Wheat (*Triticum aestivum* L.) flour free lipid fractions negatively impact the quality of sponge cake

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16 **Abstract**

17 The functionality of wheat flour lipids in sponge cakes prepared with only flour, sugar, eggs and leavening
18 agents was investigated by altering their location or content in flour. Hexane (hex) or the more polar
19 hexane:isopropanol (3:2 v/v) (hex:isoprop) were used to impact free flour lipid (FFL) or both FFL and bound
20 flour lipid (BFL) fractions, respectively. Flour from which the FFLs were removed resulted in significantly
21 improved cake volumes and crumb structures. Additional removal of part of the BFLs did not further impact
22 cake quality. Prior contact of flour with hex:isoprop followed by gently removing the solvent broke native
23 interactions between BFLs and starch or gluten and relocated more lipids than did hex. Cakes from flour
24 with relocated lipids had coarse crumb structures. Our study demonstrates that FFLs and relocated flour
25 lipids negatively impact sponge cake quality by disturbing air-liquid interface stabilization during mixing and
26 the early phases of baking.

27 **Keywords**

28 sponge cake; cake quality; non-starch flour lipids; endogenous surfactant functionality; foam formation and
29 stabilization; interfacial stability; clean label

30 **1 Introduction**

31 Cakes are, next to bread and cookies, one of the most popular baked foods worldwide. Their main
32 ingredients are flour, sugar, eggs, (fat) and leavening agents. Based on the ingredients and the mixing
33 method, cakes are classified either as batter (*e.g.* pound cake), foam (*e.g.* sponge cake) or chiffon cakes. In
34 contrast to batter cakes, foam cakes are prepared without additional fat such as margarine or shortening
35 (Wilderjans, Luyts, Brijs, & Delcour, 2013). Lipids in sponge cake batter hence only originate from egg yolk
36 and flour and are here referred to as endogenous batter lipids. The use of additional lipid-like surfactants in
37 their recipes is optional (*cf. infra*). Sponge cakes have a springy texture and are *e.g.* used in layer cakes or
38 Swiss rolls which also contain fillings such as fruit jam and toppings like whipped cream (Lai & Lin, 2006).

39 Cake making includes three stages: (i) mixing ingredients to form batter, (ii) baking batter to form cake, and
40 (iii) cooling the cake to room temperature. Traditionally, sponge cake batter is prepared by mixing the
41 ingredients in different steps, *i.e.* with a multi-stage mixing method. Initially, air is incorporated by beating
42 sugar and whole eggs or egg whites into a foam, followed by gently folding in the remaining ingredients to
43 minimize air loss (Conforti, 2006; Shepherd & Yoell, 1976). During mixing, it is necessary to form a uniform
44 batter and to entrap high levels of finely distributed gas cells. Doing so yields (sponge) cakes of high quality
45 (Conforti, 2006). With increasing temperatures during baking, the gas cells in the batter expand because of
46 more gas being present and because of the implications of the ideal gas law. The former is the result of
47 water evaporation and release of carbon dioxide from the leavening agents (Wilderjans, Luyts, Brijs, &
48 Delcour, 2013). Because no additional gas cells are formed during baking, the degree to which air initially is
49 incorporated and the way the gas cells initially are distributed determine leavening, crumb structure and
50 cake texture (Brooker, 1993; Conforti, 2006; Handleman, Conn, & Lyons, 1961).

51 Sponge cake batter is a foam and thus intrinsically unstable. As a result, the incorporated gas cells need to
52 be stabilized against coalescence and disproportionation during and after mixing and during the initial
53 phases of baking until cake structure is set (Lai & Lin, 2006; Murray, 2007; Sahi & Alava, 2003; Wilderjans,
54 Luyts, Brijs, & Delcour, 2013). In a liquid foam structure, the air-liquid interface is stabilized by surface-
55 active molecules which lower the interfacial tension by orienting their hydrophilic and hydrophobic regions

56 to reach a thermodynamically favorable situation (Hasenhuettl & Hartel, 2008). Generally, proteins and low
57 molecular weight surfactants (LMW-S) – both of which can be of endogenous or exogenous origin – are the
58 two main classes of surface-active components in food systems (Mackie & Wilde, 2005; Sahi & Alava, 2003).

59 Proteins undergo considerable changes in three-dimensional structure during adsorption at an interface
60 where, in essence, they form a viscoelastic layer (Mackie & Wilde, 2005; Murray, 2007). In contrast, the
61 more surface-active LMW-S are more mobile and form fluid-like interfacial films (Damodaran, 2005;
62 Murray, 2007). When interfacial films stretch, local thinning occurs, the local LMW-S concentration
63 decreases and interfacial tension rises. To restore equilibrium conditions, LMW-S adsorb from the bulk at
64 the interface or diffuse laterally across it. The latter also drives the aqueous phase to move towards the
65 thin film and restores equilibrium film thickness. This is the Gibbs-Marangoni mechanism (Hasenhuettl &
66 Hartel, 2008). Interface stabilization by proteins occurs more slowly but, once formed, the film surrounding
67 the gas cells is more stable. Conversely, interface stabilization by LMW-S occurs very fast, but the formed
68 film is typically not viscoelastic and therefore less stable (Damodaran, 2005; Murray, 2007).

69 When both proteins and LMW-S are present, as is the case in many food systems, they compete at the
70 interface and stabilization is not very efficient due to the incompatibility of the mechanisms whereby they
71 stabilize interfaces. A mixed interface can be formed either by simultaneous adsorption of proteins and
72 LMW-S, by penetration of LMW-S into a protein film or, *vice versa*, by penetration of proteins into an LMW-
73 S film (Mackie & Wilde, 2005; Murray, 2007). Mackie, Gunning, Wilde, & Morris (1999) introduced the
74 mechanism of organic displacement. In this mechanism, LMW-S adhere to the interface at voids in the
75 viscoelastic protein layer. As regions of LMW-S expand, the interface becomes weaker, protein regions are
76 compressed and proteins eventually are expelled from the interface (Mackie, Gunning, Wilde, & Morris,
77 1999).

78 Cake production and quality are highly impacted by the use of LMW-S such as emulsifiers and foaming
79 agents (Hasenhuettl & Hartel, 2008; Sahi & Alava, 2003). Combined, their use reduces production time and
80 leads to cakes of outstanding quality (Rodríguez-García, Sahi, & Hernando, 2014). Foaming agents stabilize
81 the air-liquid interface yielding evenly dispersed gas cells throughout the batter. High levels of additional

82 LMW-S such as polyglycerol esters of fatty acids and monoacylglycerols (MAG) facilitate air incorporation
83 and allow single stage mixing in industrial sponge cake preparation (Lai & Lin, 2006; Richardson, Langton,
84 Faldt, & Hermansson, 2002; Rodríguez-García, Sahi, & Hernando, 2014).

