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## **Lipid oxidation during the beating of cake batter containing yellow pea (*Pisum sativum* L.) flour**

Svenja Krause, Eugenia Ayebea Asamoah, Gabrielle Moulin, Catherine Bonazzi,  
Barbara Rega

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HAL Authorization

1 **Title:** Lipid oxidation during the beating of cake batter containing yellow pea (*Pisum sativum* L.) flour

2

3 **Authors:**

4 Svenja Krause, Eugenia Ayebea Asamoah, Gabrielle Moulin, Catherine Bonazzi, Barbara Rega\*

5 *Université Paris-Saclay, INRAE, AgroParisTech, UMR SayFood, 91300, Massy, France*

6 \* Corresponding author.

7 E-Mail addresses: svenja.krause@inrae.fr; eugenia.asamoah@inrae.fr;

8 gabrielle.moulin@agroparistech.fr; catherine.bonazzi@inrae.fr; barbara.rega@agroparistech.fr

9

10 **Highlights:**

- 11 • Extended beating of flour and oil favors the formation of volatile compounds
- 12 • 13- and 10-lipoxygenases are the main catalysts that oxidize fatty acids
- 13 • The degree of lipid oxidation depends on the batter microstructure and mixing time
- 14 • Reactivity increases in line with the surface of lipid droplets and air bubbles

15

16 **Abstract:**

17 The aim of this study was to understand the impact of ingredients and processes on reactivity during  
18 sponge cake batter making using yellow pea flour. Numerous volatiles were identified, mainly  
19 originating from the degradation of linoleic and linolenic acid catalyzed by 10- and 13-lipoxygenases.  
20 The extent of lipid oxidation depended on the beating process, owing to the effect on local ingredient  
21 distribution and the exposure time of lipoxygenase to its substrates. Although prolonged beating after  
22 the addition of either flour or oil did not favor volatile formation, the latter led to elevated quantities of  
23 hydroperoxides. This intensified oxidation was assigned to an improved homogeneity and reduced  
24 size of lipid droplets and air cells. A sequential increase in both mixing times significantly increased the  
25 amount of volatiles, which could be attributed to a larger reaction interface and longer contact time  
26 between reactive ingredients. These results highlight the importance of parameter controls to limiting  
27 the generation of volatiles with odor activity.

28

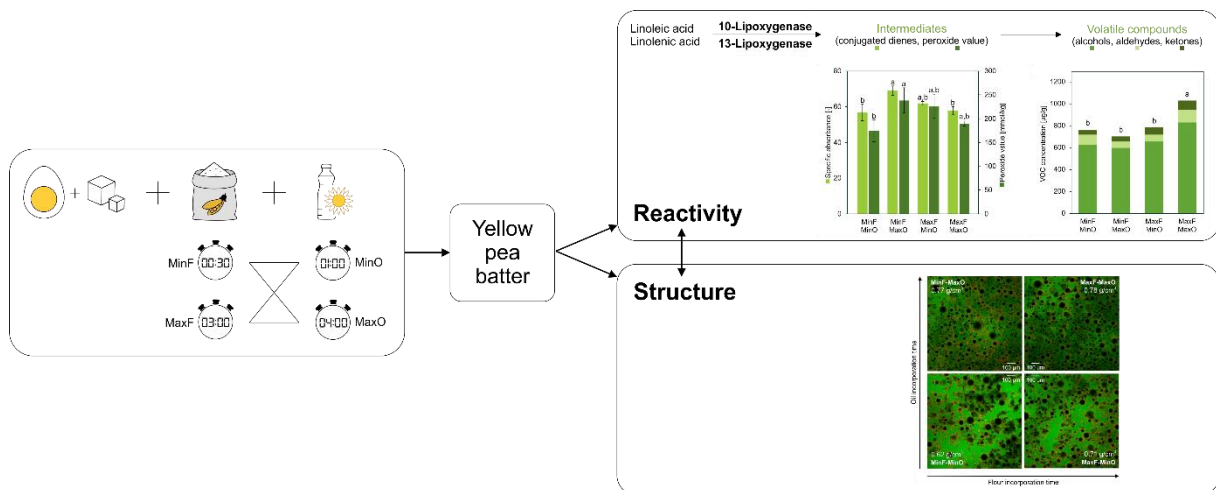
29 **Keywords:** volatile organic compounds, confocal microscopy, structure, hydroperoxide, legume

30 **Chemical compounds studied in this article:**

31 1-Hexanol (PubChem CID: 8103); 1-Penten-3-ol (PubChem CID: 12020); Hexanal (PubChem CID:  
32 6184); 3-Hexen-1-ol (PubChem CID: 10993); 2-Pentenal (PubChem CID: 12993); 1-Pentanol  
33 (PubChem CID: 6276); 1-Octen-3-ol (PubChem CID: 18827); 2-Octenal (PubChem CID: 16900); 3-  
34 Pentanone (PubChem CID: 7288); 2-Heptenal (PubChem CID: 5283316);

35

36 **Graphical abstract:**



37

38

## 39 1 Introduction

40 Growing interest in plant-based products to improve health, environmental sustainability and  
41 food security is continuously driving the incorporation of legumes in the human diet (Day, 2013; Hall,  
42 Hillen, & Garden Robinson, 2017). Among these, pea (*Pisum sativum* L.) represents a popular legume  
43 crop due to its abundant availability, low cost, attractive source of high-quality protein and low  
44 glycemic index (Dahl, Foster, & Tyler, 2012; Lam, Can Karaca, Tyler, & Nickerson, 2018; Marinangeli  
45 & Jones, 2011). Whole pea flour, in particular, can be considered an emerging ingredient for various  
46 food formulations because of its low degree of refining as opposed to isolated pea proteins.

47 Nonetheless, the applicability of such novel flour ingredients to replace traditional raw  
48 materials remains a challenge for the food industry because of their differing chemical composition  
49 which might alter reactivity during food processing, and hence product quality and safety. In the case  
50 of pea, exploitation of the benefits is especially limited because of the occurrence of inherent odor-  
51 active compounds and precursors to green-beany off-flavors (Krause, Keller, Hashemi, Descharles,  
52 Bonazzi, & Rega, 2021a). These volatile organic compounds typically originate from the lipoxygenase-  
53 catalyzed degradation of unsaturated fatty acids in the presence of oxygen (Hall *et al.*, 2017; Sessa &  
54 Rackis, 1977). Lipoxygenase (EC 1.13.11.12) is an iron-containing enzyme occurring in the raw  
55 material which introduces dioxygen into the carbon chain of fatty acids with a *cis,cis*-1,4-pentadiene  
56 system as found in linoleic and linolenic acid (Belitz, Grosch, & Schieberle, 2009). In the course of this  
57 reaction, hydroperoxides are formed and are subsequently cleaved into secondary structures, such as  
58 odorous alcohols, aldehydes and ketones, all with a probable impact on the sensory properties of the  
59 final food (Belitz *et al.*, 2009; Krause, Hashemi, Keller, Bonazzi, & Rega, 2021b).

