



Effect of barley and oat flour types and sourdoughs on dough rheology and bread quality of composite wheat bread

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ABSTRACT

The potential of sourdough to improve bread quality of barley and oat enriched wheat breads may depend on the characteristics of the added flour (cereal type, variety, extraction rate). We compared the effect of different barley flours and oat bran (substitution level 40%), unfermented and as sourdoughs (20% of total flour), on composite wheat dough and bread characteristics by combining empirical rheological analyses (DoughLab, SMS/Kieffer Dough and Gluten Extensibility Rig) with small-scale baking of hearth loaves. Whole grain barley flour sourdough increased resistance to extension (R_{max}) of the dough and improved the form ratio of hearth loaves compared to unfermented whole grain barley flour. However, sourdough showed little effect on the breads prepared with sifted barley flour or oat bran. The breads made with oat bran showed highest bread volume, lowest crumb firmness and highest β -glucan calcofluor weight average molecular weight (MW). The heat treatment of oat bran inactivated endogenous enzymes resulting in less β -glucan degradation. High MW β -glucans will increase the viscosity of the doughs water phase, which in turn may stabilise gas cells and may therefore be the reason for the higher bread volume of the oat bran breads observed in our study.

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1. Introduction

An increased consumer demand for healthy bread has led to considerable efforts to develop breads that combine health benefits with good sensory properties. The use of whole grain wheat flour is one strategy for the development of healthy breads as the consumption of whole grain has been shown to reduce the risk of colorectal cancer, cardiovascular diseases, diabetes and obesity (Slavin, 2004; Topping, 2007). It is believed that the high content of dietary fibre in whole grain plays a significant role in the health promoting effect. However, other whole grain components such as phenolics may also be involved (Slavin, 2004). Increasing the content of cereal β -glucan in the breads is another strategy to improve their nutritional quality. Cereal β -glucans are known for

their ability to lower postprandial serum glucose levels and insulin response and to lower serum cholesterol levels (Brennan and Cleary, 2005). The high content of β -glucan in barley (2.5–11.3%) and oat (2.2–7.8%) compared to wheat (0.4–1.4%) has made these two cereals increasingly interesting for bread production (Lazaridou et al., 2007). However, the incorporation of barley and oat flour or the use of whole grain flour (usually from wheat) or bran all diminish bread quality, particularly loaf volume, of wheat composite breads (Bhatty, 1986; Gill et al., 2002; Lai et al., 1989). This deteriorating effect has been shown to be more than solely the dilution of wheat gluten. Fibre, especially insoluble fibre, may mechanically interfere with gluten network formation (Gill et al., 2002; Salmenkallio-Marttila et al., 2001) and cause rupture of gas cells (Courtin and Delcour, 2002). Both soluble and insoluble fibres, tightly bind high amounts of water, which may make it less available for the development of the gluten network and may further result in less steam production during baking (Gill et al., 2002). It is therefore crucial to compensate for the high water binding capacity of cereal β -glucans when baking with barley or oat flour in order to obtain good quality oat or barley enriched wheat bread (Holtekjølén et al., 2008). On the other hand, some fibres may also have a positive effect on dough rheology and bread quality. Water extractable arabinoxylans (WE-AX) for example have a positive effect on loaf volume of wheat bread supposedly by increasing the viscosity of the liquid phase in the dough and thereby stabilising

Abbreviations: AX, arabinoxylans; DDT, dough development time; DST, dough stability time; Ext, extensibility (distance at sample rupture in extensibility test); MW, molecular weight; NSP, non-starch polysaccharides; PCA, principal component analysis; PC, principal component; R_{max} , maximum resistance to extension (maximum peak force in extensibility test); WA, water absorption; WE-AX, water extractable arabinoxylans.

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gas cells (Courtin and Delcour, 2002). The addition of barley β -glucan isolates to wheat flour of a poor bread making cultivar has also been shown to improve dough rheology and bread volume of the corresponding breads made with optimised water addition (Skendi et al., 2010).

The content and distribution in the grain of the main non-starch polysaccharides (NSP) in cereals, cellulose, arabinoxylans (AX) and β -glucans, vary depending on the cereal species. For all cereals, cellulose is mainly located in the hull and outer parts of the kernel. AX form the major component of wheat endosperm and aleurone cell walls (Izydorczyk and Biliaderis, 2007), while, in barley and oat, AX are concentrated in the aleurone layer and bran (Brennan and Cleary, 2005). About 25–30% of AX in wheat flour endosperm are water extractable. The water extractability of AX is important for their technological properties in bread making (Courtin and Delcour, 2002). While β -glucan is relatively equally distributed over the whole endosperm in barley, oat exhibits β -glucan concentration in the thick cell walls of the subaleurone layer (Lazaridou et al., 2007). The specific distribution of different fibre in the cereal grain leads to different concentrations of these polysaccharides in different grain fractions of different cereal species. This will influence the technological and physiological properties of different flours. In order to improve the quality of barley and oat enriched wheat bread, it is crucial to understand the effect of different barley and oat flour components on dough structure of wheat composite dough in the actual matrix.

Sourdough is a potential tool to improve bread quality without the use of additives. Sourdough fermentation of wheat bran has been shown to increase the bread volume of high fibre wheat bread (Salmenkallio-Marttila et al., 2001). However the use of sifted barley flour sourdough did not improve the bread volume of barley breads (60% wheat) made from sifted barley flours of different barley varieties (Marklinder et al., 1996).

The present study investigates the effects of oat bran and barley flours of different barley varieties and extraction rates, unfermented and as sourdoughs, on dough rheology and bread quality of composite wheat breads (60% wheat) by combining large deformation rheological analyses with analyses of bread characteristics. The objectives of this study were to examine the effect of different fibre composition of barley and oat flours on dough structure and bread quality of composite wheat bread and to investigate the potential of sourdough to improve the bread quality of these breads, depending on the characteristics of the flour used for substitution. The relationship between flour composition (e.g. NSP, β -glucan content, β -glucan MW, AX), rheological properties of the dough (e.g. water absorption, dough development time, maximum resistance to extension, extensibility) and bread characteristics (volume, form ratio, crumb firmness, β -glucan MW) were investigated using multivariate statistics.

