Accepted Manuscript

Changes in protein size distribution during wheat flour cake processing

M. Dewaest, C. Villemejjane, S. Berland, C. Michon, A. Verel, M.-H. Morel

PII: S0023-6438(17)30037-3

DOI: [10.1016/j.lwt.2017.01.036](https://doi.org/10.1016/j.lwt.2017.01.036)

Reference: YFSTL 5988

To appear in: *LWT - Food Science and Technology*

Received Date: 9 August 2016

Revised Date: 12 December 2016

Accepted Date: 13 January 2017

Please cite this article as: Dewaest, M., Villemejjane, C., Berland, S., Michon, C., Verel, A., Morel, M.-H., Changes in protein size distribution during wheat flour cake processing, *LWT - Food Science and Technology* (2017), doi: 10.1016/j.lwt.2017.01.036.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Comment citer ce document :

Dewaest, M., Villemejjane, C., Berland, S., Michon, C., Verel, A., Morel, M. H. (Auteur de correspondance) (2017). Changes in protein size distribution during wheat flour cake processing. *LWT - Food Science and Technology*, 79, 333-341. DOI : 10.1016/j.lwt.2017.01.036

Changes in protein size distribution during wheat flour cake processing

M. DEWAEST^{1,2}, C. VILLEMEJANE¹, S. BERLAND¹, C. MICHON¹, A. VEREL², M.-H. MOREL³

1 – UMR Ingénierie Procédés Aliments, AgroParisTech, Inra, Université Paris-Saclay, 91300 Massy, France

2 - Mondelēz International, 91400 Saclay, France

3 - INRA, UMR1208-IATE Ingénierie des Agropolymères et Technologies Emergents, Montpellier, France

e-mail address

marine.dewaest@agroparistech.fr

cindy.villemejane@agroparistech.fr

sophie.berland@agroparistech.fr

camille.michon@agroparistech.fr

aliette.verel@mdlz.com

morel@supagro.inra.fr

Corresponding author: Marie-Hélène Morel.

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

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.

Keywords: soft cake, baking, protein thermal aggregation, protein SE-HPLC, iso-peptide 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 and 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 macropolymer content would be a better parameter to predict the bread-making quality of flour (Weegels et al., 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 (Weegels et al., 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 & 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 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 et al., 2015; Wilderjans et al., 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 et al. (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, Goesart, 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 (AACC, 1983). 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 350g of flour by 11g of water and 312g 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 2.5min 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 80x45 mm (40g 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 3X10 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 10ml of 1% SDS-phosphate buffer (pH 6.8) containing 5mM iodo-acetic amide (IAM). After centrifugation (30 min, 18 000rpm) the supernatant (SDS-soluble protein extracts) was collected and stored at -20°C before SE-HPLC analysis. The pellet was re-suspended in 5ml of 1% SDS-phosphate buffer (pH 6.8) including 40mM DTE for 1h at

60°C and sonicated for 5 minutes (30% power). The supernatant (DTE-soluble protein extracts) were collected after centrifugation and stored as above. SE-HPLC analysis of the samples (20µL) was performed on an Alliance system (Waters) using a tandem of TSK G4000-SWXL (7.8 × 300mm) and TSK SWXL (6 × 40mm) columns, both from Tosoh Biosep. Elution was performed at 0.7 mL/min with 0.1% SDS, 0.1M 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 weights of the major fractions were estimated by calibrating the column with various protein standards according to Morel et al. (2000).

3. RESULTS AND DISCUSSION

3.1. SE-HPLC protein profiles of cake ingredients

Figure 1a shows the SE-HPLC profiles of the SDS-soluble proteins from flour. The chromatogram is typically divided into 5 fractions, namely F1 and F2 that include glutenin macropolymers, F3 and F4 that include mostly gliadins and fraction F5 that gathers small water-soluble proteins such as albumin and globulin (Morel et al. , 2000). The remaining proteins were then solubilized with a buffer including SDS and DTE in order to reduce the intermolecular disulfide bonds. SE-HPLC profiles of this second extract shows 3 mains peaks (Figure 1.b). The first one (F'3) can be assimilated to high-molecular-weight glutenin subunits (HMW-GS) and the second one (F'4) to the low-molecular-weight glutenin subunits (LMW-GS). Fraction F'5 would include water-soluble proteins, either chemically or physically linked to the SDS-insoluble glutenin polymers network before its reduction by DTE.

