C ^c
Motto	125	190	243
FN ^d 163	135	175	285
FN 124	183	225	250
Marder	200	285	335
FN 160	180	220	320
FN 112	225	270	300

^a Initial viscosity at 30°C.

^b Viscosity when the sample reaches 42°C.

^c Viscosity after holding at 42°C for 30 min.

^d Falling number.

TABLE IV
Area of Blue Fluorescence in Dough Sections of Total Analyzed Area

Grains	Dough Yield	Incubation Time	Area of Blue Fluorescence, %	
			After Mixing	After Incubation
Native				
Motto	175	120	13.6	11.3
Marder	175	60	13.3	11.0
Motto	180	90	11.8	10.3
Marder	180	90	11.6	9.8
Motto	185	60	12.6	10.2
Marder	185	120	12.6	10.3
Germinated ^a				
Motto, 1	175	60	13.5	13.4
Marder, 1	175	120	16.3	12.4
Motto, 1	185	120	16.9	13.7
Marder, 1	185	60	15.7	14.0
Motto, 2	175	60	9.9	8.1
Motto, 2	175	120	7.5	5.8
Marder, 2	185	60	11.6	9.9
Motto, 2	185	60	7.4	6.7

^a Numbers indicate germination batch.

ure 1 plate 8 shows the same particles of a dough where no swelling can be seen. Decrease in the blue fluorescence, as in dough of batch 2, is usually related to enzymatic hydrolysis or nonenzymatic solubilization. Both swelling and fading of cell walls can be expected to make the doughs softer.

No matter what flour doughs were prepared from, the area of blue fluorescence decreased during incubation. No systematic changes were observed in the microstructure of starch. Although the dough yield had great influence on the rheological properties of doughs, the influence on the area of fluorescent cell walls was minimal.

Microstructural examinations showed water content had a marked effect on the packing and location of starch granules. At low water content, starch granules were closely packed and mostly inside the cells (Fig. 1 plate 10). High water content, in turn, broke down cell structures, and loosely packed individual starch granules were located in the matrix (Fig. 1 plate 11). These structural changes might explain the great rheological differences between doughs with low and high water content. No fragmentation of cell walls, which is typical for high activity of endo- β -xylanase, could be detected in the micrographs.

CONCLUSIONS

The rheological properties of rye doughs are dependent on the water content, falling number, and cultivar of rye. Lower falling number is associated with softer doughs and structural changes in the cell walls. Water content has a marked effect on the packing and location of starch granules.

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