2. Experimental

2.1. Flour

All cereals and commercial flours were obtained from Lantmännen Cerealia AS (Moss, Norway). Two Norwegian barley varieties with different starch properties, one with high amylose starch (Karmose) and one with normal starch (Olve), were selected. Olve is a commercial Norwegian barley variety. Karmose is a new variety, which is of interest for the (Norwegian) baking industry due to its high β -glucan content and the health benefits of high amylose starch. High amylose starch has been shown to slow down the digestion process (Xue et al., 1996). The whole grain flours were obtained by milling the barley grains with hulls on a laboratory mill (Retsch, Model ZM1; Retsch GmbH, Haan, Germany) with a 0.5 mm

sieve. The sifted barley flours were commercially produced by roller milling and sifting by Lantmännen Cerealia AS (Kristiansand, Norway) at an extraction rate of 50.6% for Karmose and 73.5% for Olve. Commercial oat bran (from heat treated, dehulled oat grains) was milled equal to the barley grains to avoid hydration problems due to difference in particle size. The used wheat flour was commercial baking flour with strong protein quality (Regal hvetemel bakeri).

2.2. Sourdough preparation

Barley flours and milled oat bran were mixed with water (55% w/w) and fermented with a *Lactobacillus plantarum* strain (Nofima strain bank, AD2) isolated from Norwegian rye (Skrede et al., 2001). The sourdoughs were inoculated with 1×10^6 colony forming units per g sourdough and incubated at 30 °C in a heating cabinet (Termaks AS, Bergen, Norway) for 18 h. The sourdoughs were used to replace 50% of the oat bran or barley flour (corresponds to 20% of the total flour).

2.3. Physical dough measurements

2.3.1. Mixing characteristics

Farinograms were obtained with a Newport Scientific DoughLab (Perten Instruments, Stockholm, Sweden) using the 300 g mixing-bowl and a test profile corresponding to the ISO (1978) method 5530-1 for determination of water absorption. For composite wheat doughs without sourdough, 180 g wheat and 120 g oat bran/barley flour based on 14% moisture were used. For the recipes with sourdoughs half of the oat bran/barley flours were exchanged with sourdoughs and premixed with the remaining oat bran/barley flour and wheat flour for 1 min before the addition of water. The Farinograph operated at 63 rpm has been reported to be not intensive enough to develop optimal doughs for strong wheat flour (Tronsmo et al., 2002). Therefore, dough development times (DDT) were also determined at high speed (126 rpm). All dough rheology and baking experiments were performed in duplicates.

2.3.2. Dough rheology by SMS/Kieffer dough and gluten extensibility rig

Uniaxial extension measurements were carried out on the flour–water and the flour–water–sourdough doughs using the SMS/Kieffer Dough and Gluten Extensibility Rig for the TA.XT plus Texture Analyser (Stable Micro Systems Ltd., Godalming, UK). Doughs were prepared without salt and with water addition according to farinogram water absorption (500 FU) –1.5% of the water absorption of the wheat flour fraction. This was done to improve dough handling (less sticky) without impairing the comparability of the different recipes. Doughs were mixed to optimal dough development in a 10 g Mixograph (National Manufacturing, Lincoln, NE, USA). After mixing, doughs were rested for 20 min at 30 °C in a heating cabinet and moulded by hand first into a ball (10× rotations) and then into a roll (5× back and forth). The dough was placed into the teflon form, put into a plastic bag and rested for 45 min at 30 °C. The maximum peak force (resistance to extension (Rmax)) and the distance at sample rupture (extensibility (Ext)) were detected for four to eight dough strips per dough and the corresponding averages calculated.

2.4. Experimental baking

Hearth loaves were baked according to a small-scale straight-dough baking test described by Faergestad et al. (2000) with adjustments. Water addition was according to farinogram water absorption (500 FU) –1.5% of the water absorption of the wheat flour fraction. Mixing time was according to farinogram mixing

time at 126 rpm for the flour–water and flour–water–sourdough dough. The DoughLab was used for preparing the doughs. Based on flour weight at 14% moisture, 3.5% fat (vegetable fat and oil, A/S Pals, Oslo, Norway), 1.25% NaCl and 1% dry yeast (Saf Instant, S.I. Lesaffre, France) were added to the dough. Dough temperature after mixing was 27 ± 0.5 °C. The doughs were rested for 20 min in a fermentation cabinet (Lillinord A/S, Odder, Denmark) at 27 °C and 60% RH, divided into three 150 g pieces and moulded in the extensiograph (Brabender, Duisburg, Germany). The moulded dough was proved in a proving cabinet (Lillinord A/S, Odder, Denmark) at 37.5 °C and 70% RH for 40 min. The proven dough was baked in a rotating hearth oven (Revent type 626 G EL IAC, Revent international, Upplands Väsby, Sweden) for 20 min. During the first 35 s, steam (1.5 L water) was injected and the temperature was reduced from 250 °C to 220 °C immediately after the loaves were put into the oven. All recipes were baked twice and in random order.

After cooling to room temperature for 1 h, height and width were measured with the length indicator PAV-caliper (Compac type 532, Geneva, Switzerland). The form ratio was calculated by dividing the height by the width. Weight was measured using a balance (Mettler Toledo, Schwerzenbach, Switzerland). Loaf volumes were determined by laser technology using a TexVol volume measuring instrument (TexVol BVM – L 370, TexVol instruments AB, Viken, Sweden). For crumb firmness measurements, two of the three bread loaves were sliced 25 mm above the bottom. The bottom piece was measured with crust intact using a TA.XT2 Texture Analyser (Stable Micro Systems) fitted with a 35-mm diameter aluminium plunger according to AACC (2000) approved method 74-09. The loaves were frozen at -20 °C for later analysis of β -glucan molecular weight distribution.

