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Wheat starch swelling, gelatinization and pasting: Effects of enzymatic modification of wheat endogenous lipids

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ABSTRACT

Starch is widely used in food industry because of its unique gelling, thickening and stabilizing capacities. These characteristics are impacted by added surfactants. However, less is known about whether and, if so, how wheat endogenous lipids impact the swelling behaviour of starch granules. We here used three different lipases (Lecitase Ultra, Lipopan F and Lipolase) with known impact on the endogenous lipid composition and two surfactants (diacetyl tartaric esters of mono- and diacylglycerols and sodium stearoyl lactylate) for studying the impact of (endogenous) lipids on starch rheology and carbohydrate leaching. The study revealed that although amylose-lipid inclusion complex formation affects wheat starch swelling and carbohydrate leaching, there is no causal relation between the two latter phenomena. Both their location and type affect the impact of lipids on starch swelling. Next to the complex forming ability of lipid(-like) components, their ability to shield starch granules from water by forming lipophilic layers also affects starch granule swelling because it delays water absorption and increases starch granule rigidity.

1. Introduction

Starch is used in a broad range of food recipes because of its unique gelling, thickening and stabilizing capacities. It occurs as water-insoluble semi-crystalline granules (Eliasson & Gudmundsson, 1996; French, 1973) which contain amylose (AM) and amylopectin (AP) (respectively 22–28% and 78–72% for wheat starch) (Lineback, 1984; Zobel, 1988). When starch granules are heated in water, they absorb water and swell. While below the gelatinization temperature this process is reversible, above this temperature irreversible changes result in loss of crystallinity and granule disruption (Atwell, Hood, Lineback, Varriano-Marston, & Zobel, 1988; Delcour & Hoseney, 2010), the extent of which amongst others depends on the water level and the presence of components such as sugar or salt (Lelievre, 1976; Wootton & Bamunuarachchi, 1980). Gelatinization and pasting involve (i) granule swelling, (ii) leaching of carbohydrate material (mainly AM), (iii) the formation of a three-dimensional starch network of leached material as well as (iv) interactions between granule remnants and the leached material (Atwell et al., 1988). Upon cooling, a gel is formed because leached AM crystallizes into double helices in the continuous phase. The latter phenomenon is referred to as gelation (Delcour & Hoseney, 2010).

In wheat flour, starch granules are surrounded by amyloplast membrane remnants (Hargin & Morrison, 1980) which originate from lipid bilayer membranes that surround the amyloplasts in which starch is synthesized and stored during kernel development (Bechtel & Wilson, 2003). The bilayer membranes mainly consist of galacto- and phospholipids. However, during seed desiccation, amyloplast lipid bilayer membranes are (at least partially) degraded (Tan & Morrison, 1979). The presence of lipids at the starch granule surface may well impact the behaviour of AM and AP prior to and during gelatinisation and pasting. Within this context, the impact of surfactants commonly used in bread making (Eliasson, 1985; Gudmundsson & Eliasson, 1990; Krog, 1973; Van Steertegem, Pareyt, Brijs, & Delcour, 2013) on starch pasting has been studied profoundly, and was mainly attributed to formation of AM-lipid inclusion (AM-L) complexes. However, not much is

Abbreviations: AM, amylose; AM-L, amylose-lipid inclusion; AP, amylopectin; C*, close packing concentration; CHL, carbohydrate leaching; DATEM, diacetyl tartaric esters of mono- and diacylglycerols; DSC, differential scanning calorimetry; EP, enzyme protein; FFA, free fatty acids; MAG, monoacylglycerols; RVA, Rapid Visco Analyser; SSL, sodium stearoyl lactylate; SP, swelling power; TAG, triacylglycerols.

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known about the impact of wheat flour endogenous lipids on gelatinization and pasting of starch in wheat flour. It is also unclear whether the lipids associated with the starch granule surface induce effects similar to those of lipids not occurring at the starch granule surface, *e.g.* those present in spherosomes or associated with gluten in flour.

To study whether selective alteration of the endogenous lipid population affects wheat flour behaviour in a starch gelatinization, pasting and gelation cycle, three different lipases with known impact on the lipid composition, *i.e.* Lecitase Ultra, Lipopan F and Lipolase (Gerits, Pareyt, & Delcour, 2014) were applied. Lipases selectively alter endogenous lipids and thereby increase the amount of surfactants present without altering other flour constituents. An overview of the action mechanisms and cleavage sites of different lipases is given in Gerits, Pareyt, Decamps, and Delcour (2014). More in particular, Lecitase Ultra and Lipopan F mainly hydrolyse galactolipids and to a lesser extent phospholipids in the bound lipid fraction, thereby increasing the level of 'lyso' lipids and free fatty acids (FFA). Lipolase mainly acts on triacylglycerols (TAG) in the free lipid fraction, and thereby releases FFA. Free and the bound lipid fractions are defined based on their sequential extraction from wheat flour or dough with hexane (free lipids) and water saturated butanol (bound lipids) (Gerits, Pareyt, & Delcour, 2014). The changes in Rapid Visco Analyser (RVA) profiles and differential scanning calorimetry (DSC) AP crystallite thermograms upon lipase use were compared to those observed upon addition of surfactants [diacetyl tartaric esters of mono- and diacylglycerols (DATEM) and sodium stearoyl lactylate (SSL)]. Both surfactants are able to form lamellar mesophases in water at room temperature (Krog, 1981). The swelling power (SP) and carbohydrate leaching (CHL) of starch in wheat flour either supplemented with the lipases or surfactants or not, were analysed as well.

