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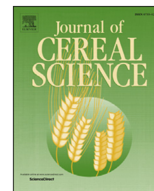

Relationships between lipase-treated wheat lipid classes and their functional effects in wheat breadmaking

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ABSTRACT

Fractionation of lipase-treated wheat lipids showed that polar lipase-reaction products were responsible for their positive functional effects in breadmaking. The results allowed the conclusion that an optimal baking lipase preferably hydrolyzes monogalactosyl diglycerides and N-acyl phosphatidyl ethanolamine, but has only moderate activity towards digalactosyl diglycerides. Synergistic effects of digalactosyl diglycerides and their products digalactosyl monoglycerides (molar proportion 1:1) provide even a greater bread volume increase. Reconstitution baking (defatted flour plus lipid or lipid fraction added) partly provided contradictory results. 100% polar lipase-treated lipids were less active than untreated polar dough lipids plus polar flour lipids. Combinations of non-polar and polar lipase-treated lipids were even more active than the polar ones alone. It appears that the polar reaction products of baking lipases need the presence of unmodified lipids or non-polar lipids to provide optimal functional effects. The present data suggests that synergistic effects between different lipid classes might play a key role in the mechanism of action of baking lipases.

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1. Introduction

Despite the fact that lipids represent a minor fraction of wheat flour, the composition and structure of wheat flour lipids strongly influence the end-use quality of the bread. Lipases have been applied over the past two decades in the process of breadmaking to improve dough processing and the bread quality (Poulsen et al., 1998; Primo-Martín et al., 2008; Moayedallaie et al., 2010; Colakoglu and Özkaya, 2012; Gerits et al., 2014a). Lipases can be used to partially or completely replace synthetic emulsifiers like DATEM which are extensively used in the bread industry to provide increase in bread oven rise and specific volume (Pareyt et al., 2011).

The mechanisms underlying the functional effects of lipases are linked to the hydrolysis of one or more fatty acid moieties from non-polar and polar lipids to generate more polar lipids leading to improved surface activity and, thus, stronger functional effects of these endogenous lipids. Analysis of lipase-treated wheat lipids revealed that the hydrolysis of galactolipids and phospholipids are most important to improve the final the bread volume (Gerits et al., 2013, 2014b; Schaffarczyk et al., 2014). De Stefanis and Ponte (1976) postulated that glycolipids are more active in baking than phospholipids. The non-polar lipids, especially the triglycerides (TG) and the generated free fatty acids (FFA), both remain in the free lipid fraction and are extractable with hexane. The polar lipids belong to the bound fraction, which is entrapped in the gluten network. Gerits et al. (2015) showed that dough liquor recovered from lipase-treated dough contained higher lipid levels than that of dough liquor recovered from control dough. The liquid film as part of dough liquor is considered the medium for incorporation and growth of gas cells in dough (MacRitchie, 1976; Primo Martin et al., 2006; Salt et al., 2006). This means that the higher the concentration of surface-active polar lipids in the dough liquor induced by lipase, the higher the impact on bread volume.

Abbreviations: ASG, acylated sterol glucosides; BU, Brabender units; DCM, dichloromethane; DG, diglycerides; DATEM, diacetyl tartaric acid esters of mono- and diglycerides; DGDG, digalactosyl diglycerides; DGMG, digalactosyl monoglycerides; dm, dry matter; FFA, free fatty acids; HPLC-ELSD, high performance liquid chromatography coupled with an evaporative light scattering detector; HPTLC, high performance thin layer chromatography; KLU, kilo lipase-units; LPC, lysophosphatidyl choline; MG, monoglycerides; MBT, micro-baking test; MGDG, monogalactosyl diglycerides; MGMG, monogalactosyl monoglycerides; NALPE, N-acyl-lysophosphatidyl ethanolamine; NAPE, N-acyl-phosphatidyl ethanolamine; PC, phosphatidyl choline; SD, standard deviation; SG, sterol glucosides; TG, triglycerides; WSB, water-saturated 1-butanol.

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Gerits et al. (2015) suggested that dough rheology is only impacted by the lipid composition readily available at the start of mixing and not by the lipase-reaction products after fermentation. Since dough rheology examined by Kieffer rig extension tests was not affected by lipases, the effect of lipases has to be caused by a direct stabilization mechanism of the liquid film surrounding the gas cells. The most probable mechanism of stabilization of the gas cells by surface-active lipids is the formation of condensed monolayers at the gas–liquid interface. These condensed monolayers generate substantial elastic restoring forces, which resist destabilization of liquid lamellae when changes in the interfacial areas occur (Sroan and MacRitchie, 2009). Lipids that give expanded monolayers are relatively compressible and lead to instability in the liquid lamellae. It was speculated that lipids forming a hexagonal phase I type phase, for example FFA, are able to emulsify the deleterious lipids thereby preventing their adsorption into the interface and helping in building of expanded monolayers.

It was shown that an overdose of lipase caused bread volume decrease (Gerits et al., 2014b; Schaffarczyk et al., 2014). Lipid analysis indicated extensive lipid hydrolysis caused by the high enzyme concentrations. This denotes the importance of the optimum lipid composition by selective breakdown of lipids to optimize bread volume increase. Moreover, lipases probably catalyze the transition of lipid classes promoting the hexagonal II mesophase to lipid classes promoting the lamellar and hexagonal I mesophases, which improve stabilization of gas cells. Overdosing of lipases decreases the level of lipid classes building condensed monolayers resulting in the destabilization of gas cells (Gerits et al., 2014b). Further, overdosing induces earlier termination of oven rise due to gas cell coalescence (Gerits et al., 2015).

