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Partial purification of components in rye water extractables which improve the quality of oat bread

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ABSTRACT

Unlike wheat bread, the dough of which has a visco-elastic network and high gas-holding capacity, oat bread generally has a low volume and a dense structure. We showed earlier that including rye water-extractable components in an oat bread batter recipe increases loaf volume by *ca.* 30% (Pauly and Delcour, submitted as back-to-back publication). We here report on efforts to identify the active factor(s). Anion exchange chromatography allowed enriching the active factor(s). This and the fact that only a limited volume increase was observed when oat batter was supplemented with boiled rye extract indicate that proteins are likely the most important components responsible for the volume increase. While the most active factor(s) had a pI below 4.5, components with pI values between 4.5 and 8.5 also contributed to oat loaf volume. Alkaline rye components (pI > 8.5) or rye arabinoxylan had no impact. Rye water-extractable components smaller than 6–8 kDa also had a positive impact on loaf volume.

1. Introduction

Celiac disease is an autoimmune-mediated disease triggered by ingesting wheat gluten or related proteins. Its reported prevalence has increased over the last 30 years. It probably affects more than 1% of the world population (Lohi et al., 2007; Hüttner and Arendt, 2010). The present only effective treatment is a life-long and strict adherence to a gluten-free diet. Although initially developed for patients suffering from celiac disease, gluten-free diets are also followed *e.g.* as part of a life-style choice. In the United States, only 7% of the gluten-free products on the market are purchased by celiac patients (Watson, 2012). Furthermore, consumers nowadays increasingly choose bread prepared from cereals other than wheat because of nutrition related reasons (*e.g.* levels of dietary fiber, essential amino acids, minerals) (Dewettinck et al., 2008). However, the absence of wheat gluten presents technological challenges. Indeed, wheat gluten has unique visco-elastic properties which confer upon bread its desired structure (Delcour et al., 2012). The quality of non-wheat bread products is, hence, much inferior to that of their counterparts containing gluten. Non-wheat bread has low loaf volume, an uneven distribution of large gas cells in the crumb and firm and crumbly texture (Hager et al., 2012; Houben et al., 2012). To overcome such quality deficiencies, additives are frequently used in non-wheat bread recipes. Hydrocolloids (*e.g.* xanthan gum, cellulose derivatives such as hydroxypropyl methyl cellulose), surfactants (*e.g.* diacetyl tartaric esters of mono- and diacylglycerols, sodium stearoyl lactylate) and enzymes (*e.g.* amylases, transglutaminases) are well-studied additives in non-wheat bread making (Renzetti et al., 2008, 2010; Hüttner and Arendt, 2010; Sciarini et al., 2012).

Cereal water extractables contain several components which may well increase batter stability. Non-starch polysaccharides may act by virtue of their water binding and viscosity increasing capacity (Courtin and Delcour, 2002; Izydorczyk and Dexter, 2008). Also, much as some surfactants, some water-extractable surface active proteins can stabilize gas cells (MacRitchie, 1976; Pauly et al., 2014). Cereal aqueous extracts also contain various enzymes.

In a previous paper, we included water extracts from barley, rye, oat and wheat flour in simple oat bread recipes (Pauly and Delcour, submitted as back-to-back publication). Oat contains high levels of the dietary fiber (1 → 3, 1 → 4)-β-D-glucan (further referred to as β-glucan) (Shewry et al., 2008). Sufficient high intake of β-glucan

Abbreviations: AEC, anion exchange chromatography; CEC, cation exchange chromatography; dm, dry matter; E_{440}, extinction at 440 nm; E_{590}, extinction at 590 nm; MM, molecular mass; MWCO, molecular weight cut-off; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; Tris, tris-(hydroxymethyl)-aminomethane.

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can reduce postprandial blood glucose, insulin and serum cholesterol levels (Braaten et al., 1994a, 1994b; Kerckhoffs et al., 2003). Other oat nutrients include essential amino acids, vitamins, minerals and antioxidants (Dewettinck et al., 2008). Oat can also be used as raw material for gluten-free products, although some controversy exists on the matter (Hüttner and Arendt, 2010). Replacing an equal weight of oat flour by rye extract [5.0% on dry matter basis (dm)] resulted in a larger loaf volume increase (+27%) than did replacing oat flour by barley (+21%), wheat (+14%) or oat (+5%) extracts in a similar way. Furthermore, we showed that replacing only 2.0% dm oat flour by rye extract made crumb significantly softer, while it had no impact on crumb mean cell area and number of cell per surface unit. Amongst the four extracts, that from rye contained the highest arabinoxylan levels and enzyme activities. High β-glucan levels and extract viscosity are probably not the main factors contributing to the volume increasing effect of rye extract (Pauly and Delcour, submitted as back-to-back publication).

In the present paper, we contribute to the identification of the active component(s) in the rye extract responsible for oat loaf volume increases. To that end, the rye extract is treated in order to enrich the active component(s) and the impact on oat loaf volume is evaluated. We here report on the outcome of our work.