The SDS-soluble whole egg protein chromatogram (Figure 1.c) presents 7 peaks but was arbitrarily divided into the same 5 fractions used above, allowing for an estimate of the relative quantitative contribution of egg and wheat proteins in the standard cake recipe, within the same molecular weight ranges. The nature of the egg proteins as deduced from their molecular weight is presented in Table1.

As shown in figure 1.e, emulsifier mostly includes water-soluble whey proteins and contributes very faintly to the total protein profile of the recipe.

3.2. SE-HPLC protein profiles of cake batter

Experimental profiles of the first and second sequential protein extracts of standard batter and each ingredient are represented on figure 2a,b. Theoretical first and second SE-HPLC profiles were obtained by summing the profiles of the ingredients containing proteins (Figure 1).

For both SDS-soluble and SDS-insoluble extracts, the real chromatogram superimposed with the theoretical one, except for the exclusion peaks (F1 and F'1, eluting at 8.5 min). Formation of some protein aggregates may have occurred during the freeze-drying step that was used to prepare dry extracts of batter and egg but omitted for flour and emulsifier.

Since the cake batter SE-HPLC profile coincided with the sum of the SE-HPLC profiles of its protein-contributing ingredients, it can be deduced that neither wheat nor egg proteins underwent polymerization or depolymerization during the mixing step. No covalent bond formation or rupturing occurred during mixing. In addition, no SE-HPLC profile change occurred during batter resting prior to baking (results not shown). During batter mixing and resting, glutenin macropolymers initially contained in flour remained unchanged in terms of molecular size distribution in contrast to bread dough (Mecham et al., 1962; Weegels et al., 1997). The lower mixing energy input (lower mixing speed, time, and dough viscosity compared to bread dough) and the fact that gluten proteins are far more diluted in cake batter may account for this lack of significant effect.

3.3. Evolution of cake batter protein profile during the baking step

3.3.1. Evolution of cake batter protein SDS extractability during baking.

Proteins from egg-free, flour-free and standard batters samples collected during baking were sequentially extracted and analyzed by SE-HPLC. Sequential extraction enables protein to be classified into SDS-soluble and DTE-soluble proteins while the last pellet gathers the SDS-unextractable proteins (Fi). Figure 3 presents the evolutions of these three protein fractions during baking for the three cake recipes.

SDS-extractable proteins follow a three-phase evolution during baking. First, from room temperature up to 60–70°C, whatever the batter formula, the total amounts of SDS-soluble and SDS-insoluble proteins

remain stable. Then, between 60–70°C and 90°C, SDS-soluble protein content drops, while both SDS-insoluble and unextractable protein contents increase. Finally, once the baking temperature exceeds 90°C, the SDS-soluble protein content remains stable.

Wilderjans, Pareyt, Goesaert, Brijs, & Delcour (2008) also found that cake batter baking resulted in loss of protein extractability in SDS buffer. They assumed that in cake batter, thermal aggregation starts between 60 and 70°C and achieved as temperature exceeds 90°C.

Many authors reported the decrease in protein SDS extractability of wheat and egg proteins during thermal treatments. The decrease is attributed to protein unfolding, exposure of hydrophobic zones and formation of covalent bonds, mostly disulfide bonds (Ma and Holme, 1982; Singh and MacRitchie, 2004; Singh, 2005; Van der Plancken et al., 2006). Adding DTE to the extracting solvent allowed almost total protein extraction, which is consistent with the prominent role play by disulfide bonds during baking. Hence in the case of standard batter (Figure 3.a and 3.c), whatever the baking time, the un-extractable protein fraction remains small, and almost all the aggregated protein can be recovered after sonication, disulfide bond reduction and protein denaturation with SDS.

