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Free wheat flour lipids decrease air-liquid interface stability in sponge cake batter

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Abbreviations used

CF, control flour; DGDG, digalactosyldiacylglycerols; DTT, dithiothreitol; FFA, free fatty acids; FRL, flour with relocated lipids; HPLC, high performance liquid chromatography; LPC, lysophosphatidylcholines; MGDG, monogalactosyldiacylglycerols; NAPE, *N*-acyl phosphatidylethanolamines; PC, phosphatidylcholines; PDF, partially defatted flour; PE, phosphatidylethanolamines; SDS, sodium dodecyl sulfate; SDS EP, the percentage of protein extractable in SDS medium; SE, size-exclusion; STG, steryl glycosides; TAG, triacylglycerols

Abstract

The impact of free wheat flour lipids on the air-liquid interface stability during sponge cake making was investigated. Therefore, the molecular population at the air-liquid interface in batters prepared with flour of which part of the lipids had been either relocated or removed prior to batter preparation was determined. Surface-active molecules were isolated from batter using a foam separation protocol. Diluted batter was whipped and the resulting foam was used as model system for the air-liquid interface in sponge cake batter. ***Relocating flour lipids*** prior to batter making enabled them to adsorb at the air-liquid interface in the foam. This limited the degree of protein adsorption at the air-liquid interface, but it did not impact the composition of the adsorbed protein population. ***Removing flour lipids*** prior to batter making resulted in foam containing relatively higher levels of lipids mainly originating from egg yolk. Prior removal of flour lipids impacted neither foam protein content nor foam protein composition. The resultant molecular population improved air-liquid interface stability in sponge cake batter. Thus, free wheat flour lipids and wheat flour lipids set free by solvent treatment decrease air-liquid interface stability in sponge cake batter mainly because they limit protein adsorption and, as such, interfere with the protein-dominated interface.

Keywords

sponge cake; batter foaming properties; foam separation; air-liquid interface stability; non-starch wheat flour lipids; surface-active molecules; free flour lipids; food foams

1 Introduction

Flour, sugar and eggs are the main ingredients of sponge cakes. Such cakes are classified as foam-type cakes and used in Swiss rolls and layer cakes (Lai & Lin, 2006). To produce sponge cake with fine crumb and high volume, gas cell incorporation during mixing and gas cell stability after mixing and during early baking are essential (Lai & Lin, 2006; Pycarelle, Bosmans, Nys, Brijs & Delcour, 2020; Pycarelle et al., 2019; Rodríguez-García, Sahi & Hernando, 2014; Sahi & Alava, 2003). Gas cells are stabilized by a layer of surface-active molecules adsorbed at the air-liquid interface. These are typically low-molecular weight surfactants, which are mostly lipids or lipid-like molecules, and proteins (Mackie & Wilde, 2005). Even though surface-active molecules have a critical role during sponge cake making, recent literature studies dealing with sponge cakes do not focus on them. They mainly examine the impact of new ingredients (e.g. pea protein, olive stone powder or broccoli leaf powder) on batter/cake quality (Bustillos, Jonchere, Garnier, Reguerre & Della Valle, 2020; Jahanbakhshi & Ansari, 2020; Krupa-Kozak et al., 2019).

Surface-active proteins and lipids orient their hydrophobic sites towards the gas phase and their hydrophilic ones to the aqueous phase to reduce interfacial tension. However, the mechanism whereby proteins and lipids stabilize interfaces is entirely different. Stabilization by the former mostly relies on formation of a strong viscoelastic layer at the interface, while that by the latter is based on rapid diffusion to and/or molecular redistribution at the interface (*i.e.* Gibbs-Marangoni mechanism). Lipids are thus rather mobile at the air-liquid interface while proteins are not (Damodaran, 2005; Mackie & Wilde, 2005). In sponge cake batter, proteins and lipids from flour and egg adsorb at the air-liquid interface (Pycarelle et al., 2020).

Wheat flour ***proteins*** (10 – 12% of flour) can be fractionated by successive extractions with water, dilute salt solution, aqueous alcohol and dilute acid or alkali resulting in extractable albumins, globulins, gliadins and glutenins, respectively (Osborne, 1907; Veraverbeke & Delcour, 2002).

Egg white dry matter almost solely consists of protein of which roughly half is ovalbumin (Powrie & Nakai, 1985). In contrast, *egg yolk* dry matter contains *ca.* 33% protein such as livetins and vitellins (Huopalahti, López-Fandiño, Anton & Schade, 2007; Powrie & Nakai, 1985).

Wheat flour contains 2.0 – 3.0% ***lipids*** that are subdivided in starch (*ca.* 40%) and non-starch (*ca.* 60%) lipids based on their location. The former reside inside the starch granules as amylose-lipid complexes and are only extracted after substantial swelling or disruption of starch granules. Extraction of flour non-starch lipids requires none of the above and is performed at room temperature (Chung, Ohm, Ram, Park & Howitt, 2009; Finnie, Jeannotte & Faubion, 2009; Morrison, 1978; Pareyt, Finnie, Putseys & Delcour, 2011). Non-starch wheat flour lipids consist of *ca.* 43% non-polar lipids [*e.g.* triacylglycerols (TAG) and free fatty acids (FFA)] and *ca.* 57% polar lipids. The latter are further divided into galactolipids [*e.g*. digalactosyldiacylglycerols (DGDG) and monogalactosyldiacylglycerols (MGDG)] and phospholipids [*e.g. N*-acyl phosphatidylethanolamines (NAPE) and phosphatidylcholines (PC)] (Finnie et al., 2009; Janssen et al., 2018; Morrison, Mann, Soon & Coventry, 1975; Pareyt et al., 2011). Based on their extractability at room temperature, flour non-starch lipids are classified as free and bound lipids. They are extracted with non-polar (*e.g.* hexane) and more polar solvents (*e.g.* water-saturated butanol), respectively. Free flour lipids are primarily non-polar but also contain small levels of polar lipids, while bound flour lipids are mainly polar (Chung et al., 2009; Pareyt et al., 2011). However, the extraction efficiency also depends on the binding between lipids and gluten and starch (Melis, Pauly, Gerits, Pareyt & Delcour, 2017).

Egg white contains a negligible portion of lipids, whereas *egg yolk* dry matter contains *ca.* 66% lipids. TAG (66%), phospholipids (28%) and cholesterol (5%) make up the bulk of the egg yolk lipids. The main egg yolk phospholipids are PC, phosphatidylethanolamines (PE) and lysophosphatidylcholines (LPC). Most of these occur as low density lipoproteins, *i.e.* spherical structures (average diameter 35 – 40 nm) with a core containing TAG and cholesterol esters surrounded by phospholipids, proteins and cholesterol (Anton et al., 2003; Kamat et al., 1972; Powrie & Nakai, 1985). Low density lipoproteins stabilize air-liquid interfaces (Dauphas, Beaumal, Riaublanc & Anton, 2006; Martinet, Saulnier, Beaumal, Courthaudon & Anton, 2003).

