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A lipase based approach for studying the role of wheat lipids in bread making

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ABSTRACT

While endogenous wheat lipids exert a major effect on bread quality, little is known on the way they impact on bread loaf volume (LV). Here we altered wheat flour lipid composition during bread making using lipases *in situ*. Lipopan F, Lecitase Ultra, and surfactants increased LV to similar extents. The increases in bread LV as a result of these enzymes were related to decreased levels of galactolipids and phospholipids and concomitant increased 'lyso'-lipid as well as free fatty acid (FFA) levels. The FFA formed were transferred to the free lipid fraction, while the 'lyso'-lipids remained in the bound lipid fraction. For optimal gas cell stabilisation, an equilibrium between the lipid classes present and hence, the type of mesophase formed, is essential. Sufficient levels of lipids forming lamellar mesophases and lipids forming hexagonal I mesophases, which respectively form condensed monolayers or emulsify (deleterious) non-polar lipids in dough liquor, are needed.

1. Introduction

Endogenous wheat lipids, although a minor fraction of the kernel and the derived flour, are important in bread making (Chung, Ohm, Ram, Park, & Howitt, 2009; Pareyt, Finnie, Putseys, & Delcour, 2011). During dough mixing, lipids redistribute as observed from decreased levels of free and concomitant increased levels of bound lipids. The phenomena occurring have been referred to as lipid binding (Carr, Daniels, & Frazier, 1992; Chung, 1986; Olcott & Mecham, 1947; Wootton, 1966). As described recently by Finnie, Jeanotte, Morris, Giroux and Faubion (2010) and Gerits, Pareyt, and Delcour (2013), during dough development lipids are rubbed from the surface of the starch granules and thereby become trapped in (Marion, Le Roux, Akoka, Tellier, & Gallant, 1987) or interact with (McCann, Small, Batey, Wrigley, & Day, 2009) the gluten network. This can positively impact the gluten network (strength) (Köhler, 2001; Pomeranz & Chung, 1978). That way, lipids can indirectly stabilise the gas cells in dough. This is very important as dough's gas-holding capacity is one of, if not the most important feature in bread making as it is associated with an airy crumb structure and a good bread loaf volume (LV) (Eliasson & Larsson, 1993). However, next to their impact on the gluten network, (polar) lipids also exert a direct effect on gas cell stabilisation. Several authors (Gan, Angold, Williams, Ellis, Vaughan, & Galliard, 1990; Gan, Ellis, & Schofield, 1995; Sroan, Bean, & MacRitchie, 2009; Sroan & MacRitchie, 2009) have suggested a dual mechanism whereby gas cells are stabilised during fermentation and the early baking phase. It is based on the cooperative (and successive) effect of (i) the gluten network and (ii) a liquid lamella surrounding the gas cells. Indeed, in optimally mixed dough, the gluten network holds the gas cells and, at that stage of the bread making process, is the primary (or even sole) responsible for their stabilisation. However, already after 15 min of fermentation and during early baking discontinuities appear in the gluten network (Gan et al., 1990). From that moment onwards, a thin liquid lamella around the gas cell, stabilised by adsorbed surface active proteins and/or (polar) lipids, aids in further gas cell stabilisation and, hence, provides a secondary stabilisation mechanism (Sroan & MacRitchie, 2009). Surface active proteins and (polar) lipids stabilise the gas cells in a different way: whereas the former form viscoelastic films, the latter act through the Gibbs–Marangoni mechanism within a liquid lipid membrane. When both components are present, they compete

Abbreviations: ASE, accelerated solvent extractor; DAG, diacylglycerols; DATEM, diacetyl tartaric esters of mono- and diglycerides; DGDG, digalactosyldiacylglycerols; DGMG, digalactosylmonoacylglycerols; dm, dry matter; EP, enzyme protein; FFA, free fatty acids; HPLC–ELSD, high performance liquid chromatography coupled with evaporative light scattering detection; LPC, lysophosphatidylcholine; LV, loaf volume; MAG, monoacylglycerols; MGDG, monogalactosyldiacylglycerols; MGMG, monogalactosylmonoacylglycerols; NAPE, N-acyl phosphatidylethanolamine; NAL-PE, N-acyl lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SSL, sodium stearoyl lactylate; TAG, triacylglycerols; WSB, water saturated butan-1-ol.

