



# Impact of water-extractable components from different cereals on the quality of oat bread



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## ABSTRACT

Loaf volume and crumb structure of oat bread are not comparable to those of bread from wheat flour. Hydrocolloids, surfactants and/or enzymes are often included in oat batter recipes for quality enhancement reasons. In this study, we examined the impact of water-extractable components from barley, oat, rye and wheat flour on oat bread quality. We speculated that such water extracts contain components which also would enhance the quality of oat bread. As expected, extract protein, non-starch polysaccharide, lipid and enzyme levels varied widely amongst the different cereal flours used. The extracts also varied in foaming properties and extract viscosities. Rye flour contained the highest level of water-extractable components. Inclusion of rye aqueous extract resulted in the largest loaf volume increase and in softer crumb than noted for control oat bread. Rheofermentometer analyses showed that the moment of gas cell opening was delayed when rye extract was added, indicating improved batter gas cell stabilization, while collapse during baking was not affected. The oat bread improving effect of the rye extract is likely due to a combination of the impact of different of its constituents such as enzymes and surface active components.

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## 1. Introduction

Bread is an important staple food in the Western world. It is almost exclusively prepared from wheat (*Triticum aestivum* L.) because of the unique properties of its proteins. Wheat flour can form a cohesive, visco-elastic dough with gas retention properties superior to those of dough or batter from other cereals. Today, there is an increasing consumer demand for bread from cereals other than wheat. Such breads have a better nutritional profile (levels of dietary fiber, essential amino acids, minerals, ...) than those from wheat. In addition, since the only available therapy for celiac disease and other gluten-related disorders is lifelong avoidance of gluten (a term in this context referring to proteins from wheat, rye and barley), alternative raw materials for producing bread need to be used. One such example is oat. It contains high levels of the dietary fiber mixed linkage (1 → 3, 1 → 4)-β-D-glucan (further referred to as β-glucan) (Shewry et al., 2008) and is a raw material

for producing gluten-free products, although some controversy exists on this matter (Hüttner and Arendt, 2010). However, the absence of gluten leads to substantial technological and quality challenges in the production of oat bread, such as small loaf volume, an uneven distribution of large gas cells in the crumb and firm and crumbly texture.

Oat batter is in essence a foam. The bubbles incorporated during the mixing stage are nucleation sites for the carbon dioxide produced by yeast during fermentation which causes expansion and batter rise. However, foams are intrinsically unstable due to gas cell coalescence, disproportionation, drainage and creaming (Gan et al., 1995; Mills et al., 2003). While drainage and creaming do not occur in visco-elastic wheat bread dough over the time scale of bread making (Mills et al., 2003), they need to be taken into account when considering the stability of non-wheat batter. Indeed, such batter is much less viscous than wheat dough. To increase batter viscosity and, hence, improve gas retention and bread quality, non-wheat batter often contains added hydrocolloids (Lazaridou et al., 2007; Hüttner and Arendt, 2010). In addition, surface active components are required for forming and stabilizing foam structures. They reduce the interfacial tension and thereby facilitate air incorporation. In essence, there are two types of surface active agents each

Abbreviations: AX, arabinoxylan; dm, dry matter; E<sub>440</sub>, extinction at 440 nm; E<sub>590</sub>, extinction at 590 nm; Tris, tris-(hydroxymethyl)-aminomethane.

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with an own stabilization mechanism. Proteins, on the one hand, stabilize interfaces by forming an adsorbed, visco-elastic layer in which neighboring molecules interact with each other. The mechanical properties of such visco-elastic layers have been thought to affect foam stability (Wilde et al., 2004). On the other hand, low molecular weight surfactants such as polar lipids act by the Gibbs-Marangoni mechanism which relies on the stabilization of the interface by a highly fluid layer of lipids (Mills et al., 2003). Whenever deformation of the layer decreases the local lipid concentration, the lipid molecules migrate to the depleted area to restore the concentration gradient. Stabilization of gas cells in non-wheat batters is often improved by surfactants such as diacetyl tartaric esters of mono- and diacylglycerols and sodium stearyl lactylate (Sciarini et al., 2012). Another approach to improve oat bread quality is by using enzymes, and, more in particular, cross-linking enzymes (transglutaminases and oxidases) (Renzetti et al., 2008, 2010; Sciarini et al., 2012), amylases (Sciarini et al., 2012) and peptidases (Renzetti et al., 2010).

Aqueous extracts of cereals contain several components which may well increase batter stability. First, non-starch polysaccharides may act by virtue of their water-binding and viscosity increasing capacity. Second, much as some surfactants, some water-extractable surface active proteins can stabilize gas cells. Finally, cereal aqueous extracts can contain different enzymes. For wheat, it has been shown that their water-extractable components contribute to the quality of wheat bread (MacRitchie, 1976; Pauly et al., 2014b). The impact of such components have never been investigated for bread made from non-wheat flour.

Against the above background, this work aimed at studying how aqueous extracts from different cereal flour samples influence oat bread quality (i.e. specific volume, crumb texture and structure). We here report on the outcome of our work.

