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## From flours to cakes: Reactivity potential of pulse ingredients to generate volatile compounds impacting the quality of processed foods

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1 **Title:** From flours to cakes: Reactivity potential of pulse ingredients to generate volatile  
2 compounds impacting the quality of processed foods

3  
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11  
12 **Highlights:**

- 13 • Pulses form a larger quantity and variety of process-induced volatiles than wheat
- 14 • Lipoxygenase plays a major role in lipid oxidation during batter beating
- 15 • Pea flour made under N<sub>2</sub> is highly prone to lipid oxidation during product making
- 16 • Pea cakes are the richest in oxidation markers typically linked to green odors
- 17 • Lentil and chickpea flours induce most odor-active Maillard compounds

18  
19 **Abstract:**

20 This study investigated the impact of substituting wheat with pulse flours (lentil, chickpea,  
21 lupin, green and yellow pea) on reactivity during different steps of sponge cake development.  
22 Pulses exhibited a greater ability to generate volatiles with probable odor activity. Batter  
23 beating initiated lipid oxidation which depended on lipoxygenase activity and the fatty acid  
24 profile of the flours. Among the pulses, pea batters were richest in oxidation markers whereas  
25 lupin was least reactive, probably due to thermal pre-treatment. Baking triggered  
26 caramelization and Maillard reactions, notably with the pulse products which were  
27 particularly enriched in pyrazines and furanic compounds. Principle component analysis  
28 revealed that pea cakes were associated with oxidation markers that typically possess green-  
29 beany flavors, while Maillard markers known to impart nutty, roasted notes were assigned to  
30 lentil and chickpea cakes. These findings highlight the importance of ingredient type and its  
31 pre-processing in the development of quality-related markers for gluten-free products.

32  
33 **Keywords:**

34 Lipid oxidation, Maillard reaction, gluten-free, hexanal, flavor, baking

## 35 **1 Introduction**

36 In recent years, the high prevalence of protein malnutrition in the rapidly growing  
37 global population is promoting the development of healthy and nutritious eating practices  
38 based on plant foods. Owing to their sustainability, low cost and nutritional value, pulses  
39 represent a popular source of vegetable protein (Hall *et al.*, 2017; Sozer *et al.*, 2017). Apart  
40 from their protein contents, these commodities not only provide dietary fiber and minerals but  
41 also possess a low glycemic index (Hall *et al.*, 2017), thus making them promising ingredients  
42 for diverse formulations, including baked goods.

43 Wheat-based bakery products have been part of the human diet for centuries.  
44 However, the associated ingestion of gluten can cause diverse clinical disorders, such as  
45 celiac disease, wheat allergy and non-celiac gluten sensitivity (Laleg *et al.*, 2016).  
46 Consequently, the market for gluten-free foods is expanding and the creation of value-added  
47 pulse products is being increasingly spotlighted.

48 Despite the appealing composition and health-promoting benefits of pulses,  
49 exploitation of their advantages is impeded by their unpleasant green-beany off-flavor (Murat  
50 *et al.*, 2013). The lipoxygenase-catalyzed oxidation of unsaturated fatty acids is considered to  
51 be a major contributor to the release of odor-active compounds during harvest and storage and  
52 has been reviewed for pea, chickpea, lentil and lupin (Azarnia *et al.*, 2011; Jakobsen *et al.*,  
53 1998; Kaczmarek *et al.*, 2018; Murat *et al.*, 2013; Paucean *et al.*, 2018; Xu *et al.*, 2019). By  
54 contrast, the literature lacks information on the ability of pulses to generate aroma markers  
55 and contribute to overall flavor during the development of pulse-based foods subjected to  
56 thermomechanical treatments.

57 Sponge cakes are baked systems of particular interest when studying the link between  
58 quality and reactivity because of their complex composition (Cepeda-Vázquez *et al.*, 2019;  
59 Maire *et al.*, 2013). They are prepared in a two-step process of batter beating and baking,  
60 which can favor the oxidative and thermal degradation of lipids, proteins and sugars (Maire *et al.*  
61 *et al.*, 2013; Xu *et al.*, 2019). Replacing wheat with pulse ingredients might therefore affect the  
62 presence of precursors likely to undergo changes *via* lipid oxidation, Maillard reaction and  
63 caramelization. It was therefore considered to be interesting, to evaluate the reactivity  
64 potential of pulse flours to generate volatiles that could impact food quality during the  
65 different steps of cake development compared to a wheat reference. Moreover, the occurrence  
66 of newly-formed compounds was studied, alongside other cake quality criteria.

## 67 2 Materials and Methods

### 68 2.1 Ingredients

69 Sucrose was purchased from Tereos (Lille, France), sunflower oil from Lesieur  
70 (Asnières-sur-Seine, France), whole pasteurized eggs from Ovoteam (Locmine, France),  
71 wheat flour from Grands Moulins de Paris (Ivry-sur-Seine, France), green lentil flour and  
72 chickpea flour from Celnat (Saint-Germain Laprade, France) and lupin flour and green pea  
73 flour from Moulin Meckert-Diemer (Krautwiller, France). Two yellow pea flours (Improve,  
74 Dury, France) were used, which had either been produced during exposure to air or under  
75 nitrogen.

### 76 2.2 Chemicals

77 Furfural-d<sub>4</sub> (≥96%) and 2-methylpyrazine-d<sub>6</sub> (98.4%) were purchased from C/D/N  
78 Isotopes Inc. (Pointe-Claire, Quebec, Canada), hexanal-d<sub>12</sub> (≥96%), Celite® 545, iodine and  
79 glyceryl triheptadecanoate (≥96%) from Sigma Aldrich (Saint-Quentin- Fallavier, France),  
80 petroleum ether, isopropanol, heptane, sodium hydroxide, methanol, glacial acetic acid,  
81 sodium thiosulfate pentahydrate, isooctane, potassium iodide and iodine (0.02M) from Carlo  
82 Erba (Val de Reuil, France) and hydrochloric acid (37%) from Prolabo (Fontenay-sous-Bois,  
83 France).

### 84 2.3 Batter and cake formulations

85 Eggs (45% w/w) and sucrose (25% w/w) were whipped together for 10 min  
86 (maximum speed) using a mixer equipped with a vertical whisk (KitchenAid Artisan  
87 5KSM150, St. Joseph, Michigan, USA). Non-sifted wheat or pulse flour (25% w/w) was  
88 gradually incorporated within 90 s (minimum speed) and the mixture was beaten for further  
89 30 s. Thereafter, sunflower oil (5% w/w) was added within 15 s (minimum speed) and the  
90 whole batter was whipped for a further 60 s. Each formulation was prepared in triplicate. A  
91 portion of each batter was immediately stored at -20 °C until reaction marker analysis in order  
92 to slow down oxidation reactions, whereas the remainder was used for immediate pH and  
93 density measurements.

94 Sponge cakes were generated from a new batch of batter (total 630 g), where 21  
95 aluminum molds (9.8×6.2×3.3 cm<sup>3</sup>) were each filled with 25 g of batter and baked at 170 °C  
96 for 25 min in an insulated convection oven (Bongard, Wolfisheim, France) guaranteeing  
97 thermal homogeneity (Fehaili *et al.*, 2010). While 15 cakes were frozen immediately at -20 °C  
98 in hermetically sealed glass jars until reaction marker analysis, the remaining six were cooled  
99 in order to characterize density, moisture content and pH. Each batch of 21 cakes was only

100 made once because a preliminary test had confirmed insignificant variability between three  
101 batches using the same flour ( $p < 0.05$ ) in terms of selected analyses described in the sections  
102 below.

## 103 **2.4 Cake grinding**

104 Prior analysis of moisture content and pH, the six cakes cooled to ambient temperature  
105 were ground after density measurement for 1 min at turbo speed using a Moulinex AT710131  
106 (SEB, Ecully, France). By contrast, a different protocol was applied to the cakes used to  
107 analyze reaction markers in order to ensure similar particle size distribution and limit the  
108 formation or loss of volatiles induced by friction heat. Accordingly, 15 frozen cakes from one  
109 baking were milled in batches of five for 20 s at 6000 rpm using a Grindomix GM200 knife  
110 mill equipped with a stainless-steel bowl and titanium blades (Retsch GmbH, Haan,  
111 Germany) and stored at  $-20\text{ }^{\circ}\text{C}$ .

