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Changes in protein size distribution during wheat flour cake processing

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ABSTRACT

Aggregation of egg and wheat proteins during cake mixing and baking was monitored by SE-HPLC after sequential extraction of dough and baked cakes in SDS-buffer first and then in SDS/DTE buffer. Three cake recipes were compared, including either only egg, only flour, or both flour and egg proteins. Dough mixing did not result in any changes in protein solubility or size distribution. Baking promoted protein aggregation and quickly resulted in the solubility loss of all proteins within the first extracting solvent with the exception of wheat omega gliadins. Upon baking similar kinetics of proteins solubility loss in SDS-buffer were observed regardless of cake recipes. Extraction of the remaining SDS-insoluble proteins with SDS/DTE buffer allowed total protein recovery but only in the case of cakes made on the basis of only flour. For cakes including eggs and despite the presence of DTE a disulfide reducing agent, very large polymers were release into solution, contrarily to the only flour cakes where only small polypeptides (>70,000 g/mol) were mostly recovered. Protein sequential extraction combined with SE-HPLC analysis highlighted the critical role of egg proteins in triggering wheat and egg proteins complexation into SDS-insoluble aggregates stabilized through disulfide but also non-reducible covalent bonds.

1. Introduction

Like bread, cakes are among the cereal products mainly composed of wheat flour (30–40% dry weight), and characterized by a soft and elastic alveolar crumb. The setting of the alveolar structure takes place during baking, as the viscous and foamy cake batter transforms into an expanded and porous soft gel.

Although it has long since been established that both proteins and starch play a major role in cereal product texture (Donelson & Wilson, 1960), their respective roles still require investigation to fully understand the mechanisms involved.

In bread-making, proteins only come from wheat flour, and interact during mixing, resting and baking to form a gluten network. Mixing flour/water blends allows for protein hydration while mechanical shear enables gluten network development by stretching and distributing the gluten evenly within the dough. Gluten network is mainly responsible for dough viscoelasticity, gas retention capacity and, after cooling, crumb elasticity. Dough mixing energy input, as well as the quality of the flour proteins, impacts the structure of the gluten network and the subsequent physical and chemical properties of the dough. Several authors have shown that gluten proteins, *i.e.* gliadins and glutenins, are responsible for the bread-making quality of flour. Since gluten proteins represent 80–85% of the total flour proteins, the flour protein content is most often considered as the first wheat flour quality criterion in bread-making. However, the glutenin macro-polymer content would be a better parameter to predict the bread-making quality of flour (Weegels, van de Pijpekamp, Graveland, Hamer, & Schofield, 1996).

In cake batter, gluten proteins are more diluted into the matrix, and the mixing step is far shorter. Thus, it is still unclear if a gluten network is formed or even initiated in cake batter during mixing. In particular the impact of cake batter mixing on flour protein size distribution remains undocumented, while for bread-making a strong depolymerization effect of mixing is well established

Abbreviations: BCS, sodium bicarbonate; SAPP, sodium acid pyrophosphate; SDS, Sodium Dodecyl Sulfate; IAM, iodoacetamide; DTE, dithioerythritol.

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(Weegels, Hamer, & Schofield, 1997, 1996). Moreover, in cake-making, wheat flour is not the only ingredient providing proteins: eggs also matter and represent from 30 to 50% of the total protein content of pound cakes. Egg proteins are known to be thermosensitive, and thus are supposed to play a major role in cake texture setting. In 1976, Shepherd and Yoell proposed a model cake structure in which starch granules play the role of bricks, and egg proteins the role of mortar. This mortar would be a composite, involving ovalbumin as a flexible cementing agent, and ovomucin as a non-denaturable fibrous protein, but its structure remains unclear (Donovan, 1977). More recently, it has been shown that cake cell walls are be made of a combination of a protein network involving both egg and gluten proteins, built upon baking, and a starch gel, set during cooling (Deleu, Wilderjans, Van Haesendonck, Courtin, Brijs, Delcour, 2015; Wilderjans, Luyts, Goesaert, Brijs, & Delcour, 2010). However, the specific roles of egg proteins and gluten in cake batter and then crumb structure are still currently unclear.

The SE-HPLC technique, combined to protein SDS extraction, was successfully developed by Singh, Donovan, Batey, & MacRitchie (1990) for wheat flour protein characterization. Morel, Dehlon, Autran, Leygue, and Bar-L'Helgouac'h (2000), improved the technique's reproducibility and proposed to divide the SE-HPLC profile of flour SDS soluble proteins into 5 fractions, which are respectively large-size glutenin macropolymers (F1: 800,000–1,700,000 g/mol), small glutenin macropolymers (F2: 800,000–120,00 g/mol), ω gliadin (F3), and then γ and α/β gliadins (F4), and latterly salt-water-soluble wheat proteins (Morel et al., 2000). In 1999, Huebner, Bietz, Nelsen, Bains, & Finney used SE-HPLC to evaluate the cookie-making quality of several soft wheat flours. They assumed that, despite finding no strong correlation between flour protein composition and cookie quality, soft flours with higher contents of glutenin macropolymers give cookies an overall better quality. As observed for bread, glutenin macropolymers may play a role in biscuit quality. In 2008, Wilderjans, Pareyt, Goesaert, Brijs, & Delcour used SE-HPLC to compare the protein extractabilities of batter and cake according to their gluten contents. They found that when more gluten is added to cake batter, protein SDS extractability decreases further after baking while cake quality increases. Gluten may therefore be presumed to play a key role in cake texture.