2.5. Starch, non-starch polysaccharides, β -glucan and arabinoxylan content

A mixed-linkage β -glucan assay kit (Megazyme International Ltd., Wicklow, Ireland) was used for total β -glucan determination and insoluble β -glucan determination after removal of soluble β -glucan. The soluble β -glucan fraction was extracted using distilled water (37 °C) with continuous stirring for 2 h + 1 h + 1 h with intermittent centrifugation (3000 rpm, 10 min; Heraeus Multifuge 4 KR, Heraeus, Hanau, Germany). The amount of soluble β -glucan was calculated as the difference between the content of total and insoluble β -glucans. Total starch contents were also measured enzymatically by using a total starch assay kit from Megazyme (Megazyme International Ltd.). Total dietary fibre was measured as NSP according to the method described by Englyst et al. (1994) with gas chromatographic detection of the constituent sugars released by acid hydrolysis. AX content was determined as the sum of arabinose and xylose content from the NSP analysis. All analyses were based on dry weight and carried out in duplicate (β -glucan, starch) or triplicate (NSP).

2.6. β -glucan molecular weight

For β -glucan calcofluor weight average molecular weight (MW) determination, β -glucans were extracted as follows. To 200 mg sample (flour, oat bran or ground and freeze dried bread) 10 mL 50% ethanol was added and the mixture boiled for 15 min. After centrifugation (2500 g, 15 min; Heraeus Multifuge 4 KR), the supernatant was discarded and 20 mL 2.5 mM CaCl₂ added to each sample. Fifty μ l thermostable α -amylase (Termamyl, Novozymes A/S, Denmark) was added and the samples were boiled for 90 min. After cooling, samples were centrifuged (2500 g, 15 min; Heraeus Multifuge 4 KR) and supernatants collected. Another 10 mL of

2.5 mM CaCl₂ was added and the mixtures were boiled for an additional 60 min. After cooling, samples were centrifuged (2500 g, 15 min) and supernatants combined with the previously obtained supernatants. One ml of each sample was centrifuged (12 000 rpm, 10 min) and supernatants were diluted 1:1 with eluent (50 mM Na₂SO₄ with 0.02% NaN₃). β -glucan MW standards with average MWs of 40 000, 123 000, 183 000, 245 000 and 359 000 were obtained from Megazyme. The standards were mixed with eluent in Precellys CK14 homogenisation tubes (Bertin Technologies, Montigny le Bretonneux, France), solubilised using the Precellys 24 homogeniser (Bertin Technologies), centrifuged (12 000 rpm, 10 min; IEC Centra-M2 Centrifuge, International Equipment Company Inc., Chattanooga, US) and diluted with eluent to give a final concentration of 250 μ g/mL.

The HPLC system consisted of two pumps (DIONEX P680, Instrumentteknikk, Oslo, Norway) one delivering the eluent (50 mM Na₂SO₄ with 0.02% NaN₃) at a flow rate of 0.5 mL/min and the other one delivering calcofluor (Megazyme International Ltd.) solution (25 mg/L in 0.1 M tris(hydroxymethyl)aminomethane) (Sigma, Schnellendorf, Germany) at a flow rate of 0.25 mL/min. A Spectaphysics AS3500 auto injector was coupled to a pre-column (Shodex OHPack SB-LG) and two serially connected columns (Shodex OHPack SB-806-HQ and SB-804-HQ). A T-valve placed in the oven containing the columns (40 °C) delivered the calcofluor post column. Injection volume was 20 μ L and a fluorescent detector (Shimadzu RF-10A, Shimadzu Europa, Duisburg, Germany) was used with the wave length $\lambda_{\text{ex}} = 415$ nm and $\lambda_{\text{em}} = 445$ nm for detection. The HPLC system was controlled with Chromeleon 6.80 (DIONEX, Sunnyvale, CA, USA). A calibration curve was established for each run with the β -glucan MW standards from Megazyme by linear regression using the software WINGPC-6.2 (DIONEX) and used for calculation of weight average MW under the assumption of linearity within the range of analysis. The calculated weight average MW only includes β -glucan molecules large enough to be detected with calcofluor and therefore does not represent the actual average MW of the samples but the calcofluor average MW.

2.7. Statistical analysis

The relationship between the flour, dough and bread characteristics of the different barley flours and oat bran, and wheat–barley or oat bran mixed doughs and breads were studied by a principal component analysis (PCA) on standardised data using the Unscrambler software package (Version 9.8.CAMO A/S, Trondheim, Norway). For a better overview, analysis was performed on average values of each sample, which corresponded very well with the analysis performed on all replicates. The pure wheat sample was excluded from the PCA.

Significance testing of DoughLab and SMS/Kieffer rig and bread quality data was performed by General Analysis of Variance (General AOV/AOCV) using Statistic 9 (Analytical Software, Tallahassee, FL, USA), followed by Tukey's Multiple Comparisons Test. Simple correlation analysis (Pearson correlation) of dough rheology and bread quality parameters was performed using Minitab (version 15; Minitab Inc, State College, PA, USA). Significant differences were declared at $p < 0.05$.

3. Results and discussion

3.1. Flour characteristics

Table 1 shows the flour characteristics of the 6 flours used in this study. Oat bran had a slightly lower content of NSP than the two sifted barley flours, while the whole grain barley flours showed the highest content of NSP. The total β -glucan content of the barley

Table 1
Flour and sourdough characteristics.

	NSP ^a [%]	Total β -glucan ^b [%]	Soluble β -glucan ^b [%]	Arabinoxylan ^a [%]	Starch ^b [%]	Soluble β -glucan calcofluor average MW ^b [kDa]		
						Flour	Sourdough	
							30 min	18 h
Wheat flour	3.9 \pm 0.1	na	na	2.5 \pm 0.0	74.5 \pm 0.3	nd	na	na
Sifted Karmose	12.2 \pm 0.1	5.3 \pm 0.1	3.1 \pm 0.2	4.6 \pm 0.0	65.5 \pm 0.1	527 \pm 10	440 \pm 2	85 \pm 14
Whole grain Karmose	19.0 \pm 0.3	5.5 \pm 0.4	2.6 \pm 0.2	8.3 \pm 0.1	55.8 \pm 1.0	559 \pm 7	115	50 \pm 13
Sifted Olve	11.4 \pm 0.1	5.3 \pm 0.1	4.0 \pm 0.2	4.2 \pm 0.1	66.4 \pm 2.3	548 \pm 1	452 \pm 6	112 \pm 9
Whole grain Olve	19.5 \pm 0.1	5.6 \pm 0.0	4.5 \pm 0.0	8.8 \pm 0.12	56.5 \pm 1.3	562 \pm 3	174 \pm 14	62 \pm 8
Oat bran	10.4 \pm 0.3	8.5 \pm 0.0	5.5 \pm 0.1	2.9 \pm 0.1	57.4 \pm 0.3	630 \pm 7	546 \pm 9	488 \pm 0

[%] weight percent per dry weight flour/bran.

na = not analysed.

nd = not detectable.