60 Lipid oxidation plays a particular role in food systems that contain a complex mixture of  
61 different ingredients that undergo beating. This process is characteristic of the production of baked  
62 goods such as sponge cakes, which can be prepared using pea flour (Krause *et al.*, 2021a). The basis  
63 for these products is a foam obtained by whisking egg and sugar, into which flour particles are gently  
64 dispersed and oil droplets emulsified. During this mixing process, air and thus oxygen is entrapped in  
65 the batter matrix, which can trigger the oxidative degradation of unsaturated fatty acids (Krause *et al.*,  
66 2021a; Maire, Rega, Cuvelier, Soto, & Giampaoli, 2013). The occurrence of this phenomenon when  
67 using pulse flours, and particularly pea flour, compared to traditional wheat flour was demonstrated in  
68 our previous publication (Krause *et al.*, 2021a). It is therefore of considerable importance to gain a

69 clearer understanding of the options available to control lipid oxidation during batter making so as to  
70 improve the sensory quality of cakes based on pulse flours. The aforementioned study also showed  
71 that the existing processing strategy of producing pea flour under nitrogen did not mitigate oxidative  
72 reactions but, on the contrary, it intensified them (Krause *et al.*, 2021a). A new approach was pursued  
73 subsequently, which consisted in varying the process conditions during batter preparation and thus  
74 identifying the importance of individual process steps in the development of lipid oxidation markers  
75 during batter making using yellow pea flour. To achieve this, the mixing times after the incorporation of  
76 flour and oil were separately or jointly extended and their impact on the release of intermediate and  
77 final lipid oxidation products was analyzed. At the same time, process-induced modifications to the  
78 batter microstructure were recorded so that their relevance to the degree of lipid oxidation could be  
79 determined.

80

## 81 2 Material and Methods

### 82 2.1 Ingredients

83 Yellow pea flour (23.8% protein, 47.6% starch, 8.8% moisture, 8.2% dietary fiber, 3.5% ash)  
84 was supplied by Cargill (Vilvoorde, Belgium). Sucrose was purchased from Tereos (Lille, France),  
85 sunflower oil from Lesieur (Asnières-sur-Seine, France) and whole pasteurized liquid eggs from  
86 Ovoteam (Locmine, France).

### 87 2.2 Chemicals

88 Furfural-d<sub>4</sub> (≥96%) and 2-methylpyrazine-d<sub>6</sub> (98.4%) were obtained from C/D/N Isotopes Inc.  
89 (Pointe-Claire, Quebec, Canada), hexanal-d<sub>12</sub> (≥96%), Celite® 545, iodine (≥99.8%) and aluminum  
90 oxide from Sigma Aldrich (Saint-Quentin-Fallavier, France), isopropanol, *n*-heptane, sodium thiosulfate  
91 pentahydrate, isooctane, glacial acetic acid, potassium iodide and iodine (0.02 M) from Carlo Erba  
92 (Val de Reuil, France) and DyLight 488 and Bodipy 655/676 from Thermo Fisher Scientific (Illkirch,  
93 France).

### 94 2.3 Purification of sunflower oil

95 In order to evidence the progress of lipid oxidation during batter preparation, the sunflower oil  
96 was purified by stripping its tocopherols as described by Pernin, Bosc, Soto, Le Roux, and Maillard  
97 (2019). In brief, 1 L sunflower oil was filtered three times at reduced pressure (960 mbar) through 200  
98 g aluminum oxide activated overnight at 200 °C. The success of stripping was verified by recording the  
99 absorbance at 290-299 nm in isooctane using a fluorescence spectrophotometer (Cary Eclipse,  
100 Agilent Technologies, Les Ulis, France). The absence of absorbance bands within this wavelength  
101 range indicated the effective removal of tocopherols. The stripped oil was characterized by  
102 significantly lower peroxide values (2.31±0.26 mmol O<sub>2</sub>/kg) and specific absorbances values for  
103 conjugated dienes at 234 nm (1.52±0.04) compared to the untreated oil (3.11±0.08 mmol O<sub>2</sub>/kg and  
104 2.62±0.14, respectively) because stripping purifies oils from polar compounds in general. Immediately  
105 after preparation, the stripped oil was stored in glass bottles under nitrogen and in the dark at -20 °C  
106 until use within two weeks.

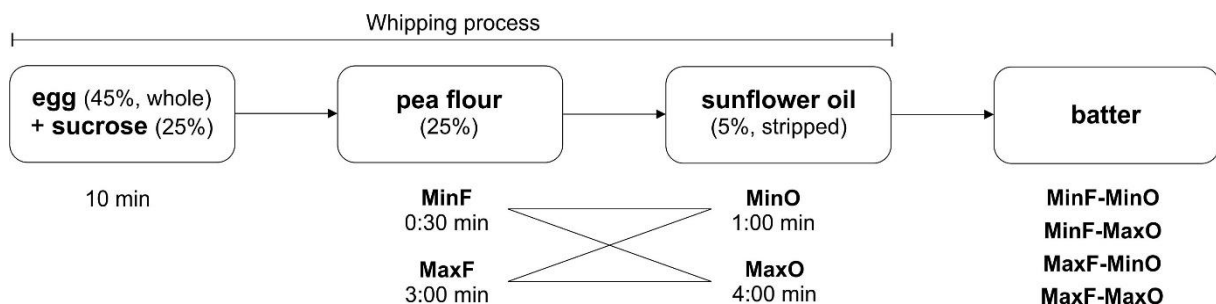
### 107 2.4 Experimental design for batter preparation

108 Sponge cake batters were prepared according to the procedure published by Krause *et al.*  
109 (2021a). Egg (45% w/w) and sucrose (25% w/w) were thus whipped for 10 min into a stable foam, to

110 which non-sifted pea flour (25% w/w) was added gently within 90 s. After mixing for 30 s, sunflower oil  
 111 was slowly incorporated within 15 s and the batter then beaten for 60 s (**Fig. 1**). This mixing protocol is  
 112 denoted below as *MinF–MinO*.

113 Because of the known presence of relevant precursors or catalysts for lipid oxidation in flour  
 114 and oil, it was deemed interesting to extend the mixing times after the incorporation of each ingredient.  
 115 The aim was to alter not only the exposure time of the lipoxygenase contained in the flour to  
 116 unsaturated fatty acids but also the amount of air, and thus oxygen, embedded in the batter, in order  
 117 to identify the relevant process parameters that favor lipid oxidation and their effects on batter  
 118 microstructure. A preliminary test was thus performed, during which beating after the addition of flour  
 119 was prolonged from 30 s to 3 min (*MaxF*), followed by the extension of mixing after the addition of oil  
 120 from 1 min to 4 min (*MaxO*). Over both periods, batter density was monitored in order to select  
 121 maximal beating times after adding flour and oil that would be sufficiently long to modify air entrapment  
 122 and exposure time of the enzyme to its substrates while concurrently avoiding collapse of the batter  
 123 structure. Interestingly, batter density changed only slightly over both time periods when compared to  
 124 *MinF–MinO*. For this reason, an experimental plan was set up in which four batters were prepared by  
 125 crosslinking the maximal limits thus selected with the minimum conditions described in *MinF–MinO*  
 126 (**Fig. 1**). Each batter was only made once because a preliminary test had confirmed insignificant  
 127 variability ( $p < 0.05$ ) between three independently prepared *MinF–MinO* batters in terms of the analyses  
 128 described in the sections below. In order to prevent further oxidative reactions after making, the  
 129 batters were frozen immediately at  $-20\text{ }^{\circ}\text{C}$  under nitrogen in glass vessels sealed with septum-  
 130 containing lids until analysis.