Recently, our team demonstrated that the air-liquid interface in sponge cake batter is dominated by proteins (Pycarelle et al., 2020). More specifically, it was shown that wheat flour α- and γ-gliadins have higher affinity for the air-liquid interface in sponge cake batter than other flour proteins and that intermolecular disulfide bonds between proteins such as ovalbumin are formed at the air-liquid interface (Pycarelle et al., 2020).

In other work by our group, it was hypothesized that free flour lipids negatively impact air-liquid interface stability in sponge cake batter (Pycarelle et al., 2019). It was reasoned that these lipids easily adsorb at such interfaces which in turn decreases the protein-to-lipid ratio of the molecular population at the interface and hence decreases its stability (Pycarelle et al., 2019). The present study was set up to investigate the molecular population at the air-liquid interface in batters prepared from sugar, eggs, leavening agents and flour in which lipid content or location had been varied.

Control flour (CF) was subjected to prior solvent treatment with hexane:isopropanol (3:2 v/v) at room temperature to extract part of the non-starch lipids (i.e. free flour lipids and part of the bound flour lipids) (Pycarelle et al., 2019). When the extracted lipids were removed, partially defatted flour (PDF) was obtained. When the extracted lipids were added back to the flour pellet remaining after the solvent treatment, flour with relocated lipids (FRL) was obtained. Evidently, lipids removed from PDF equaled those relocated in FRL (see section 2.2.2). CF, FRL and PDF were then used to prepare sponge cake batter from which surface-active molecules were isolated using a foam separation protocol (Pycarelle et al., 2020). In short, diluted sponge cake batter was whipped to form foam. Thereafter, foam samples were taken at two points in time and their chemical composition was determined. How partial relocation or removal of flour lipids prior to batter making impacts the composition of these foam samples will provide information about the role of flour lipids at the air-liquid interface in sponge cake batter.

This is, to the best of our knowledge, the first time that an in-depth study on the role of flour lipids at the air-liquid interface in a complex food system such as cake is performed. Since these lipids are endogenously present in flour, insights gained in this study may very well support the development of clean label food products and in that way meet recent consumer demands.

2 Materials and Methods

2.1. Chemicals and materials

Wheat flour [Halm commercial brand, 14.0% moisture content and 10.4% protein (N x 5.7) content] was from Paniflower (Merksem, Belgium). Sodium acid pyrophosphate (number 15) and sodium bicarbonate were

from Budenheim (Budenheim, Germany). Rice starch (11.8% moisture content) was from Beneo (Wijgmaal, Belgium). Eggs were purchased at a local supermarket, kept at 3 °C and used before their "best before" date. Isopropanol was from Honeywell Riedel-de Haën (Seelze, Germany), isooctane from Merck (Darmstadt, Germany) and hexane, chloroform and methanol from Thermo Fisher Scientific (Geel, Belgium). All solvents were of high performance liquid chromatography (HPLC) grade. Chemicals were of analytical grade and from Sigma-Aldrich (Bornem, Belgium) unless indicated otherwise.

2.2. Methods

2.2.1 Moisture and protein contents

The AACC method 44-15.02 (AACCI, 1999) was used to determine moisture contents of rice starch and flours. Protein content (N X 5.7) of CF was determined using an automated Dumas protein analysis system (VarioMax Cube N, Elementar, Hanau, Germany) which is based on the AOAC method 990.03 (AOAC, 1995). Above analyses were performed in triplicate.

2.2.2 Relocating lipids in and removing lipids from wheat flour

Flour lipids were relocated in or removed from CF by solvent treatment as in Pycarelle et al. (2019). Treatments resulting in FRL and PDF are briefly summarized below. Both flour samples were prepared in duplicate.

FRL was prepared by shaking CF in hexane:isopropanol (3:2 v/v) at room temperature, removing the supernatant containing the extracted lipids by Büchner filtration over paper and repeating said extraction three times. The obtained defatted solids were spread in glass containers, pooled supernatants containing extracted lipids were added onto the solids and the solvent was allowed to evaporate under a fume hood overnight. Lipids were physically separated from the defatted solids during the solvent treatment, as such relocating (part of) the flour lipids. The next day FRL was milled, sieved and stored at 7 °C until further use.

To obtain PDF, the above procedure was repeated without adding lipid extracts back to the flour solids. Instead, flour solids were dried on filter paper overnight under a fume hood, milled and sieved the next day.

As hexane:isopropanol (3:2 v/v) is rather polar, free flour lipids and part of the bound flour lipids were relocated in or removed from the flour. Lipid contents and compositions of CF, FRL and PDF have been reported in detail (Pycarelle et al., 2019) and are repeated below when needed.

2.2.3 Batter preparation

Sponge cake batters were made from flour, sugar, egg white, egg yolk, leavening agent, rice starch and deionized water. Mixing was in five steps. First, egg white and egg yolk were beaten separately, each with part of the sugar. Egg white and egg yolk foams were then carefully mixed and finally flour was added in two steps. For a detailed description the reader is referred to Pycarelle et al. (2019). The quantity of deionized water was adapted to correct for varying flour and rice starch moisture contents and to obtain batter moisture contents of 35.8%. One batter was prepared from each batch of FRL and PDF obtained in section 2.2.2. Batters from CF were prepared in duplicate. In what follows, sponge cake batters prepared with CF, FRL or PDF are denoted as CF, FRL and PDF batter, respectively.

2.2.4 Batter foaming properties

Foaming properties of CF, FRL and PDF batter were determined by first diluting batter with deionized water to remove the gas cells from it. The degree of dilution was the same for all samples (1:5.6 batter dry matter:water w/w) (Pycarelle et al., 2020). The suspension was then whipped to form foam as in Pycarelle et al. (2020). In short, the diluted batter was homogenized with a magnetic stirrer and transferred to graduated glass cylinders. The latter were covered with Parafilm M and kept in a water bath at 25 °C for at least 30 min. Next, foam was formed by rotating a propeller in the suspension. Foam volume was monitored over 60 min. Foaming capacity is the foam volume measured 60 s after the end of whipping and foam stability the percentage of the initial foam volume left after 60 min. For each individual batter, foaming properties were determined in triplicate. Photographs were taken at two points in time (see section 2.2.5) to record foam coarsening and drainage. The former is observed as coalescence of gas cells on top of the foam and the latter equals the loss of bulk fluid (*i.e.* fluid residing in between the gas cells) from the foam to the liquid phase due to gravitation.