## 112 **2.5 Chemical composition**

### 113 *2.5.1 Moisture, ash, protein, free amino acid, starch and fiber contents*

114 The moisture contents of the ingredients and cakes were determined in triplicate by  
115 differential weighing before and after drying for 24 hours at  $105\text{ }^{\circ}\text{C}$ . The ash content of eggs  
116 and flours were analyzed by dry combustion for twelve hours at  $550\text{ }^{\circ}\text{C}$ , while their protein  
117 contents were determined using the Kjeldahl method (NF EN ISO 5983-2) with a nitrogen-to-  
118 protein conversion factor of 6.25 for pulses and 5.7 for wheat. The flours were further  
119 characterized in terms of total starch (EC 152/2009), dietary fiber (AOAC 985.29) and free  
120 amino acids (ISO 13903:2005 and ISO 13904:2016). The ash, protein, total starch, dietary  
121 fiber and free amino acid contents were determined in duplicate by Improve (Dury, France).

### 122 *2.5.2 pH value*

123 The pH of 2 g batter or cake was analyzed in triplicate in 20 mL distilled water using a  
124 SensION<sup>TM</sup>+ PH3 meter (Hach Lange, Lognes, France).

## 125 **2.6 Physical properties**

### 126 *2.6.1 Density*

127 Batter density was determined in triplicate by slowly drawing the batter up into 10 mL  
128 open-mouth syringes to avoid gas cell destruction. After weighing, density was calculated  
129 from the ratio of batter mass to syringe volume, previously defined using water.

130 Similarly, the density of six pre-weighed cakes was obtained by calculating the mass  
131 to volume ratio. The latter was determined using a laser-based scanner (VolScan Profiler,  
132 Stable Micro Systems, Surrey, UK).

### 133 2.6.2 *Color*

134 The CIEL\*a\*b\* color parameters of three thawed cakes were recorded at illuminant  
135 D65 and a 10° observer angle using a CD-6834 spectro-guide sphere gloss colorimeter (BYK-  
136 Gardner, Geretsried, Germany). Color was measured at three points on the cake crust, then the  
137 cake was halved in a horizontal plane and three further points were analyzed on the crumb of  
138 the lower half.

## 139 2.7 **Analysis of lipid oxidation**

### 140 2.7.1 *Lipoxygenase (LOX) activity*

141 LOX activity was determined externally by Eurogerm (Saint-Apollinaire, France)  
142 according to the method described by Delcros *et al.* (1998). Briefly, crude enzyme extracts  
143 were prepared in triplicate by blending 2 g flour with 20 mL phosphate buffer (pH 7.5). After  
144 centrifugation, 20-200 µL enzymatic extract was mixed with linoleic acid in phosphate buffer  
145 (pH 6.5) and saturated with air. LOX activity was expressed as nmol oxygen consumed per  
146 second.

### 147 2.7.2 *Oil extraction*

148 To analyze lipid oxidation precursors and markers, oil was extracted from the products  
149 using accelerated solvent extraction adapted from Maire *et al.* (2013). Samples (in triplicate)  
150 were mixed with Celite® 545 at ratios (w/w) of 1/1, 3/2, 10/9 and 14/5 for eggs, flours,  
151 batters and cakes, respectively, and extracted at 40 °C with heptane/isopropanol (5/1 v/v) for  
152 25 min using a Dionex ASE 350 (Thermo Fisher Scientific, Waltham, MA, USA). The  
153 solvents were evaporated at 40 °C under a vacuum; yield was then determined by differential  
154 weighing before extraction and after evaporation. The oil was stored under nitrogen at -20 °C  
155 until analysis.

### 156 2.7.3 *Fatty acid (FA) profile*

157 The FA profile of extracted oils was determined by gas chromatography coupled with  
158 mass spectrometry (GC-MS) after transmethylation as described by Roman *et al.* (2013) with  
159 slight modifications. Approximately 30 mg extracted oil (in triplicate) was dissolved in 1 mL  
160 petroleum ether containing 3 mg internal standard heptadecanoic acid. After gentle mixing for  
161 10 s, 200 µL NaOH in methanol (2 M) was added and the mixture was shaken for 10 s. The

162 tubes were then incubated in a water bath at 52 °C under constant agitation for 40 s. The  
163 reaction was stopped by the addition of 400 µL HCl in methanol (1 M). Samples were  
164 allowed to stand for 2 min for phase separation before the upper phase was transferred into  
165 GC vials.

166 Fatty acid methyl esters (FAME) were eluted on a TRACE GC Ultra gas  
167 chromatograph (Thermo Fisher Scientific) equipped with an SP-2560 GC column (100  
168 m×250 µm×0.2 µm, Supelco, Bellefonte, PA, USA) using helium as the carrier gas (1.20  
169 mL/min). A 1 µL sample was injected at 250 °C with a split flow of 448 mL/min. The  
170 FAMEs were separated by increasing the temperature by 5 °C/min increments from 100 °C  
171 (held for 4 min) to 200 °C (held for 8 min) and then to 250 °C (held for 12 min). Mass spectra  
172 ( $m/z$  40-400) were detected at 250 °C using an ISQ single quadrupole mass spectrometer  
173 (Thermo Fisher Scientific). Peaks were identified by comparing their mass spectra and  
174 retention times with those of a FAME standard mixture containing 37 compounds (Supelco)  
175 subjected to the same procedure. Relative FAME quantities were calculated by relating the  
176 integrated areas under the peaks to those of the internal standard.

#### 177 2.7.4 Conjugated dienes (CD)

178 The UV-spectrophotometric measurement of CD in sunflower and extracted oils was  
179 performed in triplicate according to Roman *et al.* (2013) with some modifications. 10-20 mg  
180 oil was weighed into 10 mL volumetric flasks, filled quantitatively with isooctane and  
181 vortexed at maximum speed for 10 s. Depending on the oxidation status of the sample, a  
182 second dilution (0.5-5 mL in 10 mL isooctane) was necessary to ensure absorbance within the  
183 linear range of the spectrophotometer. The absorbance of the dilutions was recorded against  
184 isooctane in 1 cm quartz cuvettes at 234 nm using a Specord 210 Plus (Analytik Jena, Jena,  
185 Germany). The CD content was expressed as a specific absorbance, defined as the absorbance  
186 of 1 g oil in 100 mL isooctane at 234 nm.

#### 187 2.7.5 Peroxide value (PV)

188 The PV of sunflower and extracted oils was analyzed in triplicate by adapting the  
189 method described by Roman *et al.* (2013). 0.2 g oil was weighed into 75 mL amber beakers  
190 and dissolved in 16 mL glacial acetic acid/isooctane (3/2 v/v) containing 0.002% (w/v) iodine.  
191 1 mL saturated potassium iodide solution was added and the mixture was blended for 90 s. 9  
192 mL distilled water was added and the released iodine was titrated potentiometrically with  
193 sodium thiosulfate solution (5 mM). Water was used for the blank titration. The PV was  
194 calculated according to the equation Eq. (1).

$$PV \left[ \frac{mmol}{kg} \right] = \frac{(V_{Oil} - V_{Blank}) \cdot c_{Thio} \cdot 2000}{m_{Oil}} \quad (1)$$

195 where  $V_{Oil}$  and  $V_{Blank}$  are the volumes of sodium thiosulfate required to reach the equilibrium  
 196 point of the oil or blank sample [mL],  $c_{Thio}$  is the molarity of the sodium thiosulfate solution  
 197 [mM] and  $m_{Oil}$  is the weight of the oil [g].

### 198 2.7.6 Acid value

199 The acid value of extracted oils from the flours was analyzed in triplicate according to  
 200 the standard method NF EN ISO 660:2020 with slight modifications. Briefly, 50 mg oil was  
 201 dissolved in 20 mL neutralized ethanol/petroleum ether (1/1 v/v) and the acids present titrated  
 202 with ethanolic potassium hydroxide (KOH) solution (5 mM). The acid value was expressed as  
 203 the mass of KOH required to neutralize 1 g fat using the equation Eq. (2).

$$Acid\ value = \frac{M_{KOH} \cdot c_{KOH} \cdot V_{KOH}}{m_{Oil}} \quad (2)$$

204 where  $M_{KOH}$  is the molar mass of KOH [g/mol],  $c_{KOH}$  is the concentration of the KOH  
 205 solution [mol/L],  $V_{KOH}$  is the volume of KOH solution required to reach the equilibrium point  
 206 [mL] and  $m_{Oil}$  is the weight of the oil [g].