In the case of the gluten-free cake recipe (Figure 3.b) even after both sonication and DTE reduction, proteins become less and less extractable as baking time increases while the unextractable protein fraction increases. It may indicate that, when cake batter contains only egg proteins, disulfide bonds are not the only covalent bonds at play during baking, and/or that the interactions are so tight that the reducing conditions do not allow the formation of small SDS-soluble aggregates. Baking of the standard and gluten-free batters did not lead to similar accumulation of unextractable proteins suggesting the formation of flour and egg proteins mixed aggregates.

3.3.2. Proteins involved in cake protein aggregates.

Figure 4.a shows the SE-HPLC profiles of the SDS-soluble proteins from egg-free cakes sampled at different baking times. After 12.5min of baking, no more change in SDS-soluble profiles occurs and only traces of protein from F3, F4 and F5 fractions remain. All fractions drop during baking, but at different extents and rates (Fig. 4b). Above 60–70°C, fraction F2 rapidly decrease and disappear above 90°C. Fraction F1 starts to decrease above 80°C, and totally disappears above 92°C. Fractions F3 and F5 decrease between 66°C and 92°C but do not completely disappear. Fraction F4 decrease is slower, incomplete as well, and ends later than the other fractions do. These results are consistent with those reported in the literature for heated hydrated gluten (Schofield et al., 1983; Singh and MacRitchie, 2004).

Glutenin polymers (F1+F2) are found to be the first to aggregate while gliadin (F4), except omega-gliadin (F3), reacts at higher temperature ($>90^{\circ}\text{C}$). Omega-gliadins which are devoid of cysteine residues remain SDS-soluble even after prolonged heating.

Slightly higher aggregation temperatures was observed for egg-free batter compared to heat treated wet gluten. It is likely that flour dilution with large amounts of sugar and water lower protein reactivity in cake batter (Donovan, 1977). Although Schofield, Bottomley, Timms, & Booth (1983) did not observe globulin/albumin aggregation during heating of wet gluten, Rosell et al. (2013) found that albumin and globulin partially co-aggregate with gluten proteins in flour dough during baking, which is consistent with the current results.

Results obtained for the gluten-free cake are showed in figures 4.c and 4.d. The protein signal comes from flour and egg proteins. The low density lipoprotein (LDL) amounts for more than 50% of the total egg proteins followed by ovalbumin (15%), high density lipoprotein (HDL, 12.5%) and livetin (7.5%). Phosvitin, ovotransferin and ovomucoid amount roughly for 3% while lysozyme and ovomucin contribute for less than 1% of total egg proteins.

All protein fractions decrease during baking, except fraction F1 which shows a transient increase around 65°C . Fraction F2, which may correspond to both yolk high density lipoprotein (HDL) and low density lipoprotein (LDL), disappears in two steps; the LDL peak starts to decrease between 65°C and 83°C while the HDL peak starts to decrease above 83°C . Both totally disappear above 90°C . Ovotransferrin (F3), starts to decrease between 65°C and 83°C and totally disappears above 90°C . Fraction F4, which probably corresponds to ovalbumin, ovoglobulin, livetin and ovomucoid, disappears in two steps; first the ovalbumin-ovoglobulin-livetin peak rapidly decreases when temperature reaches 65°C , as observed for F3, while the ovomucoid peak remains stable up to 83°C before totally disappearing once temperature exceeds 90°C . F1, which corresponds to the larger soluble egg proteins such as ovomucin, acts the same way, being stable up to 83°C before totally disappearing beyond 90°C . F5, which corresponds to the smaller egg proteins such as lysozyme, only changes slightly. For all fractions, after 15min of baking (*i.e.* 8 min spent at 90°C), no more change in SDS-soluble profiles was observed.

Although, to our knowledge, no aggregation kinetics study was made on whole eggs, these results are consistent with those found in the literature on, respectively, egg yolk and albumen: Donovan (1977), found that egg albumen aggregation is mainly driven by ovotransferrin insolubilization first and, later, ovalbumin. More recently, Kiosseoglou & Paraskevopoulou (2005) have reported that, in yolk, LDL and livetin are more thermosensitive than HDL.