131



132

133 **Fig. 1.** Schematic illustration of batter preparation using different incorporation times for flour and oil.

134

## 135 **2.5 Physical and chemical characterization of batters**

### 136 *2.5.1 Batter density*

137 Batter density was determined in triplicate directly after preparation at room temperature by  
138 slowly drawing it up into 10 mL open-mouth syringes to prevent the destruction of gas cells. After  
139 weighing, density was calculated from the ratio of the mass of the batter to the volume of the syringes  
140 which was previously been defined using water.

### 141 *2.5.2 Microstructure analysis by Confocal Laser Scanning Microscopy (CLSM)*

142 The microstructure of the batters was analyzed using a Leica TC2 SP8 confocal laser  
143 scanning microscope (Wetzlar, Germany). 1 g freshly prepared product was stained with 3  $\mu$ L of a  
144 mixture of DyLight 488 and Bodipy 665/676 (1/1 v/v) in order to the label proteins and lipids in green  
145 and red, respectively. After excitation at 488 nm (green) and 638 nm (red) using two lasers, the  
146 emitted fluorescence was detected in two channels within the wavelength ranges of 497-601 nm and  
147 643-765 nm. Images were acquired at 10 $\times$  magnification using a scan speed of 400 Hz, 70.7  $\mu$ m  
148 pinhole and xyz scan mode.

### 149 *2.5.3 pH value*

150 To determine the pH, 2 g batter were dispersed in triplicate in 20 mL distilled water and the pH  
151 value recorded using a Sension+ PH3 meter (Hach, Loveland, CO, USA).

## 152 **2.6 Analysis of lipid oxidation reaction**

### 153 *2.6.1 Lipoxygenase (LOX) activity*

154 LOX activity of the pea flour was determined in triplicate by adapting the method described by  
155 Delcros, Rakotozafy, Boussard, Davidou, Porte, Potus, and Nicolas (1998). Briefly, 20 mL phosphate  
156 buffer (pH 7.5) was homogenized with 2 g flour and centrifuged, and 20-200  $\mu$ L of the supernatant was  
157 mixed with linoleic acid in phosphate buffer (pH 6.5). LOX activity was expressed as nmol oxygen  
158 consumed per second.

### 159 *2.6.2 Fatty acid profile, conjugated dienes and peroxide value*

160 The oxidation status of the batters was monitored by measuring conjugated dienes, peroxide  
161 values and the profile of fatty acids after the extraction of fatty matter according to the accelerated  
162 solvent extraction procedure described by Krause *et al.* (2021a). In brief, the batters were pounded in  
163 a mortar with Celite® 545 (10/9 w/w), transferred into 34 mL stainless-steel extraction cells and

164 extracted for 25 min at 40 °C with a binary mixture of heptane and isopropanol (5/1 v/v) using a  
165 Dionex ASE 350 (Thermo Fisher Scientific, Waltham, MA, USA). After solvent evaporation at reduced  
166 pressure and 40 °C, the oil was deep-frozen at -20 °C under nitrogen until analysis of the reaction  
167 markers.

168 **Conjugated dienes** were determined in triplicate by adapting the method described by  
169 Roman, Heyd, Broyart, Castillo, and Maillard (2013). Firstly, dilutions of 10 mg extracted oil in  
170 isooctane (1:100-1:200) were prepared and the mixtures vortexed for 10 s. Absorbance was then  
171 recorded against isooctane in 1 cm quartz cuvettes at 234 nm using a Specord 210 (Analytik Jena,  
172 Savigny-le-Temple, France). The content in conjugated dienes was expressed as specific absorbance,  
173 which was defined as the absorbance of 1 g oil in 100 mL isooctane at 234 nm.

174 The **peroxide value** was analyzed in triplicate by iodometric titration according to Krause *et al.*  
175 (2021a). In brief, 200 mg extracted oil were dissolved in glacial acetic acid/isooctane (3/2 v/v, 16 mL),  
176 followed by the addition of saturated potassium iodide solution (1 mL) and distilled water (9 mL).  
177 Titration of the released iodine was performed using sodium thiosulfate (5 mM). The peroxide value  
178 was expressed as millimole of hydroperoxide per kg of oil.

179 The **profile of polyunsaturated fatty acids** was analyzed in triplicate as reported by Krause  
180 *et al.* (2021a). In short, 30 mg extracted oil were dissolved in petroleum ether (1 mL) containing  
181 heptadecanoic acid as standard, and the fatty acids were esterified at 52 °C for 40 s using NaOH in  
182 methanol (2 M, 200 µL). The reaction was stopped by HCl in methanol (1 M, 400 µL) and the fatty acid  
183 methyl esters (FAMES) present in 1 µL of the upper phase were separated on a TRACE GC Ultra gas  
184 chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a SP-2560 GC column  
185 (100 m × 250 µm × 0.2 µm, Supelco, Bellefonte, PA, USA). The oven program was set as follows:  
186 100 °C isotherm (4 min); 100 °C to 200 °C at 5 °C/min; 200 °C isotherm (8 min); 200 °C to 250 °C at 5  
187 °C/min, 250 °C isotherm (12 min). Helium carrier gas was used at a split flow of 448 mL/min. For  
188 detection, an ISQ single quadrupole mass spectrometer (Thermo Fisher Scientific) was operated at  
189 250 °C, scanning masses from  $m/z$  40 to 400. Compounds were identified by comparing the mass  
190 spectra and retention times with those of a standard mixture containing 37 compounds (Supelco)  
191 subjected to the same procedure. Relative FAME quantities were calculated using the internal  
192 standard.

193 2.6.3 Profiles of volatile organic compounds (VOCs)

194 VOCs were extracted by Headspace-Solid Phase Micro Extraction (HS-SPME) using the  
195 optimized procedure reported by Krause *et al.* (2021a). Two grams of batter were weighed in triplicate  
196 in 20 mL glass vials, spiked with 100  $\mu$ L of an aqueous solution containing 778 mg/L of the deuterated  
197 standard hexanal-d<sub>12</sub> and sealed using aluminum caps with PTFE/silicone septa. After overnight  
198 storage at 4 °C to equilibrate the standard within the sample, the vials were incubated at 50 °C for 18  
199 min in a Triplus autosampler (Thermo Fisher Scientific). Volatiles were absorbed on a  
200 DVB/CAR/PDMS fiber (50/30  $\mu$ m, 2 cm, Supelco) for 42 min at 50 °C and finally desorbed for 2 min at  
201 250 °C in a TRACE GC Ultra (10 mL/min) injector port.

202 The separation of VOCs was achieved on a ZB-wax capillary column (30 m  $\times$  0.25 mm  $\times$   
203 0.5  $\mu$ m) by increasing the temperature from 40 °C (maintained for 5 min) to 240 °C at 4 °C/min. Helium  
204 was used as the carrier gas (1.2 mL min). Mass spectrometry was performed using an ISQ single  
205 quadrupole mass spectrometer (Thermo Fisher Scientific) operated in the EI mode at 70 eV, scanning  
206 in full scan (TIC,  $m/z$  33-300) and SIM mode for selected molecules. VOCs were identified by  
207 matching (i) experimental mass spectra with MS libraries (NIST 08, Wiley 8) and (ii) by comparing  
208 experimental retention indices obtained using a series of *n*-alkanes with the reference values of the  
209 NIST database. Hexanal was quantified by comparing the peak areas with those of the internal  
210 standard hexanal-d<sub>12</sub>. Similarly, relative quantities were determined for the remaining compounds  
211 (alcohols, aldehydes and ketones).