^a Mean values \pm standard deviation of three replicates.

^b Mean values \pm standard deviation of two replicates.

flours (5.3–5.6%) did not vary much depending on the barley variety or the extraction rate reflecting the fact that β -glucan is relatively equally distributed over the whole barley endosperm (Lazaridou et al., 2007). However, Olve showed a higher content of soluble β -glucan than Karmose both for the sifted and the whole grain flour. Even though oat bran showed a lower NSP content, the content of total β -glucan in oat bran was considerably higher compared to the barley flours. Oat bran is prepared from dehulled oat and contains high amounts of the subaleurone layer, which is especially rich in β -glucan (Lazaridou et al., 2007). It is interesting to note that the β -glucan from oat bran also showed a higher calcofluor weight average molecular weight (MW) than the barley flours. There was also a small difference in β -glucan MW between the sifted and whole grain barley flours, with higher values for the whole grain flours. All the barley flours and especially the whole grain barley flours showed higher contents of AX than oat bran. For oat bran the sum of β -glucan and AX exceeds the amount of NSP. However, the arabinose content determined by gas chromatography during NSP analysis was not corrected for the presence of arabinogalactan peptide, which may have led to slightly overestimated AX contents.

3.2. Principal component analysis – interrelationships between flour, dough and hearth bread characteristics

PCA was performed on all flour, dough and bread characteristics of the composite wheat breads prepared with the different barley flours or oat bran to get an overview of possible interrelationships and the most influential parameters. In order to reveal differences among the different barley flours and oat bran, the highly influential pure wheat sample was excluded from the analysis. The samples containing oat bran are located on the left side of the score plot (Fig. 1A), while the barley samples are grouped to the right. The loading plot (Fig. 1B) shows that the first principal component, which mainly separates the oat bran from the barley samples is dominated by flour β -glucan content (soluble and total), β -glucan MW (both bread and flour), water absorption (WA) and bread volume on one side and bread crumb firmness on the other side. The first principal component explains 40% of the variation seen in flour, dough and bread characteristics. A simple correlation analysis (Pearson correlation) revealed a significant positive correlation between WA and total β -glucan content of the flour (0.66) and β -glucan MW in bread (0.70). Interestingly there was no correlation between WA and total fibre or AX content. Bread volume was positively correlated with β -glucan MW in bread (0.83) and flour β -glucan content (0.67) and negatively correlated with bread crumb firmness (–0.83), NSP (–0.70) and AX content (–0.70). β -Glucan

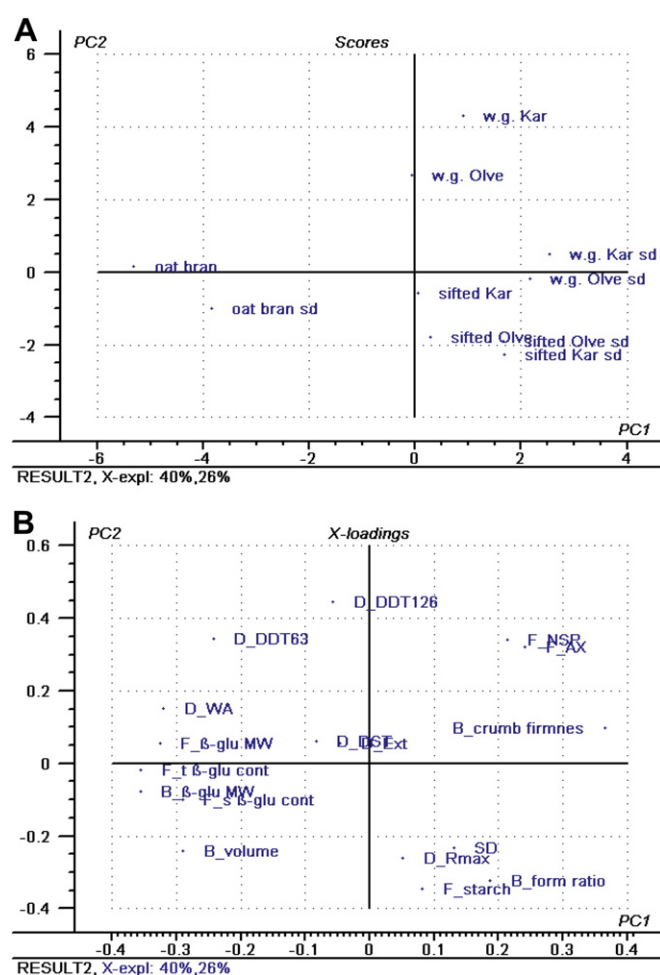


Fig. 1. Scores (A) and loadings (B) for the 2 first principal components in PCA of dough and bread characteristics of wheat composite breads. Loadings: SD = sourdough content. Flour characteristics: F_NSP = total fibre content as NSP, F_AX = arabinoxylan content, F_t β -glu cont = total β -glucan content, F_s β -glu cont = soluble β -glucan content, F_ β -glu MW = β -glucan calcofluor average MW in flour. Dough characteristics: D_WA = water absorption, D_DDT63 = dough development time at low speed (63 rpm), D_DDT126 = dough development time at high speed (126 rpm), D_DST = dough stability time, D_Rmax = resistance to extension, D_Ext = extensibility. Bread characteristics: B_volume = bread volume, B_form ratio = bread form ratio, B_crumb firmness = bread crumb firmness, B_beta-glu = bread beta-glucan calcofluor average MW. Scores: Kar = Karmose; w.g. = whole grain; sd = sourdough; samples with sourdough contain 20% barley or oat bran sourdough, 20% barley flour or oat bran and 60% wheat flour; samples without sourdough contain 40% barley flour or oat bran and 60% wheat flour.