85 In the European Union, food labels must mention additives according to their functional category (*e.g.*
86 sweetener) and by their specific name or E-number, *i.e.* a number referring to its specific function (Bearth,
87 Cousin, & Siegrist, 2014; Van Loco, Vandevijvere, Cimenci, Vinkx, & Goscinny, 2015). As many consumers do
88 not trust E-number additives (Bearth, Cousin, & Siegrist, 2014), the demand for clean label food products
89 which still are of outstanding quality rapidly increases. However, in the specific case of sponge cake
90 systems, profound knowledge concerning the role of lipids and proteins from egg and flour which can act as
91 surface-active components is lacking. An in-depth investigation on the current role of these endogenous
92 surface-active components during sponge cake making is therefore indispensable.

93 In this work, we focused on **flour lipids**, a fraction of the endogenous batter lipids present in sponge cakes
94 produced from flour, sugar, eggs and leavening agents only. They make up 2.0 to 3.0% of flour dry matter
95 (dm) and have been classified as starch (*ca.* 40%) or non-starch lipids (*ca.* 60%). It is typically stated that the
96 former are only extractable at temperatures exceeding the gelatinization temperature, while the latter are
97 extractable at room temperature (Chung, Ohm, Ram, Park, & Howitt, 2009; Pareyt, Finnie, Putseys, &
98 Delcour, 2011). Flour non-starch lipids are a heterogeneous group composed of *ca.* 43% non-polar (neutral)
99 lipids and *ca.* 57% polar lipids (Pareyt, Finnie, Putseys, & Delcour, 2011). They are typically classified as free
100 and bound flour lipids (BFLs) based on their extractability at room temperature. Free flour lipids (FFLs) are
101 extracted with a non-polar solvent (*e.g.* hexane). They primarily contain non-polar lipids such as
102 triacylglycerols (TAG) and free fatty acids (FFA) but also small amounts of more polar lipids. After extracting
103 the FFLs, BFLs are extracted using a more polar solvent [*e.g.* water saturated butanol (WSB)]. They include
104 polar lipids such as galacto- and phospholipids (Chung, Ohm, Ram, Park, & Howitt, 2009; Pareyt, Finnie,
105 Putseys, & Delcour, 2011). In wheat, oil bodies or spherosomes, *i.e.* TAG surrounded by a monolayer of
106 polar lipids and oleosin proteins, mostly occur in the germ and aleurone and to a smaller extent in the
107 endosperm (Chung, Ohm, Ram, Park, & Howitt, 2009; Hargin, Morrison, & Fulcher, 1980; Huang, 1992).

108 During milling the endosperm lipids and a small portion of the germ lipids are transferred to the flour
109 (Morrison, 1978).

110 Even though lipids are only a minor component of flour, they are of great importance for bread loaf volume
111 and crumb structure (Gan, Angold, Williams, Ellis, Vaughan, & Galliard, 1990; Gerits, Pareyt, & Delcour,
112 2014; Schaffarczyk, Ostdal, Matheis, & Koehler, 2016; Sroan & MacRitchie, 2009). In the case of cake
113 systems, some work has already dealt with lipids in chlorinated flour and how they contribute to cake
114 quality (Clements & Donelson, 1982; Johnson, Hosene, & Varriammarston, 1979). However, to the best of
115 our knowledge, the importance of unmodified flour lipids as endogenous surface-active components for
116 foam formation and stabilization in cake batter systems and for cake quality has never been investigated.

117 The present work aimed at studying the role of flour lipids as surface-active components in sponge cakes
118 made from flour, sugar, eggs and leavening agents only. To that end, flour was treated with hexane
119 (hereafter referred to as hex) or hexane:isopropanol (3:2 v/v) (hereafter referred to as hex:isoprop) to
120 impact the flour lipid population. These solvents were chosen because they differ in polarity, are easy to
121 evaporate and extract little if any protein (results not shown). Because, unlike what is the case in bread
122 making, wheat flour gluten development is not a determining factor during batter preparation and cake
123 structure consists of networks containing gelatinized starch and both flour and egg proteins (Deleu,
124 Wilderjans, Van Haesendonck, Brijs, & Delcour, 2016; Wilderjans, Luyts, Brijs, & Delcour, 2013), it is
125 reasonable to expect only limited solvent effects when producing sponge cake from flour treated with
126 these solvents. In our work, we used two different experimental approaches. First, we brought flour in
127 contact with these solvents. Thereafter, they were evaporated without prior removal of the extracted lipids
128 as a way to relocate lipids. In a second approach, the same solvents were used to defat the flour. The
129 impact of both flour lipid relocation and defatting on sponge cake (batter) quality was then examined.
130 Batter density, batter viscosity, cake volume, cake texture and crumb structure were evaluated. The work
131 not only extends knowledge concerning flour lipid functionality in complex food foams, it can also be
132 valuable for developing clean label alternative ingredients for sponge cake making (*cf. supra*).

133 2 Materials and methods

134 **2.1 Chemicals and materials**

135 Wheat flour (Halm flour) [14.0% moisture content (MC) and 10.4% protein (N x 5.7) content] was from
136 Paniflower (Merksem, Belgium). Rice starch (11.8% MC) was from Beneo (Wijgmaal, Belgium). Sodium acid
137 pyrophosphate (number 15) and sodium bicarbonate were from Budenheim (Budenheim, Germany). Eggs
138 were from a local supermarket, stored at 3 °C and used before their expiry date. Hex, chloroform and
139 methanol were from Thermo Fisher Scientific (Geel, Belgium), isoprop from Honeywell Riedel-de Haën
140 (Seelze, Germany), butanol from Chem-Lab (Zedelgem, Belgium) and isooctane from Merck (Darmstadt,
141 Germany). All solvents were of high performance liquid chromatography (HPLC) grade. Acid washed silicon
142 dioxide was from Sigma-Aldrich (Bornem, Belgium).

143 **2.2 Methods**

144 *2.2.1 Flour moisture and protein contents*

145 Flour MC was determined in triplicate according to AACCI approved method 44-15.02 (AACCI, 1999).
146 Protein content was determined in triplicate using an adaptation of the Association of Official Analytical
147 Chemists (AOAC) official method 990.03 (AOAC, 1995) to an automated Dumas protein analysis system
148 (VarioMax Cube N, Elementar, Hanau, Germany).