Figures 4e and 4f show the results obtained for the standard cake batter. As before all protein fractions tend to disappear during baking, but not at the same rate. Fraction F2 rapidly decreases above 60°C to totally disappear beyond 90°C. Fractions F3, F4 and F5 severely drop between 60 and 90°C as well but do not disappear at the end of baking. Fraction F1 decreases later, once temperature rises above 81°C, and a few SDS-soluble proteins remain present after baking. Above 90°C, all remaining fractions do not evolve or drop slightly. After baking, the SDS-soluble protein extract mostly includes fractions F3, F4 and F5, as observed for the egg-free recipe.

Whatever the recipe, most of the changes in protein SDS extractability occur between 65 and 90°C. In the case of the standard cake, protein SDS solubility might start slightly earlier, as soon as the temperature reaches 60°C. The early stages of cake protein aggregation mostly involved glutenin, ovalbumin, ovotransferrin, LDL, livetin and lysozyme. Gliadin, HDL and ovomucin then aggregated as well. Once the temperature reaches 90°C, a comparison of the baking profiles of standard cake batter formula and egg-free batter formula (Figures 4.a and 4.e) shows that the SDS-soluble protein profiles are quite similar. Regardless of the presence or absence of egg proteins, the same flour proteins, in the same temperature/time ranges will aggregate.

3.3.3. Protein aggregate size evolutions during baking.

Figure 5 shows the SE-HPLC SDS-insoluble protein profiles (second extract in SDS/DTE) after different baking times for the egg-free (a), gluten-free (c) and standard (e) cake recipes. Evolutions of the SE-HPLC fractions are represented as well (figures 5b, 5d, and 5f). Areas numbered from F'1 to F'5 consist in the DTE-reduced protein fractions as defined in section 3.1, and Fi represents unextractable proteins. For sake of clarity and since both F'1 and F'2 fractions include covalently linked protein species in contrast with F'3, F'4 and F'5 which include the constitutive egg or gluten protein monomers, their evolutions are grouped in a F'1+F'2 curve.

Figure 5 reveals strong differences between the three different recipes in contrast with what was observed for the SDS-soluble proteins (Figure 4). After extraction with DTE and sonication, the SDS-insoluble proteins from egg-free samples are at first brought into solution in the form of monomeric polypeptides: HMW-glutenin subunits (F'3), LMW-glutenin subunits and gliadin (F'4) and smaller polypeptides (see section 3.1) (Figure 5.a). Nevertheless, between 12 to 17 min baking and while the majority of the SDS-soluble proteins have been insolubilized (Figure 4b), the SE-HPLC profile continues to evolve before stabilizing. Accumulation of large protein species (F'1+F'2) occur at the expense of monomeric species from fractions F'4 and F'5 (Figure 5b). This is consistent with the multi-steps

aggregation mechanism of gluten protein postulated by several authors (Weegels et al., 1997; Singh & MacRitchie, 2000). First, heating would induce the formation of disulfide bonds by oxidation of thiol groups, mostly between glutenin polymers, resulting in the formation of SDS-insoluble aggregates that could entrap some gliadin. Then gliadin, except ω -gliadin which have no cysteine residue, will unfold and form disulfide bonds through sulfhydryl-disulphide exchanges with the firstly formed SDS-insoluble glutenin aggregates (Domenek et al., 2002). Aggregates grow in size since the number of disulfide bonds stabilizing their structure increases. These highly crosslinked aggregates would resist more to the disrupting effect of DTE and sonication leading to an increase of the (F'1+ F'2) fraction.