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for the gas cell interface and impair each other's ability to stabilise gas cells (Mills, Wilde, Salt, & Skeggs, 2003). Hence, not only the gluten network rheology, but also the type of components at the interface as well as their surface active properties are important for proper gas cell stabilisation. However, although both the impact of lipids on gluten network strength including that of polar lipids and surface active proteins on gas cell stabilisation have been studied profoundly (Krog, 1981; Köhler, 2001; Selmair & Koehler, 2009), little is known about the stabilisation effects exerted by the different wheat endogenous (polar) lipid classes.

Lipases have gained interest in the bread making industry as alternatives for surfactants, generally (incorrectly) referred to as emulsifiers in literature dealing with bread making. Lipases hydrolyse the endogenous wheat lipids in dough to form surface active lipids (Aravindan, Anbumathi, & Viruthagiri, 2007; Colakoglu & Özkaya, 2012; Moayedallaie, Mirzaei, & Paterson, 2010). In fact, lipase addition allows modifying the lipid population *in situ*, without altering other flour components. We here studied how the different wheat endogenous lipid classes affect bread LV by including lipases with different specificities in a straight dough recipe. Advantages of such an approach are that (i) it is free from impacts of extraction solvent(s) on (other) flour constituents (in particular gluten proteins) and (ii) when in contact with the enzymes lipids still occur at their native (endogenous) position. The latter is not the case when using fractionation–reconstitution (i.e. defatting followed by re-addition) (MacRitchie & Gras, 1973).

According to De Maria, Vind, Oxenboll, and Svendsen (2007), the 'perfect' lipase would be one with optimal activity on the (tri)acylglycerol, phospholipid and galactolipid substrates in flour, and result in a gas cell stability similar to that brought about by surfactants. However, it is not clear which lipase currently can be considered as 'perfect' and to what extent each of the lipid classes should be hydrolysed. Against this background, it seemed logical to include bread making trials with two surfactants, i.e. diacetyl tartaric esters of mono- and diglycerides (DATEM) or sodium stearoyl lactylate (SSL), both of which are regularly used in bread making to positively impact bread LV. Free and bound lipid fractions of fermented control dough pieces and dough pieces containing added lipases in their formula were analysed with high pressure liquid chromatography coupled with evaporated light scattering detection (HPLC–ELSD). Bread properties, in particular LV, were analysed as well. Taken together, the present study not only demonstrates the specific action of the different lipases during bread making, but also relates (changes in) the properties of the lipid population to bread LV. We here report on the outcome of our work.

2. Materials and methods

2.1. General

Grains from soft wheat cultivar Claire were obtained from Limagrain (Rilland, The Netherlands) and conditioned to 16.0% moisture before milling with a Bühler (Uzwil, Switzerland) MLU-202 laboratory mill, of which the milling flow scheme is depicted in Delcour, Vanhamel, and De Geest (1989). Milling yield of straight grade flour was 72.7%, its moisture and protein contents were respectively 14.1% and 10.6% [on dry matter (dm) basis]. The latter were determined with an American Association of Cereal Chemists International (AACC-I) approved method 44-19.01 (AACC-I, 1999) and an adaptation of the AOAC official method (AOAC, 1995) to an automated Dumas protein analysis system (EAS Vario Max CN, Elt, Gouda, The Netherlands) with 5.7 as nitrogen to protein conversion factor.