149 *2.2.2 Relocating lipids in wheat flour with hexane or hexane:isopropanol (3:2 v/v)*

150 **Control flour** (CF) was treated with hex or hex:isoprop by shaking a quantity containing 400 g dm in 800 mL
151 solvent for 60 min at 150 rotations/min (rpm) at room temperature. The supernatant was removed by
152 Büchner filtration over paper. The solids were suspended in 800 mL extraction solvent and the extraction
153 was repeated. After four such extraction steps in total, the solids were spread out in glass containers and
154 the pooled lipid extracts were added. The solvent was evaporated under a fume hood overnight at room
155 temperature. This protocol evidently did not remove but only relocated (part of the) flour lipids (*cf.* section
156 3.1). The next day the resulting **flour with relocated lipids** (FRL) was milled with a laboratory mill (model
157 A10, IKA, Staufen, Germany), sieved (mesh size of 400 µm), and stored at 7 °C until further use. In what
158 follows, the subscripts hex and hex:isoprop are used to specify the solvents used for this procedure. Thus,

159 e.g. flour containing relocated lipids as a result of treatment with hex is abbreviated as FRL_{hex}. FRL_{hex} was
160 prepared once, while FRL_{hex:isoprop} was prepared in duplicate.

161 2.2.3 *Extracting lipids from wheat flour with hexane or hexane:isopropanol (3:2 v/v)*

162 To extract flour lipids, the abovementioned procedure (cf. section 2.2.2) was repeated without adding the
163 lipid extracts back to the flour solids. Flour solids were dried on filter paper overnight under a fume hood
164 and are referred to as **defatted flour** (DF) which was also milled and sieved as described in section 2.2.2. As
165 above, subscripts are used to specify the extraction solvents and DF_{hex} was prepared once, while
166 DF_{hex:isoprop} was prepared in duplicate.

167 2.2.4 *Flour lipid analysis*

168 FFL and BFL **contents** of CF, FRL_{hex}, FRL_{hex:isoprop}, DF_{hex} and DF_{hex:isoprop} were determined in triplicate as in
169 Gerits, Pareyt, & Delcour (2013). In brief, 0.86 g dm flour was homogenized with 26.0 g of acid washed
170 silicon dioxide (50 – 70 mesh particle size) and poured into 22 mL extraction cells. FFLs and BFLs were
171 sequentially extracted at 40 °C with an accelerated solvent extractor 200 (Dionex, Amsterdam, The
172 Netherlands) at increased pressure (6.90 MPa). Other settings were as in Gerits, Pareyt, & Delcour (2013).
173 Hex and WSB were used to extract FFLs and BFLs, respectively. To avoid confusion with the hex treatments
174 (cf. sections 2.2.2 and 2.2.3), the hex solvent used to afterwards determine FFL content is referred to as
175 hex'. The hex' and WSB extracts were collected in test tubes and the solvents evaporated with a rotational
176 vacuum concentrator (Martin Christ, Osterode am Harz, Germany). Hex' was evaporated at 40 °C and 800
177 Pa, and WSB at 60 °C and 100 Pa. BFLs were purified according to Bligh and Dyer (1959). Dry lipid extracts
178 were accurately weighed and stored at -80 °C under nitrogen atmosphere in amber colored vials until
179 analysis. The term total lipid content here refers to the sum of FFLs and BFLs.

180 FFL and BFL **composition** of CF, DF_{hex} and DF_{hex:isoprop} were determined by injecting the lipid extracts onto
181 an HPLC system (Shimadzu, Kyoto, Japan) connected to an evaporative light scattering detector (model
182 3300, Alltech, Deerfield, IL, USA) as described by Melis, Pauly, Gerits, Pareyt, & Delcour (2017) which itself
183 is based on Gerits, Pareyt, & Delcour (2013). Optimal separation and detection of wheat flour lipid classes

184 were performed using a quaternary gradient and by altering the gain during HPLC analysis, respectively.
185 Cholesterol was used as internal standard. Lipid extracts from 0.86 g dm flour (*cf. supra*) were dissolved in
186 1.00 mL isooctane and, depending on the extract, injection volumes and cholesterol levels were adapted to
187 facilitate accurate detection of all lipid classes. Injection volumes for FFLs and BFLs were 0.5 μ L (containing
188 2.5 μ g cholesterol) and 3.0 μ L (containing 3.0 μ g cholesterol), respectively. Data acquisition and analysis
189 were performed with Shimadzu LCSolution version 1.23 SP1.

190 2.2.5 Batter preparation and cake baking

191 Sponge cake batter was prepared using an in-house developed multi-stage mixing method. It contained
192 sieved flour (284.0 g, 14.9% MC), sugar (247.0 g), egg white (176.0 g), egg yolk (64.0 g), sodium bicarbonate
193 (4.8 g), sodium acid pyrophosphate (6.6 g), rice starch (30.8 g, 10.0% MC) and deionized water (90.6 g).
194 Ingredients were mixed with a Hobart (Troy, OH, USA) N50 5-Quart Mixer. In a first step, egg white and one
195 third of the sugar were beaten with a wire whip for 90 s (speed 3). Secondly, egg yolk, the remaining sugar,
196 water, rice starch and 50% of the sieved flour were mixed with a wire whip (3 min, speed 3) and, thereafter,
197 blended with the egg white mixture (wire whip, 30 s, speed 1). Next, 25% of the sieved flour, sodium acid
198 pyrophosphate and sodium bicarbonate were added. To minimize air loss, a flat beater instead of a wire
199 whip was used (10 s, speed 1). Finally, the remaining flour was added (flat beater, 10 s, speed 1), the
200 material adhering to the edges and at the bottom of the mixing bowl was scraped loose and the batter was
201 homogenized one last time (flat beater, 15 s, speed 1).

202 CF batter was prepared in triplicate, FRL_{hex} and DF_{hex} batters were prepared once and FRL_{hex:isoprop} and
203 DF_{hex:isoprop} batters in duplicate from separately made flours (*cf. sections 2.2.2 and 2.2.3*). Four aluminum
204 baking trays (Gents Bakkershuis, Gent, Belgium, length x width x height: 19.0 x 8.6 x 5.0 cm) were filled with
205 125.0 g of each batch of sponge cake batter. Baking was for 30 min in a conventional plate oven (Hein
206 Condilux, Hein, Strassen, Luxembourg) with top and bottom temperatures set to 180 and 160 °C,
207 respectively. Cakes were cooled for 2 h at 23 °C and then stored in plastic bags until further analysis.