## 207 2.8 Analysis of volatile organic compounds (VOCs)

### 208 2.8.1 Internal deuterated standard solutions

209 For each food matrix (flours/batters/cakes), an aqueous solution containing furfural-d<sub>4</sub>,  
 210 2-methylpyrazine-d<sub>6</sub> and hexanal-d<sub>12</sub> at concentrations of 14/91/15 mg/L, 0.1/89/3 mg/L and  
 211 139/778/411 mg/L, respectively, were prepared and stored at -20 °C until use.

### 212 2.8.2 HeadSpace-Solid Phase Micro Extraction (HS-SPME) of volatile compounds

213 For the GC-MS analysis of VOCs, preliminary experiments were performed to  
 214 optimize the sample, equilibrium, extraction and injection conditions. Accordingly, 2 g  
 215 thawed flour, batter or ground cake were weighed in triplicate into 20 mL transparent vials,  
 216 spiked with 100 µL of their respective standard solution and enclosed with aluminum  
 217 polytetrafluoroethylene-coated silicon septa. To ensure that the VOCs of the sample and  
 218 added standards reached equilibrium in the headspace, the vials were stored overnight at 4 °C.  
 219 The next day, they were incubated at 50 °C for 18 min in a TriPlus autosampler (Thermo  
 220 Fisher Scientific). A DVB/CAR/PDMS fiber (50/30 µm, 2 cm, Supelco) was then inserted for  
 221 42 min at 50 °C. The compounds were thermally desorbed for 2 min (250 °C) and transferred  
 222 into the injection port of a TRACE GC Ultra gas chromatograph at a split flow of 10 mL/min.



### 223 2.8.3 Determination and analysis of VOCs

224 Separation of the volatiles was performed on a ZB-wax capillary column  
225 (30 m×0.25 mm×0.5 μm) using helium as the carrier gas (1.2 mL/min). The initial oven  
226 temperature of 40 °C (held for 5 min) was increased to 240 °C at 4 °C/min. Mass spectra were  
227 recorded in the electron impact ionization mode at 70 eV by means of an ISQ single  
228 quadrupole mass spectrometer. Data were acquired by applying both full scan (TIC,  $m/z$  33-  
229 300) and SIM mode using specific  $m/z$  values for selected compounds. Identification was  
230 performed using Xcalibur 2.2 (Thermo Fisher Scientific) coupled with the MS libraries NIST  
231 08 and Wiley 8, as well as by calculating the retention indices of the compounds using a  
232 series of *n*-alkanes (C5-C21), which were compared with the values reported on the NIST  
233 database (detailed information in **Table S1**). For VOC quantification, the areas under the TIC  
234 or SIM peaks were integrated and related to those of the internal standards. Hexanal, furfural  
235 and 2-methylpyrazine were quantified using their deuterated standards. The relative  
236 quantification of other compounds was performed using 2-methylpyrazine- $d_6$  for nitrogenous,  
237 furfural- $d_4$  for furanic and hexanal- $d_{12}$  for the remaining compounds.

### 238 **2.9 Statistical analysis**

239 One-way analysis of variance (ANOVA) was performed and significant differences  
240 were evaluated by Tukey's test at  $p < 0.05$  using SAS studio version 3.8 (SAS Institute Inc.,  
241 Cary, NC, USA). The data acquired is reported with two significant figures. Principal  
242 component analysis (PCA) was conducted using XLSTAT 2020.1 (Addinsoft, Paris, France).

### 243 3 Results and Discussion

244 Wheat, lupin, lentil, chickpea, green pea, yellow pea and yellow pea processed under  
245 nitrogen are abbreviated to W, LU, LE, CP, GP, YP and YPN, respectively. The letters “F”,  
246 “B” and “C” indicate whether the flour, batter or cake was being examined.

#### 247 3.1 Volatile compounds identified in the flours

248 **Figure 1** shows the profiles of VOCs extracted from the flours, batters and cakes  
249 made using wheat or pulse flours. An overview of all compounds and their quantities can be  
250 found in **Table S2**.

251 The identification of the VOCs present in the flours is an important prerequisite to  
252 understand their potential to form molecules during cake development. As shown in **Figure 1**,  
253 the pulse flours, and particularly YPF/YPNF, contained significantly higher relative VOC  
254 amounts compared to WF, with the exception of LUF. Moreover, distinct differences in the  
255 VOC composition between the raw materials could be determined.

256 In WF, monoterpenes were observed as the dominant group of volatiles (**Figure 1**),  
257 which was not the case for the pulse flours, even though high levels were also detected. The  
258 molecules found included limonene,  $\beta$ -myrcene,  $\beta$ -pinene, 3-carene and linalool, which might  
259 have been products of endogenous isoprenoid biosynthesis or carotenoid degradation (Azarnia  
260 *et al.*, 2011; Jakobsen *et al.*, 1998; Paucean *et al.*, 2018).

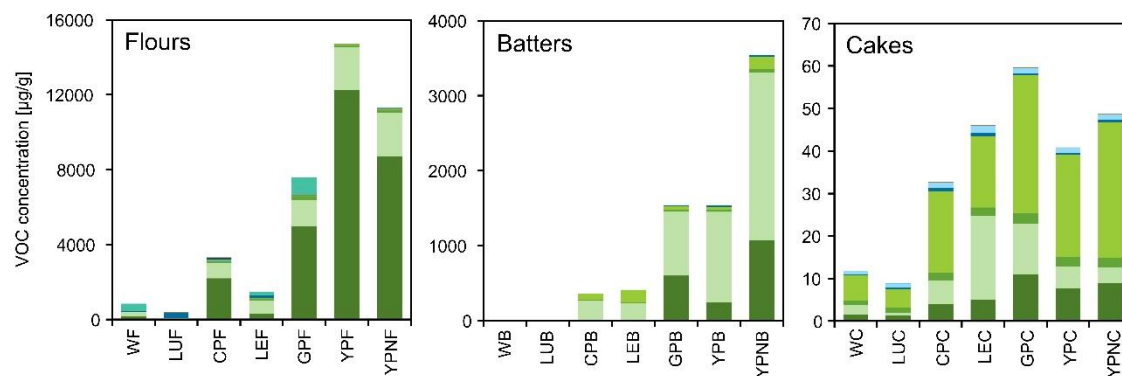
261 By contrast, the most abundant constituents of CPF, GPF and YPF/YPNF included a  
262 complex mixture of alkanes (**Figure 1**). These compounds might have been produced by the  
263 oxidation of lipids (Paucean *et al.*, 2018; Stevenson *et al.*, 1996) or they could have migrated  
264 from the packaging (Chang *et al.*, 2016; Rivas-Cañedo *et al.*, 2009).

265 In LEF, however, the main group of volatiles was alcohols. Relative to WF and LUF,  
266 the concentrations determined were significantly higher, but not when compared to CPF and  
267 the pea flours (**Figure 1**). Ethanol generally accounted for a large share of alcohols in all the  
268 flours, and particularly in pea cultivars (**Table S2**). This might suggest microbial spoilage, as  
269 the compound can be produced from the amino acid alanine *via* the Ehrlich pathway (Pico *et*  
270 *al.*, 2015). The presence of 2-methyl-1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol and  
271 2-phenylethanol in the different flours could have confirmed fungal infection as they can form  
272 *via* the same mechanism from valine, leucine, isoleucine and phenylalanine, respectively  
273 (Burdock, 2010; Pico *et al.*, 2015). The comparatively low proportions of these molecules in  
274 LUF, however, could indicate that this flour or its seeds had been subjected to thermal  
275 treatment, thereby suppressing microbial growth.

276 According to the literature, the remaining alcohols can be assumed as being more  
277 likely to derive from lipid oxidation, although some have also been described as fungal  
278 metabolites (Azarnia *et al.*, 2011; Maire *et al.*, 2013). Among these, 1-hexanol was one of the  
279 most abundant, particularly in LEF, GPF and YPF. Other alcohols typical of lipid oxidation,  
280 such as 1-penten-3-ol, 2-hexen-1-ol and 3-hexen-1-ol (Kochhar, 1996), were exclusively  
281 detected in LEF. Moreover, this flour contained two aldehyde oxidation markers, namely  
282 hexanal and 2-hexenal (Belitz *et al.*, 2009), the latter of which was not detected in any other  
283 flour, thereby emphasizing the higher degree of oxidation of LEF. The opposite could be  
284 concluded for CPF based on the absence of aldehydes.

285 Interestingly, no difference in alcohols and aldehydes profiles could be measured  
286 between the two yellow pea flours. Both flours were produced from the same raw material,  
287 but were processed and stored under exposure to air (YPF) or under nitrogen (YPNF). From  
288 this it could be deduced that lipid oxidation in this ingredient tended to be initiated during  
289 harvest and transport rather than during seed grinding or storage.