Egg proteins behave differently (Figures 5.c, 5d). In the very early stage of baking, *i.e.* from 65 to 80°C, monomeric polypeptides (F'3, F'4, F'5) are recovered, indicating that disulfide bond formation is at first involved in egg protein thermal aggregation (Figures 4c, 4d). This is consistent with the literature on both egg yolk and albumen (Kiosseoglou and Paraskevopoulou, 2005; Van der Plancken et al., 2006). Since ovalbumin is the only egg protein to present free sulfhydryl groups, but not the only one to be insolubilized in this temperature range (see section 3.2), it can be assumed that sulfhydryl/disulfide bond exchanges occur and mixed egg proteins aggregates are formed. Then, above 80°C, large DTE-resistant aggregates accumulates (F'1 + F'2), at the expense of the monomeric species (F'3, F'4, F'5). Further baking leads to the drop of these large aggregates while the amount of unextractable protein (Fi) sharply increases. This proves the formation of DTE and sonication resistant aggregates connected through covalent bonds possibly of iso-peptide type (Mohammadi Nafchi et al., 2013). Indeed, since the recipe contains glucose syrup and egg-lysine-rich proteins, Maillard reaction can occur to form cross-links which will not be broken in presence of either denaturant or reducing agents. It is noteworthy to underline that the longer the baking the more densely cross-linked the protein aggregates will be; contrarily to what was observed for egg-free recipe where an equilibrium situation was observed after 17 min baking.

As for the egg-free formula, DTE-soluble protein profiles of the uncooked standard cake samples (Figures 5.e and 5f) contain three main peaks (F'3, F'4 and F'5), which likely derive from flour proteins, and a small exclusion peak which can be attributed to egg protein aggregates (F'1). During the early stages of baking and as observed for the previous recipes, F'3, F'4 and F'5 increase indicating that the first formed SDS-insoluble protein aggregates can be readily disrupted by DTE and sonication into the constitutive egg and flour protein monomers. Then above 90°C, F'4 (mostly ovalbumin, gliadin and LMW-glutenin) and to a smaller extent F'3 (mostly HMW-glutenin) and F'5 decrease, at the benefit of F'1 + F'2 (Figure 5f). The SDS-insoluble protein aggregates become more and more resistant to the disrupting action of DTE and sonication. However, contrarily to the gluten-free recipe, the amount of unextractable protein (Fi)

remained low. This would indicate that less iso-peptide bonds takes place between the egg proteins when flour proteins are also present. Contrarily to the egg-free recipe, F'1 + F'2 keeps growing all along baking, while accumulation of unextractable proteins remains even below the range observed for the egg-free samples. All these results support a mixed reactivity between wheat and egg proteins aggregates above 90°C. During standard cake baking, protein aggregation would proceed in two steps. From 65°C to 90°C, egg and flour protein would form homo-aggregates mostly stabilized by inter-chain disulfide bonds. Egg proteins would then support crosslinking between both types of proteins aggregates through the formation of iso-peptide bonds. In the full cake recipe, egg proteins will contribute to reinforce the protein connectivity and are likely to impact the crumb mechanical strength.

4. CONCLUSION

Following the changes in protein solubility and size distribution during cake mixing and baking was made possible by using SE-HPLC and sequential protein extraction. Flour and egg proteins interactions during cake processing were deduced from the study of standard, egg-free and gluten-free recipes. It was shown that mixing did not result in gluten network development contrarily to what is commonly observed during bread dough mixing. Baking induced protein aggregation but gluten and egg proteins did not react in the same way when present separately or combined. Below 90°C, egg and wheat proteins aggregated mainly through a thiol/disulfide exchange mechanism but did not seem to interact together. The formed SDS-insoluble protein aggregates were brought into solution and analyzed by SE-HPLC after their disruption by sonication in the presence of SDS and DTE. By this way it was possible to demonstrate that the SDS-insoluble aggregates structure continued to evolve all along baking. Hence, the egg SDS-insoluble aggregates from the gluten-free recipe became less and less sensitive to DTE and sonication, indicating the formation of permanent iso-peptide bonds between egg proteins. In the same time/temperature baking range, the gluten SDS-insoluble aggregates from egg-free recipe remained sensitive to DTE suggesting that covalent disulfide bonds were mainly accountable for protein aggregation all along cake baking. An intermediate situation was observed for the standard recipe indicating that above 90°C egg and wheat protein involved in separated aggregates started to interact and formed mixed and DTE-resistant aggregates. In cake recipe, egg proteins would contribute to increase the crosslinking density of the gluten protein aggregates all along the baking stage.