2.2.5 Foam separation

To enrich and isolate surface-active molecules from sponge cake batter the abovementioned foaming protocol (see section 2.2.4) was used (Pycarelle et al., 2020). After diluted batter had been whipped for 150 s, foam was separated at 210 s (T1) and 15 min (T2) after the start of whipping (Figure 1A). At every point in time foam samples were taken from at least four cylinders resulting from one individual batter. Foam fractions were pooled, frozen with liquid nitrogen and freeze-dried. Diluted batters were frozen and freeze-dried in the same way to allow proper comparison of the chemical compositions of batter and its foam fractions. All freeze-dried samples were gently ground with a mortar and pestle.

2.2.6 Protein content of batter and foam made thereof

Protein contents (N x 5.98) of freeze-dried batters and foams were determined in triplicate (Pycarelle et al., 2020) based on the AOAC method 990.03 (AOAC, 1995). The latter was adapted to an automated Dumas protein analysis system (1108 Elemental Analyzer, Carlo Erba, Hindley Green, UK) for microgram scale samples.

2.2.7 Lipid content of batter and foam made thereof

Lipid contents (expressed as a percentage of sample dry matter) of freeze-dried batters and foams were determined as in Pycarelle et al. (2020). In brief, lipids were extracted by shaking the sample in chloroform:methanol (2:1 v/v) twice at room temperature. The solvent was evaporated from the pooled supernatants, the resulting extracts purified, accurately weighed and stored at -80 °C under nitrogen atmosphere until further analysis (see section 2.2.9). Lipids were extracted at least in duplicate from two independent freeze-dried batter or foam samples (see sections 2.2.3 and 2.2.5). Coefficients of variation were lower than 4%.

2.2.8 Protein composition of batter and foam made thereof

Protein compositions of freeze-dried batters and foams were determined by extracting them with (i) 0.05 M sodium phosphate buffer (pH 6.8) containing 2.0% (w/v) sodium dodecyl sulfate (SDS) (*i.e.* SDS medium) and (ii) the same buffer also containing 2.0% (w/v) dithiothreitol (DTT, VWR International, Leuven, Belgium) (*i.e.* SDS+DTT medium) and then separating them with size-exclusion (SE)-HPLC (Pycarelle et al., 2020). SDS

disrupts non-covalent interactions (Turro, Lei, Anantnapadmanabhan & Aronson, 1995), while DTT breaks up disulfide bonds. The latter leads to solubilization of cross-linked protein molecules (Konigsberg, 1972). Proteins were extracted in duplicate from two independent freeze-dried batter or foam samples (see sections 2.2.3 and 2.2.5). Samples contained 1.0 mg protein/mL extraction solvent. Extracts were loaded onto an HPLC system. Details on the latter and on HPLC analyses can be found in Pycarelle et al. (2020).

With the data obtained, the percentage of protein extractable in SDS medium (SDS EP) was calculated (Equation 1). Total areas were integrated from 0 to 11 min to avoid interference of the elution of DTT.

$$\text{SDS EP (\%)} = \frac{\text{total area of sample SDS extract}}{\text{total area of sample SDS + DTT extract}} \times 100$$

2.2.9 Lipid composition of batter and foam made thereof

Lipid composition of freeze-dried batters and foams were determined using HPLC with evaporative light scattering detection (model 3300, Alltech, Deerfield, IL, USA) as in Pycarelle et al. (2020) but with slightly altered lipid concentrations and injection volumes. Extracted lipids (see section 2.2.7) and the internal standard lauryl laurate (Larodan, Solna, Sweden) were dissolved in isooctane (3.0 mg lipids/mL and 500 µg lauryl laurate/mL). Samples were injected twice – 1.0 µL to accurately detect PC and TAG, and 5.0 µL to detect all other lipid classes – onto an HPLC column (Chromolith Performance-Si column, 100 × 4.6 mm, Merck). Data acquisition and analysis were performed with LCSolution version 1.23 SP1 (Shimadzu, Kyoto, Japan). Lipid levels (expressed as the area under the curve relative to that of the internal standard lauryl laurate) can only be compared semi-quantitatively within one lipid class. Results thus only show higher or lower levels of one lipid class for different samples.

2.2.10 Statistical analyses

Student's t-test was applied to determine significant differences between two groups. For variables with more than two groups a one-way analysis of variance (ANOVA) was performed. Where appropriate, a Tukey multiple comparison test was executed to verify significance of differences between mean values. Tests were performed with JMP Pro 12 software (SAS Institute, Cary, NC, USA) at a significance level (α) of 0.05.

3 Results

3.1 Batter foaming properties

Foaming capacities of CF and ***FRL batter*** did not significantly differ (Figure 1). For both samples, foam volume decreased over time because bulk fluid drained to the liquid phase and the foam itself was destabilized. The former was evidenced by the increase in liquid phase volume over time (Figure 1B), the latter by foam coarsening (indicated with arrows in Figure 1B and 1C). As foam volume decreased to the same extent for foams prepared from CF or FRL batter, foam stabilities (71 ± 5% and 70 ± 3%, respectively) were also similar. Thus, use of FRL instead of CF for producing sponge cake batter had no clear impact on batter foaming properties.

Foam prepared from ***PDF batter*** had a significantly higher initial volume than foams prepared from CF or FRL batter (Figure 1A). Partial removal of flour lipids prior to batter making did not significantly impact foam stability (73 ± 1%). However, because more liquid was initially retained in foam from PDF batter than in foams from CF or FRL batter, initial draining (until 10 min after the start of whipping) occurred more quickly in the former. In contrast, between 10 and 60 min after the start of whipping, foam from PDF batter only lost 10 ± 1% of its volume while the corresponding losses for CF and FRL batter were 19 ± 8% and 19 ± 4% (Figure 1A). Also, less coarsening was observed for the former (Figure 1B and 1C). Overall, decreasing flour lipid content from 1.57% to 0.44% of flour dry matter (Pycarelle et al., 2019) improved foaming properties of sponge cake batter.

3.2 Quantification of proteins and lipids in batter and foam made thereof

Foams prepared from CF, FRL and PDF batter were isolated at 210 s (T1) and 15 min (T2) after the start of whipping (Figure 1A). In this section, the quantification of proteins and lipids in batters and foams is described while the next section (section 3.3) deals with their characterization.

Protein and lipid contents of ***FRL*** batter evidently did not differ from those of CF batter. Both had a protein-to-lipid ratio of 2.7 ± 0.1 (Table 1). When isolated at T1, foam from FRL batter contained significantly less

protein and more lipid than foam from CF batter (Table 1) which evidently resulted in a lower protein-to-lipid ratio for the former. Thus, in foam isolated at T1, as a result of prior relocation of free flour lipids and part of the bound flour lipids (Pycarelle et al., 2019), foam from FRL batter contained more surface-active lipids than foam from CF batter. Presumably, prior relocation enabled their rapid adsorption at the air-liquid interface which in turn limited the extent of protein adsorption. However, when isolated at T2, protein and lipid contents and protein-to-lipid ratios were similar for both samples (Table 1). Thus, although prior relocation of wheat flour lipids resulted in different protein and lipid contents in foam isolated at T1, these differences were no longer observed for foam isolated at T2 because of the increased protein content. It is plausible that over time proteins displaced surface-active lipids while adsorbing at the air-liquid interface. Proteins indeed diffuse to and adsorb at an interface more slowly than lipids (Damodaran, 2005), and may insert themselves in a lipid layer (Mackie & Wilde, 2005).