has been associated with negative effects on bread volume due to its high water binding capacity, which results in less available water for gluten network formation and less steam production during baking (Gill et al., 2002). However in our study, high bread volume is correlated with high β -glucan content in the flour and high β -glucan MW in the bread. All breads in our study were prepared with optimised water addition (according to dough consistency of 500 FU). This may have eliminated the problems caused by the high water binding capacity of β -glucan. By contributing to the viscosity of the water phase in the dough, β -glucans may have stabilised gas cells and thus improved the gas retaining capacity of the dough in the way reported for WE-AX in wheat dough (Courtin and Delcour, 2002).

The second principal component mainly separates the barley samples according to whole grain and sourdough content (Fig. 1A) and explains 26% of the variation seen in flour, dough and bread characteristics. According to the loadings plot (Fig. 1B), the whole grain barley samples are associated with high NSP and AX content and high dough development time at high speed (DDT126). The sifted barley samples and the whole grain barley samples containing sourdough are grouped together. They are associated with bread form ratio, maximum resistance to extension of the dough (Rmax), sourdough content and starch content of the flour. Correlation analysis revealed a significant positive correlation between Rmax and bread form ratio (0.73). Even though there were no significant correlations between sourdough content and bread form ratio or Rmax, sourdough clearly improved the dough and bread characteristics of the whole grain barley samples. This strong influence on the whole grain barley samples contributed to the grouping of sourdough with Rmax and bread form ratio in the loading plot indicating positive relation. There was no correlation between the starch content and Rmax or bread form ratio. However, starch content was negatively correlated to DDT126, which explains its position in the loadings plot.

3.3. Large deformation rheological analyses using the DoughLab

The substitution of wheat with 40% oat or barley significantly increased the WA with 8–11.8%, compared to wheat flour alone (Table 2). This is probably due to the high content of fibre in oat bran and the barley flours. Fibre can bind high amounts of water and will therefore increase the WA. Interestingly, oat bran showed a significantly higher WA than the barley flours even though the

total amount of fibre (measured as NSP) was lower in oat bran (Table 1). However, the PCA loading plot shows a positive relation between the content and MW of β -glucan and WA along the first PC, while NSP and AX are located in the opposite direction along the first PC. This indicates that the content and MW of β -glucan is more important for WA than other fibres and oat bran had a much higher content of β -glucan than the barley flours. A significant decrease in WA by 6.8–7.5% was observed with the use of barley flour sourdoughs, while oat bran sourdough significantly increased the WA (absolute increase of 0.7%) compared to the respective unfermented flours (Table 2). The decrease in WA for the barley flour sourdoughs is likely related to the degradation of soluble fibre e.g. β -glucan during the fermentation. A 69–80% drop in β -glucan MW was observed in the barley sourdoughs (Table 1). During processing of commercial oat bran, the endogenous enzymes are inactivated by heat treatment. This restricted the growth of the starter culture (pH drop only to 4.7 compared to 3.7–3.6 for the barley flour sourdoughs, data not shown) presumably due to a lack of available polysaccharide degradation products. The heat treatment likely limited the enzymatic degradation of β -glucan to a 10% reduction in MW during sourdough fermentation of oat bran (Table 1).

Dough development time (DDT) both at low (63 rpm) and high speed (126 rpm) significantly increased with the substitution of wheat flour with whole grain barley flour and oat bran, but remained at a similar level as for wheat flour with the substitution with sifted barley flours (Table 2). This is consistent with Bhatti (1986), who reported little to no difference in mixograph peak time with the substitution of wheat by 5–20% hull-less barley flour and Jacobs et al. (2008), who showed that a fibre rich barley fraction increased farinograph DDT compared to the wheat control. For pure wheat flour, DDT reflects the total energy amount that is needed to develop the gluten network. In the presence of fibre rich flours, the gluten network formation will be influenced by water availability and mechanical disturbance due to insoluble fibre. The constant DDT seen in our study, when wheat was substituted with sifted barley flours (Table 2), might be due to a combined effect of gluten dilution and an increased content of soluble fibre. The former is reported to shorten DDT, while the latter might increase DDT due to the competition for water. Interestingly we found a significant positive correlation between WA and DDT63 (0.73), but not for DDT126. It is possible that differences in DDT63 in our study mostly reflect the delay of gluten network formation due to high water binding capacity of the present fibres, while DDT126 may mostly

Table 2
DoughLab mixing characteristics of dough and dough rheology by SMS/Kieffer dough and gluten extensibility rig.^a