Until now, it is not clear, whether and to what extent each endogenous lipid class should be hydrolyzed by a baking lipase to yield a lipid mixture providing the best gas cell stability. Altogether, in-depth understanding of lipase-mediated reaction products is missing and especially information regarding the functional effects of lipid classes generated by the action of lipases is still very scarce. None of the studies published so far used fractionation of lipid classes before and after lipase addition to show the effect of individual lipid classes on the baking performance. Against this background, the aim of this study was to determine the relationships between specific wheat lipid classes and their functional effects in the baked product. Different lipid mixtures and fractionated polar and non-polar fractions should be analyzed for their functional effects using a recently established micro-reconstitution baking test with 10 g 2-propanol defatted wheat flour (Schaffarczyk et al., 2016). To determine the relation between lipid mixtures and their functional effects in breadmaking, combinations of polar and non-polar lipids as well as of specific isolated wheat lipid classes were quantitated by high-performance liquid chromatography with evaporative light scattering detection (HPLC-ELSD).

2. Materials and methods

2.1. Wheat flour

'Kolibri' flour, a commercial flour obtained from a mixture of wheat cultivars (Meneba, Rotterdam, The Netherlands, 2014 harvest, containing no additives), was characterized as follows: The moisture and ash contents of the flour were determined according to ICC Standards 110/1 (ICC, 1976) and 104/1 (ICC, 1990), respectively. The protein content ($N \times 5.7$) was determined by the Dumas method by means of a TruSpec N nitrogen analyzer (Leco, Kirchheim, Germany). Analytical characteristics of the flour were 13.5% moisture, 9.7% protein (dry matter (dm)), and 0.59% ash (dm). Further analytical and functional data of the flour are reported elsewhere (Schaffarczyk et al., 2016).

2.2. Chemicals and reagents

Fresh baker's yeast (*Saccharomyes cerevisiae;* standard yeast for breadmaking applications) was obtained from Wieninger GmbH (Passau, Germany). Lipid standards were as described in Schaffarczyk et al. (2014). Silica gel (0.063–0.200 mm) and concentrating zone-HPTLC plates (20 × 20 cm) coated with silica gel G 60 (20 × 5 cm) on glass were from VWR Merck (Darmstadt, Germany). The following lipase samples were used: Commercial enzyme granulates Lipopan F-BG (25.0 KLU/g) and Lipopan Xtra-BG (7.2 KLU/g), Novozymes A/S, Bagsvaerd, Denmark; commercial enzyme granulate Panamore Golden, DSM, Delft, The Netherlands; experimental sample enzyme granulate EL 2013 000 405, AB Enzymes, Darmstadt, Germany. All solvents used were HPLC or LC-MS grade and purchased from Sigma–Aldrich (Steinheim, Germany).

2.3. Defatting of flour

Flour (1000 g) and 2-propanol (2.5 L) were stirred for 1 h at 20 °C. After centrifugation (20 min, 4 °C, 3550 × *g*), the solvent was removed by filtration on a glass filter (porosity 3; pore size 16–40 μm). The flour was re-extracted twice (2 × 2.5 L, 30 min). The defatted flour was spread out on a flat glass tray and left to dry in a fume hood for one week.

2.4. Lipid extraction from flour

Flour lipids were extracted from flour using water-saturated 1-butanol (WSB) as described by Schaffarczyk et al. (2014).

2.5. Lipid extraction from dough

Dough was prepared by upscaling the recipe based on 10 g of flour described by Koehler and Grosch (1999). Flour (298.8 g), NaCl (6 g), and Lipopan Xtra (0 and 39 mg; 0 and 130 mg/kg flour) were mixed for 1 min in a Farinograph (300 g Z-blade mixer; Brabender, Duisburg, Germany) at 22 °C. Water (179.4 mL) was added within 25 s and mixing was continued until the optimum consistency of the dough (550 Brabender Units (BU) at 7 min) was reached. The dough was allowed to rest for 20 min at 30 °C in a water-saturated atmosphere. The dough was then reshaped on a dough rounder (Type 440; Brabender, Duisburg, Germany) for ten cycles and the resulting spherical dough piece was rolled (PTFE cylinder, diameter 5 cm, length 30 cm) to yield an oval dough piece of 5 mm thickness. The dough piece was folded twice to ¼ of its original size and reshaped on the dough rounder for 20 cycles. After proofing (30 °C, 38 min) the dough was frozen with liquid N_2, freeze-dried, and milled using an ultracentrifugal mill ZM 200 (200 μm mesh size, Retsch, Haan, Germany). The milled freeze-dried dough was stirred in WSB (1.3 L, 16 h, 20 °C) to extract lipids. After centrifugation for 20 min (3550 × *g*, 4 °C), the supernatant was filtered through a fluted filter paper. The solvent was evaporated to dryness under reduced pressure and stored under Ar atmosphere at −75 °C until further analysis.

2.6. Separation of non-polar and polar lipids by batch procedure

The silica gel (G 60, 0.040–0.063 mm) was activated as follows: Silica gel (200 g) was dried overnight at 105 °C and left to cool in a desiccator. After adding water (10 mL) the material was shaken for 20 min. Flour lipids, dough lipids or lipase-treated dough lipids (15 g) were dissolved in diethyl ether (100 mL) and added to activated silica gel (200 g). To extract the non-polar lipids, diethyl

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