In light of these investigations, SE-HPLC appears to be a promising tool for monitoring protein changes (polymerization and aggregation) during soft cake processing steps (mixing and baking) and therefore, a better understanding of the specific roles of flour and egg proteins in cake structure building. The aim of this study was to validate SE-HPLC analysis of cake proteins as an effective tool to study the interactions between egg and flour proteins during cake batter mixing and baking.

2. Materials and method

2.1. Materials

Apache bread wheat flour (13.5% moisture content, 8.6% protein content d.b., 0.42% ash content), pasteurized eggs (10.6% protein w.b., 76% water content), emulsifiers (containing 9% of milk proteins, d.b.), sucrose, glucose syrup (19.7% moisture content), rapeseed oil, glycerol (0.5% moisture content), salt and raising agents were all provided by Mondelēz International. Native wheat starch (12.2% moisture content, 0.4% gluten) was supplied by Cargill, USA. Moisture contents were determined according to AACC Approved Method 44-19. The protein contents of flour, eggs and emulsifiers were determined from triplicate measurements using Kjeldhal procedure. Nitrogen-to-protein conversion factors of 5.7 for wheat flour samples and 6.25 for egg and emulsifier samples were applied.

2.2. Cake batter preparation

The standard recipe was prepared as follows: 35.0% of wheat flour, 13.5% of eggs, 8.0% of rapeseed oil, 10.5% of additional water, 12% of sucrose, 12% of glucose syrup, 6% of glycerin, 1.6% of emulsifiers, 0.2% of salt and 1.2% of raising agents. The egg-free cake batter was prepared with the same ingredients except that eggs were replaced by their water equivalents in order to keep constant the batter moisture content. The gluten-free recipe was prepared by replacing the 350 g of flour by 11 g of water and 312 g of native wheat starch, in order to keep constant both starch and water contents in the gluten-free cake batter. All the cake batters were prepared at 20 °C using a planetary Hobart N50 5-Quart mixer with a flat beater paddle (Hobart Corporation, Troy, OH, USA). Powders were homogenized before adding all soluble ingredients except rapeseed oil. Mixing was started for 2.5 min at medium speed, before adding the oil and mixing one minute more.

2.3. Cake batter baking kinetics

After a resting time of 30min, the cake batter was poured into 15 metallic pans of 80 × 45 mm (40 g of batter each) and baked at 180 °C for 25min. The batters' core temperatures all increased from 20 °C to 90 °C in approximately 10min, then remained at 90 °C due to their high internal moisture contents. The crumb temperature increased a little faster in the case of the gluten-free batter (90 °C after 7.5min of baking), probably due to the lower batter viscosity and the lower air bubble content. Every 5min, 3 cakes were removed from the oven, one for temperature measurements and two for HPLC analysis sampling. In order to limit thermal fluctuation during baking, the number of sampling (and so the oven door opening) was limited to five. Two different batter batches were prepared in order to obtain 3 × 10 samples, covering a 25 min baking period.

2.4. SE-HPLC characterization

The batter protein extraction procedure was adapted from Morel et al. (2000). Eighty milligrams of freeze-dried and grinded cake batter were dispersed for 80 min at 20 °C, in 10 ml of 1% SDS-phosphate buffer (pH 6.8) containing 5 mM iodo-acetic amide (IAM). After centrifugation (30 min, 18 000 rpm) the supernatant (SDS-soluble protein extracts) was collected and stored at −20 °C before SE-HLPC analysis. The pellet was re-suspended in 5 ml of 1% SDS-phosphate buffer (pH 6.8) including 40 mM DTE for 1 h at 60 °C and sonicated for 5 min (30% power). The supernatant (DTE-soluble protein extracts) were collected after centrifugation and stored as above. SE-HPLC analysis of the samples (20 μL) was performed on an Alliance system (Waters) using a tandem of TSK G4000-SWXL (7.8 × 300 mm) and TSK SWXL (6 × 40 mm) columns, both from Tosoh Biosep. Elution was performed at 0.7 mL/min with 0.1% SDS, 0.1 M sodium phosphate buffer (pH 6.8) and 214 nm was used for protein detection. The total protein extraction yield was estimated from the sum of the area of the SDS-soluble and the DTE-soluble chromatography profiles after correction for the difference in solid to solvent ratios. The un-extractable protein fraction remaining in the last pellet was estimated in reference to the results obtained for the mixed batter for which the extraction yield was set to 100%. Cake ingredients (flour, egg and emulsifier) were similarly analyzed, except that solid to solvent ratios corresponding to their specific contribution to the total dry mass of the standard batter recipe were used for extraction. The apparent molecular