	Water absorption [%]	Dough development time at 63 rpm [min]	Dough development time at 126 rpm [min]	Dough stability time [min]	Rmax ^e [mN]	Ext ^f [mm]
Wheat ^b	61.2 ± 0.2 ^I	2.5 ± 0.1 ^{FG}	2.9 ± 0.0 ^E	14.7 ± 0.2 ^A	378 ± 9 ^A	29.3 ± 1.3 ^A
<i>Substitution with flour</i>						
Sifted Karmose ^c	69.2 ± 0.0 ^E	2.7 ± 0.0 ^F	3.2 ± 0.1 ^D	6.6 ± 0.1 ^B	125 ± 17 ^{BCDE}	29.2 ± 2.4 ^A
Whole grain Karmose ^c	71.1 ± 0.1 ^C	6.5 ± 0.1 ^C	5.8 ± 0.0 ^A	4.9 ± 0.2 ^B	97 ± 7 ^E	20.1 ± 1.7 ^B
Sifted Olve ^c	69.9 ± 0.1 ^D	2.3 ± 0.2 ^{GH}	2.9 ± 0.0 ^E	6.1 ± 0.2 ^B	144 ± 7 ^{BC}	19.6 ± 2.8 ^B
Whole grain Olve ^c	70.1 ± 0.0 ^D	6.9 ± 0.0 ^B	5.1 ± 0.1 ^B	5.9 ± 0.3 ^B	114 ± 7 ^{CDE}	21.3 ± 1.8 ^B
Oat bran ^c	73.0 ± 0.1 ^B	7.5 ± 0.0 ^A	3.9 ± 0.0 ^C	5.7 ± 0.1 ^B	122 ± 11 ^{BCDE}	21.3 ± 1.9 ^B
<i>Substitution with sourdough</i>						
Sifted Karmose sd ^d	63.9 ± 0.4 ^H	1.7 ± 0.1 ^I	2.7 ± 0.0 ^E	4.9 ± 0.3 ^B	149 ± 8 ^{BC}	16.7 ± 1.1 ^B
Whole grain Karmose sd ^d	64.7 ± 0.1 ^G	4.3 ± 0.2 ^E	3.2 ± 0.1 ^D	7.1 ± 0.2 ^B	151 ± 7 ^B	15.6 ± 1.1 ^B
Sifted Olve sd ^d	65.5 ± 0.1 ^F	1.7 ± 0.0 ^I	1.4 ± 0.0 ^E	2.5 ± 0.6 ^C	103 ± 5 ^{DE}	20.5 ± 4.4 ^B
Whole grain Olve sd ^d	62.4 ± 0.1 ^I	2.0 ± 0.0 ^{HI}	3.2 ± 0.1 ^D	6.3 ± 0.5 ^B	148 ± 7 ^{BC}	17.1 ± 1.2 ^B
Oat bran sd ^d	73.7 ± 0.2 ^A	5.5 ± 0.1 ^D	2.9 ± 0.0 ^E	6.9 ± 1.6 ^B	139 ± 7 ^{BCD}	15.5 ± 1.3 ^B

^a Mean values ± standard deviation of two replicates. Mean values followed by a common letter within the same column are not significantly different ($p < 0.05$).

^b 100% wheat.

^c 60% wheat, 40% barley flour or oat bran.

^d 60% wheat, 20% barley flour or oat bran, 20% barley flour or oat bran added as sourdough.

^e Maximum resistance to extension.

^f Extensibility.

reflect the disturbance of the gluten network by insoluble fibre. DDT126 was significantly higher for the whole grain barley samples than for oat bran, while oat bran showed the highest DDT63 amongst all the unfermented substituted samples. The inclusion of sourdough led to a significant decrease in DDT both at low (63 rpm) and high (126 rpm) speed for all barley flours and oat bran compared to the respective unfermented flours. The decrease was most pronounced for the whole grain barley flours. The partial degradation of fibre during the sourdough fermentation of the barley flours may have resulted in higher water availability for gluten network formation. Sourdough fermentation may also have softened bran particles as reported for wheat bran sourdough by Salmenkallio-Marttila et al. (2001) resulting in less mechanical disturbance of the gluten network formation. The lower pH of the doughs containing barley sourdoughs may also have contributed to the observed decrease in DDT63 and DDT126 as the addition of organic acids to wheat flours has been reported to decrease DDT (Galal et al., 1978; Wehrle et al., 1997).

The dough stability time (DST) decreased significantly by 8.6–9.8 min with the substitution of wheat by barley flours or oat bran (Table 2). No significant differences were found among the DSTs of the different barley flours or between the different barley flours and oat bran. The use of sourdough did not influence DST significantly except for sifted barley flour from the variety Olve. Doughs containing sourdough made with sifted barley flour from the variety Olve showed a significantly lower DST than doughs prepared with unfermented sifted barley flour from the variety Olve. Throughout all experiments the use of sourdough made from sifted barley flour from the variety Olve led to changes in dough rheology and bread quality that were quite different and often opposed to the changes observed with all other sourdoughs. This difference could not be related to any of the studied differences in composition of the sifted barley flour from the variety Olve and remains unclear. Except for oat bran, the doughs containing sourdough had a significantly lower pH than the doughs prepared with the unfermented flours (data not shown). At low pH the cohesion of the protein network is weakened due to the overall positive charge of the proteins (Bennett and Ewart, 1962), which will result in a lower DST (Clarke et al., 2002; Galal et al., 1978). However, other effects of the sourdough fermentation like a particle softening effect and the degradation of fibre may have counteracted the effect of the pH drop in our experiment.

3.4. Large deformation dough rheology by SMS/Kieffer rig

The substitution of wheat flour with barley flour or oat bran led to a significant decrease of R_{max} for all doughs (Table 2). Compared to this decrease with wheat flour substitution, the differences between the different barley flours and oat bran were small. The whole grain barley flours showed a tendency towards lower R_{max} values than the sifted barley flours. Wheat flour substitution furthermore led to a significant reduction of the dough extensibility (Ext), except for sifted barley flour from the variety Karmose (Table 2). There were no significant differences in Ext among the other barley flours and oat bran. The observed decrease in R_{max} and Ext with wheat flour substitution can be explained by the dilution of wheat gluten and the increased content of fibre that may disrupt the gluten network in the mixed wheat flour – barley or oat doughs. Even though not significant, the lower R_{max} values for the whole grain barley flours are likely related to the high fibre content of these flours.