290 With regard to aldehydes, LUF occupied a special position. Unlike the other flours,  
291 LUF contained 2- and 3-methylbutanal (**Table S2**), which are both indicators of heat  
292 treatment, generated by Strecker degradation of either isoleucine or leucine (Cepeda-Vázquez  
293 *et al.*, 2018). The assumption of the heat exposure of LUF was affirmed by the identification  
294 of furanic compounds, which made up 75% of the VOCs. These included furfural, a known  
295 marker of the Maillard reaction (Belitz *et al.*, 2009). In addition, LUF contained traces of  
296 pyrazines (0.01-0.03 µg/g) and ketones linked to the Maillard reaction such as 2,3-  
297 butanedione, 2,3-pentanedione and 3-hydroxy-2-butanone, which validated the thermal  
298 processing of lupin seeds/flour.



Sample	VOC concentration [ $\mu\text{g/g}$ sample]											
	Alkanes	Alcohols	Ketones	Aldehydes	Furanic	Nitrogenous	Acids/Esters	Pyrones	Sulfurous	Terpenes	Total	
Flour	W	160.2 $\pm$ 5.5 <sup>c</sup>	235 $\pm$ 40 <sup>c</sup>	16.2 $\pm$ 2.5 <sup>d</sup>	6.19 $\pm$ 0.32 <sup>b</sup>	45 $\pm$ 12 <sup>c</sup>	0.013 $\pm$ 0.011 <sup>b</sup>	n.d.	n.d.	n.d.	381 $\pm$ 14 <sup>b</sup>	843 $\pm$ 54 <sup>d</sup>
	LU	23.1 $\pm$ 2.1 <sup>c</sup>	30.9 $\pm$ 1.3 <sup>c</sup>	29.6 $\pm$ 1.4 <sup>d</sup>	2.054 $\pm$ 0.014 <sup>c</sup>	291 $\pm$ 11 <sup>a</sup>	0.0575 $\pm$ 0.0038 <sup>a</sup>	11.45 $\pm$ 0.66 <sup>c</sup>	n.d.	n.d.	0.54 $\pm$ 0.14 <sup>d</sup>	389 $\pm$ 11 <sup>d</sup>
	CP	2200 $\pm$ 550 <sup>c</sup>	835 $\pm$ 100 <sup>b,c</sup>	69.8 $\pm$ 9.7 <sup>d</sup>	n.d.	27.4 $\pm$ 1.3 <sup>c,d</sup>	0.0178 $\pm$ 0.0067 <sup>b</sup>	n.d.	n.d.	126 $\pm$ 17 <sup>a</sup>	57.8 $\pm$ 9.2 <sup>d</sup>	3310 $\pm$ 680 <sup>c,d</sup>
	LE	293 $\pm$ 29 <sup>c</sup>	729 $\pm$ 99 <sup>b,c</sup>	105.4 $\pm$ 1.8 <sup>b,c</sup>	12.9 $\pm$ 1.8 <sup>a</sup>	144.6 $\pm$ 5.0 <sup>b</sup>	0.022 $\pm$ 0.012 <sup>b</sup>	n.d.	n.d.	29.0 $\pm$ 1.1 <sup>b</sup>	174 $\pm$ 66 <sup>c</sup>	1490 $\pm$ 180 <sup>d</sup>
	GP	4990 $\pm$ 180 <sup>b,c</sup>	1390 $\pm$ 320 <sup>b</sup>	218 $\pm$ 39 <sup>a</sup>	6.871 $\pm$ 0.048 <sup>b</sup>	5.02 $\pm$ 0.44 <sup>e</sup>	0.0090 $\pm$ 0.0037 <sup>b</sup>	42.95 $\pm$ 0.86 <sup>a</sup>	n.d.	n.d.	922 $\pm$ 21 <sup>a</sup>	7570 $\pm$ 480 <sup>b,c</sup>
	YP	12200 $\pm$ 2000 <sup>a</sup>	2280 $\pm$ 480 <sup>a</sup>	124 $\pm$ 20 <sup>b,c</sup>	14.54 $\pm$ 0.36 <sup>a</sup>	14.66 $\pm$ 0.43 <sup>d,e</sup>	0.0077 $\pm$ 0.0046 <sup>b</sup>	30.6 $\pm$ 8.6 <sup>b</sup>	n.d.	n.d.	21.7 $\pm$ 4.9 <sup>d</sup>	14700 $\pm$ 2400 <sup>a</sup>
	YPN	8700 $\pm$ 4700 <sup>a,b</sup>	2320 $\pm$ 480 <sup>a</sup>	152 $\pm$ 35 <sup>b</sup>	13.4 $\pm$ 1.3 <sup>a</sup>	32.6 $\pm$ 3.4 <sup>c,d</sup>	0.0124 $\pm$ 0.0055 <sup>b</sup>	41.2 $\pm$ 3.5 <sup>a,b</sup>	n.d.	15.0 $\pm$ 7.3 <sup>b</sup>	22.9 $\pm$ 2.8 <sup>d</sup>	11300 $\pm$ 4500 <sup>a,b</sup>
Batter	W	0.62 $\pm$ 0.43 <sup>c</sup>	4.83 $\pm$ 0.37 <sup>c</sup>	n.d.	0.531 $\pm$ 0.019 <sup>d</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.98 $\pm$ 0.62 <sup>c</sup>
	LU	3.85 $\pm$ 0.29 <sup>c</sup>	1.580 $\pm$ 0.027 <sup>c</sup>	0.598 $\pm$ 0.034 <sup>d</sup>	0.1292 $\pm$ 0.0074 <sup>d</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.20 $\pm$ 0.24 <sup>c</sup>
	CP	9.5 $\pm$ 4.8 <sup>c</sup>	255 $\pm$ 19 <sup>c</sup>	9.28 $\pm$ 0.64 <sup>c,d</sup>	89.2 $\pm$ 4.9 <sup>b</sup>	n.d.	n.d.	89.2 $\pm$ 4.9 <sup>b</sup>	n.d.	n.d.	n.d.	363 $\pm$ 29 <sup>c</sup>
	LE	0.84 $\pm$ 0.40 <sup>c</sup>	232 $\pm$ 26 <sup>c</sup>	7.4 $\pm$ 1.1 <sup>d</sup>	172 $\pm$ 10 <sup>a</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	413 $\pm$ 31 <sup>c</sup>
	GP	600 $\pm$ 100 <sup>a,b</sup>	857 $\pm$ 74 <sup>b</sup>	20.81 $\pm$ 0.76 <sup>b,c</sup>	48.5 $\pm$ 2.9 <sup>c</sup>	12.9 $\pm$ 1.9 <sup>a</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	1540 $\pm$ 140 <sup>b</sup>
	YP	247 $\pm$ 69 <sup>b,c</sup>	1200 $\pm$ 170 <sup>b</sup>	28.8 $\pm$ 3.6 <sup>b</sup>	37.7 $\pm$ 2.9 <sup>c</sup>	21.0 $\pm$ 6.8 <sup>a</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	1540 $\pm$ 100 <sup>b</sup>
	YPN	1070 $\pm$ 480 <sup>a</sup>	2240 $\pm$ 520 <sup>a</sup>	50.4 $\pm$ 8.2 <sup>a</sup>	159.1 $\pm$ 9.4 <sup>a</sup>	21.4 $\pm$ 3.7 <sup>a</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	3540 $\pm$ 930 <sup>a</sup>
Cake	W	1.456 $\pm$ 0.014 <sup>d</sup>	2.299 $\pm$ 0.071 <sup>e</sup>	1.143 $\pm$ 0.032 <sup>c</sup>	5.84 $\pm$ 0.34 <sup>a</sup>	0.197 $\pm$ 0.011 <sup>f</sup>	0.790 $\pm$ 0.025 <sup>d</sup>	n.d.	n.d.	n.d.	n.d.	11.72 $\pm$ 0.46 <sup>e</sup>
	LU	1.35 $\pm$ 0.10 <sup>d</sup>	0.593 $\pm$ 0.026 <sup>f</sup>	1.168 $\pm$ 0.034 <sup>c</sup>	4.37 $\pm$ 0.14 <sup>f</sup>	0.472 $\pm$ 0.013 <sup>d</sup>	0.8409 $\pm$ 0.0069 <sup>d</sup>	n.d.	0.00542 $\pm$ 0.00078 <sup>a</sup>	n.d.	n.d.	8.81 $\pm$ 0.27 <sup>e</sup>
	CP	3.993 $\pm$ 0.053 <sup>c</sup>	5.48 $\pm$ 0.15 <sup>c</sup>	2.013 $\pm$ 0.058 <sup>b</sup>	19.06 $\pm$ 0.43 <sup>c</sup>	0.713 $\pm$ 0.028 <sup>b</sup>	1.211 $\pm$ 0.010 <sup>b,c</sup>	0.1579 $\pm$ 0.0070 <sup>b</sup>	0.112 $\pm$ 0.085 <sup>a</sup>	n.d.	n.d.	32.74 $\pm$ 0.84 <sup>d</sup>
	LE	5.13 $\pm$ 0.71 <sup>c</sup>	19.56 $\pm$ 0.81 <sup>a</sup>	2.00 $\pm$ 0.13 <sup>b</sup>	16.74 $\pm$ 0.35 <sup>d</sup>	0.770 $\pm$ 0.021 <sup>a</sup>	1.69 $\pm$ 0.12 <sup>a</sup>	0.241 $\pm$ 0.021 <sup>a</sup>	n.d.	n.d.	n.d.	46.1 $\pm$ 1.9 <sup>b</sup>
	GP	10.98 $\pm$ 0.61 <sup>a</sup>	11.68 $\pm$ 0.11 <sup>b</sup>	2.527 $\pm$ 0.042 <sup>a</sup>	32.47 $\pm$ 0.33 <sup>a</sup>	0.4312 $\pm$ 0.0029 <sup>d,e</sup>	1.261 $\pm$ 0.039 <sup>b,c</sup>	0.1453 $\pm$ 0.0091 <sup>b</sup>	n.d.	n.d.	n.d.	59.49 $\pm$ 0.48 <sup>a</sup>
	YP	7.7 $\pm$ 1.1 <sup>b</sup>	5.15 $\pm$ 0.11 <sup>c</sup>	2.180 $\pm$ 0.030 <sup>b</sup>	24.11 $\pm$ 0.48 <sup>b</sup>	0.415 $\pm$ 0.018 <sup>e</sup>	1.171 $\pm$ 0.051 <sup>c</sup>	0.1136 $\pm$ 0.0071 <sup>c</sup>	n.d.	n.d.	n.d.	40.9 $\pm$ 1.6 <sup>c</sup>
	YPN	8.9 $\pm$ 1.2 <sup>b</sup>	3.666 $\pm$ 0.055 <sup>d</sup>	2.390 $\pm$ 0.071 <sup>a</sup>	31.80 $\pm$ 0.65 <sup>a</sup>	0.592 $\pm$ 0.017 <sup>c</sup>	1.356 $\pm$ 0.012 <sup>b</sup>	0.1419 $\pm$ 0.0059 <sup>b,c</sup>	n.d.	n.d.	n.d.	48.8 $\pm$ 1.6 <sup>b</sup>