ACKNOWLEDGEMENTS

Joëlle Bonicel (UMR IATE 1208) and Anne-Flore Monnet (Mondelez) are warmly thanked for their technical support.

REFERENCES

- Deleu, L.J., Wilderjans, E., Van Haesendonck, I., Courtin, C.M., Brijs, K., Delcour, J.A. (2015). Storage induced conversion of ovalbumin into S-ovalbumin in eggs impacts the properties of pound cake and its batter. *Food Hydrocoll.*, 49, 208–215.
- Domenek, S., Morel, M.-H., Bonicel, J., Guilbert, S. (2002). Polymerization Kinetics of Wheat Gluten upon Thermosetting. A Mechanistic Model. *J. Agric. Food Chem.*, 50, 5947–5954.
- Donelson, D.H., Wilson, J.T. (1960). Studies on the effect of flour-fraction interchange upon cake quality.pdf. *Cereal Chem.*, 37, 683–710.
- Donovan, J.W. (1977). A study of the baking process by differential scanning calorimetry. *J. Sci. Food Agric.*, 28, 571–578.
- Huebner, F.R., Bietz, J.A., Nelsen, T., Bains, G.S., Finney, P.L. (1999). Soft Wheat Quality as Related to Protein Composition. *Cereal Chem.*, 76, 650–655.
- Kiosseoglou, V., Paraskevopoulou, A. (2005). Molecular interactions in gels prepared with egg yolk and its fractions. *Food Hydrocoll.*, 19, 527–532.
- Ma, C.-Y., Holme, J. (1982). Effect of Chemical Modifications on Some Physicochemical Properties and Heat Coagulation of Egg Albumen. *J. Food Sci.*, 47, 1454–1459.
- Mecham, D.K., Sokol, H.A., Pence, J.W. (1962). Extractable protein and hydration characteristics of flours and doughs in dilute acid. *Cereal Chem.*, 39, 81-93.
- Mine, Y. (2008). *Egg Bioscience and Biotechnology*. Wiley-Interscience.
- Mohammadi Nafshi, A., Tabatabaei, R.H., Pashania, B., Rajabi, H.Z., Karim, A.A. (2013). Effects of ascorbic acid and sugars on solubility, thermal, and mechanical properties of egg white protein gels. *Int. J. Biol. Macromol.*, 62, 397–404.
- Morel, M.-H., Dehlon, P., Autran, J.C., Leygue, J.P., Bar-L'Helgouac'h, C. (2000). Effects of Temperature, Sonication Time, and Power Settings on Size Distribution and Extractability of Total Wheat Flour Proteins as Determined by Size-Exclusion High-Performance Liquid Chromatography. *Cereal Chem.*, 77, 685–691.