2. Materials & methods

2.1. Materials

Grains from soft wheat cultivar Claire were from Limagrain (Rothwell, UK) and conditioned to 16.0% moisture before milling with a Bühler (Uzwil, Switzerland) MLU-202 laboratory mill using the milling diagram outlined in Delcour, Vanhamel, and De Geest (1989). The milling yield of straight grade flour was 69.4%, and its moisture and protein contents were respectively 14.8% and 10.3% [on dry matter basis]. The latter were determined with AACCI Approved Method 44-19.01 (AACCI, 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, respectively. Its lipid composition is depicted in Gerits, Pareyt, and Delcour (2013). The enzyme preparations were kindly donated by Novozymes (Bagsvaerd, Denmark). Lipopan F, a *Fusarium oxysporum* enzyme preparation, is used in bread making as a source of lipase and phospholipase activities. Lecitase Ultra is a phospholipase used in edible oil degumming. It is the result of combining homologous genes encoding *Thermomyces lanuginosus* lipase and *Fusarium oxysorum* phospholipase (De Maria, Vind, Oxenboll, Svendsen, & Patkar, 2007). Lipolase, a recombinant *T. lanuginosus* lipase is used in detergent systems (Aravindan, Anbumathi, & Viruthagiri, 2007). Lecitase Ultra, Lipopan F and Lipolase had lipase activities against *p*-nitrophenyl palmitate of 0.15 units (U), 56.50 U and 0.12 U, respectively, with one U being the amount (in μmole) of *p*-nitrophenol released per minute and per mg enzyme under the conditions of the assay (Gerits, Pareyt, Decamps, et al., 2014; Gerits, Pareyt, & Delcour, 2014). How the lipases affect wheat lipid composition during bread making was studied in Gerits, Pareyt, and Delcour (2014). DATEM and SSL were from Puratos (Groot-Bijgaarden, Belgium). All solvents used were from VWR (Haasrode, Belgium) unless specified otherwise and of at least analytical grade.

2.2. Methods

2.2.1. Dough making

Dough was made according to Shogren and Finney (1984) on 10 g scale but without 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 water added and the optimal mixing time were determined by Mixograph analysis (National Manufacturing) according to AACCI Approved Method 54-40.02 (AACCI, 1999) and were respectively 5.1 ml and 150 s. Lipases (Lecitase Ultra, Lipopan F or Lipolase), SSL or DATEM were included in the recipe in levels of 0.5 and 5 mg enzyme protein (EP) lipase/kg flour and 0.5% surfactant (dry powder, on flour basis), respectively.

2.2.2. Differential scanning calorimetry

DSC analysis was performed with a Q1000 DSC (TA instruments, New Castle, DE, USA). At least three fermented (120 min at 30 °C, to allow for enzyme activity) dough samples (4.0–7.0 mg) were accurately weighed into aluminium pans (Perkin Elmer, Waltman, MA, USA). Deionized water was added in a ratio of 1:3 w/w sample dry matter: water. The pans were hermetically sealed and equilibrated at 0 °C before heating to 140 °C at 4 °C/min (together with an empty reference pan). The system was calibrated with indium. Onset and conclusion temperatures and enthalpies (J/g sample) of AP crystallite melting and dissociation of the AM-L complexes [96–100 °C for amorphous and 105–125 °C for semi-crystalline complexes (Karkalas, Ma, Morrison, & Pethrick, 1995)] of fermented control dough or fermented dough containing lipase (0.5 and 5.0 mg EP/kg flour) or surfactant (0.5% on flour basis) were calculated with TA Instruments Universal Analysis software.

2.2.3. Rapid visco-analysis

Swelling, pasting and gelation of starch in wheat flour was studied with a Rapid Visco Analyser (RVA-4D, Newport Scientific, Sydney, Australia). Flour suspensions [12.0% dm, *i.e.* above the close packing concentration (C*, *cfr. infra*), in deionized water (total weight 25.0 g)] were prepared in duplicate with or without added lipases (0.5, 1.0 and 5.0 mg EP/kg flour) or surfactants [0.5 and 1.5% (on flour basis) DATEM or SSL]. The suspensions were subjected to a time–temperature profile which consisted of an incubation step of 10 min at 30 °C (to allow for lipase activity), a heating step to 95 °C at 3.25 °C/min, an isothermal step at 95 °C for 5 min, a cooling step to 50 °C at 4.5 °C/min and a final isothermal step at 50 °C for 10 min. The stirring speed was 160 rpm.

2.2.4. Determination of swelling power and carbohydrate leaching

Swelling power (SP) of wheat flour at three different temperatures and with or without addition of 5.0 mg EP Lecitase Ultra or Lipolase/kg flour or 1.5% (on flour basis) SSL or DATEM were determined according to Eerlingen, Jacobs, Block, and Delcour (1997). Prior to analysis, wheat flour suspensions (100 mg in 9.0 ml deionized water) were incubated at room temperature for 120 min to allow for lipase action. Thereafter, the suspensions were heated at 45 °C, 75 °C or 95 °C for 30 min, with shaking every 5 min. Samples were allowed to cool for 5 min and centrifuged for 30 min at 1000 *g* at 20 °C. Analyses were performed in triplicate. Carbohydrate leaching (CHL) was determined on the supernatant as in Dubois, Gilles, Hamilton, Rebers, and Smith (1956) and expressed as a percentage of total dry matter starch. SP and the C*,