299

300 **Figure 1.** Profile of VOCs [ $\mu\text{g/g}$  sample] extracted from flours, batters and cakes. Compounds are categorized into alkanes (■) alcohols (■), ketones (■), aldehydes (■), furanic compounds (■),  
 301 nitrogenous compounds (■), pyrones (■), acids/esters (■), terpenes (■) and sulfurous compounds (■). n.d.: not detected. Relative amounts were estimated based on the internal deuterated  
 302 standards hexanal-d<sub>12</sub>, furfural-d<sub>4</sub> and 2-methylpyrazine-d<sub>6</sub>. Different letters within a column of one food matrix indicate significantly different means ( $p < 0.05$ ). W = wheat, LU = lupin, CP =  
 303 chickpea, LE = lentil, GP = green pea, YP = yellow pea and YPN = yellow pea processed and stored under nitrogen.

### 304 **3.2 Changes to the VOC profile during batter beating**

305 The distribution of dry ingredients in the moist matrix, along with air entrapment  
306 during batter whipping, seemed to initiate reactions. This could be seen from the marked  
307 changes in VOC profiles compared to the flours (**Figure 1**). The highest levels of compounds  
308 were extracted from batters made with green and yellow pea, while the lowest quantities were  
309 recorded in WB and LUB. In terms of the number of VOCs, the batters could be ranked in a  
310 similar order (**Table S2**).

311 Alcohols represented the dominant group of VOCs in all batters with the exception of  
312 LUB, where the profile was primarily characterized by hydrocarbons. The highest relative  
313 amounts were determined in pea batters, the alcohol levels in which exceeded those in WB by  
314 up to 460× (**Figure 1**). Taking account of the fact that the batters only contained 25% flour,  
315 and that the contributions of inherent egg and oil volatiles to the VOC profile were negligible  
316 (Maire *et al.*, 2013), it emerged that the amounts of alcohols increased sharply during batter  
317 beating. Most of these alcohols were associated with FA breakdown, attributable to the  
318 incorporation of air. 1-Hexanol was one of the most abundant compounds in the batters  
319 (**Table S2**), which was found at concentrations up to 35× higher than those detected in the  
320 flours when considering their 25% content. The widely accepted pathway for 1-hexanol  
321 production involves the oxidation of linoleic acid under the formation of hexanal, which is  
322 reduced to 1-hexanol by the action of an alcohol dehydrogenase (Jakobsen *et al.*, 1998). In  
323 addition, several other alcohols were generated, some of which are also believed to originate  
324 from linoleic acid degradation. These included 1-octen-3-ol and 1-pentanol, which appeared  
325 at high levels, particularly in GPB, YPB and YPNB (**Table S2**). These batters were moreover  
326 highly enriched in 1-penten-3-ol, which was more likely to have been produced from linolenic  
327 acid oxidation catalyzed by an alcohol dehydrogenase (Murray *et al.*, 1976). Among the  
328 yellow pea batters, YPNB stood out for its significantly higher concentrations of lipid  
329 oxidation markers compared to YPB (**Figure 1**). This might indicate that producing pea flour  
330 under nitrogen protected unsaturated FAs from oxidation. Consistent with this, it could be  
331 hypothesized that any contact with atmospheric oxygen would cause an instant reaction,  
332 leading to VOC formation.

333 In addition, large quantities of ethanol were measured in the batters, which probably  
334 derived from the raw materials or arose in the course of fermentation processes during batter  
335 preparation. Indeed, it was the principal alcohol in both WB and LUB (**Table S2**), suggesting  
336 their low susceptibility to lipid oxidation.

337 LEB and CPB could be discriminated from the other batters as having higher aldehyde  
338 than alcohol contents (**Figure 1**). Hexanal, in particular, was of considerable importance as its  
339 level increased by up to 80× compared to the respective flours, when considering that only  
340 25% flour was used during batter making. High values were also extracted from YPB, GPB  
341 and CPB, which thus greatly exceeded those in WB and LUB, where only traces of aldehydes  
342 were found. Apart from hexanal, the oxidative degradation of linoleic acid also seemed to  
343 have caused the release of 2,4-nonadienal, 2-nonenal, 2-heptenal, 2-octenal, pentanal and  
344 heptanal (Belitz *et al.*, 2009; Murray *et al.*, 1976), most of which were solely identified in  
345 LEB. The same was true for the breakdown products of linolenic acid, which included 2-  
346 hexenal, 2,4-heptadienal and 2-pentenal (Murray *et al.*, 1976). Based on these findings, it  
347 could be assumed that CPF, and notably LEF, contained alcohol dehydrogenases that were  
348 either at lower levels or with weaker activities, thus transforming fewer aldehydes into  
349 alcohols, unlike peas.

350 The greater vulnerability of unsaturated FAs to oxidative degradation in LEB, CPB,  
351 GPB and YPB/YPNB was further substantiated by the presence of ketones such as 3-  
352 octanone, that were lacking in LUB and WB. After alcohols and aldehydes, these molecules  
353 made up the third largest group of VOCs. Finally, furanic components were also found, but  
354 only accounted for a maximum of 1% of volatiles (**Figure 1**).