Lipid content of ***PDF*** batter was evidently lower than that of CF and FRL batter (Table 1). As a result, PDF batter had a higher protein-to-lipid ratio (3.1 ± 0.1) than the latter two (2.7 ± 0.1). Foam made from PDF batter isolated at T1 or T2 had the same protein content and a lower lipid content than foam made from CF batter separated at the same points in time (Table 1). That foam protein content was not affected by prior flour defatting suggests that the removed flour lipids do not impact the tendency of proteins to adsorb at the air-liquid interface of foam made from sponge cake batter.

3.3 Characterization of proteins in batter and foam made thereof

In this section and section 3.4 preferential enrichment of certain protein and lipid types in foams from CF, FRL or PDF batter is discussed. To identify differences in protein composition between foam samples, proteins of foam samples were extracted in SDS medium and analyzed with SE-HPLC (see section 2.2.8). The elution profiles of the resultant SDS medium extracts were divided into five regions (indicated I tot V in Figure 2). In earlier work of our group (Pycarelle et al., 2020), the focus was on region III which mainly contains ovalbumin (*i.e.* the main egg white protein) and region IV which primarily represents wheat flour α- and γ-gliadins. Both regions also contain other flour and egg proteins.

Figures 2A and 2B clearly show that prior relocation of lipids in flour did not impact the protein composition of foam made from sponge cake batter. This was further evidenced by the areas of SDS extracts which were similar for foam samples from either CF or FRL batter (Table 2). This also indicates that the used solvent treatment hardly affected protein functionality at the air-liquid interface.

Irrespective of whether CF or ***FRL*** had been used, proteins extracted in SDS medium from the respective foam samples at different moments in time showed that, as time of sampling progressed, the areas of regions III decreased while those of region IV increased (Figure 2A). It follows from the latter that wheat flour α- and γ-gliadins have higher affinity for the air-liquid interface than most other proteins in the batter (Pycarelle et al., 2020). It has already been reported earlier that wheat flour α- and γ-gliadins have a higher affinity for the air-liquid interface than other flour proteins (Keller, Orsel & Hamer, 1997; Li, Dobraszczyk & Wilde, 2004; Thewissen, Celus, Brijs & Delcour, 2011).

The decrease in regions II, III and V has been explained as resulting from disulfide bond formation between proteins (*e.g.* ovalbumin) at the air-liquid interface and from drainage of protein from the foam to the liquid phase (Pycarelle et al., 2020). To examine whether disulfide bonds had been formed, proteins were extracted in SDS+DTT medium (see section 2.2.8). The inclusion of DTT in the SDS medium resulted in higher levels of protein extractable from foams made from CF batter (Table 2) and confirmed that disulfide bonds had been formed at the air-liquid interface. Similar observations were made for foam from FRL batter.

Irrespective of whether CF or FRL had been used for batter preparation, SDS EP contents of foams made thereof significantly decreased over time (Table 2). Moreover, at each point in time SDS EP contents of foams from CF or FRL batter were similar. The protein populations in those foams evolved towards one with relatively less protein extractable in SDS medium and relatively more protein extractable in SDS+DTT medium which was due to (i) disulfide bond formation between proteins such as ovalbumin adsorbed at the air-liquid interface and/or (ii) drainage of some protein from the foam. Possibly, also protein aggregates resulting from intermolecular disulfide bond formation contributed to proteins being withheld in the foam over time.

Figure 2C shows the SE-HPLC elution profiles of SDS medium extracts of freeze-dried ***PDF*** batter and foam made thereof. Much as observed for foams from CF or FRL batter, region III clearly decreased and region IV

clearly increased when samples were withdrawn later in time. All in all, use of PDF instead of CF or FRL for batter preparation did not severely alter the protein composition in foams made from those batters.

3.4 Characterization of lipids in batter and foam made thereof

Figure 3 shows lipid compositions of CF, FRL or PDF batters and foams made thereof. Lipid classes were grouped based on their origin into those mainly present in flour (Figure 3A) or mainly present in egg yolk (Figure 3B).

Irrespective of whether CF or ***FRL*** had been used to prepare sponge cake batter, lipid composition in foam made thereof at T1 did not differ from that in the batter itself. Over time, levels of DGDG, MGDG and steryl glycosides (STG) (significantly) increased (Figure 3A), while those of PC, PE and cholesterol (significantly) decreased (Figure 3B) in foams made from CF or FRL batter. Even if some of the present results revealed no statistically significant differences, this was clearly so in previous work for foam made from CF batter (Pycarelle et al., 2020). It is therefore concluded that in CF batter lipids mainly present in flour have a higher affinity for the air-liquid interface than those mainly present in egg yolk (Pycarelle et al., 2020) and that the tendency of flour and egg lipids to adsorb at the interface is not altered by relocating flour lipids prior to sponge cake batter making. Indeed, foams from FRL and from CF batter isolated at the same point in time had similar lipid populations (Figure 3). FFA, PC and cholesterol levels in foam from FRL batter isolated at T1 significantly differed from those in foam from CF batter isolated at the same point in time (indicated with an asterisk in Figure 3). For said foams isolated at T2, only PC and cholesterol levels significantly differed (indicated with an asterisk in Figure 3B).

Evidently, lower levels of DGDG, MGDG, NAPE, STG and FFA were present in ***PDF*** batter than in CF batter due to prior partial defatting of CF (white bars in Figure 3A). Since PDF contained lower FFA levels than CF (Pycarelle et al., 2019), it was quite unexpected that FFA levels in foam made from PDF batter reached values similar to those in foam from CF batter (Figure 3A). In contrast to what was observed for foams from CF and FRL batters, PC and PE levels in foam from PDF batter remained constant instead of (significantly) decreasing over time (Figure 3B). Furthermore, PC, PE and cholesterol levels were higher in foam from PDF batter

isolated at T2 than those in foam from CF batter isolated at the same point in time. PC, PE and cholesterol, which are mostly part of egg yolk (Anton et al., 2003), are thus more abundant in foam when part of the flour lipids are no longer present.