212 **2.7 Statistical analysis**

213 One-way analysis of variance (ANOVA) was performed and significant differences evaluated  
214 by Tukey's test at  $p < 0.05$  using XLSTAT 2020.5.1 (Addinsoft, Paris, France).  
215

## 216 3 Results and Discussion

### 217 3.1 VOCs as indicators of lipid oxidation during batter beating

218 **Table 1** shows the volatile organic compounds extracted from the yellow pea batters prepared  
219 using different mixing times after flour and oil incorporation.

220 A total of 18 different VOCs were identified in the batter produced according to the standard  
221 protocol (*MinF–MinO*), the majority of which are alcohols (10), followed by ketones (5) and aldehydes  
222 (4). From a quantitative point of view, alcohols were observed as the dominant group of volatiles  
223 (626 µg/g), and thus accounted for 82% of the total VOC concentration (760 µg/g). The remaining fifth  
224 was mostly composed of aldehydes (91 µg/g), while ketones contributed only marginally (43 µg/g).

225 According to the general consensus in the literature, most of the alcohols, aldehydes and  
226 ketones detected were typical markers of the oxidative degradation of unsaturated fatty acids.  
227 Nevertheless, molecules such as ethanol also appeared at high concentrations (226 µg/g), which were  
228 assumed to have emerged from the flour or during fermentation reactions. Among the lipid oxidation-  
229 derived compounds, 1-penten-3-ol and 1-hexanol were the most abundant in *MinF–MinO*, each  
230 reaching values of around 160 µg/g (**Table 1**). The formation pathway for 1-penten-3-ol likely involves  
231 the oxidation of linolenic acid to 13-hydroperoxide, which in turn can be cleaved to form 1-penten-3-ol  
232 (Fisher, Grimes, & Fall, 2003; Gardner, Grove, & Salch, 1996; Salch, Grove, Takamura, & Gardner,  
233 1995; Shen, Tieman, Jones, Taylor, Schmelz, Huffaker, Bies, Chen, & Klee, 2014). Alternatively, de  
234 Lumen, Stone, Kazeniak, and Forsythe (1978) proposed its generation from 16-hydroperoxide deriving  
235 from the same fatty acid, which could be isomerized enzymatically into 1-penten-3-one, followed by a  
236 reduction to 1-penten-3-ol catalyzed by an alcohol dehydrogenase (Ma, Boye, Azarnia, & Simpson,  
237 2016). By contrast, 1-hexanol has been reported to arise from linoleic acid after peroxidation to the  
238 corresponding 13-hydroperoxide, from which hexanal can be released upon cleavage (Belitz *et al.*,  
239 2009; Xu, Jin, Lan, Rao, & Chen, 2019). In a subsequent step, this aldehyde can be further  
240 transformed into 1-hexanol in the presence of an alcohol dehydrogenase (Belitz *et al.*, 2009;  
241 Jakobsen, Hansen, Christensen, Brockhoff, & Olsen, 1998). The occurrence of this consecutive  
242 reduction reaction in *MinF–MinO* was reflected by the lower relative concentration of hexanal  
243 compared to 1-hexanol (**Table 1**). Apart from the compounds mentioned above, other volatiles,  
244 similarly associated with the degradation of the 13-hydroperoxides described were also found. These  
245 included 3-hexen-1-ol (linolenic acid), 2-pentenal (linolenic acid) and 1-pentanol (linoleic acid), which

246 however accumulated in considerably smaller amounts (1-51  $\mu\text{g/g}$ ) (**Table 1**) (Frankel, 2012; Luning,  
247 Carey, Roozen, & Wichers, 1995; Przybylski & Eskin, 1995; Schaich, 2013). Furthermore, Sakouhi,  
248 Saadi, Herchi, Alibi, Luigi, and Boukhchina (2016) and Sánchez-Ortiz, Pérez, and Sanz (2013) had  
249 suggested that the ketone 3-pentanone, which totaled 32  $\mu\text{g/g}$ , might have been produced through a  
250 similar reaction scheme.

251 Moreover, trace quantities of 1-octen-3-ol and 2-octenal were detected (1-6  $\mu\text{g/g}$ ), the  
252 synthesis of which is typically based on the oxidation of linoleic acid (Frankel, 2012; Kakumyan &  
253 Matsui, 2009) Unlike the previous volatiles, this oxidative breakdown takes place under the formation  
254 of an intermediate non-conjugated 10-hydroperoxide (Belitz *et al.*, 2009; Luning *et al.*, 1995).

255 Interestingly, oxidation products of monounsaturated oleic acid were also present in low  
256 concentrations (1-4  $\mu\text{g/g}$ ) (**Table 1**), comprising 2-heptanone, 1-octanol and 2-octanone (Cao, Zou,  
257 Deng, Fan, Li, Li, & Deng, 2014; Kochhar, 1996; Przybylski *et al.*, 1995).

258 In our previous publication on the ability of diverse pulse flours to generate VOCs during  
259 sponge cake making (Krause *et al.*, 2021a), we reported a significantly higher susceptibility of pulse  
260 flours to oxidative deterioration compared to wheat flour, which is traditionally used in bakery products.  
261 We postulated that LOX is the main catalyst of lipid oxidation during batter beating when using pulse  
262 flours, which could be attributed to a positive correlation between enzyme activity and VOC formation:  
263 while the sum of oxidation-derived alcohols, aldehydes and alcohols was negligible (<1  $\mu\text{g/g}$ ) in batters  
264 made with wheat flour, in which LOX was practically inactive (<20 nkat/g), those in batters based on  
265 flours from chickpeas, lentils or peas were several hundred higher because of LOX activities of up to  
266 4350 nkat/g. In the present study, an average LOX activity of 5379 nkat/g yellow pea flour as well as a  
267 total relative VOC amount of 760  $\mu\text{g/g}$  were determined, of which around 70% were assumed to most  
268 likely originate from lipid oxidation. This corresponded to a concentration of typical oxidation volatiles  
269 that was more than 500 times higher compared to the wheat flour batter of our previous study (Krause  
270 *et al.*, 2021a). It was thus hypothesized that LOX might likewise have played a crucial role in VOC  
271 development through fatty acid breakdown in the present yellow pea batter. This enzyme targets the  
272 *cis,cis*-1,4-pentadiene system of polyunsaturated fatty acids, such as linoleic and linolenic acid,  
273 thereby introducing dioxygen into the carbon chain to form the corresponding hydroperoxides. In  
274 accordance with Krause *et al.* (2021a), the oxygen solubilized in the batter during beating seemed to

275 be sufficient to trigger an instant reaction as soon as the dry ingredients were dispersed in the moist  
276 batter matrix.