### 355 **3.3 Factors influencing reactivity potential during batter beating**

#### 356 *3.3.1 Amount of incorporated air*

357 The generation of VOCs during batter preparation was largely attributed to the  
358 oxidation of unsaturated FAs. In order to understand whether the amount of air incorporated  
359 was correlated with the potential of the flours to form VOCs, the density of the batters was  
360 analyzed and associated with the VOC quantities. As shown in **Table 1**, YPNB and GPB  
361 appeared to be the most voluminous, while LUB was the densest. Since the former products  
362 were distinctively enriched in VOCs compared to the latter (**Figure 1**), it was supposed that  
363 enhanced air enclosure promoted oxidative reactions. However, this hypothesis was refuted  
364 by the behavior of YPB, which contained similar amounts of volatiles as GPB but displayed  
365 significantly higher density. Furthermore, its density did not differ significantly from that of  
366 WB, LEB, CPB and LUB, but far exceeded their VOC concentrations. This led to the  
367 conclusion that the amount of incorporated air was not the principal cause of lipid oxidation,  
368 so it appeared essential to address the enzymatic activity of the different flours.

369 **Table 1.** Batter densities, LOX activities, profile of unsaturated fatty acids and acid value in flours.

Flour	Batter density [g/cm <sup>3</sup> ]	LOX activity [nkat/g flour]	Unsaturated fatty acids [g/100 g fat]			Acid value
			C18:1	C18:2	C18:3	
W	0.6046 ± 0.0077 <sup>b</sup>	<20 <sup>d</sup>	5.61 ± 0.25 <sup>d</sup>	24.1 ± 1.2 <sup>d</sup>	1.88 ± 0.18 <sup>d</sup>	23.22 ± 0.74 <sup>b</sup>
LU	0.645 ± 0.027 <sup>a</sup>	<1 <sup>d</sup>	37.0 ± 1.3 <sup>a</sup>	12.07 ± 0.31 <sup>e</sup>	5.29 ± 0.15 <sup>b,c</sup>	4.512 ± 0.089 <sup>d</sup>
CP	0.618 ± 0.013 <sup>a,b</sup>	2510 ± 140 <sup>c</sup>	18.25 ± 0.80 <sup>b</sup>	38.14 ± 0.35 <sup>a</sup>	1.35 ± 0.24 <sup>d</sup>	5.01 ± 0.27 <sup>d</sup>
LE	0.603 ± 0.013 <sup>b</sup>	3200 ± 250 <sup>b</sup>	15.26 ± 0.51 <sup>c</sup>	22.77 ± 0.86 <sup>d</sup>	4.50 ± 0.58 <sup>c</sup>	42.522 ± 0.038 <sup>a</sup>
GP	0.560 ± 0.023 <sup>c</sup>	3870 ± 110 <sup>a</sup>	15.550 ± 0.070 <sup>e</sup>	28.22 ± 0.18 <sup>c</sup>	5.63 ± 0.31 <sup>a,b</sup>	10.59 ± 0.49 <sup>e</sup>
YP	0.621 ± 0.017 <sup>a,b</sup>	4350 ± 300 <sup>a</sup>	19.80 ± 0.39 <sup>b</sup>	35.51 ± 0.82 <sup>b</sup>	6.050 ± 0.060 <sup>a,b</sup>	10.94 ± 0.52 <sup>c</sup>
YPN	0.533 ± 0.028 <sup>c</sup>	4340 ± 160 <sup>a</sup>	19.70 ± 0.69 <sup>b</sup>	34.54 ± 0.95 <sup>b</sup>	6.42 ± 0.36 <sup>a</sup>	10.31 ± 0.46 <sup>e</sup>

370 Different letters within a column indicate significantly different means ( $p < 0.05$ ). W = wheat, LU = lupin, CP = chickpea,  
 371 LE = lentil, GP = green pea, YP = yellow pea and YPN = yellow pea processed and stored under nitrogen.

372

### 373 3.3.2 LOX activity and FA profile

374 Unlike the other batters, LUB and WB contained fewer lipid oxidation markers than  
 375 their respective flours used at a proportion of 25%. As discussed in **Section 3.2**, these  
 376 molecules are well-known breakdown products of the polyunsaturated FAs linoleic and  
 377 linolenic acid. Owing to their 1-*cis*,4-*cis*-pentadiene system, they are suitable substrates for  
 378 LOX, catalyzing their conversion into unstable hydroperoxides, which are subsequently  
 379 transformed into volatiles (Belitz *et al.*, 2009). Because of the low level of oleic acid  
 380 degradation products, it was assumed that enzyme-mediated lipid oxidation was the main  
 381 pathway through which VOCs were released. This was confirmed by analyzing the LOX  
 382 activities of the flours at a pH similar to that of the batters (**Table 1**). While the enzyme was  
 383 practically inactive in WF and LUF, its activity in the other flours ranged from 2511 nkat/g  
 384 (CPF) to 4351 nkat/g (YPF). This finding provided a substantiated explanation for the low  
 385 concentrations of lipid oxidation markers in the batters made with LUF or WF. In the former  
 386 case, the suppression of LOX could indicate heat-induced denaturation during seed/flour  
 387 roasting, which could be supported by the presence of thermal reaction markers (e.g.  
 388 pyrazines). However, this assumption was not applicable to WF owing to its lack of such  
 389 molecules. But for the other flours, a logical link between LOX activity and volatiles could be  
 390 established: the more the enzyme was active, the more VOCs were identified.

391 The high levels of linoleic and linolenic acid in the pea flours, combined with their  
 392 striking LOX activity, could further underline their pronounced susceptibility to oxidation  
 393 (**Table 1**). Although these flours were characterized by a significantly lower acid value than  
 394 wheat, which reflects the quantity of free FA that can be easily oxidized, they had a higher  
 395 ability to undergo oxidation and form volatile compounds. This implies, that the fewer FA  
 396 freely available in the pea flours were probably polyunsaturated fatty acids, which were

397 rapidly oxidized by the highly active LOX. Interestingly, however, CPF and LEF had a  
398 weaker ability to generate oxidation markers despite high concentrations of polyunsaturated  
399 fatty acids (**Table 1**). In addition, a large number of these seemed to be freely available, in  
400 particular in LEF, however were much less degraded compared to the pea flours. This could  
401 not only be related to reduced LOX activity but potentially also to a high antioxidative  
402 capacity, as described in the literature (Hall *et al.*, 2017). In light of their reported elevated  
403 polyphenol contents, a deceleration of FA breakdown could be imagined (Hall *et al.*, 2017).

#### 404 **3.4 Development of density and color in cakes during baking**

405 Baking induces starch gelatinization and protein denaturation that lead to the setting of  
406 a solid cake structure. This affects the density of the cakes, which is considered a good  
407 indicator of cake quality. As depicted in **Figure 2**, the complete substitution of WF with the  
408 different pulse flours results in the successful production of sponge cakes with a slightly  
409 higher volume. These findings coincide with the results published by de la Hera *et al.* (2012),  
410 who reported an increase in sponge cake volume after the incorporation of lentil flour. The  
411 authors assigned this behavior to the elevated level of protein, which has important  
412 emulsifying properties. However, other studies have demonstrated the opposite effect with  
413 chickpea (Gómez *et al.*, 2008) or green pea flour (Monnet *et al.*, 2020). These differences  
414 could be related to the different recipes chosen. Monnet *et al.* (2020) observed cracks on the  
415 surface of their cakes, indicating crust rupture. Unlike the present study, the authors used  
416 baking powder in their formula, which presumably led to an additional formation of gas  
417 during cake production. The resulting increase in internal pressure caused the crust to burst  
418 and allowed the vapor to escape, triggering shrinkage.

419 According to Gómez *et al.* (2012), cake shrinkage can also be correlated to the  
420 proportion of the starch fraction. In addition to proteins, these macromolecules play a decisive  
421 role in the formation of a structural framework during baking based on their heat-induced  
422 gelatinization, which in turn affects viscosity and thus the ability to retain air incorporated in  
423 the batter (Monnet *et al.*, 2020; Ronda *et al.*, 2011). With a reduced starch-to-protein ratio, the  
424 gas cells cannot be sufficiently stabilized during batter solidification, which therefore causes  
425 the development of a dense cake. This phenomenon might be used to describe the  
426 extraordinary behavior of LUC, which was characterized by the highest density (**Figure 2**,  
427 **Table 2**). In addition, the elevated fiber content in this flour (**Table 2**) might have contributed  
428 to the reduced volume owing to its effect on the gelatinization and pasting properties of the



429 starch and disorganization of the starch-protein network, being accompanied by a lower  
 430 capacity for gas retention (Aydogdu *et al.*, 2018; BeMiller, 2011; Sasaki *et al.*, 2000).

