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The impact of disulfide bond dynamics in wheat gluten protein on the development of fermented pastry crumb

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ABSTRACT

Gluten proteins functionality during pastry production was examined by including redox agents in the ingredient bill. Addition of reducing and oxidizing agents respectively increased and decreased dough height during fermentation. The presence of large gas bubbles in the samples with oxidizing agents may have caused a 'stacking'-effect and a more effective dough lift. During baking, the level of extractable proteins decreased to comparable values for all samples, except when potassium iodate (KIO_3) was used in the recipe. As a result of its use, a lower level of gliadin was incorporated into the gluten polymer and dough layers tended to 'slide' apart during baking, thereby causing collapse. Most likely, KIO_3 caused glutenin oxidation within each individual dough layer to such extent during the dough stage that insufficient thiol groups were available for forming dough layer interconnections during baking, after margarine melting. Furthermore, addition of redox agents impacted the product's crumb structure.

1. Introduction

Gluten proteins make up 80–85% of the total wheat flour protein (Lagrain, Brijs, & Delcour, 2008). They consist of monomeric gliadin with a molecular weight (MW) of 30,000–60,000 and polymeric glutenin proteins with MWs ranging from 80,000 to several million (Delcour et al., 2012; Shewry, Halford, & Tatham, 1992; Wieser, 2007). Gliadins are a mixture of proteins which are subdivided into α-, γ- and ω-gliadin (Lagrain et al., 2008). The respectively 6 and 8 cysteine (CSH) residues of α- and γ-gliadin are involved in 3 and 4 intramolecular disulfide (SS) bonds. ω-Gliadins lack CSH residues (Lagrain et al., 2008; Shewry & Tatham, 1997). Glutenin polymers consist of high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits (GS) which are linked together by SS bonds and are set free under reducing conditions (Bruneel, Lagrain, Brijs, & Delcour, 2011). Glutenin not only contains these intermolecular SS bonds, it also contains intramolecular SS bonds (Delcour et al., 2012; Gianibelli, Larroque, MacRitchie, & Wrigley, 2001). The intermolecular SS bonds are of utmost importance in the development of a three-dimensional network (Oak et al., 2006). Two types of reactions are important for protein network formation during bread baking: (1) the oxidation of free thiol (SH) groups to SS bonds, which increases the MW of the glutenin aggregates (Bruneel et al., 2011; Wieser, 2003), and (2) SH-SS exchange reactions, which involve cleavage and reformation of SS bonds (Schofield, Bottomley, Timms, & Booth, 1983). The latter are initiated either by LMW SH compounds or by free SH groups in gluten proteins (Dong & Hoseney, 1995). Heat-induced SH/SS interchange reactions start at temperatures of 50 °C for isolated gluten in excess water (Schofield et al., 1983) and yield intermolecular SS bonds between glutenin. At higher temperatures, SH/SS interchange reactions also occur between gliadin and glutenin. These incorporate gliadin into the gluten network structure and occur at temperatures exceeding 70 °C for isolated gluten (Schofield et al., 1983) and 90 °C (Lagrain et al., 2008) or even 100 °C (Singh & MacRitchie, 2004) during bread baking. As they lack cysteine residues, ω-gliadins are not incorporated in the gluten network through SS bonds (Schofield et al., 1983; Singh, 2005).

Abbreviations: AA, ascorbic acid; CSH, L-cysteine; EA & G, extractable albumin and globulin proteins; EGLI, extractable gliadin proteins; EGLU, extractable glutenin proteins; EP, extractable proteins; GS, glutenin subunits; HMW, high molecular weight; LMW, low molecular weight; SDS, sodium dodecyl sulfate; SH, sulfhydryl group; SS, disulfide

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The role of gluten proteins in laminated dough systems used for producing puff and fermented pastry products has not been studied in detail. Laminated dough is made up of alternating layers of dough and bakery fat (Hay, 1993). Successive sheeting and folding of a margarine sheet between two dough layers yields a multi-layered system (Bent, 2007; Cauvain & Young, 2009).

In bread making, the ability of the gluten network to retain gas during fermentation and baking is crucial for proper dough expansion (Gan, Ellis, & Schofield, 1995; Scanlon & Zghal, 2001). Indeed, the gluten network is the primary mechanism which stabilizes the expanding gas bubbles (Sroan & MacRitchie, 2009). However, in fermented pastry production, another mechanism of retaining gas is said to be in play, although to date no concrete evidence for it has been provided (Deligny & Lucas, 2015). It invokes that steam is entrapped in the layered structure during baking because the fat layers are impermeable to water vapour (Deligny & Lucas, 2015; Wickramarachchi, Sissons, & Cauvain, 2015). However, as recently reviewed and discussed (Ooms, Pareyt, Brijs, & Delcour, 2016; Wickramarachchi et al., 2015), this explanation somewhat ignores the many physical and chemical transformations during pastry baking. Indeed, based on what is known for bread dough, gluten proteins may also play a vital role in gas retention during pastry baking. In this context, Hay (1993) already reported a positive relation between flour protein content and the height and specific volume of puff pastry.