277 Pea may contain three, four or five isoenzymes of LOX, which were grouped by Fischer,  
278 Cachon, and Cayot (2020) in one heat-stable LOX with a pH optimum above 8 and 13-regiospecificity  
279 (LOX-1) as well as three heat-labile LOX with optimal activity at pH 6-7 but different regiospecificities  
280 of 9, 10 and 13 (LOX-3, LOX-5 and LOX-2, respectively). The remaining LOX-4 has not yet been  
281 identified. Owing to the quantitative dominance of 1-hexanol and 1-penten-3-ol in pea batter, which  
282 has been shown to originate from the 13-hydroperoxides of linoleic and linolenic acids, it is reasoned  
283 that the activity of a 13-LOX was of major importance. Because the pH values of the batters were  
284 around 7.2 (**Table 3**), it could be imagined that LOX-1 and/or LOX-2 were active. This was further  
285 corroborated by the presence of traces of other molecules at the same time, which also arose from  
286 these 13-hydroperoxides. On the other hand, however, the occurrence of volatiles formed *via* 10-  
287 hydroperoxides might also indicate the catalysis of fatty acid oxidation by a 10-LOX (LOX-5).

288 Although LOX activity is generally assumed to be the main driver for lipid oxidation during  
289 batter beating, small amounts of VOCs arising from oleic acid were monitored. Based on the lack of a  
290 *cis,cis*-1,4-pentadiene system in this fatty acid, which is a necessary requirement for LOX catalysis, it  
291 was concluded that non-enzymatic lipid oxidation had taken place, but only to a limited extent.  
292 Ongoing autoxidation was supported by the presence of 2-heptenal (**Table 1**), which has been  
293 reported to arise from linoleic acid 12-hydroperoxide. To date, no 12-LOX has been detected in pea,  
294 so the enzymatically-controlled formation of 12-hydroperoxide, and hence 2-heptenal could be ruled  
295 out.

296 **Table 1.** Occurrence of volatile organic compounds [ $\mu\text{g/g}$  batter] identified in yellow pea batters prepared using different mixing times after the addition of flour  
 297 and oil (mean  $\pm$  SD,  $n = 3$ ). Relative amounts are estimated based on the internal deuterated standard hexanal- $\text{d}_{12}$ .

Compound	Selected ion ( $m/z$ )	Experimental retention index	Reference retention index <sup>1</sup>	Relative concentration of volatile organic compounds [ $\mu\text{g/g}$ batter]			
				MinF–MinO	MaxF–MinO	MinF–MaxO	MaxF–MaxO
<b>Alcohols</b>							
Ethanol	45	944	942	225.94 $\pm$ 18.32 <sup>a</sup>	214.26 $\pm$ 47.84 <sup>a</sup>	237.26 $\pm$ 55.72 <sup>a</sup>	248.86 $\pm$ 15.55 <sup>a</sup>
1-Propanol	59	1047	1049	5.59 $\pm$ 0.39 <sup>b</sup>	5.75 $\pm$ 0.83 <sup>b</sup>	5.11 $\pm$ 0.48 <sup>b</sup>	7.56 $\pm$ 0.10 <sup>a</sup>
1-Butanol	56	1153	1147	4.47 $\pm$ 0.49 <sup>a</sup>	2.76 $\pm$ 0.65 <sup>b</sup>	2.73 $\pm$ 0.48 <sup>b</sup>	4.26 $\pm$ 0.08 <sup>a</sup>
1-Pentanol	70	1257	1255	51.19 $\pm$ 3.66 <sup>b</sup>	44.31 $\pm$ 6.68 <sup>b</sup>	41.42 $\pm$ 4.93 <sup>b</sup>	64.06 $\pm$ 1.20 <sup>a</sup>
1-Hexanol	69	1360	1360	159.36 $\pm$ 8.20 <sup>a,b</sup>	131.09 $\pm$ 22.66 <sup>b</sup>	122.56 $\pm$ 15.65 <sup>b</sup>	190.28 $\pm$ 2.62 <sup>a</sup>
1-Octanol	56	1567	1569	0.83 $\pm$ 0.04 <sup>b</sup>	0.78 $\pm$ 0.13 <sup>b</sup>	0.70 $\pm$ 0.10 <sup>b</sup>	1.23 $\pm$ 0.03 <sup>a</sup>
1-Penten-3-ol	57	1169	1165	161.16 $\pm$ 15.30 <sup>c</sup>	234.98 $\pm$ 20.27 <sup>b</sup>	170.89 $\pm$ 18.14 <sup>c</sup>	287.72 $\pm$ 9.86 <sup>a</sup>
1-Octen-3-ol	99	1457	1458	5.65 $\pm$ 0.35 <sup>a</sup>	4.42 $\pm$ 0.45 <sup>b</sup>	4.34 $\pm$ 0.65 <sup>b</sup>	6.64 $\pm$ 0.19 <sup>a</sup>
3-Pentanol	59	1047	1049	10.64 $\pm$ 0.96 <sup>b</sup>	15.61 $\pm$ 2.83 <sup>a,b</sup>	11.23 $\pm$ 2.75 <sup>b</sup>	17.56 $\pm$ 0.83 <sup>a</sup>
3-Hexen-1-ol	82	1392	1394	1.47 $\pm$ 0.17 <sup>a</sup>	1.48 $\pm$ 0.34 <sup>a</sup>	1.64 $\pm$ 0.27 <sup>a</sup>	1.79 $\pm$ 0.11 <sup>a</sup>
<i>Total</i>				626.28 $\pm$ 47.89 <sup>b</sup>	655.44 $\pm$ 102.68 <sup>a,b</sup>	597.88 $\pm$ 99.16 <sup>b</sup>	829.97 $\pm$ 30.57 <sup>a</sup>
<b>Aldehydes</b>							
Hexanal	56	1091	1091	83.43 $\pm$ 3.79 <sup>b</sup>	55.36 $\pm$ 2.03 <sup>c</sup>	50.22 $\pm$ 3.89 <sup>c</sup>	104.25 $\pm$ 12.35 <sup>a</sup>
2-Pentenal	84	1137	1132	2.76 $\pm$ 0.31 <sup>c</sup>	4.24 $\pm$ 0.04 <sup>b</sup>	2.83 $\pm$ 0.51 <sup>c</sup>	5.83 $\pm$ 0.77 <sup>a</sup>
2-Heptenal	83	1332	1332	3.79 $\pm$ 0.24 <sup>b</sup>	2.69 $\pm$ 0.35 <sup>c</sup>	2.72 $\pm$ 0.20 <sup>c</sup>	5.47 $\pm$ 0.26 <sup>a</sup>
2-Octenal	70	1437	1437	1.15 $\pm$ 0.04 <sup>b</sup>	0.85 $\pm$ 0.08 <sup>c</sup>	0.79 $\pm$ 0.07 <sup>c</sup>	1.54 $\pm$ 0.16 <sup>a</sup>
<i>Total</i>				91.13 $\pm$ 4.38 <sup>b</sup>	63.13 $\pm$ 2.49 <sup>c</sup>	56.57 $\pm$ 4.67 <sup>c</sup>	117.08 $\pm$ 13.54 <sup>a</sup>
<b>Ketones</b>							
2-Butanone	72	911	900	1.77 $\pm$ 0.13 <sup>b</sup>	1.97 $\pm$ 0.13 <sup>b</sup>	1.65 $\pm$ 0.21 <sup>b</sup>	2.51 $\pm$ 0.14 <sup>a</sup>
2-Heptanone	58	1189	1185	3.68 $\pm$ 0.23 <sup>b,c</sup>	4.69 $\pm$ 0.43 <sup>b</sup>	3.52 $\pm$ 0.53 <sup>c</sup>	6.50 $\pm$ 0.28 <sup>a</sup>
2-Octanone	58	1292	1297	2.00 $\pm$ 0.09 <sup>b</sup>	2.40 $\pm$ 0.24 <sup>b</sup>	1.99 $\pm$ 0.36 <sup>b</sup>	3.30 $\pm$ 0.06 <sup>a</sup>
3-Pentanone	86	985	986	32.09 $\pm$ 2.51 <sup>c</sup>	54.48 $\pm$ 4.46 <sup>b</sup>	37.54 $\pm$ 3.77 <sup>c</sup>	64.28 $\pm$ 1.70 <sup>a</sup>
3-Octanone	99	1261	1261	3.22 $\pm$ 0.22 <sup>b</sup>	3.07 $\pm$ 0.44 <sup>b</sup>	2.68 $\pm$ 0.42 <sup>b</sup>	4.26 $\pm$ 0.04 <sup>a</sup>
<i>Total</i>				42.76 $\pm$ 3.18 <sup>c</sup>	66.60 $\pm$ 5.70 <sup>b</sup>	47.39 $\pm$ 5.29 <sup>c</sup>	80.85 $\pm$ 2.23 <sup>a</sup>
<b>Total</b>				760.17 $\pm$ 55.46 <sup>b</sup>	785.17 $\pm$ 110.89 <sup>b</sup>	701.84 $\pm$ 109.12 <sup>b</sup>	1027.90 $\pm$ 46.35 <sup>a</sup>