431 In addition to density, the use of pulse flours was associated with modifications to the  
 432 color properties of the cake, which is another decisive criterion for the cake quality (**Figure**  
 433 **2**). Pulse flours induced a significant reduction in lightness  $L^*$  of the crumb compared to WF,  
 434 which was most pronounced with LEF. In contrast, redness  $a^*$  was significantly increased  
 435 except with GPC, for which negative  $a^*$  values were measured. This implied a greenish hue  
 436 of the crumb, probably due to the presence of chlorophyll in the raw material. Additionally,  
 437 color generally shifted toward more yellow tints, as perceptible from the higher  $b^*$  values,  
 438 which could be assigned to the high proportion of carotenoids. It has been reported that  
 439 temperatures  $\geq 120^\circ\text{C}$  and water activities  $\leq 0.6$  are required to trigger the color formation of  
 440 baked goods. Working on similar sponge cakes, Srivastava *et al.* (2018) measured  
 441 temperatures lower than  $120^\circ\text{C}$  in the crumb, while those at the surface were sufficiently high  
 442 to initiate browning reactions, thereby causing darkening of the crust. As visible in **Figure 2**,  
 443 the  $L^*$ ,  $a^*$  and  $b^*$  values were very similar in the different products, especially for the crust.  
 444 This could be explained by the Maillard reaction which leads to the formation of brown  
 445 pigments capable of masking the differences due to the flours in the crust.

446

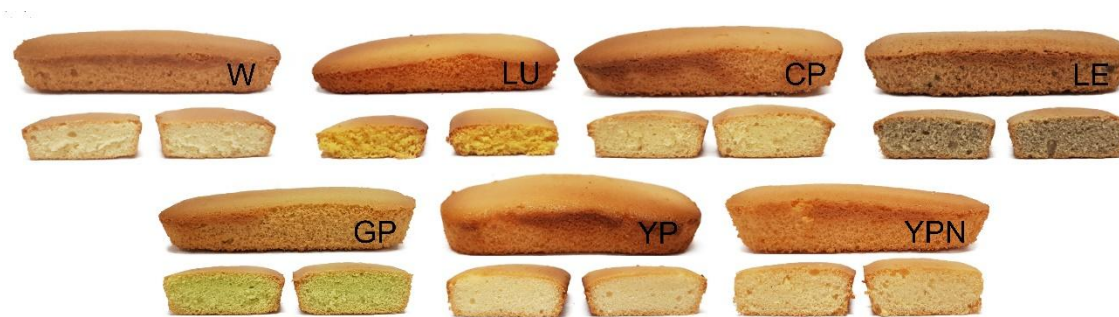
447 **Table 2.** Chemical composition [g/100 g] of different flours.

Flour	Dry matter	Ash	Starch	Protein	Fiber	Fat
W	87.463 ± 0.028 <sup>c</sup>	0.76 ± 0.40 <sup>d</sup>	70.2 ± 7.4 <sup>a</sup>	8.65 ± 0.36 <sup>e</sup>	2.9 ± 1.2 <sup>c</sup>	1.112 ± 0.031 <sup>e</sup>
LU	92.90 ± 0.11 <sup>a</sup>	4.79 ± 0.40 <sup>a</sup>	<0.4 <sup>c</sup>	42.4 ± 1.3 <sup>a</sup>	26.7 ± 3.3 <sup>a</sup>	11.93 ± 0.12 <sup>a</sup>
CP	91.787 ± 0.047 <sup>a,b</sup>	3.60 ± 0.40 <sup>b,c</sup>	39.3 ± 4.3 <sup>b</sup>	22.19 ± 0.67 <sup>c</sup>	13.6 ± 2.4 <sup>b</sup>	6.354 ± 0.018 <sup>b</sup>
LE	91.134 ± 0.013 <sup>a,b</sup>	3.79 ± 0.40 <sup>b</sup>	40.7 ± 4.4 <sup>b</sup>	27.64 ± 0.83 <sup>b</sup>	8.9 ± 2.0 <sup>b,c</sup>	1.746 ± 0.043 <sup>d</sup>
GP	90.091 ± 0.039 <sup>b,c</sup>	2.67 ± 0.40 <sup>c</sup>	51.9 ± 5.5 <sup>b</sup>	18.68 ± 0.56 <sup>d</sup>	8.2 ± 1.9 <sup>b,c</sup>	1.652 ± 0.056 <sup>d</sup>
YP	90.11 ± 0.17 <sup>b,c</sup>	2.69 ± 0.030 <sup>c</sup>	48.8 ± 5.5 <sup>b</sup>	22.98 ± 0.10 <sup>c</sup>	8.8 ± 2.0 <sup>b,c</sup>	2.064 ± 0.024 <sup>c</sup>
YPN	90.13 ± 0.12 <sup>b,c</sup>	2.68 ± 0.030 <sup>c</sup>	48.6 ± 5.2 <sup>b</sup>	22.98 ± 0.10 <sup>c</sup>	8.1 ± 1.9 <sup>b,c</sup>	2.024 ± 0.022 <sup>c</sup>

448 Different letters within a column indicate significantly different means ( $p < 0.05$ ). W = wheat, LU = lupin, CP = chickpea, LE  
 449 = lentil, GP = green pea, YP = yellow pea and YPN = yellow pea processed and stored under nitrogen.

450

451 In addition to their attractive appearance, the developed cakes were also characterized  
 452 by an improved nutritional quality compared to the wheat-based reference. This could be  
 453 concluded from the particular chemical composition of the flours used, which led to pulse-  
 454 based cakes with elevated levels of protein and fiber as well as lower levels of starch and thus  
 455 glycemic index.



Cake	Density [g/cm <sup>3</sup> ]	Dry matter [g/100 g]	pH value	Crumb color			Crust color		
				L*	a*	b*	L*	a*	b*
W	0.3423 ± 0.0094 <sup>b</sup>	95.36 ± 0.10 <sup>d</sup>	7.55 ± 0.10 <sup>a</sup>	87.32 ± 0.83 <sup>a</sup>	1.40 ± 0.16 <sup>d</sup>	22.78 ± 0.71 <sup>f</sup>	64.9 ± 2.1 <sup>d</sup>	14.15 ± 0.61 <sup>b</sup>	31.27 ± 0.57 <sup>d</sup>
LU	0.4087 ± 0.034 <sup>a</sup>	97.10 ± 0.14 <sup>a</sup>	6.180 ± 0.046 <sup>e</sup>	74.7 ± 2.2 <sup>e</sup>	7.24 ± 0.87 <sup>a</sup>	50.15 ± 0.67 <sup>a</sup>	69.7 ± 3.9 <sup>a</sup>	14.1 ± 1.7 <sup>b</sup>	40.7 ± 2.0 <sup>a</sup>
CP	0.309 ± 0.011 <sup>c</sup>	96.19 ± 0.46 <sup>c</sup>	6.91 ± 0.10 <sup>d</sup>	83.82 ± 0.63 <sup>c</sup>	2.82 ± 0.19 <sup>b</sup>	29.46 ± 0.72 <sup>b</sup>	67.7 ± 2.4 <sup>a,b</sup>	14.23 ± 0.73 <sup>b</sup>	33.12 ± 0.47 <sup>b</sup>
LE	0.305 ± 0.010 <sup>c</sup>	95.46 ± 0.26 <sup>c,d</sup>	6.847 ± 0.026 <sup>d</sup>	64.9 ± 1.1 <sup>f</sup>	1.92 ± 0.32 <sup>c</sup>	15.66 ± 0.81 <sup>g</sup>	61.5 ± 1.6 <sup>e</sup>	9.1 ± 1.1 <sup>c</sup>	26.13 ± 0.99 <sup>e</sup>
GP	0.2971 ± 0.0048 <sup>c</sup>	96.333 ± 0.090 <sup>b</sup>	7.157 ± 0.065 <sup>b</sup>	78.9 ± 1.1 <sup>d</sup>	-4.58 ± 0.27 <sup>e</sup>	28.01 ± 0.76 <sup>c</sup>	65.3 ± 1.7 <sup>c,d</sup>	9.1 ± 1.3 <sup>c</sup>	31.56 ± 0.52 <sup>c,d</sup>
YP	0.2944 ± 0.0043 <sup>c</sup>	95.521 ± 0.073 <sup>c,d</sup>	6.950 ± 0.035 <sup>c,d</sup>	85.9 ± 1.6 <sup>b</sup>	2.65 ± 0.43 <sup>b</sup>	25.63 ± 0.65 <sup>e</sup>	67.3 ± 3.6 <sup>b,c</sup>	14.6 ± 1.2 <sup>a,b</sup>	32.11 ± 0.36 <sup>c</sup>
YPN	0.2810 ± 0.0054 <sup>c</sup>	93.71 ± 0.28 <sup>e</sup>	7.10 ± 0.10 <sup>b,c</sup>	83.4 ± 1.6 <sup>c</sup>	2.52 ± 0.30 <sup>b</sup>	26.25 ± 0.82 <sup>d</sup>	65.9 ± 3.2 <sup>b,c,d</sup>	15.3 ± 1.2 <sup>a</sup>	33.23 ± 0.44 <sup>b</sup>

456

457

458

**Figure 2.** Appearance and characteristics of sponge cakes prepared using different flours. Different letters within a column indicate significantly different means ( $p < 0.05$ ). W = wheat, LU = lupin, CP = chickpea, LE = lentil, GP = green pea, YP = yellow pea and YPN = yellow pea processed and stored under nitrogen.