4 Discussion

4.1 Relation between batter foaming properties and batter density

Densities of CF, FRL and PDF batters were previously reported in Pycarelle et al. (2019). They were measured by accurately determining the weight of 100 mL batter contained in a 100 mL graduated plastic cylinder. Foaming capacities examined in this study were essentially in line with batter densities previously observed (Pycarelle et al., 2019). Indeed, the lowest density was measured for PDF batter (0.65 ± 0.01 g/cm³) and matched with the highest foaming capacity observed here. However, the used foaming protocol did not pick up the slightly higher density of FRL batter (0.76 ± 0.00 g/cm³) compared to that of CF batter (0.74 ± 0.01 g/cm³) probably because of batter dilution prior to foaming. Batter dilution lowers the bulk concentration of both surface-active protein and lipid but not the protein-to-lipid ratio in the bulk. However, prior dilution of sponge cake batter may affect the protein-to-lipid ratio at the air-liquid interface in the foam. As stated above, prior dilution lowers the bulk lipid concentration. As a result, less lipid molecules adsorb at the air-liquid interface in the foam than at the air-liquid interface in the batter. Hence, because of the dilution step lipids occupy less area of the air-liquid interface and as a result proteins may occupy more of that interfacial area. This most likely masked the negative impact of relocated flour lipids on air incorporation during batter preparation.

Based on the above, it is concluded that foaming properties greatly reflect batter density. Furthermore, it was recently shown that foam stability correlates well with the molecular population that covers the air-liquid interface (Graetz et al., 2020). It was also demonstrated that this experimental setup can be used for isolating surface-active molecules present in sponge cake batters (Pycarelle et al., 2020). As a result, it is reasonable to state that the used foaming protocol is a relevant model system for studying the molecular population at air-liquid interfaces in such batters.

4.2 Impact of prior relocation of wheat flour lipids on air-liquid interface stability in sponge cake batter

As a result of prior flour lipid relocation, at T1 more lipid had adsorbed at the air-liquid interface in foam from FRL batter than at the interface in foam from CF batter (see section 3.2). These results further substantiate the earlier hypothesis that prior solvent treatment of flour makes part of its lipids more available for adsorption at air-liquid interfaces (Pycarelle et al., 2019). Because during the solvent treatment hydrophobic, electrostatic or hydrogen bonds between flour lipids and other flour constituents are broken, relocated lipids occur in a more free state in FRL (Pycarelle et al., 2019). They then limit the extent of protein adsorption (see section 3.2). This is likely the reason why slightly less air is incorporated in FRL batter than in CF batter (Pycarelle et al., 2019).

Protein populations in foam from CF and FRL batter at T1 did not noticeably differ from each other (see section 3.3). Thus, the relative tendency of some protein types to adsorb at the air-liquid interface in foam made from sponge cake batter is not altered by prior relocation of flour lipids.

When gas cells expand during early baking (Godefroidt, Ooms, Pareyt, Brijs & Delcour, 2019) the area of that interface evidently increases, local thinning occurs and interfacial tension rises (Hasenhuettl & Hartel, 2008). Since equilibrium conditions have to be restored in rather short time scales, it is reasonable to state that foam from FRL batter at T1 is a good model for the air-liquid interface during early baking. Similar to what is the case during mixing, relocated flour lipids probably also limit the extent of protein adsorption during early baking. In that way, they hamper the formation of a viscoelastic protein layer and hence interfacial stability. As a result, gas cells likely are destabilized (because of disproportionation and/or coalescence) before the cake structure sets and coarse crumb is formed (Sahi & Alava, 2003). This reasoning is in line with the observation that FRL sponge cakes have a coarse crumb (Pycarelle et al., 2019).

That smaller lipid molecules generally adsorb faster at air-liquid interfaces than larger protein molecules (Bos & van Vliet, 2001) may explain the negative impact of relocated flour lipids on air-liquid interface stability at relatively short time scales (*i.e.* during mixing and when gas cells expand during early baking). However, the adsorption rate of surface-active molecules at interfaces not only depends on molecular size but also on *e.g.*

pH, molecule concentration, protein-to-lipid ratio in the continuous phase and the viscosity of the continuous phase (Bos & van Vliet, 2001; Damodaran, 2005). In this context, it is of note that in emulsions the protein-to-lipid ratio at the oil-liquid interface is lower when it is higher prior to homogenization with the oil (Damodaran, 2005).

At T2, foam from FRL batter had the same protein and lipid contents as that made from CF batter at the same time (see section 3.2). Moreover, their lipid and protein compositions also did not differ (see sections 3.3 and 3.4). This indicates that after some time the air-liquid interface in FRL batter is occupied by a mixed molecular population similar to that in CF batter. Hence, at longer time scales flour lipids probably have a smaller impact on air-liquid interface stability than at shorter time scales.

4.3 Impact of prior removal of wheat flour lipids on air-liquid interface stability in sponge cake batter

PDF batter and foam made thereof contained less lipid but the same concentration of protein as CF batter and foam samples made from the latter, respectively (see section 3.2). When isolated at T1 or T2, foam protein compositions did not largely differ between both samples (see section 3.3). Hence, partial removal of flour lipids prior to sponge cake batter preparation did not impact the adsorption of proteins at the air-liquid interface in foam isolated from PDF batter.

Evidently, the level of lipids mainly originating from flour were lower in foams from PDF batter than in those made from CF or FRL batter (see section 3.4). In contrast to what was the case for foams made from CF batter (Pycarelle et al., 2020) or FRL batter, PC and PE were retained in foam from PDF batter (see section 3.4). Prior removal of flour lipids clearly increased the level of those lipids adsorbed at the air-liquid interface in foam made from PDF batter at T2. PC and PE mainly occur as spherical low density lipoproteins in egg yolk (Anton et al., 2003; Kamat et al., 1972; Powrie & Nakai, 1985). Since these structures are important for air incorporation during sponge cake batter preparation (Kamat et al., 1972; Kamat, Lawrence, Hart & Yoell, 1973) and stabilize air-liquid interfaces (Dauphas et al., 2006; Martinet et al., 2003), the increased adsorption of PC and PE at longer time scales (*i.e.* T2) may aid gas cell stability after mixing and during early baking.

Based on the above results, it is concluded that the molecular population at the air-liquid interface in foam from PDF batter consists mainly of egg yolk lipids and the same flour and egg proteins which also adsorb at the air-liquid interface in foam from CF batter. Foaming properties of PDF batter are thus likely improved (see section 3.1) because flour lipids do not interfere with the protein-dominated air-liquid interface as previously hypothesized (Pycarelle et al., 2019). Especially the removal of free flour lipids is beneficial in this context. Indeed, bound flour lipids, as their name implies, are associated with other flour components and, when not removed by the solvent treatment, are unable to diffuse to and adsorb at the interface (Pycarelle et al., 2019). Furthermore, it seems reasonable that during baking the air-liquid interface in PDF batter is also more stable than that in CF or FRL batter as flour lipids can no longer rapidly adsorb at expanding interfaces (see section 4.2). Indeed, partial removal of flour lipids not only improves air incorporation during mixing but probably also gas cell stability during further cake making. This is deduced from the high volume and fine crumb of cakes made with PDF as previously observed (Pycarelle et al., 2019).