208 2.2.6 Batter properties

209 **Batter density** was determined in duplicate by weighing the batter contained in a plastic cylinder of 100 mL
210 (Wilderjans, Pareyt, Goesaert, Brijs, & Delcour, 2008). A rheometer (Brookfield DV-III Ultra, Brookfield
211 Engineering, Middleboro, MA, USA) equipped with a Helipath stand was used to determine **apparent batter**
212 **viscosity**. Batter was poured into a 150 mL glass beaker to a height of 80 mm and levelled off at the top.
213 Viscosity measurements were with a T-E spindle rotating at 1 rpm and moving into and out of the sample at
214 0.37 mm/s over a distance of 40 mm. Because the spindle continuously cut into fresh material as it rotated,
215 channeling – which often occurs when conventional spindles are used to measure high viscosities – was
216 avoided. The apparent viscosity reported is the average viscosity registered during the experiment. Three
217 samples of a single batter were analyzed. **Gas cell size and distribution** in cake batter were qualitatively
218 evaluated with light microscopy images taken with a Nikon (Melville, NY, USA) ECLIPSE 80i normal light
219 microscope equipped with a charge-coupled device camera. To ensure visualization of intact gas cells, a
220 drop of cake batter was placed on a cover glass fixed to a metal holder (thickness of 2.0 mm). Next, another
221 cover glass not in contact with the batter was placed on top of the metal holder. This sealed the batter
222 between two cover glasses without exerting pressure on it. Microscopy images were taken in bright field
223 mode, 10 times magnified and captured with NIS-Elements BR software (Nikon).

224 2.2.7 *Cake properties*

225 **Cake volume** (in mL) was measured with a VolScan Profiler (Stable Micro Systems, Godalming, UK) with
226 surface scans made from top to bottom every 2 mm. **Crumb hardness, cohesiveness** and **springiness** were
227 determined with an Instron (Norwood, MA, USA) 3342 texture analyzer equipped with a cylindrical probe
228 (diameter of 75 mm) and a 50 N load cell. A cylindrical crumb sample (height of 25 mm and diameter of 30
229 mm) was taken from the center of the cake and subjected to two compression-decompression cycles in
230 which the force-time curve was monitored. Cake crumb was compressed until 50% of the initial sample
231 height at 2.0 mm/s. The waiting time between compressions was 3 s. Hardness was the maximal force
232 necessary to compress cake crumb by 50%. The hardness data were corrected for cake density and are
233 referred to as corrected hardness expressed in $\text{N} \cdot \text{cm}^3/\text{g}$. Crumb cohesiveness is the area beneath the curve
234 of the second compression-decompression cycle divided by that beneath the curve of the first
235 compression-decompression cycle. Springiness equals the ratio of the time needed for the second

compression to that needed for the first and shows the extent to which a deformed sample returns to its original shape. In total, at least eight crumb samples for every batter replicate were analyzed. **Crumb structure** was analyzed as in Wilderjans, Pareyt, Goesaert, Brijs, & Delcour (2008) with small adaptations. Two slices from the center of each cake [thickness of 1.0 cm, cut with an electrical slicer (Affettatrice Slicer 30N, Galesecca, Italy)] were scanned in 256 grey levels with a resolution of 300 dots/inch on a flatbed scanner (HP Scanjet 3800, Hewlett-Packard Company, Beijing, China). A field of view of 35 x 35 mm was chosen at the center of the slice. Images were stored in bmp format. They consisted of 412 columns by 412 rows of picture elements (pixels) and were converted to binary images with the Otsu thresholding algorithm using ImageJ. Finally, the binary images were used to determine the crumb grain parameters mean cell size and number of cells/cm² using the image processing toolbox of Matlab R2016b (Mathworks, Natick, MA, USA).

2.2.8 Statistical analyses

For several variables, mean values based on at least three measurements were compared using one-way analysis of variance (ANOVA) performed with JMP Pro 12 (SAS Institute, Cary, NC, USA). Significant differences between mean values were verified with a Tukey multiple comparison test at a significance level (α) of 0.05.

3 Results

3.1 Flour lipid analysis

Table 1 lists FFL (*i.e.* lipids extracted with hex'), BFL (*i.e.* lipids extracted with WSB) and total lipid contents of all flour samples used in this study (*cf.* section 2.2.4).

Total lipid content of CF was in line with earlier data (Chung, Ohm, Ram, Park, & Howitt, 2009; Gerits, Pareyt, & Delcour, 2013; Morrison, 1978; Morrison, Mann, Soon, & Coventry, 1975). In total, hex and hex:isoprop extracted 59.9% (or 0.9% of flour dm) and 72.0% (or 1.1% of flour dm) of total extractable lipids, respectively (Table 1). Similar trends were recently observed for solvent extractions at ambient temperature by Bahrami, Yonekura, Linforth, da Silva, Hill, Penson, et al. (2014). They showed that

261 increased solvent polarity results in increased extraction of polar lipids and that hex:isoprop extracts
262 significantly more galactolipids than hex.

263 As expected, FFL and BFL content of FRL_{hex} were not significantly ($\alpha = 0.05$) different from those of CF. This
264 was also the case for FRL_{hex:isoprop}. However, some lipids in FRLs no longer occurred at their original location
265 as interactions between lipids and other flour components were broken as a result of the solvent
266 treatment. Hex only breaks hydrophobic interactions, whereas a more polar solvent such as hex:isoprop
267 also breaks up electrostatic interactions and/or hydrogen bonds (Melis, Pauly, Gerits, Pareyt, & Delcour,
268 2017). Apparently, disruption of bonds by hex:isoprop did not result in an increased level of FFLs. As
269 expected, DF_{hex} and DF_{hex:isoprop} no longer contained FFLs. The BFL content of DF_{hex:isoprop} was lower than
270 that of DF_{hex}. However, that hex had already removed a significant portion of the BFL rather came as a
271 surprise. DF_{hex} and DF_{hex:isoprop} still contained 66.7% and 48.3% of the BFL fraction of CF, respectively.

272 Flour lipids removed by hex or hex:isoprop were identified indirectly by examining the remaining lipids in
273 DF_{hex} and DF_{hex:isoprop} by HPLC. It needs to be stressed that one can only compare results within one lipid
274 class and that lipid levels between different flour samples cannot be compared in absolute terms. This
275 means that for a given lipid class it is only possible to conclude that levels are lower or higher in different
276 flour samples. Regardless of the solvent (hex or hex:isoprop) used for defatting, the DF samples contained
277 only a negligible amount of FFLs, *i.e.* extracted with hex' (Table 1). This decrease was clear for FFA and TAG
278 in the FFL fraction (Figure 1A). FFLs of CF also contained low levels of polar lipids such as
279 digalactosyldiacylglycerols (DGDG), *N*-acyl phosphatidylethanolamines (NAPE) and
280 phosphatidylethanolamines (PE). As a result of defatting with hex or hex:isoprop, DGDG and NAPE were no
281 longer detected. Next to that, both solvents decreased PE levels to a similar extent (results not shown). It is
282 known that PE occur as a part of oil bodies in wheat (Huang, 1992). Since levels of DGDG, NAPE and PE
283 were very low, they are not taken into account in the further discussion.