298 Different letters within a column indicate significantly different means ( $p < 0.05$ ).

299 <sup>1</sup> National Institute of Standards and Technology (NIST), 2021

### 300 3.2 Importance of mixing time on VOC quantities

301 As shown in **Table 1**, extending mixing times after the addition of either flour or oil from 30 s to  
302 3 min (*MaxF–MinO*) and from 1 to 4 min (*MinF–MaxO*), respectively, as well as their combined  
303 extension (*MaxF–MaxO*) led to the release of identical volatile compounds but in different quantities.

304 In general, the individual extensions of either flour or oil beating times by 2.5 or 3 min,  
305 respectively, resulted in batters characterized by total VOC concentrations that did not differ  
306 significantly from that determined in *MinF–MinO* (785 µg/g and 702 µg/g vs. 760 µg/g). Apart from this,  
307 the percentage distribution of volatile levels between the chemical classes of alcohols, aldehydes and  
308 ketones remained almost constant. It was thus deduced that both mixing times were insufficient to  
309 drive the formation of volatile compounds. However, closer inspection of *MaxF–MinO* revealed that  
310 molecules known to originate from the oxidative degradation of linolenic acid (i.e. 1-penten-3-ol and 2-  
311 pentenal) appeared to be formed in increasing quantities (**Table 1**). Considering the proportions of the  
312 individual ingredients, as well as their fat content and fatty acid profile, it emerged that linolenic acid  
313 mainly derived from the egg and flour. This indicated that extending the mixing time after adding the  
314 flour increased the probability of contact between the enzyme and linolenic acid, thus leading to a  
315 higher proportion of linolenic acid-derived volatiles. This assumption was corroborated by non-  
316 significant differences in the levels of linolenic acid oxidation markers in *MinF–MaxO* compared to  
317 *MinF–MinO* (**Table 2**). During the short mixing after flour addition (30 s), competition for the active site  
318 of the LOX only occurred between the linoleic and linolenic acids arising from the egg and flour. The  
319 subsequent incorporation of oil rich in linoleic acid thus diluted the amount of linolenic acid in the  
320 batter. The extended exposure of LOX to this complex mixture of fatty acids therefore led to a similar  
321 VOC profile as that seen with the standard protocol.

322 A different picture emerged when both periods were extended at the same time, as the *MaxF–*  
323 *MaxO* batter thus produced was considerably enriched in all the lipid oxidation markers identified. In  
324 the case of alcohols, this increase was mainly ascribed to 1-penten-3-ol, reaching values around 288  
325 µg/g, which corresponded to an approximately 80% increment compared to *MinF–MinO* (**Table 1**).  
326 Unlike the standard batter, this compound clearly dominated the profile of volatile compounds in  
327 *MaxF–MaxO*. Nevertheless, 1-hexanol and its precursor hexanal concurrently accumulated at high  
328 relative concentrations (190 and 104 µg/g, respectively), thus markedly contributing to the total VOC  
329 content. Interestingly, substantial increases in the amounts of minor compounds by up to 111% (2-

330 pentenal) were likewise recorded. These changes were not only assigned to molecules known to  
 331 originate from the LOX-mediated oxidation of linoleic and linolenic acid, but also to those arising from  
 332 the autoxidation of oleic acid. It was therefore concluded that the combined prolongation of mixing  
 333 times after addition of both flour and oil, reaching a total of 5.5 min, was sufficient to prompt not only  
 334 enzymatic but also non-enzymatic lipid oxidation.

### 335 3.3 Evolution of lipid oxidation as a function of mixing time

336 The GC-MS analysis of VOCs contained in the diverse batters revealed that extended mixing  
 337 times for both flour and oil (*MaxF–MaxO*) favored molecule generation when compared to *MinF–MinO*  
 338 (**Section 3.2**). By contrast, non-significant modifications were observed following the separate  
 339 extension of beating times after the incorporation of either flour or oil (*MaxF–MinO* and *MinF–MaxO*,  
 340 respectively). Volatile compounds, however, are the end products of fatty acid degradation, for which  
 341 reason they are often described as secondary markers of oxidation. In fact, the oxidative breakdown of  
 342 fatty acids takes place *via* intermediate products, or hydroperoxides, which are commonly referred to  
 343 as primary lipid oxidation markers. Investigation of these intermediate structures can therefore serve to  
 344 gain greater insight into the oxidation status of the batters and reveal differences that are not visible  
 345 from the analysis of secondary oxidation products alone (Maire *et al.*, 2013).

346

347 **Table 2.** Peroxide value, specific absorbance of conjugated dienes and profile of polyunsaturated fatty  
 348 acids of yellow pea batters prepared using different mixing times after the addition of flour and oil  
 349 (mean  $\pm$  SD,  $n = 3$ ).