5 Conclusions

This study provides insights on the role of flour lipids at the air-liquid interface in sponge cake batter. Flours were used in which lipids were relocated or from which part of the lipids were removed prior to batter preparation. The molecule population at the air-liquid interface in batters prepared with different flour samples was determined using a foam separation protocol. The insights obtained allowed validating the hypothesis that free flour lipids negatively impact air-liquid interface stability during sponge cake making.

Free flour lipids, either natively free or freed by solvent treatment, impact the composition of the molecular population adsorbed at the air-liquid interface in sponge cake batter. They mainly impact air-liquid interface stability at shorter time scales (i.e. during and after mixing and during early baking) because they adsorb faster thereat than surface-active proteins. Their adsorption affects that of egg lipids and limits the degree of protein adsorption at that interface. The latter most likely hinders the formation of a viscoelastic protein layer and reduces interfacial stability. Free flour lipid adsorption did however not impact the type of protein adsorbed at the air-liquid interface.

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Figure captions

Figure 1: Foaming properties of sponge cake batter prepared from (i) control flour (CF), (ii) flour containing relocated lipids (FRL) and (iii) partially defatted flour (PDF). (A) Diluted sponge cake batter was whipped for 150 s after which foam volume was monitored over a 60 min time period. Vertical bars indicate standard deviations. (B) Side and (C) top view pictures of foams prepared from CF, FRL and PDF batters taken 210 s (T1) and 15 min (T2) after the start of whipping. Arrows indicate foam coarsening. Graduated markings on cylinders differ in number but not in size.

Figure 2: SE-HPLC chromatograms of proteins extracted with a sodium dodecyl sulfate (SDS) containing medium from (A) sponge cake batter prepared from control flour (CF) and foam made thereof, (B) sponge cake batter prepared from flour containing relocated lipids (FRL) and foam made thereof and (C) sponge cake batter prepared from partially defatted flour (PDF) and foam made thereof. Foams were made by whipping diluted batter for 150 s. Foam was isolated at 210 s (T1) and 15 min (T2) after the start of whipping. Molecular weight markers include trypsin inhibitor from soy bean (20.1 kDa), chicken egg ovalbumin (45 kDa), bovine serum albumin (66 kDa) and phosphorylase b (97 kDa).

Figure 3: Lipid composition of sponge cake batter prepared from control flour (CF, black), flour containing relocated lipids (FRL, grey) and partially defatted flour (PDF, white) and foams made thereof. Foams were made and isolated as in Figure 2. (A) Lipid classes mainly present in flour. (B) Lipid classes mainly present in egg yolk. Vertical bars indicate standard deviations. Bars with the same color and the same letter within one lipid class are not siginificantly different ($\alpha > 0.05$). Significant differences ($\alpha < 0.05$) between foams prepared from CF or FRL batter separated at the same point in time or between foams from CF or PDF batter separated at the same point in time are indicated by an asterisk. DGDG: digalactosyldiacylglycerols, MGDG: monogalactosyldiacylglycerols, NAPE: *N*-acyl phosphatidylethanolamines, FFA: free fatty acids, TAG: triacylglycerols, PC: phosphatidylcholines, PE: phosphatidylethanolamines, CHO: cholesterol.