284 The BFLs (*i.e.* extracted with WSB) were less efficiently extracted with hex or hex:isoprop than the FFLs.
285 Prior defatting with hex or hex:isoprop did not result in an overall decrease of the non-polar lipid levels in

the BFL fraction (Figure 1A: FFA (bound), TAG (bound) and MAG (bound)). FFA were extracted with neither of these solvents. TAG levels were decreased due to prior defatting with hex while the use of hex:isoprop resulted in an unexpected increase. In contrast to the above, prior defatting with both solvents did result in a significant decrease of MAG, which was larger in the case of DF_{hex:isoprop} than in that of DF_{hex} (Figure 1A). In contrast to what was found for the level of non-polar lipids in the BFL fraction, the concentration of almost all polar lipids analyzed in this fraction was lower in the DF samples than in CF samples, and this irrespective of the solvent used (Figure 1B). Unexpectedly, hex removed a significant portion of monogalactosylmonoacylglycerols (MGMG), DGDG, NAPE, *N*-acyl lysophosphatidylethanolamines (NALPE) and phosphatidylcholines (PC). This means that, at room temperature (*cf.* defatting procedure described in section 2.2.3), hex already extracted part of the BFL fraction which is typically extracted with WSB at elevated temperatures and pressures (*cf.* flour lipid analysis described in section 2.2.4). Because of its polarity (Bahrami, et al., 2014), hex:isoprop extracted significantly higher levels of all galactolipids, NALPE and PC than did hex. NAPE, PE and lysophosphatidylcholines (LPC) were as efficiently extracted with hex:isoprop as with hex (Figure 1B). Logically, the lipids relocated by prior solvent treatment equaled those that were removed by defatting.

As briefly mentioned in the introduction, *ca.* 40% of wheat flour lipids are starch lipids. They natively occur as amylose-lipid complexes in starch granules and mostly consist of LPC (Chung, Ohm, Ram, Park, & Howitt, 2009; Morrison, 1978). Unexpectedly, a significantly lower LPC level was noted after extraction with the more polar hex:isoprop. It is typically believed that starch lipids are only extractable at temperatures higher than the starch gelatinization temperature with more polar solvents such as butanol (Morrison, 1978; Pareyt, Finnie, Putseys, & Delcour, 2011). However, during high temperature extraction starch granules only swell and get disrupted but do not gelatinize (Finnie, Jeannotte, & Faubion, 2009). Hence, prolonged extractions at 20 °C, as was here the case, that cause starch granule swelling and disruption possibly extract part of the starch lipids (Morrison, 1978; Morrison, Mann, Soon, & Coventry, 1975). The decreased level of LPC in DF_{hex:isoprop} (Figure 1B) can thus be resulting from the extraction of LPC residing either in and/or outside the starch granule.

312 From the above, it follows that hex mainly removed or relocated non-polar but also some polar FFLs. The
313 more polar solvent hex:isoprop removed or relocated non-polar and some polar FFLs which also hex did but
314 also an additional amount of polar BFLs (Figure 1 and Table 1). Presumably, the additionally extracted polar
315 lipids strongly interacted with starch or gluten in CF and, thus, some of the lipids in FRL samples interacted
316 differently with flour constituents than when present in CF. In this context, it is useful to note that
317 FRL_{hex:isoprop} contained a higher level of relocated flour lipids than FRL_{hex}.

318 **3.2 The impact of relocating lipids in wheat flour with hexane or hexane:isopropanol (3:2** 319 **v/v) on batter and cake properties**

320 Flour lipid functionality in sponge cake was investigated by first treating flour with hex or hex:isoprop. The
321 former solvent mostly altered the location of FFLs (Table 1) [primarily non-polar but also some polar lipids
322 (Figure 1)] (Chung, Ohm, Ram, Park, & Howitt, 2009; Pareyt, Finnie, Putseys, & Delcour, 2011). The latter
323 not only impacted FFLs but also a considerable part of the BFL fraction. Since the lipids that were relocated
324 by prior solvent treatment were those that were removed by defatting, it can be concluded that
325 hex:isoprop treatment resulted in a higher level of relocated flour lipids than did hex treatment (Table 1),
326 mainly because of increased levels of polar lipids in the former case (Figure 1) (*cf.* section 3.1).

327 The use of FRL instead of CF for sponge cake making resulted in slightly increased batter densities. Thus,
328 slightly less air was incorporated in batter prepared with FRL samples than in that prepared with CF. As
329 densities of batters prepared with CF and FRL samples were quite comparable, they also yielded cakes of
330 comparable volume (Table 2). Light microscopy images revealed a heterogeneous gas cell distribution for
331 batter prepared with either CF or FRL_{hex:isoprop} (Figure 2). In batter from the latter also slightly less gas cells
332 were observed, which was in line with its batter density being higher than that of batter prepared with CF.
333 The lower amount of incorporated air probably contributed to a lower apparent batter viscosity than that
334 of batter prepared with CF (Table 2). Rodríguez-García, Sahi, & Hernando (2014) earlier noted lower
335 viscosities for batters with higher densities. Because they altered batter density by only varying mixing
336 times, batters had identical compositions and batter viscosity could be related to how much air was
337 incorporated. However, the authors ascribed the differences in viscosity to differences in gluten

development rather than to differences in the amount of gas cells incorporated and their size distribution. In contrast, an in-depth rheological study of Chesterton, Meza, Moggridge, Sadd & Wilson (2011) related batter viscosity to air incorporation. Batter viscosity co-determines gas cell stabilization. Indeed, a low batter viscosity increases the likelihood of coalescence and bubble rise (Sahi & Alava, 2003; Wilderjans, Luyts, Brijs, & Delcour, 2013) resulting in heterogeneous gas cell distribution and low levels of gas cells in the batter and consequently also in cakes with low volumes and coarse crumb structures (Rodríguez-García, Sahi, & Hernando, 2014; Sahi & Alava, 2003).