Batter	Peroxide value [mmol O <sub>2</sub> /kg fat]	Conjugated dienes [-]	Linoleic acid [g/100 g fat]	Linolenic acid [g/100 g fat]
MinF–MinO	175 $\pm$ 23 <sup>b</sup>	56.8 $\pm$ 4.6 <sup>b</sup>	21.7 $\pm$ 2.3 <sup>a</sup>	0.35 $\pm$ 0.05 <sup>a,b</sup>
MinF–MaxO	238 $\pm$ 27 <sup>a</sup>	69.1 $\pm$ 2.7 <sup>a</sup>	18.5 $\pm$ 1.2 <sup>a</sup>	0.27 $\pm$ 0.04 <sup>a,b</sup>
MaxF–MinO	225 $\pm$ 25 <sup>a,b</sup>	61.9 $\pm$ 1.1 <sup>a,b</sup>	22.4 $\pm$ 5.0 <sup>a</sup>	0.38 $\pm$ 0.05 <sup>a</sup>
MaxF–MaxO	190 $\pm$ 5 <sup>a,b</sup>	57.9 $\pm$ 2.1 <sup>b</sup>	20.4 $\pm$ 1.2 <sup>a</sup>	0.25 $\pm$ 0.05 <sup>b</sup>

350 Different letters within a column indicate significantly different means ( $p < 0.05$ ).

351

352 Analysis of the peroxide value and absorbance of conjugated dienes are appropriate tools to  
 353 determine primary oxidation markers. **Table 2** depicts the outcomes with the four yellow pea batters

354 using both techniques. At closer inspection, it became apparent that the two approaches described a  
355 similar trend: while *MaxF–MinO* and *MaxF–MaxO* could not be discriminated from the standard *MinF–*  
356 *MinO*, a significant accumulation of primary markers was measurable in the case of *MinF–MaxO*. This  
357 finding indicated intensified lipid oxidation in the batter produced using a longer beating time after oil  
358 addition, which contrasted with the results of the VOC analysis. It was therefore assumed that lipid  
359 oxidation occurred during the initial stage and the beating time was insufficiently long to convert the  
360 hydroperoxides thus generated into volatiles. Conversely, this appeared to be the case for the batter  
361 with maximum mixing duration (*MaxF–MaxO*) as it was characterized by a significantly higher quantity  
362 of VOCs (**Table 1**) and a reduced concentration of hydroperoxides (**Table 2**), thus implying the  
363 successful transformation of primary into secondary markers. The greater susceptibility of  
364 polyunsaturated fatty acids to oxidative degradation during prolonged flour and oil mixing was likewise  
365 noticeable from the significantly lower content of linolenic acid in *MaxF–MaxO* compared to the other  
366 products (**Table 2**).

#### 367 **3.4 Correlation between mixing time and local distribution of reactive ingredients**

368 Cake batter is considered as a liquid foam that consists of a continuous aqueous phase  
369 containing proteins in which lipids are emulsified and both gas cells and starch granules are dispersed.  
370 In order to prevent foam collapse, the stabilization of these air bubbles is achieved by surface-active  
371 proteins and lipids, which are naturally present in the ingredients; i.e. egg, flour and oil (Godefroidt,  
372 Ooms, Pareyt, Brijs, & Delcour, 2019). The microstructure of batters is determined by the beating  
373 protocol applied. Modifications to mixing might therefore be accompanied by changes to the  
374 distribution of air cells, water-soluble proteins (e.g. enzymes) and lipid droplets, which could affect  
375 their availability to participate in lipid oxidation. In addition, the duration of beating is typically  
376 correlated with the amount of air, and thus oxygen, entrapped in the continuous phase of the batter  
377 (Bousquière, Michon, & Bonazzi, 2017; Monnet, Jeuffroy, Villemejeane, & Michon, 2020), thereby  
378 potentially impacting the degree of oxidative fatty acid degradation. For this reason, it was of particular  
379 importance to investigate changes in the structural properties of the batters as a function of mixing  
380 times after the incorporation of flour and oil.

381 **Fig. 2** shows CLSM images of the four batters using red and green dyes to stain lipids and  
382 proteins, respectively. In the case of *MinF–MinO*, a non-homogenous distribution of proteins and lipids  
383 could be seen. Moreover, dark disks of various diameters were observable, representing the

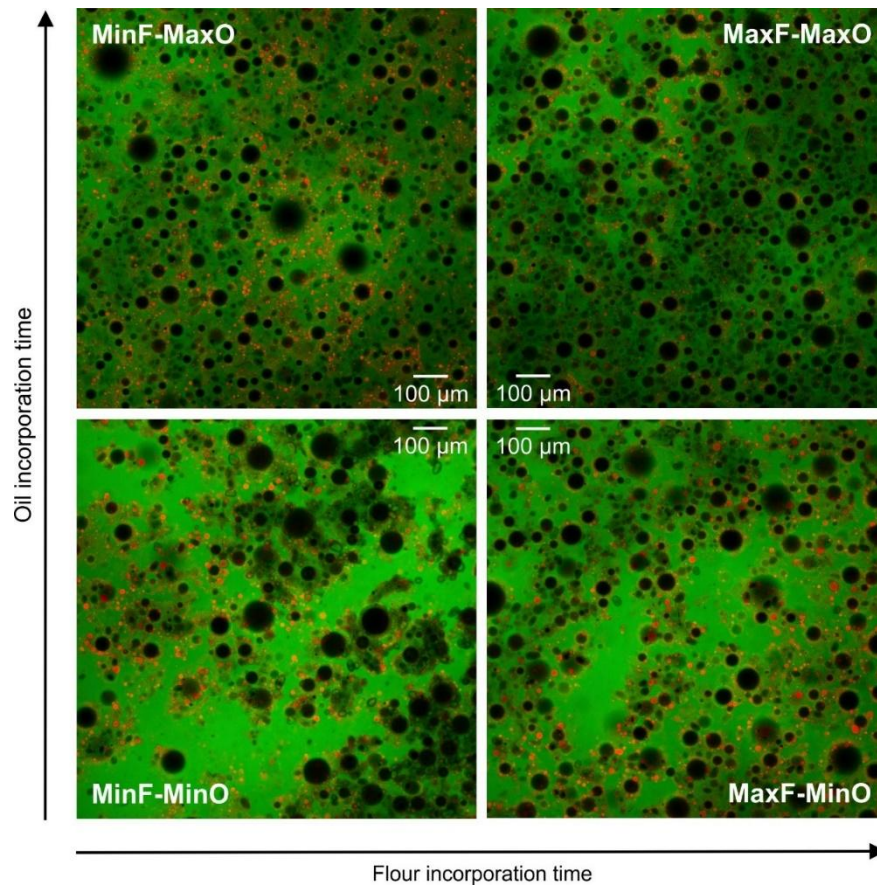
384 incorporated air bubbles that caused a batter density of 0.62 g/cm<sup>3</sup> (**Table 3**). This large and uneven  
385 size distribution of gas cells might indicate only partial stabilization by surface-active compounds such  
386 as lipids, which appeared to be incompletely emulsified as visible from the occurrence of large lipid  
387 droplets.

388 A similar image was captured when increasing the mixing time after flour addition from 30 s to  
389 3 min (*MaxF–MinO*). Compared to the standard batter, however, *MaxF–MinO* was characterized by a  
390 significantly higher batter density of 0.71 g/cm<sup>3</sup> (**Table 3**), indicating a loss of previously entrapped air.  
391 According to Bousquières *et al.* (2017), this might be explained by the mechanical disruption of air  
392 cells by the solid flour particles.