## 2. Materials and methods

### 2.1. Materials

Barley grains from cultivar Explorer were from Cargill (Herent, Belgium). They were conditioned to 16.0% moisture and subsequently milled with a Bühler (Uzwil, Switzerland) MLU-202 laboratory mill. Flour [12.4% moisture, 7.1% protein on dry matter (dm) basis, 1.3% dm arabinoxylan (AX), 2.0% dm  $\beta$ -glucan, 1.6% dm lipids and 0.75% dm ash] yield was 43.6%. Commercial oat flour (10.1% moisture, 12.4% dm protein, 1.5% dm AX, 2.9% dm  $\beta$ -glucan, 6.6% dm lipids and 1.67% dm ash) from heat treated oats was from Raisio Nutrition (Raisio, Finland), rye flour (12.9% moisture, 7.2% dm protein, 5.1% dm AX, 1.5% dm  $\beta$ -glucan, 1.3% dm lipids and 0.82% dm ash) was from Koopmans (Leeuwarden, The Netherlands) and wheat flour (13.5% moisture, 13.2% dm protein, 2.2% dm AX, 0.2% dm  $\beta$ -glucan, 1.4% dm lipids and 0.55% dm ash) from Dossche Mills (Deinze, Belgium). Their compositions were determined as described in Section 2.2.2. Dry yeast was from Puratos (Groot-Bijgaarden, Belgium). Sugar and salt were commercial food grade products. All other chemicals, solvents and reagents were from Sigma-Aldrich (Bornem, Belgium) and were analytical grade, unless specified otherwise.

### 2.2. Experimental

#### 2.2.1. Extraction of flour water soluble components

Barley, oat, rye or wheat flour was extracted with deionized water (ratio 3:20 flour:water) during 15 min at room temperature under continuous shaking (150 rpm). After centrifugation (5000 g, 15 min, 7 °C), the supernatants were freeze-dried. Preliminary experiments showed that freeze-drying did not affect the functionality of the extracts in oat bread making (data not shown).

#### 2.2.2. Composition of flour samples and extracts

Moisture and ash contents of flour were determined according to AACCI Approved Methods 44–19.01 and 08–01.01 (AACCI, 1999) and protein levels using the Dumas combustion method, an adaptation of the AOAC Official Method (AOAC, 1995) to an automated 1108 Elemental Analyser (Carlo Erba, Hindley Green, United Kingdom). Nitrogen to protein conversion factors were 6.25 for barley flour, 5.70 for rye and wheat flour and 5.80 for oat flour. Carbohydrate levels and compositions were determined with gas chromatography following acid hydrolysis and conversion to alditol peracetates (Courtin et al., 1999). AX level was calculated as 0.88 times the sum of xylose and arabinose levels.  $\beta$ -Glucan levels were colorimetrically measured according to AACCI Approved Method 32–23.01 (AACCI, 1999). Flour total lipid was extracted with water-saturated butanol as in Pauly et al. (2014a) followed by a Bligh and Dyer (1959) purification step and gravimetric quantification. Lipid contents of the flour extracts were determined according to Bligh and Dyer (1959). Chloroform (2.0 mL), methanol (2.0 mL) and milli-Q water (1.8 mL) were sequentially added to 300 mg freeze-dried extract. After each addition, the solution was vortexed. After centrifugation (540 g, 20 min), the lower lipid containing chloroform phase was retained, while the upper-methanol-water phase containing mainly protein was discarded. As the middle phase still contained some lipid, it was extracted twice more. Chloroform was evaporated from the combined lower chloroform phases and lipid levels were then determined gravimetrically. All analyses were done at least in triplicate.

#### 2.2.3. Enzymatic activities

Enzymatic activities were determined at pH 5.4 (i.e. average batter pH during fermentation; Section 2.2.6) and 30 °C (i.e. fermentation temperature).  $\alpha$ -Amylase, endoxylanase and endo- $\beta$ -glucanase activity levels were determined in triplicate with the Amylazyme, Xylazyme AX and  $\beta$ -Glucazyme methods (Megazyme, Bray, Ireland), respectively. Freeze-dried extracts (50 mg) were suspended in 10.0 mL sodium maleate buffer (25 mM, pH 5.4) containing 5.0 mM  $\text{CaCl}_2$  for the Amylazyme method or sodium acetate buffer (25 mM, pH 5.4) for the Xylazyme AX and  $\beta$ -Glucazyme methods. After centrifugation (5000 g, 15 min), the supernatants (1.0 mL) were equilibrated for 10 min at 30 °C before adding an Amylazyme, Xylazyme AX or a  $\beta$ -Glucazyme tablet. After 90 min incubation for  $\alpha$ -amylase, 18 h for endoxylanase and 5 h for endo- $\beta$ -glucanase activity levels, the reactions were stopped by adding 10.0 mL of a tris-(hydroxymethyl)-aminomethane (Tris) solution (2.0% or 1.0% for the Amylazyme and  $\beta$ -Glucazyme or Xylazyme AX methods, respectively). The solutions were vigorously vortexed, filtered through a MN 615 filter (Macherey-Nagel, Düren, Germany) and their extinction at 590 nm ( $E_{590}$ ) measured. Control samples were extract suspensions incubated without the respective tablets. Corrections were made for nonenzymatic color release by the respective tablets. Activities were expressed as  $\alpha$ -amylase, endoxylanase and endo- $\beta$ -glucanase units per gram dm extract. One unit is the enzyme activity which increases  $E_{590}$  by 1.00 per hour of incubation under the conditions of the test.

Endopeptidase activity levels were determined in triplicate using azocasein substrate. Freeze-dried extracts (50 mg) were suspended in 10.0 mL 25 mM sodium acetate buffer (pH 5.4). After centrifugation (5000 g, 15 min), 250  $\mu\text{L}$  supernatant was combined with 350  $\mu\text{L}$  1.4% (w/v) azocasein. After 24 h incubation at 30 °C, 500  $\mu\text{L}$  cooled (7 °C) 10.0% (w/v) trichloroacetic acid was added and precipitated proteins were removed by centrifugation (9600 g, 10 min). Finally, 500  $\mu\text{L}$  0.5 M sodium hydroxide was added to 500  $\mu\text{L}$  supernatant and the extinction at 440 nm ( $E_{440}$ ) was measured. Activity was expressed as endopeptidase units per gram dm. One unit is the enzyme activity which increases  $E_{440}$  by 1.00

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