Relocating FFLs resulted in sponge cakes with a significantly higher springiness, but similar corrected hardness and cohesiveness readings than those of sponge cakes prepared with CF (Table 2). Next to that, Figure 3A shows that sponge cakes prepared with FRL_{hex} had a slightly coarser crumb structure than sponge cakes prepared with CF. Corrected hardness, cohesiveness and springiness of sponge cakes prepared with FRL_{hex:isoprop} did not significantly differ from those of sponge cakes prepared with FRL_{hex}, but were higher than those of CF (Table 2). Although changes in texture as a result of flour treatment were significant, it should be noted that the impact of relocating flour lipids on cake texture was rather small. Use of FRL_{hex:isoprop} clearly resulted in an even more coarse crumb structure than in the case of cakes prepared with FRL_{hex} (Figure 3). Moreover, crumb of the former cake contained less gas cells per area unit than that of cakes prepared with CF (Table 2). This was probably caused by a lower initial amount of gas cells in the batter prepared with FRL_{hex:isoprop} than in that prepared with CF (Figure 2). However, coalescence and loss of gas cells after mixing and during baking may also have contributed to the crumb being coarser. Overall, there was a trend towards an increased corrected hardness for sponge cakes prepared with FRLs which is possibly explained by their coarser crumb structure. Indeed, in coarse crumb, the cell wall material is divided among a lower amount of air cells and therefore cell walls are thicker.

These results indicate that mostly FFLs participate in stabilizing the air-liquid interface in sponge cakes prepared with CF. Indeed, use of FRL_{hex} did not severely alter batter or cake properties (Table 2 and Figure 3). Next to that, use of FRL_{hex:isoprop} resulted in a more coarse cake crumb than that of cakes prepared with

363 FRL_{hex} (Table 2 and Figure 3), even if in the former flour only 0.16% of polar lipids (expressed on dm flour)
364 (Table 1 and Figure 1) had been relocated more than in the latter. We hypothesize from this finding that in
365 sponge cake batter prepared with FRL_{hex:isoprop} the BFL fraction, which mainly contains polar lipids natively
366 associated with starch or gluten in CF, disturb stabilization of the air-liquid interface because they are
367 relocated as a result of the solvent treatment (*cf.* section 3.1). In conclusion, this section shows that only
368 native FFLs and flour lipids relocated by a solvent treatment impact interfacial stabilization in sponge cake
369 batter.

370 **3.3 The impact of removing lipids in wheat flour with hexane or hexane:isopropanol (3:2 v/v)** 371 **on batter and cake properties**

372 The use of DFs for sponge cake making resulted in more air incorporation and thus in decreased batter
373 densities (Table 2). Sponge cakes prepared with DFs had higher cake volumes than those prepared with CF
374 or FRL samples (Table 2). This shows again that initial air incorporation is important for sponge cake quality
375 and that batter density is inversely related to sponge cake volume (Rodríguez-García, Sahi, & Hernando,
376 2014; Sahi & Alava, 2003). Batter prepared from DF_{hex:isoprop} had a higher apparent viscosity and larger gas
377 cells with a more homogeneous gas cell distribution than batters prepared from CF or FRL_{hex:isoprop} (Table 2
378 and Figure 2). As mentioned in the previous section, increased batter viscosity enhances gas cell
379 stabilization because gas cells are more fixed in the batter and coalescence and bubble rise are minimized.
380 Next to that, a more homogeneous gas cell distribution evidently reduces disproportionation, as
381 differences in gas cell size enhance this phenomenon (Sahi & Alava, 2003). Overall, these characteristics
382 indicate an increased stability of the gas cells in sponge cake batter prepared with DF_{hex:isoprop} than in
383 batter prepared with CF. As additional removal of polar lipids by hex:isoprop impacted neither batter
384 density nor cake volume readings (Table 2), the same reasoning presumably holds for cakes prepared with
385 DF_{hex}.

386 Cakes prepared with DF samples tended to have lower springiness and finer crumb than those produced
387 with FRL samples (Table 2 and Figure 3). Because cakes prepared with DF had higher volumes, their crumb

cell walls contained less material per surface area resulting in a more brittle structure with lower springiness. Although cake hardness was corrected for density, cakes prepared with DF samples tended to be softer than those prepared with FRL samples which again indicates differences in cell wall structure. An inverse relationship between sponge cake volume and springiness was already reported by Sahi & Alava (2003) in their study on emulsifier functionality in sponge cake making. However, as mentioned for FRLs (*cf.* section 3.2), use of DF also had only a small impact on cake texture.

In general, removing flour lipids resulted in improved batter and cake properties. Although they are a minor component in sponge cake batter – they here comprised 1.1% of its dm – they clearly have an impact on cake volume and crumb structure (Table 2 and Figure 3). It is hypothesized that removing flour lipids results in an improved stabilization of the air-liquid interface in the batter. As in section 3.2, these results also indicate that mostly FFLs interfere with the stabilization of the air-liquid interface in batter prepared with CF because removing an additional 0.16% of polar lipids (expressed on flour dm) (Table 1 and Figure 1) did not further alter sponge cake (batter) quality. This also means that polar lipids in the BFL fraction do not interfere with interface stabilization in sponge cake batter prepared with CF because they are associated with flour constituents.

4 Discussion

This work was conducted on sponge cakes prepared from flour, sugar, eggs and leavening agents only. Hence, the surface-active components (*i.e.* proteins and LMW-S) that possibly contributed to foam formation during mixing and foam stabilization after mixing and during baking were those from flour and eggs. To ensure sufficient air incorporation, sponge cake batter was prepared using a multi-stage mixing method (*cf.* section 2.2.5).

In a first step, egg white and sugar were whipped into a foam. Because of the minimal level of lipids in egg white, lipid interactions were limited at this stage and the air-liquid interface was stabilized by a viscoelastic egg protein layer. Secondly, air was also incorporated when mixing egg yolk, the remaining sugar and 50% of the flour. Because this mixture contained a variety of surface-active components from egg yolk (*i.e.* egg

413 yolk protein and low density lipoproteins) and flour (*i.e.* flour proteins and lipids), the interface at that
414 stage can reasonably be expected to be a mixed one. Afterwards, both mixtures were homogenized and the
415 remaining flour was added. It is reasonable to expect that the interface in the final batter was also a mixed
416 one. Even though batter lipids were added to the egg white foam after it had been beaten, it is likely that
417 they diffused and adsorbed at defects in the egg white protein layer at the interface, a phenomenon
418 referred to as orogenic displacement (*cf. supra*) (Mackie, Gunning, Wilde, & Morris, 1999). Due to the
419 incompatibility of the stabilization mechanisms of proteins and lipids, this mixed interface is less efficiently
420 stabilized than an interface containing only egg white proteins (Murray, 2007).

421 Relocating and removing flour lipids negatively and positively impacted batter and cake properties,
422 respectively (*cf.* section 3.2 and 3.3). Relocating lipids apparently decreased the stability of the air-liquid
423 interface while removing them had the opposite effect.