- 390 Nau, F., Guérin-Dubiard, C., Baron, F., Thapon, J.L. (2010). *Science et Technologie de l'oeuf, Volume 2 : De*
391 *l'Oeuf aux Ovoproduits*. Lavoisier.
- 392 Rosell, C.M., Altamirano-Fortoul, R., Don, C., Dubat, A. (2013). Thermomechanically Induced Protein
393 Aggregation and Starch Structural Changes in Wheat Flour Dough. *Cereal Chem.*, 90, 89–100.
- 394 Schofield, J.D., Bottomley, R.C., Timms, M.F., Booth, M.R. (1983). The effect of heat on wheat gluten and
395 the involvement of sulphhydryl-disulphide interchange reactions. *J. Cereal Sci.*, 1, 241–253.
- 396 Shepherd, I.S., Yoell, R.W. (1976). *Cake emulsions*. Food Emuls.
- 397 Singh, H. (2005). A study of changes in wheat protein during bread baking using SE-HPLC. *Food Chem.*,
398 90, 247–250.
- 399 Singh, H., MacRitchie, F. (2004). Changes in proteins induced by heating gluten dispersions at high
400 temperature. *J. Cereal Sci.*, 39, 297–301.
- 401 Singh, N.K., Donovan, G.R., Batey, I.L., MacRitchie, F. (1990). Use of Sonication and Size-Exclusion High
402 Performance Liquid Chromatography in the Study of Wheat Flour Proteins. I. Dissolution of Total
403 Proteins in the Absence of Reducing Agents. *Cereal Chem.*, 67, 150–161.
- 404 Van der Plancken, I., Van Loey, A., Hendrickx, M.E. (2006). Effect of heat-treatment on the physico-
405 chemical properties of egg white proteins: A kinetic study. *J. Food Eng.*, 75, 316–326.
- 406 Weegels, P.L., Hamer, R.J., Schofield, J.D. (1997). Depolymerisation and Re-polymerisation of Wheat
407 Glutenin During Dough Processing. II. Changes in Composition. *J. Cereal Sci.*, 25, 155–163.
- 408 Weegels, P.L., van de Pijpekamp, A.M., Graveland, A., Hamer, R.J., Schofield, J.D. (1996).
409 Depolymerisation and Re-polymerisation of Wheat Glutenin During Dough Processing. I.
410 Relationships between Glutenin Macropolymer Content and Quality Parameters. *J. Cereal Sci.*, 23,
411 103–111.
- 412 Wilderjans, E., Luyts, A., Goesaert, H., Brijs, K., Delcour, J.A. (2010). A model approach to starch and
413 protein functionality in a pound cake system. *Food Chem.*, 120, 44–51.
- 414 Wilderjans, E., Pareyt, B., Goesaert, H., Brijs, K., Delcour, J.A. (2008). The role of gluten in a pound cake
415 system: A model approach based on gluten–starch blends. *Food Chem.*, 110, 909–915.

ACCEPTED MANUSCRIPT

Figure 1 : SE-HPLC protein profiles of cake ingredients containing proteins. SDS-soluble (a) and DTE-soluble (b) protein extracts of Apache flour ; SDS-soluble (c) and DTE-soluble (d) extracts of pasteurized whole eggs ; SDS-soluble extract of emulsifier (e). No DTE-soluble protein extract was found for emulsifier. Sample mass was adjusted to the amount theoretically found in 80 mg of the standard cake recipe (dry basis)

Figure 1: SE-HPLC chromatograms of SDS-soluble (a) and SDS-insoluble (b) protein extracts of standard cake batter and of the cake ingredients (flour, egg and emulsifier) that include proteins. A theoretical standard cake batter chromatogram was calculated by summing, in regards to their batter proportions, the chromatograms of egg, flour and emulsifier.

Figure 3: Cake crumb protein SDS extractability of an egg-free recipe (a), a gluten-free recipe (b) and a standard recipe (c) during baking. For each baking time, unextractable protein fraction was calculated from the difference in total SE-HPLC area between the initial un-baked batter and batter at current baking time.

Figure 4 : SE-HPLC SDS-soluble protein profiles (a, c, e) and compositions (b, d, f) of egg-free, gluten-free and standard cake batters during baking. SE-HPLC protein fractions were obtained by calculating SE-HPLC fraction areas at different baking times.

Figure 5. SE-HPLC DTE-soluble protein profiles (a, c, e) and compositions (b, d, f) of egg-free, gluten-free and standard cake batters during baking. SE-HPLC protein fractions were obtained by calculating SE-HPLC fraction areas at different baking times and were summed to evaluate DTE-soluble protein composition. The unextractable protein fraction was also estimated.

- Cake protein aggregation during processing was studied using SE-HPLC
- Dough mixing does not impact the size distribution of egg and flour proteins
- Egg and flour proteins formed mixed aggregates upon baking
- Mixed aggregates are stabilized by thiol/disulfide and iso-peptide bonds
- Eggs proteins contributes to strengthen the cake protein network

ACCEPTED MANUSCRIPT

Comment citer ce document :

Dewaest, M., VILLEMEJANE, C., BERLAND, S., MICHON, C., VEREL, A., MOREL, M. H. (Auteur de correspondance) (2017). Changes in protein size distribution during wheat flour cake processing. LWT - Food Science and Technology, 79, 333-341. DOI : 10.1016/j.lwt.2017.01.036