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Impact of lipases with different substrate specificity in wheat flour separation on the properties of the resultant gluten

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ABSTRACT

Wheat gluten was isolated in a laboratory dough-batter flour separation process in the presence or absence of lipases differing in hydrolysis specificity. The obtained gluten was blended with wheat starch to obtain gluten-starch (GS) blends of which the water and oil binding capacities were investigated. Furthermore, GS blends were mixed into dough and processed into model breads, of which dough extensibility and loaf volume were measured, respectively. In comparison to GS blends prepared with control gluten, oil binding capacity was higher when GS blends contained gluten isolated with Lecitase Ultra (at 5.0 mg enzyme protein/kg flour), a lipase hydrolyzing both non-polar and polar lipids. Additionally, dough extensibility and total work needed for fracture were lower for dough prepared from GS blends containing gluten isolated with Lipolase (at 5.0 mg enzyme protein/kg flour), a lipase selectively degrading non-polar lipids. In GS blend bread making, this resulted in inferior loaf volumes. Comparable GS blend properties were measured when using control gluten and gluten isolated with YieldMAX, a lipase mainly degrading N-acyl phosphatidylethanolamine. In conclusion, properties of GS blend model systems are altered when gluten prepared in the presence of lipases is used to a degree which depends on lipase specificity and concentration.

1. Introduction

Gluten is the cohesive viscoelastic mass formed when the storage protein of wheat is mixed with water. It is also a co-product of the isolation of starch from wheat flour (Day et al., 2006; Delcour and Hoseney, 2010; Van Der Borght et al., 2005). Commercial gluten typically contains approximately 75% protein, 8% moisture and varying levels of starch, lipid and fiber. The protein fraction consists of approximately equal portions of gliadin and glutenin (Day et al., 2006). Gliadin is a monomeric protein fraction. It is extractable in aqueous ethanol. Glutenin consists of large polymers made up of disulfide-linked glutenin subunits. A significant portion of glutenin is extractable in dilute acid or alkali. Upon reduction of disulfide bonds, glutenin subunits are liberated which are, like gliadin, extractable in aqueous ethanol (Delcour and Hoseney, 2010).

In bread dough preparation, gliadin and glutenin form a viscoelastic network that is responsible for gas retention during fermentation and baking (Delcour and Hoseney, 2010). Gliadin is sticky when hydrated and shows little resistance to extension, whereas glutenin is resilient, rubbery and easily breaks upon extension (Delcour and Hoseney, 2010; Wrigley et al., 2006). Dough needs to be sufficiently extensible to respond to increases in gas pressure but also strong enough to resist collapse (Sroan et al., 2009). The balance between gliadin, responsible for dough viscosity and cohesiveness, and glutenin, providing the dough with elasticity, is of major importance in bread making (Delcour and Hoseney, 2010; Wrigley et al., 2006). In addition, gluten can bind fat and water, is insoluble in water and hydrophobic (Day et al., 2006).

Depending on the production process and, more importantly, the conditions during drying, dried and powdered gluten that either fully or only partially retains its functional properties upon hydration is produced. In literature, the term 'vital wheat gluten' is frequently used to refer to dried gluten of which the functional properties are restored upon hydration. It is an important ingredient of various baked products and cheese substitutes. 'Non-vital

Abbreviations: DATEM, diacetyltartaric acid esters of mono- and diacylglycerols; dm, dry matter; EP, enzyme protein; FFA, free fatty acids; GS, gluten-starch; NAPE, N-acyl phosphatidylethanolamine; RT, room temperature.

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wheat gluten', on the other hand, upon hydration regains the functional properties of vital wheat gluten except for its viscoelastic properties. It is used in applications where the cohesiveness of vital wheat gluten is either not important or disadvantageous, such as in various meat, fish and poultry applications, simulated meat for canned pet foods or aquaculture feed (Day et al., 2006).

Wheat flour contains approximately 2–3% lipids, of which 30–40% are starch lipids located in the granular starch and 60–70% are non-starch lipids (Pareyt et al., 2011; Pomeranz et al., 1966). Interactions between non-starch lipids and gluten protein have been widely investigated, especially in a bread making context, and it is generally accepted that they impact on gluten characteristics (Carr et al., 1992; Olcott and Mecham, 1947). During dough making, lipids strengthen the gluten network by interacting with gluten protein, a phenomenon referred to as 'lipid binding' (Chung and Tsen, 1975; Köhler, 2001; Pomeranz and Chung, 1978). However, more recent literature has shown that wheat endogenous lipids, when present at their naturally occurring levels, have no impact on dough rheology but influence bread quality through interfacial gas cell stabilization during fermentation and early baking (Sroan et al., 2009; Sroan and MacRitchie, 2009). In contrast, surfactants such as diacetyltartaric acid esters of mono- and diacylglycerols (DATEM) increase dough resistance to extension (Gerits et al., 2015; Selmair and Koehler, 2008). DATEM is commonly added during bread making to improve dough strength, stability and handling (Pareyt et al., 2011; Stampfli and Nersten, 1995).

Recently, our research group demonstrated that wheat endogenous lipids play an important role in the separation of wheat flour into gluten and starch. Indeed, lipases influence gluten agglomeration and yield to a degree which depends on their hydrolysis specificity and concentration. Lipolase, a lipase which selectively degrades non-polar lipids, can be used to improve gluten agglomeration and yield. The improving effect seems to be caused by binding of mainly free fatty acids (FFA) to gluten protein. Lecitase Ultra, which degrades both non-polar and polar lipids, or YieldMAX, which mainly degrades N-acyl phosphatidylethanolamine (NAPE), had no or a negative impact on gluten agglomeration and yield (Melis et al., 2017).