424 Relocated lipids – of which the identity depends on the polarity of the used solvent – are no longer present
425 at their native location and occur in a more free state in FRL than in CF because of broken hydrophobic,
426 electrostatic or hydrogen bonds between those lipids and flour constituents (Figure 4). As a result, they
427 disturb the native air-liquid interface composition and reduce the degree to which it is stabilized.
428 Relocating mainly FFLs (*i.e.* mostly TAG and FFA) with hex alters batter and cake properties to a limited
429 extent. Figure 4 illustrates the slightly lower quality of sponge cakes prepared with FRL_{hex} than of those
430 prepared with CF. We speculate that lipids relocated by hex, *i.e.* non-polar and some polar lipids, are
431 already sufficiently free to interfere with the air-liquid interface stabilization. Relocating lipids with
432 hex:isoprop, on the other hand, negatively impacted crumb structure (Figure 3) as it additionally released
433 lipids that are natively bound to flour constituents such as starch or gluten (Figure 4). From this, it is
434 suggested that relocating BFLs (*i.e.* polar lipids) and thereby making them more free, hampers air-liquid
435 interface stabilization during mixing and the early phases of baking. This can be caused by several
436 mechanisms. First, released lipids possibly adsorb at the interface thereby increasing the lipid-to-protein
437 ratio and disturbing stabilization mechanisms. Another possibility is that they adsorb at the interface but
438 are less efficient surfactants and/or that they expel more efficient surface-active components from the

439 interface to the bulk. In any case, this results in a reduced stabilization of the interface by the mixture of
440 proteins and lipids at that interface. When mostly FFLs had been removed by hex, stabilization of the air-
441 liquid interface was enhanced resulting in a fine crumb structure and increased cake volume (Figure 4). We
442 hypothesize that this is because of a decreased lipid-to-protein ratio at the air-liquid interface which then
443 leads to a more protein based stabilization. When FFLs and part of BFLs were removed by hex:isoprop,
444 again a higher cake volume was noted than for cakes prepared with CF. However, the former cakes were
445 not larger than those prepared with DF_{hex} (Figure 4). This further substantiated the hypothesis that only
446 FFLs disturb the stabilization of the air-liquid interface in sponge cakes prepared with CF.

447 Recently, it has been suggested that amylose-lipid complexes dissociate during bread baking resulting in
448 release of the starch internal lipid LPC (Janssen, Wouters, Pareyt, Gerits, Delcour, Waelkens, et al., 2018).
449 This release probably also occurs during sponge cake baking. At 96 – 100 °C, *i.e.* the melting temperature of
450 amorphous amylose-lipid complexes (Goderis, Putseys, Gommès, Bosmans, & Delcour, 2014), batter
451 viscosity has already increased to form a cake structure due to starch gelatinization and protein
452 polymerization (Deleu, Wilderjans, Van Haesendonck, Brijs, & Delcour, 2016; Wilderjans, Luyts, Goesart,
453 Brijs, & Delcour, 2010). Therefore, we believe that it is unlikely that LPC diffuses to the air-liquid interface, if
454 it is present at this stage, and impacts its stability.

455 It has also been shown that the impact of lipids on bread quality depends on their level and identity (Sroan
456 & MacRitchie, 2009). Because wheat flours of different origin may also vary in lipid content and
457 composition, additional work on flour samples with different lipid contents and/or compositions would be
458 appropriate.

459 A final remark is that next to flour lipids also egg lipids possibly play a role in interface stabilization. The
460 majority of the egg lipids occur as low density lipoproteins which are micelle-like structures with a core of
461 TAG and cholesterol esters surrounded by a monolayer of phospholipids and proteins. These structures can
462 stabilize foams and emulsions by adsorbing and spreading at the corresponding interfaces (Anton, 2013).

463 **5 Conclusions**

464 Although in this work wheat flour lipids made up only 1.1% of batter dm, they largely impacted cake
465 quality. Analysis of batter and cake properties of sponge cakes prepared with flour in which lipids had been
466 relocated with hex or hex:isoprop (*i.e.* FRL_{hex} and $FRL_{hex:isoprop}$) and with flour from which these lipids had
467 been removed (*i.e.* DF_{hex} and $DF_{hex:isoprop}$) revealed that FFLs had a negative impact on air-liquid interface
468 stabilization during sponge cake batter mixing and the early phases of baking. The FFL fraction consists of
469 mostly non-polar (*e.g.* TAG and FFA) and some polar lipids (*i.e.* galacto- and phospholipids). We suggest
470 that BFLs (*i.e.* mainly polar lipids) have no impact on the interface stabilization because they are associated
471 with starch or gluten and cannot interfere with the molecule population at the air-liquid interface. The
472 extent to which air-liquid interface stabilization during sponge cake making occurs is the result of an
473 interplay between proteins and lipids from both flour and eggs, and obviously is very sensitive to changes in
474 flour lipid location and composition. Changes in lipid content and composition of wheat flour as such can
475 thus also alter air-liquid interface stabilization in sponge cake batter. Therefore, studies on the impact of
476 lipid level and/or composition of wheat flour would be a valuable next step.

477 In summary, this study clearly shows that removal of FFLs is very efficient at increasing the air-liquid
478 interface stability in sponge cake batter prepared from flour, sugar, eggs and leavening agents only.
479 Subsequently, destruction and/or capture of FFLs are promising possibilities for developing sponge cakes of
480 high quality and with a cleaner label.

481 **Abbreviations used**

482 hex: hexane, hex:isoprop: hexane:isopropanol (3:2 v/v), FFL: free flour lipid, BFL: bound flour lipid,
483 LMW-S: low molecular weight surfactants, MAG: monoacylglycerols, dm: dry matter, TAG:
484 triacylglycerols, FFA: free fatty acids, WSB: water saturated butanol, MC: moisture content, HPLC:
485 high performance liquid chromatography, CF: control flour, rpm: rotations/min, FRL: flour with
486 relocated lipids, DF: defatted flour, hex': hexane used to determine FFL content, DGDG:
487 digalactosyldiacylglycerols, NAPE: *N*-acyl phosphatidylethanolamines, PE:
488 phosphatidylethanolamines, MGMT: monogalactosylmonoacylglycerols, NALPE: *N*-acyl
489 lysophosphatidylethanolamines, PC: phosphatidylcholines, LPC: lysophosphatidylcholines

490

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497 **Conflict of interest**

498 The authors declare that they do not have any conflict of interest.

499

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596

597

598 Figure Captions

599 **Figure 1:** Lipid levels [expressed as relative peak areas in arbitrary units (au)] of (A) non-polar and
 600 (B) polar lipids of control flour (CF, black) and flour defatted with hexane (hex) (DF_{hex}, dark grey) or
 601 hexane:isopropanol (3:2 v/v) (hex:isoprop) (DF_{hex:isoprop}, light grey). The terms ‘free’ and ‘bound’
 602 between brackets refer to the lipid fraction in which lipids were extracted, *i.e.* respectively with
 603 hexane (hex’) and with water saturated butanol (WSB). The presented polar lipids were all part of
 604 the bound flour lipid (BFL) fraction. Vertical bars indicate standard deviations. Values with the
 605 same letter within one lipid class are not significantly different ($\alpha = 0.05$). FFA: free fatty acids,
 606 TAG: triacylglycerols, MAG: monoacylglycerols, MGDG: monogalactosyldiacylglycerols, MGMG:
 607 monogalactosylmonoacylglycerols, DGDG: digalactosyldiacylglycerols, NAPE: *N*-acyl
 608 phosphatidylethanolamines, NALPE: *N*-acyl lysophosphatidylethanolamines, PE:
 609 phosphatidylethanolamines, PC: phosphatidylcholines, LPC: lysophosphatidylcholines.