In this study, redox agents were used to selectively impact on gluten oxidation and exchange reactions during pastry production. Ascorbic acid (AA), a widely used bread flour improving agent, increases dough strength (Aamodt, Magnus, & Faergestad, 2003). KIO_3, a fast-acting oxidizing agent, affects the flour protein SH-SS system and can thus increase gluten cross-linking and dough strength (Goesaert et al., 2005). The reducing agent CSH weakens the dough structure by destruction/reduction of intermolecular SS bonds (Joye, Lagrain, & Delcour, 2009). A similar approach has been used recently for studying the role of gluten proteins in laminated dough (Ooms et al., 2017). In this paper, we therefore focus on gluten protein functionality during fermentation and baking of (model) pastry products.

2. Materials and methods

2.1. Materials

Commercial wheat flour (protein level: 13.6%, dry basis; moisture content: 13.7%) was provided by Vandemoortele (Izegem, Belgium). Its protein content was determined with an adaptation of the AOAC Official Method 22 to an automated Dumas protein analysis system (EAS Vario Max C/N, Elt, Gouda, The Netherlands) with 5.7 as the nitrogen to protein conversion factor. Its moisture content was analysed according to AACC International Approved Method 44-15.02.23. Palm-oil based margarine (80% fat, free from emulsifiers) was used as in-dough and roll-in fat, and also provided by Vandemoortele. All reagents, solvents, and chemicals were of analytical grade and obtained from Sigma-Aldrich (Bornem, Belgium) unless indicated otherwise.

2.2. Pastry making

The laboratory scale pastry making procedure used was described in Ooms et al. (2017). Laminated dough (ideally containing 16 fat layers) was sheeted to its final thickness. Dough cylinders (18) were cut from the laminated dough sheet using a circular cutter (diameter 62.5 mm) and fermented at 32 °C and 95% relative humidity for 75 min. The fermented dough pieces were baked in a floor oven (top temperature 210 °C, bottom temperature 220 °C, 16 min). Baked products were cooled for 60 min prior to analyses.

2.3. Inclusion of redox agents in the recipe

AA and KIO_3 were directly included in the recipe [13.96 and 2.30 mmol/g protein respectively (i.e. 300 mg/kg and 60 mg/kg on flour base)]. CSH was added [2.37 mmol/g protein (i.e. 35 mg/kg on flour base)] as an aqueous solution previously sparged with N_2 to avoid oxidation and such that the same amount of water was used in the control and CSH containing recipe.

2.4. Microscopic imaging

During fermentation of control dough, samples (1 cm × 1 cm) were cut out every 5 min, snap-frozen in 2-methylbutane (Merck, Overijse, Belgium) at a temperature of −40 °C to avoid freezing artefacts, and stored at −78 °C on dry ice. Within 6 h after sample collection, the pieces were sliced using a cryo-microtome (Thermo Fisher Scientific, Waltham, MA, USA) cooled to −20 °C. They were first stuck on a sample holder using Tissue-Tek® O.C.T. Compound (Sakura Finetek Belgium, Antwerpen, Belgium) and then trimmed down to obtain a smooth sample surface. Sections of 20 µm thickness were then cut. Three slices per sample were deposited onto a glycerol-albumin-coated slide. The samples were fixated by immersing the slide into 4% paraformaldehyde fixative for 10 min. Imaging was done with a Nikon (Melville, NY, USA) ECLIPSE 80i epifluorescence microscope.

2.5. Rheofermentometer analysis

Dough development, total gas volume produced and gas volume retained in dough over time were evaluated with rheofermentometer analysis (Chopin, Villeneuve La Garenne, France). Laminated dough pieces were cut out to the exact size of the fermentation vessel, thus covering its entire base. No additional weight was placed on top of the piston. The fermentation temperature and time were 32 °C and 240 min, respectively.

2.6. Monitoring of sample baking

During baking, samples were filmed using a digital camera (Canon Powershoot S50, Machelen, Belgium) mounted on the outside of the oven. A metal yardstick was used to determine pastry height. Measurements were performed with four dough pieces per sample. Pastry crumb temperature was monitored using a Multipaq21 Temperature logger (Datapaq, Fluke Process Instruments, Cambridge, UK).

2.7. Determination of the level of proteins extractable in sodium dodecyl sulfate containing medium

Pastry crumb samples were withdrawn after 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14 and 16 min of baking, freeze-dried, ground and subsequently defatted. To that end, samples (1.0 g) were shaken with 10.0 ml hexane in a 30.0 ml glass test tube for 15 min. The hexane phase was then decanted and the procedure repeated twice. After the final extraction, samples were left under the fume hood overnight to ensure complete evaporation of remaining hexane. Proteins were extracted from the samples (1.0 mg dry matter protein/ml) with 0.05 M sodium phosphate buffer (pH 6.8) containing 2.0% sodium dodecyl sulfate (SDS). Size-exclusion high performance liquid chromatography (SE-HPLC) was conducted using an LC-2010 HT system (Shimadzu, Kyoto, Japan) with automatic injection. The extracts (60.0 µl) were loaded on a Biosep-SEC-S4000 column (Phenomenex, Torrance, CA, USA). The SDS containing extraction buffer was used as the elution solvent. The flow rate was 1.0 ml/min at 30 °C and protein detection was at 214 nm. The elution profiles were divided into three fractions using the lowest absorbance reading between the peaks as the cut-off point. The fractions were denoted as glutenin (SDS-EGLU), gliadin (SDS-EGLI) and albumin

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