To the best of our knowledge, the impact of wheat endogenous lipid hydrolysis during gluten-starch (GS) separation on gluten properties was never investigated. We here combined gluten isolated in a dough-batter wheat flour separation process in the presence or absence of the above lipases with commercial wheat starch to obtain GS blends as a model for wheat flour (Lee et al., 2001; Pauly et al., 2014; Uthayakumaran et al., 2002) and assessed whether the resultant GS blends differ in functional properties. We report on GS blend water and oil binding capacity, dough extensibility and model bread loaf volume.

2. Materials and methods

2.1. Materials

Grains from the wheat cultivar Apache were from Dossche Mills (Deinze, Belgium) and conditioned to 16.0% moisture before milling with a Bühler MLU-202 laboratory mill (Uzwil, Switzerland) with the milling scheme outlined in Delcour et al. (1989). Milling yield of straight grade flour was 76.8%. Its moisture level, determined with AACCI Approved Method 44–15.02 (1999), was 14.1%. Flour ash and protein levels [on dry matter (dm) base] were 0.54% and 9.9%, respectively. The former was determined with AACCI Approved Method 08–01.01 (1999), the latter with an adaptation of the AOAC Official Method (AOAC, 1995) to an automated Dumas protein analysis system (EAS Vario Max Cube CN, Hanau, Germany) with 5.7 as nitrogen to protein conversion factor. Lecitase Ultra, Lipolase and YieldMAX were kindly donated by Novozymes (Bagsvaerd, Denmark) in purified form and contained neither amylase, peptidase nor xylanase side activities. Lecitase Ultra is a phospholipase A_1 which is applied in degumming of edible oils (Casado et al., 2012). It results from combining homologous genes encoding *Thermomyces lanuginosus* lipase and *Fusarium oxysporum* phospholipase. It is described as having the stability of the former and the activity of the latter (Bojsen et al., 2000). Lipolase is a *T. lanuginosus* lipase (Aravindan et al., 2007) applied in the detergent industry. YieldMAX is a phospholipase A_1 from *Fusarium* sp. It is used to increase yield during cheese production (Casado et al., 2012). Lipase activities towards *p*-nitrophenyl palmitate of Lecitase Ultra, Lipolase and YieldMAX were 1.2×10^0, 3.7×10^{-1} and 5.7×10^{-4} μmol *p*-nitrophenol released/[min*mg enzyme protein (EP)], respectively (Melis et al., 2017). Commercial wheat starch was from Syral (Aalst, Belgium) and had moisture and protein levels of 12.3% and 0.46% of dm, respectively (determined as described above). Sugar, salt, fresh compressed baker's yeast (Koningsgist, AB Mauri, Dordrecht, The Netherlands) and sunflower oil were purchased at a local supermarket. Solvents, reagents and chemicals were from VWR Chemicals (Haasrode, Belgium) and at least analytical grade.

2.2. Methods

2.2.1. Wheat flour separation

Gluten was isolated in triplicate from wheat flour without (control) or with 0.5 or 5.0 mg EP Lecitase Ultra, Lipolase and YieldMAX per kg flour as in Melis et al. (2017). Briefly, wheat flour (250 g, 14.0% moisture base) was mixed for 4 min with 140 mL 15 mM NaCl [based on the Farinograph water absorption (AACCI Approved Method 54–21.02, 2011)] in a KitchenAid mixer (KPM5, St. Joseph, MI, USA) equipped with a dough hook. Lipases were added at the beginning of mixing at 0.5 and 5.0 mg EP/kg flour by dissolving them in the 140 mL 15 mM NaCl used for mixing. The mixed dough was allowed to rest for 8 min and then transformed into batter by adding 250 mL 15 mM NaCl and stirring with a flat beater for 25 min. Subsequently, 1.0 L 15 mM NaCl was added and the suspension was further stirred for 35 min. It was then sieved over a set of vibrating (100 Hz) sieves with a diameter of 20.0 cm and decreasing pore sizes (400, 250 and 125 μm). The retained gluten was washed three times with 1.0 L 15 mM NaCl in total. Gluten fractions were recovered from the three sieves, lyophilized and ground with a laboratory mill (model A10, IKA-Werke KG, Staufen, Germany) to pass a 250 μm sieve.

2.2.2. Preparation of GS blends

The above obtained gluten fractions were reconstituted in their original ratio (§ 2.2.1) and blended with commercial wheat starch to obtain GS blends with a protein level of 12.0% dm. Blending was overnight by end-over-end shaking. Three GS blends containing a specific 'type' of gluten (isolated either without or with lipase addition) were prepared as gluten was isolated in triplicate from wheat flour without (control) or with 0.5 or 5.0 mg EP Lecitase Ultra, Lipolase and YieldMAX per kg flour (§ 2.2.1).

2.2.3. Protein extractability in dilute salt and aqueous alcohol media

The extractability of GS blends in dilute salt and aqueous alcohol media was determined based on Osborne type fractionation. GS blend (containing 100 mg protein) was weighed in duplicate in centrifuge tubes containing 10 glass pearls. First, samples were mixed with 3.00 mL 50 mM sodium phosphate buffer (pH 7.6) containing 0.4 M NaCl, shaken [10 min, 150 rpm, room temperature (RT)], and centrifuged (10 min, 10,000 g, RT). The supernatant was retained. The extraction was repeated twice on the remaining pellet