610 **Figure 2:** Light microscopy images of sponge cake batters prepared with control flour (CF, left), with
 611 flour containing relocated lipids (FRL) as a result of treatment with hexane:isopropanol (3:2 v/v)
 612 (hex: isoprop, middle) or with flour defatted (DF) with hex:isoprop (3:2 v/v) (right).

613 **Figure 3:** Sponge cakes prepared with control flour (CF, left), flour containing relocated lipids (FRL,
 614 middle) and defatted flour (DF, right). In (A) flour was treated or defatted with hexane (hex) and in
 615 (B) flour was treated or defatted with hexane:isopropanol (3:2 v/v) (hex:isoprop). Color tones
 616 differ somewhat between (A) en (B) due to differences in lighting.

617 **Figure 4:** Schematic overview of the impact of treating and defatting flour at room temperature
 618 with a non-polar (*i.e.* hexane, hex) or more polar [*i.e.* hexane:isopropanol (3:2 v/v) (hex:isoprop)]
 619 solvent on a flour particle. Use of flour with relocated lipids (FRL) or defatted flour (DF) in sponge
 620 cake making resulted in cakes of different quality. Control flour (CF) contains oil bodies [*i.e.* core of
 621 triacylglycerols (TAG) surrounded by polar lipids], non-polar lipids (*e.g.* TAG or free fatty acids) and
 622 polar lipids (*i.e.* galacto- or phospholipids) associated with starch or gluten. Polar lipids such as
 623 lysophosphatidylcholine are complexed with amylose but not visualized to increase readability. In
 624 FRL, as a result of a solvent treatment, part of the flour lipids no longer occur in their original
 625 location. Relocation of flour lipids resulted in a lower cake quality. In defatted flour (DF), the lipids
 626 that were relocated due to the solvent treatment evidently were no longer present. Hex relocated
 627 or removed non-polar lipids and polar lipids that occur more freely in the flour. Hex:isoprop
 628 additionally removed polar lipids strongly associated with starch or gluten. Hence, less free flour
 629 lipids were present in DF than in CF and sponge cakes of higher quality were produced.

Table 1: Free flour lipid [FFL, extracted with hexane (hex')], bound flour lipid [BFL, extracted with water saturated butanol (WSB)] and total flour lipid content [expressed on a dry matter (dm) basis] of (i) control flour (CF), (ii) flour containing relocated lipids (FRL) as a result of treatment with hexane (hex) (FRL_{hex}) or hexane:isopropanol (3:2 v/v) (hex:isoprop) (FRL_{hex:isoprop}) and (iii) flour defatted (DF) with hex (DF_{hex}) or hex:isoprop (DF_{hex:isoprop}). Standard deviations are indicated between brackets.

Flour type	Lipid content (% dm flour)		
	FFLs	BFLs	Total lipids
CF	0.70 (0.05) ^a	0.87 (0.03) ^a	1.57 (0.07) ^a
FRL_{hex}	0.73 (0.03) ^a	0.81 (0.06) ^a	1.54 (0.04) ^a
FRL_{hex:isoprop}	0.63 (0.07) ^a	0.75 (0.13) ^a	1.38 (0.12) ^b
DF_{hex}	0.05 (0.05) ^b	0.58 (0.05) ^b	0.63 (0.01) ^c
DF_{hex:isoprop}	0.02 (0.01) ^b	0.42 (0.01) ^c	0.44 (0.02) ^d

Results in the same column with the same letter are not significantly different ($\alpha = 0.05$).

Table 2: Properties of sponge cake batters and sponge cakes prepared with (i) control flour (CF), (ii) flour containing relocated lipids (FRL) as a result of treatment with hexane (hex) (FRL_{hex}) or hexane:isopropanol (3:2 v/v) (hex:isoprop) (FRL_{hex:isoprop}) or (iii) flour defatted with hex (DF_{hex}) or hex:isoprop (DF_{hex:isoprop}). Standard deviations are indicated between brackets.

Flour type	Batter viscosity* (Pa*s)	Batter density (g/cm ³)	Cake volume (mL)	Corrected hardness (N*cm ³ /g)	Cohesiveness (%)	Springiness (%)	Cell size (mm ²)	Number of cells/cm ²
CF	160 (7) ^b	0.74 (0.01) ^b	369 (4) ^b	9.1 (0.6) ^b	0.71 (0.01) ^b	0.88 (0.01) ^b	0.30 (0.02) ^b	84 (5) ^{a,b}
FRL _{hex}	n.d.	0.76 (0.00) ^a	376 (3) ^b	9.9 (1.1) ^{a,b}	0.71 (0.01) ^{a,b}	0.90 (0.00) ^a	n.d.	n.d.
FRL _{hex:isoprop}	117 (7) ^c	0.76 (0.00) ^a	372 (6) ^b	10.0 (1.0) ^a	0.72 (0.01) ^a	0.89 (0.01) ^a	0.40 (0.03) ^a	72 (4) ^b
DF _{hex}	n.d.	0.64 (0.01) ^c	403 (5) ^a	9.1 (0.7) ^{a,b}	0.70 (0.01) ^c	0.86 (0.01) ^c	n.d.	n.d.
DF _{hex:soprop}	199 (14) ^a	0.65 (0.01) ^c	397 (9) ^a	8.0 (0.9) ^c	0.72 (0.01) ^a	0.87 (0.02) ^b	0.27 (0.02) ^b	87 (7) ^a

Results in the same column with the same letter are not significantly different ($\alpha = 0.05$). n.d.: values were not determined. *Batter viscosity was measured using a rheometer equipped with a Helipath stand and therefore batter viscosity refers to apparent batter viscosity.

Figure 1

Figure 2

Figure 3

Figure 4