Figure 1

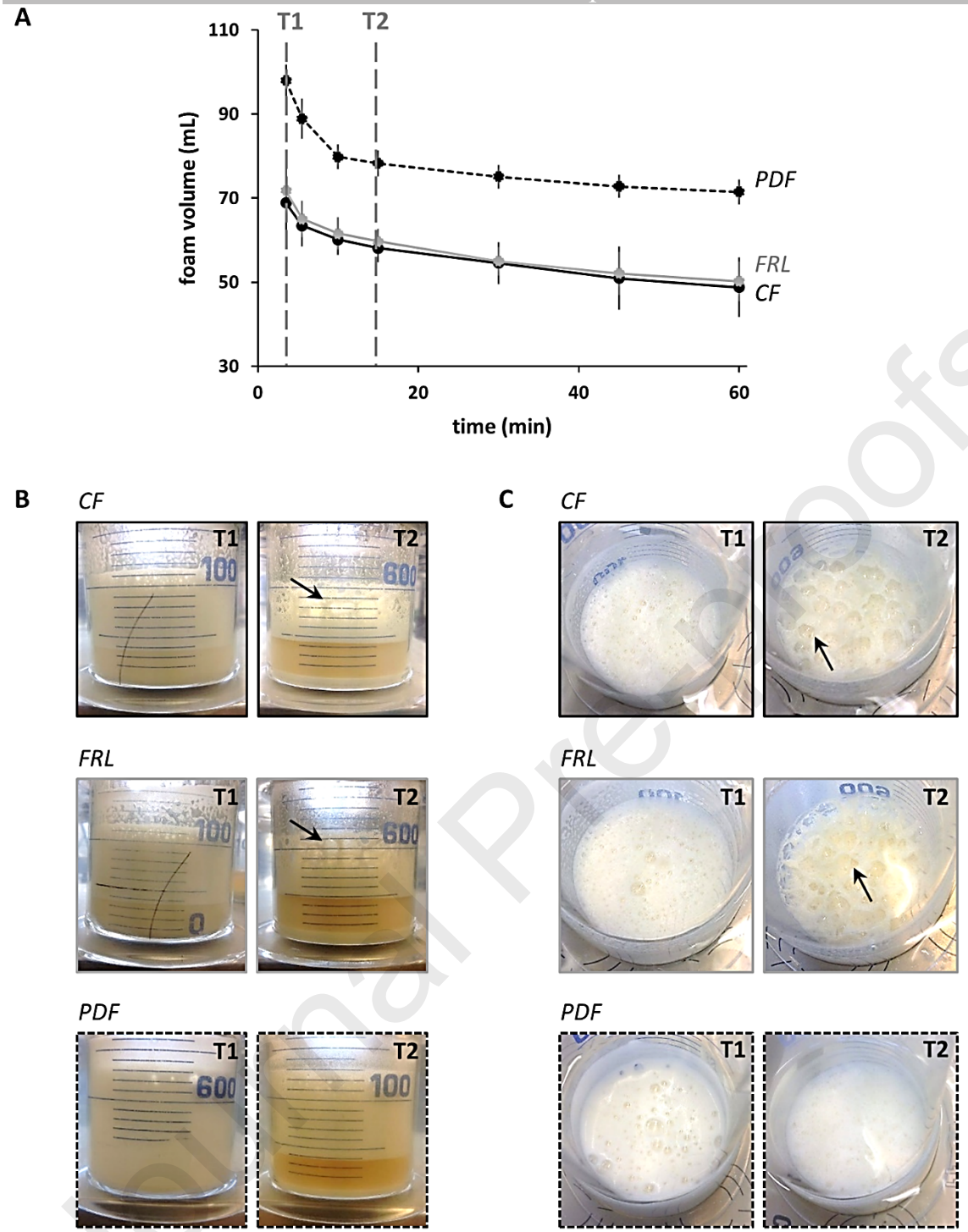


Figure 2

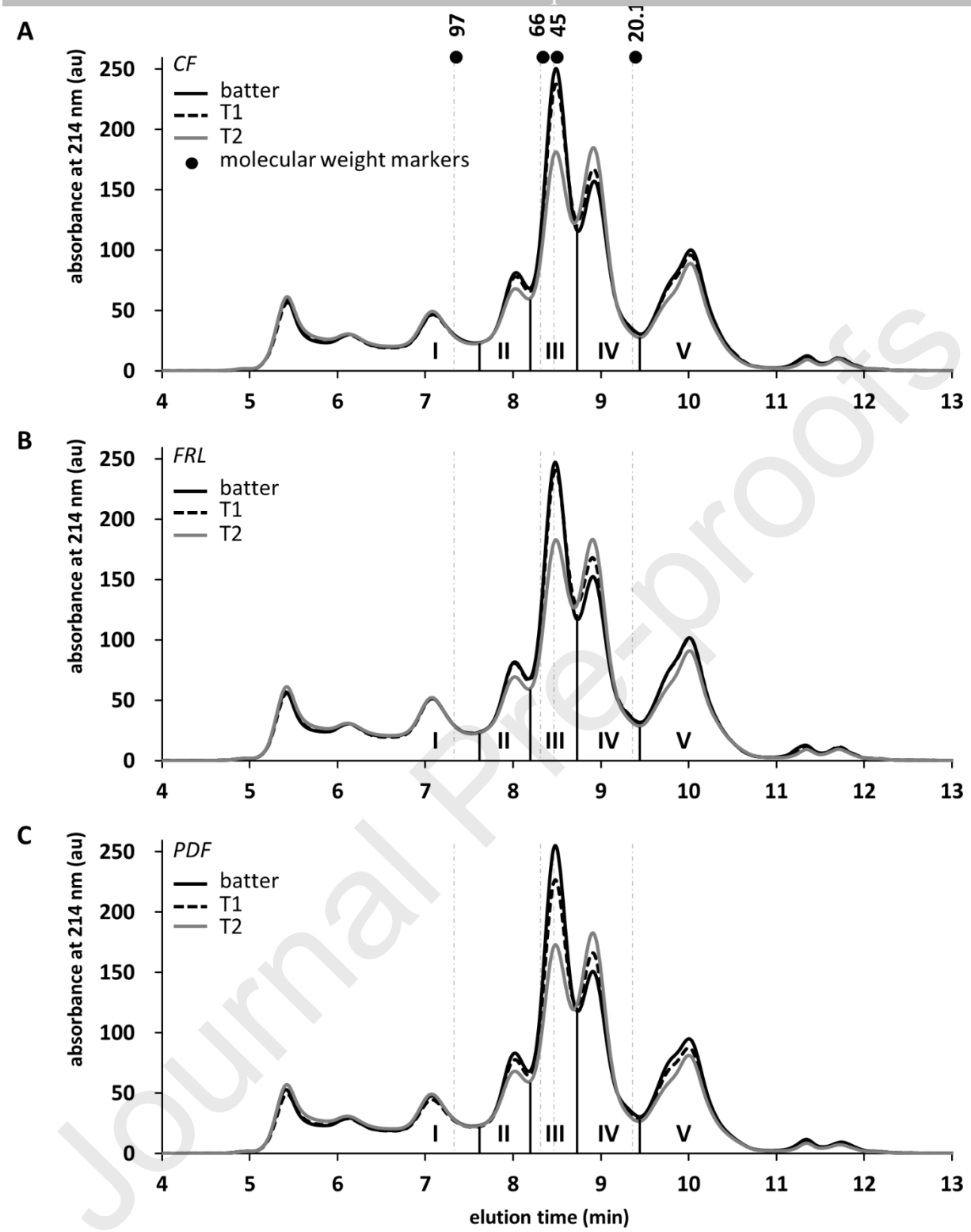


Figure 3

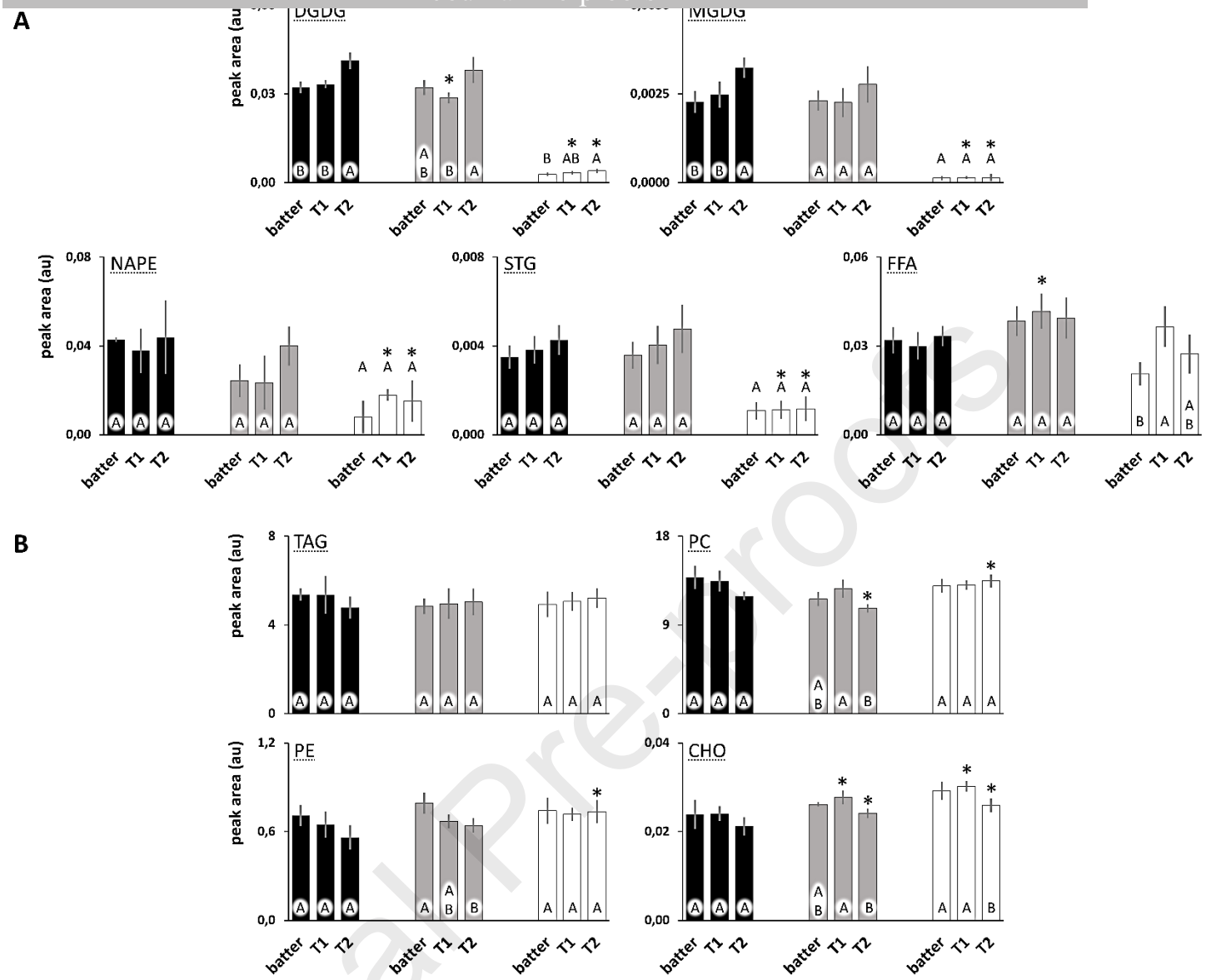


Table 1: Protein and lipid contents and protein-to-lipid ratios in sponge cake batter prepared from (i) control flour (CF), (ii) flour containing relocated lipids (FRL) and (iii) partially defatted flour (PDF) and in foams made thereof. Foams were made by whipping diluted sponge cake batter for 150 s and isolated at 210 s (T1) and 15 min (T2) after the start of whipping. Protein and lipid contents are expressed as a percentage of the sample dry matter (dm). Standard deviations are indicated between brackets.

		Batter	Foam at T1	Foam at T2
Protein content (% of dm)	CF	10.1 (0.4)^{a}	11.3 (0.3)^{B,a}	12.4 (0.3)^{A,a}
	FRL	10.0 (0.2)^{a}	10.8 (0.2)^{B,b}	11.9 (0.5)^{A,a}
	PDF	10.1 (0.1)^{a}	11.2 (0.3)^{B,a}	12.3 (0.4)^{A,a}
Lipid content (% of dm)	CF	3.8 (0.1)^{a}	3.9 (0.1)^{B,b}	4.1 (0.2)^{A,a}
	FRL	3.6 (0.1)^{a}	4.2 (0.1)^{A,a}	4.0 (0.2)^{A,a}
	PDF	3.2 (0.1)^{b}	3.3 (0.1)^{A,c}	3.4 (0.1)^{A,b}
Protein-to-lipid ratio (-)*	CF	2.7 (0.1)	2.9 (0.1)	3.0 (0.2)

FRL	2.7 (0.1)	2.6 (0.1)	3.0 (0.2)
PDF	3.1 (0.1)	3.4 (0.1)	3.6 (0.2)

Results in the same row with the same upper case letter and results in the same column with the same lower case letter are not significantly different ($\alpha > 0.05$).

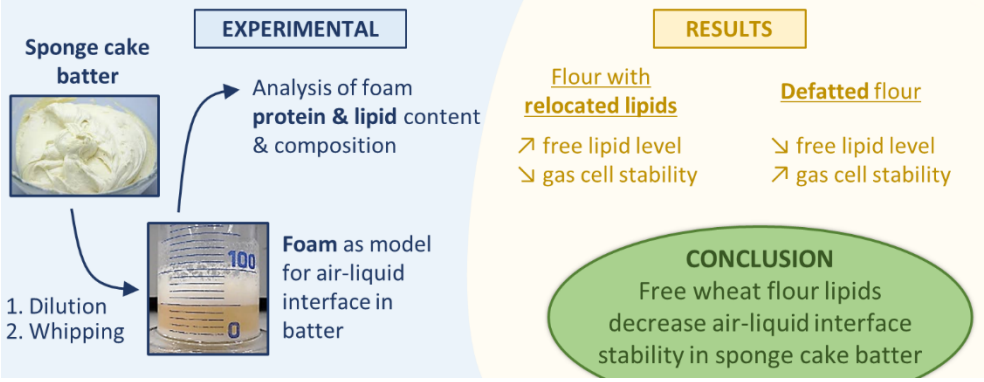
* Protein-to-lipid ratios were calculated from average protein and lipid contents. Standard deviations (indicated between brackets) were calculated according to propagation of errors. As no individual values were obtained for this parameter also no statistical analysis was performed.