393 On the other hand, the sole prolongation of mixing after oil incorporation by 3 min perceptibly  
394 modified the microstructure of the corresponding *MinF–MaxO* batter (**Fig. 2**). Despite a total batter  
395 preparation time similar to that of *MaxF–MinO*, a significantly higher density in *MinF–MaxO* was  
396 determined (0.77 g/cm<sup>3</sup>) (**Table 3**). This indicated a greater loss of air, which was presumably due to  
397 the degassing effect of the sunflower oil. This phenomenon had likewise been reported by Monnet *et*  
398 *al.* (2020) who studied the effects of the order of ingredient incorporation on the properties of cake  
399 batters. At the same time, this beating protocol appears to have led to a reduction in the size not only  
400 of air cells but also of fat droplets, which was accompanied by an enlargement of the reaction  
401 interface, promoting lipid oxidation. As a second consequence, an improved emulsification of lipids as  
402 well as a more homogenous distribution of lipids, proteins and air bubbles was achieved, which  
403 increased the likelihood of contact between LOX and its substrates. These structural properties of  
404 *MinF–MaxO* therefore provided a substantiated explanation for the elevated levels of primary lipid  
405 oxidation markers (i.e. conjugated dienes and hydroperoxides) compared to *MinF–MinO* and *MaxF–*  
406 *MinO* (**Section 3.3**).

407 As soon as the batter beating times after flour and oil addition were both extended, synergistic  
408 effects became apparent. From a structural perspective, no clear distinction between *MaxF–MaxO* and  
409 *MinF–MaxO* could be made as the distribution of macromolecules and air cells, as well as batter  
410 density, were very similar (**Table 3, Fig. 2**). However, significantly higher concentrations of secondary  
411 oxidation markers (i.e. volatile compounds) were identified in this product (**Table 2**). In addition to the  
412 surface enlargement effects, this could also be attributed to a significantly longer mixing time, which  
413 favored the enzymatically catalyzed breakdown of fatty acids. Interestingly, the increase in batter

414 density did not affect fatty acid degradation, although it reflected a reduced amount of air, and thus of  
 415 incorporated oxygen, which was required for lipid oxidation. This led to the conclusion that solubilized  
 416 oxygen was sufficient to trigger oxidation, which was in line with the results reported by Krause *et al.*  
 417 (2021a).  
 418



419  
 420 **Fig. 2.** Microstructure of yellow pea batters prepared using different mixing times after the addition of  
 421 flour and oil. Proteins are stained in green, lipids in red. Dark circles represent air bubbles.  
 422 Magnification: 10x, scale bar: 100 µm.

423  
 424 **Table 3.** Density and pH value of yellow pea batters prepared using different mixing times after the  
 425 addition of flour and oil (mean ± SD, *n* = 3).

Batter	Density [g/cm <sup>3</sup> ]	pH value [-]
MinF–MinO	0.62 ± 0.01 <sup>c</sup>	7.22 ± 0.03 <sup>a</sup>
MinF–MaxO	0.77 ± 0.02 <sup>a</sup>	7.23 ± 0.00 <sup>a</sup>

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MaxF–MinO	$0.71 \pm 0.01^b$	$7.24 \pm 0.02^a$
MaxF–MaxO	$0.78 \pm 0.02^a$	$7.21 \pm 0.01^a$

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426 Different letters within a column indicate significantly different means ( $p < 0.05$ ).

#### 427 **4 Conclusion**

428 Lipid oxidation played a crucial role in the development of sponge cake batters containing  
429 yellow pea flour, which was mainly attributable to the activity of lipoxygenase present in the flour. The  
430 batter we produced was characterized by a complex mixture of volatile organic compounds, essentially  
431 composed of alcohols, followed by ketones and aldehydes. Most of these molecules are known to  
432 originate from 13- and 10-hydroperoxides of linoleic and linolenic acid, thus suggesting the  
433 predominant activity of 13- and 10-lipoxygenases, preferably introducing dioxygen at positions 13 or  
434 10 of the fatty acid carbon chain.

435 Interestingly, the intensity of fatty acid degradation appeared to be influenced by the ingredient  
436 mixing process applied, owing to a combined impact on the local distribution of reactive ingredients  
437 and the exposure time of lipoxygenase to its substrates. When the batter beating time was increased  
438 after the addition of flour from 30 sec initially to 3 min, neither the quantities of hydroperoxides nor  
439 those of volatile compounds varied significantly; this indicated that the longer contact time between  
440 lipoxygenase and fatty acids from egg and flour was insufficient to intensify lipid oxidation. This could  
441 be assigned to the uneven partitioning and high proportion of large air bubbles and lipid droplets in the  
442 batter, which limited their probability of contact. In contrast, prolonging batter beating after the addition  
443 of oil by a similar duration of 3 min led to a significant accumulation of hydroperoxides, which implied  
444 an elevated degree of lipid oxidation. This supposedly resulted not only from improved batter  
445 homogeneity but also from a reduced size, thus enlarging the reaction surface of both lipid droplets  
446 and air bubbles, promoting chemical reactivity. Notwithstanding this, the higher potential for fatty acid  
447 breakdown after a modified oil mixing time was not reflected in a higher level of volatile compounds.  
448 This signified that during the selected extension to beating, the intermediate lipid oxidation markers  
449 had not yet been subjected to subsequent cleavage. However, this phenomenon was visible as soon  
450 as both mixing times after oil and flour incorporation were extended sequentially by a total of 5.5 min.  
451 While the corresponding batter was significantly enriched in volatile compounds, a lower level of  
452 hydroperoxides was detected, thus implying the successful transformation of primary into secondary  
453 lipid oxidation markers. Because the microstructure of this batter was similar to that obtained after  
454 prolonged oil mixing time, the greater potential to generate volatiles was also assigned to a longer  
455 contact between the enzyme and its substrates.

456           Based on these findings, it was concluded that by varying the mixing process parameters, a  
457 significant effect can be achieved on the degree of lipid oxidation during the development of legume-  
458 based products. This valuable knowledge of the opportunities to control the generation of volatiles with  
459 potential effects on the sensory attributes of the final food will help to exploit the potential of less  
460 refined legume ingredients, such as whole pea flour, and thus to contribute to the design of more  
461 sustainable foods.  
462

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581 **Key references:**

- 582 Krause *et al.* (2021a) This is our previous work on the ability of pulse flours to generate volatile  
583 compounds during sponge cake development, which raised new  
584 questions about the elevated reactivity of pea flour during food  
585 processing to which we wanted to answer with the present study. It lays the  
586 foundation for the different methods applied in this study.
- 587 Maire *et al.* (2013) This work supports the strategy to link VOC analysis with the  
588 determination of primary oxidation markers, namely conjugated dienes and  
589 hydroperoxides, to describe the oxidation status of samples and progress  
590 during sponge cake development.
- 591 Monnet *et al.* (2020) This work demonstrates the role of mixing in the structuration of batters  
592 and cakes and discusses the changes when substituting wheat flour by  
593 pea flour. It made us reflect about the importance of ingredient  
594 functionality and raised the question about their availability to participate in reactions.
- 595 Fischer *et al.* (2020) This is an excellent review that highlights different aspects of pea  
596 ingredients in comparison to soy, including a section about LOX activity,  
597 its different isoenzymes, regiospecificities and pH optima.
- 598 Belitz *et al.* (2009) This book provides in-depth information about the chemistry of food and  
599 the chemical reactions taking place during food processing, storage and  
600 handling. The principle of lipid oxidation is discussed as well as the  
601 decomposition of hydroperoxides into volatile compounds.