2. Materials and methods

2.1. Materials

Commercial oat flour (moisture, protein, and ash contents on dm respectively 10.1%, 12.4%, and 1.67%) and rye flour (corresponding data 12.9%, 7.2%, and 0.82%) were from Raisio Nutrition (Raisio, Finland) and from Koopmans (Leeuwarden, The Netherlands), respectively. Dry yeast was from Puratos (Groot-Bijgaarden, Belgium). Sugar and salt were commercial food grade products. SP Sepharose Big Beads and Q Sepharose Fast Flow ion exchange matrices for batch and column fractionations were from GE Healthcare (Uppsala, Sweden). All other chemicals, solvents and reagents were from Sigma-Aldrich (Bornem, Belgium) and were at least analytical grade, unless specified otherwise.

2.2. Experimental

2.2.1. Boiling of the rye extract

Aqueous extracts of rye flour were prepared as in Pauly and Delcour (submitted as back-to-back publication). For some experiments, the freeze-dried extract (3.0 g) was suspended in 100 mL deionized water, boiled for 15 min, cooled to room temperature and centrifuged (5000 *g*, 15 min). The supernatant was then freeze-dried.

2.2.2. Batch ion exchange fractionation

Fractionation into bound and unbound material was performed with batch anion exchange chromatography (AEC) or cation exchange chromatography (CEC) on SP Sepharose Big Beads or Q Sepharose Fast Flow, respectively. The ion exchange matrices (*ca.* 500 mL) were equilibrated with 25 mM tris-(hydroxymethyl)-aminomethane (Tris) - HCl (pH 8.5) or 25 mM sodium acetate (pH 4.5) for AEC or CEC, respectively. The suspensions of matrix and extract (3.0 g in 100 mL of these media) were shaken overnight at 6 °C and then brought onto a P1 glass filter. The matrix was rinsed with 25 mM Tris-HCl (AEC) or 25 mM sodium acetate (CEC) to remove unbound material. Bound material was eluted with 1.0 L 1.0 M NaCl. The four obtained fractions (unbound and eluate of AEC or CEC) were dialyzed [molecular weight cut-off (MWCO) 6–8 kDa] against deionized water and freeze-dried. The untreated extract was also dialyzed (MWCO 6–8 kDa) against deionized water to investigate the impact of removing small rye components.

2.2.3. Column anion exchange chromatography

Extracts [3.0 g in 150 mL 25 mM Tris-HCl (pH 8.5)] were separated by AEC on a Q Sepharose Fast Flow column (diameter 16 mm, length 230 mm). They were suspended in loading buffer [25 mM Tris-HCl (pH 8.5)] and centrifuged (5000 *g*, 15 min, 4 °C). The supernatants were loaded onto the column, equilibrated with loading buffer, at a flow rate of 0.3 mL/min. After rinsing the column with loading buffer (flow rate 1.0 mL/min) to remove unbound material (= run-through fraction), bound molecules were eluted using a gradient of 1.0 M NaCl in 25 mM Tris-HCl (pH 8.5) at a flow rate of 1.0 mL/min. The NaCl concentration was first increased linearly from 0 to 300 mM NaCl in 150 m), and then further increased to 800 mM in 500 mL. This elution gradient was chosen based on preliminary tests. The fractions detected at 280 nm were dialyzed (MWCO 6–8 kDa) against deionized water and freeze-dried.

2.2.4. Protein electrophoresis

Protein composition was analyzed with sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE). Freeze-dried extracts or extract fractions were dissolved in sample buffer [12.5 mM Tris-HCl; 4.0% (w/v) SDS; 30% (v/v) glycerol; 0.004% (w/v) bromophenol blue; pH 6.8] to obtain a protein concentration of approximately 2.0 mg/mL. After boiling (5 min) and centrifugation (10,900 *g*; 3 min), the proteins in the supernatant were separated under non-reducing conditions with a PhastSystem (GE Healthcare) according to GE Healthcare separation technique file 110. Molecular mass (MM) markers (LMW-SDS Marker Kit, GE Healthcare) were also analyzed. Gels were stained with sensitive silver staining as described in the GE Healthcare development technique file 210.

2.2.5. Enzyme activities

Enzyme activities were determined at pH 5.4 [*i.e.* average batter pH during fermentation (Pauly and Delcour, submitted as back-to-back publication)] and 30 °C (*i.e.* fermentation temperature). α-Amylase, endoxylanase and endo-β-glucanase activity levels were determined in triplicate with the Amylazyme, Xylazyme AX and β-Glucazyme methods (Megazyme, Bray, Ireland), respectively. Freeze-dried extracts (50 mg) were suspended in 25 mM 10.0 mL sodium maleate buffer (pH 5.4) containing 5.0 mM $CaCl_2$ for the Amylazyme method or 25 mM sodium acetate buffer (pH 5.4) for the Xylazyme AX and β-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 β-Glucazyme tablet, respectively. After 90 min incubation for α-amylase, 18 h for endoxylanase and 5 h for endo-β-glucanase activity levels, the reactions were stopped by adding 10.0 mL of a Tris solution (2.0% or 1.0% for the Amylazyme and β-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 non-enzymatic color release by the respective tablets. α-Amylase, endoxylanase and endo-β-glucanase activities were expressed as 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 (Brijs et al., 1999). 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 μL supernatant was combined with 350 μL 1.4% (w/v) azocasein. After 24 h