Table 2: Areas in SE-HPLC chromatograms [in arbitrary units (au)] of proteins extracted with sodium dodecyl sulfate (SDS) medium or SDS and dithiothreitol (SDS+DTT) medium and the corresponding percentages of proteins extractable in SDS medium (SDS EP) for sponge cake batter prepared from (i) control flour (CF), (ii) flour containing relocated lipids (FRL) and (iii) partially defatted flour (PDF) and for foams made thereof. Foams were made and isolated as in Table 1. Standard deviations are indicated between brackets.

		Batter	Foam at T1	Foam at T2
SDS area (au)	CF	194 (1)^{A,a}	192 (8)^{A,a}	184 (7)^{A,ab}
	FRL	194 (5)^{A,a}	196 (5)^{A,a}	185 (3)^{B,a}
	PDF	190 (4)^{A,a}	187 (1)^{A,a}	175 (2)^{B,b}
SDS+DTT area (au)	CF	222 (4)^{A,a}	221 (8)^{A,a}	222 (3)^{A,a}
	FRL	228 (5)^{A,a}	226 (6)^{A,a}	222 (2)^{A,a}
	PDF	221 (3)^{A,a}	221 (3)^{A,a}	216 (2)^{B,b}
SDS EP (%)	CF	87.3 (0.5)^{A,a}	87.2 (0.5)^{A,a}	83.1 (2.3)^{B,a}
	FRL	85.4 (2.1)^{AB,a}	86.8 (0.4)^{A,a}	83.3 (0.8)^{B,a}
	PDF	86.0 (1.0)^{A,a}	84.7 (0.7)^{A,b}	81.3 (1.1)^{B,a}

Results in the same row with the same upper case letter and results in the same column with the same lower case letter are not significantly different ($\alpha > 0.05$).

Graphical abstract



Highlights

- Prior removal of flour lipids (FLs) improves foaming of sponge cake batter
- Air-liquid interfaces in sponge cake batter from defatted flour contain less lipid
- Free FLs decrease the stability of the protein-dominated interface in batter
- Relocation of FLs makes them more available for adsorption at air-liquid interfaces
- Relocation of FLs lowers initial protein adsorption at air-liquid interfaces

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Sarah C. Pycarelle: conceptualization, methodology, investigation, validation, visualization, writing – original draft, writing – review & editing

Geertrui M. Bosmans: conceptualization, writing – review & editing

Bram Pareyt: conceptualization, writing – review & editing

Kristof Brijs: writing – review & editing, supervision

Jan A. Delcour: conceptualization, resources, writing – review & editing