Four different lipases were kindly donated by Novozymes (Bagsvaerd, Denmark) in a purified form, and without any amylase, peptidase or xylanase side activities. Lipopan F, a *Fusarium oxysporum* enzyme preparation, is used in the bread making industry as a source of both lipase and phospholipase activities. Lecitase Ultra is a phospolipase used in degumming of edible oils. It is a product of combining homologous genes encoding *Thermomyces lanuginosus* lipase and *F. oxysporum* phospholipase (De Maria et al., 2007). Lipolase, a recombinant *T. lanuginosus* lipase is used in detergents (Aravindan et al., 2007). Finally, YieldMAX is a phospholipase A_1. It is, hence, active on the sn1 acyl chain of phospholipids (Aloulou, Ben Ali, Bezzine, Gargouri, & Gelb, 2012). It originates from *Fusarium* sp. and is widely used in the dairy industry (De Maria et al., 2007). Lipopan F (56.51 U) had the highest lipase activity (determined as described below) towards *p*-nitrophenyl palmitate, followed by Lecitase Ultra (0.14 U) and Lipolase (0.12 U), which had very similar activities, and YieldMAX (5.78×10^{-2} U), which had the lowest activity towards *p*-nitrophenyl palmitate. DATEM and SSL were from Puratos (Groot-Bijgaarden, Belgium). The lipid standards needed to identify the lipids in the HPLC–ELSD method were as in Gerits et al. (2013), with exception of monogalactosylmonoacylglycerol (MGMG), which was kindly donated by Novozymes. All solvents used were HPLC-grade and from VWR (Haasrode, Belgium) or Sigma Aldrich (Steinheim, Germany), unless specified otherwise.

2.2. Dough and bread making

Bread was prepared in triplicate on a 10 g scale based on the straight dough method of Shogren and Finney (1984) but without using shortening. Flour (10.0 g on a 14.0% moisture base), water, sugar (6.0% on flour basis), compressed yeast (5.3% on flour basis) and salt (1.5% on flour basis) were mixed in a 10 g pin mixer (National Manufacturing, Lincoln, NE). The amount of water added and the optimal mixing time were determined by Mixograph analysis (National Manufacturing) according to AACC-I approved method 54-40.02 (AACC-I, 1999), and were respectively 4.8 ml and 165 s. Lipases were included in the dough recipes in levels ranging from 0 to 5.0 mg EP/kg flour. In a second set-up, DATEM or SSL were added in levels of 0.5%, 1.0% and 1.5% on flour basis.

For lipid analyses, dough samples were prepared in duplicate, fermented (126 min) and immediately frozen with liquid nitrogen, freeze dried, milled, sieved (mesh size: 250 μm) and stored at −18 °C. They are hereafter referred to as processed dough samples.

2.3. Lipid extraction and purification

Sequential free and bound lipid extraction was as in Gerits et al. (2013) with an Accelerated Solvent Extractor (ASE) 200 (Dionex, Amsterdam, The Netherlands). Processed dough sample [0.86 g dry matter (dm)] was homogenised with 26 g of sand (50–70 mesh particle size) (Sigma Aldrich, Steinheim, Germany) and subsequently poured in a 22 ml ASE extraction cell. Free lipids were extracted with hexane and bound lipids with water saturated butan-1-ol (WSB). The ASE settings were as in Gerits et al. (2013). The extracts were collected in glass test tubes and the respective extraction solvents evaporated with a Rotational Vacuum Concentrator (Q-lab, Vilvoorde, Belgium). Bound lipid extracts were purified from non-lipid material (mainly protein) as in Bligh and Dyer (1959). Finally, dry lipid extracts were stored at −80 °C in amber coloured vials under nitrogen prior to further analysis.

2.4. Lipid analyses

Lipid analyses were conducted with HPLC–ELSD as in Gerits et al. (2013), which itself was based on the method described by Graeve and Janssen (2009). In the present case, the Alltech Model 3300 ELSD (Grace, Lokeren, Belgium) detector allowed altering