The use of sourdough increased R_{max} and lowered Ext for all barley flours and oat bran except for sifted barley flour from the variety Olve compared to the unfermented flours (Table 2). The increase in R_{max} was most pronounced for the whole grain barley

samples, but significant only for the whole grain barley flour from the variety Karmose. It is interesting that the use of barley and oat sourdough increased R_{max} and decreased Ext in our study since the use of wheat sourdough has been reported in the literature to decrease R_{max} and increase Ext of the bread dough (Di Cagno et al., 2003). However, these changes have been attributed to the degradation of gluten proteins by flour proteases at low pH (Arendt et al., 2007). Barley and oat proteins are not believed to contribute to the wheat dough gluten network formation. Thus, their degradation during sourdough fermentation is unlikely to have the same impact on bread dough rheology as the degradation of wheat proteins. The tendency towards higher R_{max} and lower Ext that we observe in our study with the use of sourdough compared to unfermented flour, may be partially explained by the lower pH (4.7–4.8, data not shown) of the doughs containing barley flour sourdough (not for oat bran). Acid addition to wheat dough has been reported to increase extensigraph resistance to extension and decrease extensibility (Bennett and Ewart, 1962; Tanaka et al., 1967) presumably by increasing mutual repulsion of the positively charged proteins in the acid treated dough with the result that the proteins are unable to yield much before the dough piece ruptures (Bennett and Ewart, 1962). However it is likely that other changes during sourdough fermentation also contribute since the doughs containing oat bran sourdough did not have a low pH, but showed the same tendencies for R_{max} and Ext as the doughs containing fermented barley flours. A softening effect of sourdough on insoluble fibre particles may explain a part of the observed increase in R_{max} with sourdough fermentation especially for the whole grain barley flours. It is also possible that sourdough fermentation influenced the solubility of β -glucans and AX. Dough fermentation in the production of rye crisp bread for example has been shown to release water extractable β -glucans from insoluble matrix (Andersson et al., 2008). Both β -glucans and WE-AX have been shown to increase R_{max} and decrease Ext of wheat dough fortified with these fibres (Courtin and Delcour, 2002; Skendi et al., 2010). However, sourdough fermentation also resulted in fibre degradation as indicated by the significant decrease of β -glucan MW during fermentation of the barley flours (Table 1).

3.5. Effect of wheat flour substitution with oat and barley on bread characteristics

The substitution of wheat with barley flour or oat bran with or without sourdough resulted in a significant decrease in bread volume accompanied by a significant increase in crumb firmness (except for oat bran) compared to bread baked with wheat flour (Table 3). The breads prepared with whole grain flour from the variety Karmose had the lowest bread volume, followed by whole grain barley flour from the variety Olve, while the oat bran breads showed the highest bread volume, highest β -glucan MW and lowest crumb firmness among the composite wheat breads (Table 3). As we have seen in the PCA analysis, the oat bran samples were all associated with high β -glucan content in the flour and high β -glucan MW in flour and bread. Compared to the barley flours, oat bran contained a considerably higher amount of β -glucan (Table 1). The heat treatment of the oat bran furthermore limited the degradation of β -glucan during sourdough fermentation (Table 1) and presumably also during dough formation and baking. Contribution of β -glucan degrading enzymes from the commercial wheat flour is believed to be limited since wheat flour has been shown to have low activity of β -glucanases (Wang et al., 1998). Skendi et al. (2010) reported a positive effect of barley β -glucan addition on wheat bread specific volume, which was more pronounced for high MW β -glucan and wheat flours with weak gluten quality. Even though speculative, it seems likely that β -glucans have a potential

Table 3
Bread characteristics.^a

	Bread volume [mL]	Form ratio	Crumb firmness [N]	Soluble β -glucan calcoflour average MW [kDa]
Wheat ^b	544 \pm 7 ^A	0.6 \pm 0.02 ^A	7.7 \pm 0.5 ^C	n.a.
<i>Substitution with flour</i>				
Sifted Karmose ^c	350 \pm 5 ^{BCD}	0.58 \pm 0.01 ^{AB}	21.5 \pm 1.5 ^A	248 \pm 7 ^B
Whole grain Karmose ^c	296 \pm 1 ^E	0.51 \pm 0.04 ^B	24.4 \pm 0.6 ^A	187 \pm 2 ^{CDE}
Sifted Olve ^c	351 \pm 8 ^{BCD}	0.63 \pm 0.01 ^A	21.3 \pm 1.0 ^{AB}	206 \pm 3 ^{CD}
Whole grain Olve ^c	339 \pm 4 ^{BCD}	0.58 \pm 0.01 ^{AB}	21.8 \pm 1.0 ^A	198 \pm 6 ^{CDE}
Oat bran ^c	375 \pm 3 ^B	0.56 \pm 0.01 ^{AB}	11.6 \pm 1.0 ^C	308 \pm 8 ^A
<i>Substitution with sourdough</i>				
Sifted Karmose sd ^d	346 \pm 8 ^{BCD}	0.61 \pm 0.03 ^A	22.0 \pm 0.8 ^A	212 \pm 3 ^C
Whole grain Karmose sd ^d	323 \pm 5 ^{DE}	0.62 \pm 0.04 ^A	24.5 \pm 0.6 ^A	156 \pm 2 ^F
Sifted Olve sd ^d	320 \pm 15 ^{DE}	0.60 \pm 0.01 ^A	22.3 \pm 1.6 ^A	175 \pm 4 ^{EF}
Whole grain Olve sd ^d	330 \pm 17 ^{CDE}	0.62 \pm 0.01 ^A	23.4 \pm 2.2 ^A	178 ^{DEF}
Oat bran sd ^d	365 \pm 10 ^{BC}	0.57 \pm 0.02 ^{AB}	16.7 \pm 1.0 ^B	266 \pm 12 ^B

n.a. = not analysed.

^a Mean value \pm standard deviation of two replicates (each comprising three breads). Mean values followed by a common letter within the same column are not significantly different ($p < 0.05$).^b 100% wheat.^c 60% wheat, 40% barley flour or oat bran.^d 60% wheat, 20% barley flour or oat bran, 20% barley flour or oat bran added as sourdough.

to improve bread volume of composite wheat breads by e.g. contributing to the viscosity of the water phase of the dough and thereby stabilising gas cells in the way reported for WE-AX in wheat dough (Courtin and Delcour, 2002). However, the difference in bread quality between the oat bran and barley breads in our study may also be related to other differences in flour composition than enzyme inactivation, β -glucan content and MW.

The use of sourdough improved the bread quality of the breads made with whole grain barley flour from the variety Karmose considerably. These breads had a tendency towards higher bread volume and a significantly higher form ratio than the breads prepared with unfermented whole grain barley flour (Table 3). A high number of holes in the crust of the breads prepared with unfermented whole grain barley flour from Karmose (Fig. 2A)

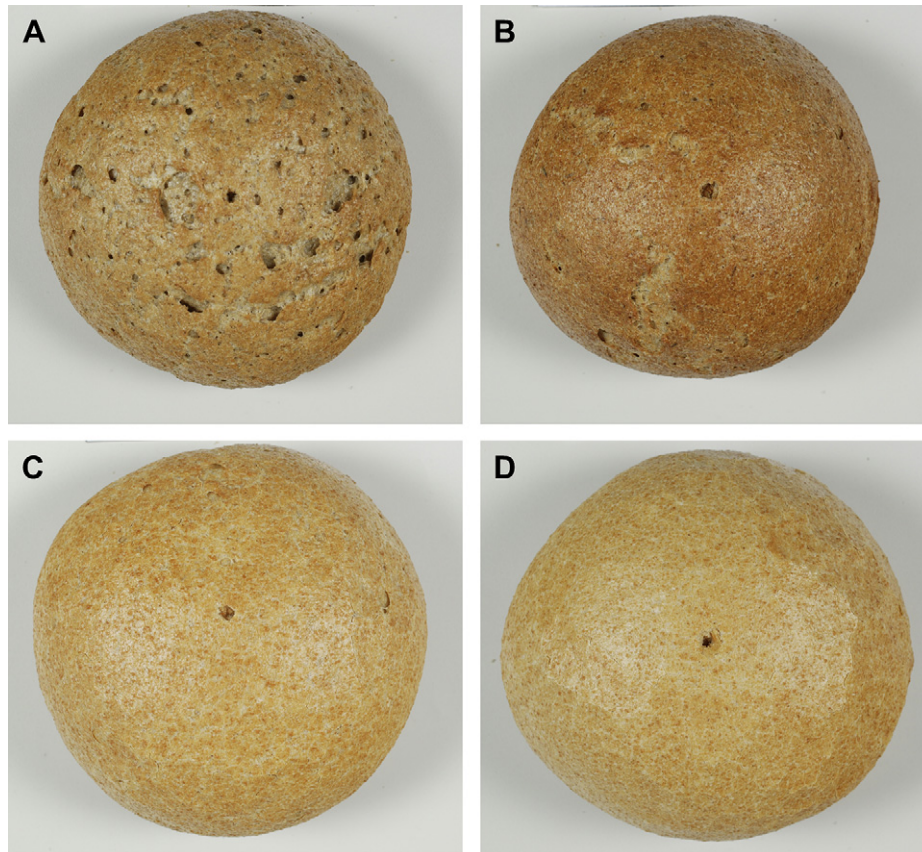


Fig. 2. A: bread made with 40% whole grain Karmose flour; B: bread made with 20% whole grain Karmose flour and 20% whole grain Karmose sourdough; C: bread made with 40% oat bran – no crust cracking; D: bread made with 20% oat bran and 20% oat bran sourdough – crust cracking.

indicated a poor gas retaining ability, which was improved by the use of sourdough (Fig. 2B). All composite wheat breads except the breads prepared with sifted barley flour from the variety Olve showed a tendency towards higher form ratio with the use of sourdough. Even though sourdough had a positive effect on bread volume for the breads made with whole grain barley flour from the variety Karmose, we found no effect or a small decrease (sifted barley flour from the variety Olve and oat bran) of bread volume with the use of sourdough for the other composite wheat breads. The positive effect of sourdough on bread quality of whole grain barley breads (especially whole grain barley flour from the variety Karmose) might be related to a particle softening effect of the fermentation as described by Salmenkallio-Marttila et al. (2001) for wheat bran. The use of sourdough further improved the form ratio of all composite wheat breads except for breads prepared with sifted barley flour from the variety Olve. It is possible that the effect of barley sourdough on bread volume depends on the flour characteristics (whole grain vs. sifted flour) as also Marklinder et al. (1996) found a small decrease in bread volume of wheat breads enriched with sifted barley flour sourdoughs compared to unfermented sifted barley flour.

The breads prepared with whole grain barley flour from the variety Karmose showed significantly lower β -glucan MW than breads prepared with sifted barley flour from Karmose. The same tendency was observed for the barley variety Olve (not significant). The β -glucan MW in the whole grain barley flour sourdoughs after 30 min fermentation (Table 1) was also considerably lower than that of the sifted barley flour sourdoughs. This may indicate a higher activity of endogenous enzymes in the whole grain barley flours compared to the sifted barley flours. The use of sourdough decreased the average MW of β -glucan in the bread significantly (with the exception of breads prepared with whole grain barley flour from the barley variety Olve) compared to the respective breads without sourdough. Even though sourdough fermentation of oat bran showed only a minor decrease of β -glucan MW (Table 1), it may have changed the β -glucan solubility, thus making it more available for degradation by wheat flour β -glucanases. However, the use of oat bran sourdough improved the appearance (crust cracking illustrated in Fig. 2C and D), led to crispier crust and improved aroma of the breads (expert baker evaluation) compared to the breads prepared with unfermented oat bran.

4. Conclusions

Sourdough improved the dough structure (Rmax) and bread quality (volume and form ratio) of breads containing whole grain barley, but showed little effect on the breads prepared with sifted barley flour or oat bran. The positive effect of sourdough on whole grain barley bread may be related to a softening effect on bran particles during fermentation resulting in less mechanical disruption of the gluten network and gas cells in the dough. Sourdough fermentation might therefore be useful for the quality improvement of whole grain breads in general.

Our results showed a positive correlation between flour β -glucan content and bread β -glucan MW on one side and bread volume on the other side. Even though this is speculative we think that β -glucans by contributing to the viscosity of the water phase of the dough have a potential to improve bread volume of composite wheat breads if the water addition to the doughs is adjusted for the high water binding capacity of the β -glucans. Apart from increasing the amount of β -glucan in the starting material, inactivation of endogenous flour enzymes might help to limit β -glucan degradation and ensure a high viscosity of the dough water phase. Enzyme inactivation would further help to maintain high MW β -glucans,

which convey considerable health benefits, in the breads and should therefore be further investigated.

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