### 459 3.5 Heat-driven generation of volatile compounds during baking

460 Apart from structure and color changes, the energy-driven degradation of molecules  
461 can be triggered during baking and involve lipid oxidation, the Maillard reaction and  
462 caramelization. As shown in **Figure 1**, the cakes were characterized by substantially lower  
463 quantities of VOCs compared to the batters, most probably due to volatilization, consecutive  
464 reactions or matrix effects. A total of 59 VOCs was detected, most of them in GPC and LEC,  
465 while LUC and WC contained the fewest (**Table S2**). Moreover, the latter two stood out for  
466 their remarkably low total VOC amounts among all products (**Figure 1**).

467 Except for LEC, aldehydes formed the main group of components in all the cakes,  
468 reaching particularly high concentrations in GPC and YPNC. Although the amounts in LEC,  
469 CPC and YPC were significantly lower, they clearly surpassed those found in WC and LUC.  
470 Among these aldehydes, hexanal predominated in most cakes (**Table S2**). This marker of  
471 linoleic acid oxidation is typically characterized by a low odor threshold, and thus is  
472 considered to be relevant regarding the perception of a grassy, beany odor (Buttery *et al.*,  
473 1998; Pozo-Bayón *et al.*, 2007). Further oxidation products resulting from linoleic acid  
474 degradation were present, such as pentanal, 2-octenal and 2,4-decadienal as well as heptanal  
475 and octanal, although the latter two might also have derived from the breakdown of oleic acid  
476 (Kochhar, 1996). The first occurrence of some of these compounds might indicate heat-  
477 triggered autoxidation of the aforementioned precursors, while others might have been  
478 retained from the batter, rendering the aldehyde profiles more complex than those of the  
479 batters. Together they may not only enhance the green scent, but also impart a fatty, nutty and  
480 toasted fragrance to the cakes, especially those based on pea (Burdock, 2010; Murat *et al.*,  
481 2013; Szczygiel *et al.*, 2017).

482 The VOC profiles of WC and LUC, on the other hand, contained fewer lipid oxidation  
483 markers but more of the aldehydes assigned to thermal amino acid degradation through the  
484 Strecker reaction (**Table S2**). This led to the assumption that the wheat and lupin products  
485 were generally less susceptible to lipid oxidation, regardless of whether they were  
486 enzymatically catalyzed or heat-induced. The globally accepted mechanism for the formation  
487 of Strecker aldehydes involves the reaction between amino acids and  $\alpha$ -dicarbonyl  
488 compounds such as the deoxyosones generated through the Maillard reaction (Belitz *et al.*,  
489 2009). The Strecker aldehydes identified comprised 2-methylpropanal, 2-methylbutanal, 3-  
490 methylbutanal, acetaldehyde and phenylacetaldehyde, which are associated with the oxidative  
491 decarboxylation of valine, isoleucine, leucine, alanine and phenylalanine, respectively (Belitz

492 *et al.*, 2009). While 2- and 3-methylbutanal totaled about 60-70% of the aldehydes in both  
493 WC and LUC, the percentage contributions in the other cakes reached only 10-19%, although  
494 in quantitative terms, the concentrations in all cakes were similar (**Table S2**). Strecker  
495 aldehydes typically have a marked malty and sweet aroma (Garvey *et al.*, 2021; Pico *et al.*,  
496 2015).

497 LEC differed from the other cakes in terms of its higher alcohol content (**Figure 1**).  
498 The majority of this class was composed of 1-hexanol, followed by ethanol and 1-penten-3-ol  
499 (**Table S2**). These three components were also most strongly represented in the other  
500 products, with 1-penten-3-ol dominating in YPC/YPNC and ethanol in GPC, LUC and WC.  
501 According to the literature, these compounds exhibit more than 1000× higher odor threshold  
502 values in water than aldehydes (Belitz *et al.*, 2009; Buttery *et al.*, 1998). For this reason, they  
503 may have contributed only marginally to the overall cake aroma despite their relatively  
504 elevated concentrations (**Table S2**).

505 The same conclusion could be drawn for ketones, which were present at much lower  
506 concentrations than alcohols (**Figure 1**). Unlike the batters, the VOC profiles of the cakes  
507 were enriched in markers characteristic of Maillard and caramelization reactions, including 1-  
508 hydroxy-2-propanone (sweet, burnt), 3-hydroxy-2-butanone (buttery), 2,3-butanedione  
509 (buttery) and 2,3-pentanedione (buttery) (**Table S2**). Yaylayan *et al.* (2000) proposed two  
510 pathways for the generation of 2,3-butanedione, including either glucose alone or in  
511 combination with glycine *via* pyruvaldehyde. Subsequently, 2,3-butanedione can be reduced  
512 to form 3-hydroxy-2-butanone (Yaylayan *et al.*, 2000). Moreover, these authors suggested  
513 that 2,3-pentanedione is produced from pyruvaldehyde, followed by chain elongation with  
514 two carbon atoms from amino acids like alanine (Yaylayan *et al.*, 2000). For 1-hydroxy-2-  
515 propanone, it was assumed that it could derive from isomerized 1-deoxyosone or dehydrated  
516 Amadori product through retro-aldol cleavage (Yaylayan *et al.*, 2000).

517 Apart from ketones, a large number of other browning reaction products were  
518 detected, including furanic compounds such as furfural, 5-methylfurfural and 2-  
519 furanmethanol, which were found at higher concentrations in the pulse cakes than in WC  
520 (**Figure 1, Table S2**). According to the literature, furfural can be generated at elevated  
521 temperatures *via* 1,2-enolization from pentoses or glucose through either caramelization or the  
522 Maillard reaction (Belitz *et al.*, 2009; Yaylayan *et al.*, 2000). The latter pathway involves  
523 condensation of the sugars with free amino acids, followed by further enolization and  
524 deamination reactions, giving rise to 3-deoxyosones from which furfural can be released  
525 (Srivastava *et al.*, 2018). The generation of 2-furanmethanol from 3-deoxyosone has also been

526 reported (Hollnagel *et al.*, 2002). Furanic molecules are generally associated with a burnt,  
527 caramel-like scent (Cepeda-Vázquez *et al.*, 2018; Garvey *et al.*, 2021). However, other  
528 molecules from this class and not originating from thermal degradation reactions but lipid  
529 oxidation (such as 2-pentylfuran) were present, which could have contributed to the green,  
530 nutty notes of the cakes (except LUC).

531 Nitrogenous compounds, i.e. pyrazines and pyrroles, represented another class of  
532 VOCs typically arising from Maillard reactions. Pyrazine formation is widely explained by a  
533 condensation reaction between two  $\alpha$ -aminocarbonyl compounds, which themselves are  
534 generated next to Strecker aldehydes through Strecker degradation from the reaction between  
535  $\alpha$ -dicarbonyl compounds and amino acids (Belitz *et al.*, 2009). Among the different cakes, the  
536 highest levels were found in LEC, and the lowest in LUC and WC (**Figure 1**) Despite their  
537 low levels, their percentage contributions to all the VOCs in WC and LUC were the highest.  
538 The structures identified ranged from simple pyrazine to its single or multiple alkyl-  
539 substituted or amidated form. In addition, pyrrole and its acetylated derivative were detected.  
540 In all cases, 2,5-dimethylpyrazine accounted for more than half of the nitrogenous VOCs,  
541 followed by 2-methylpyrazine (**Table S2**). These heterocyclic compounds are known to have  
542 roasted, nutty and earthy smells (Garvey *et al.*, 2021; Pico *et al.*, 2015; Pozo-Bayón *et al.*,  
543 2007).

### 544 **3.6 Monitoring lipid oxidation throughout cake development**

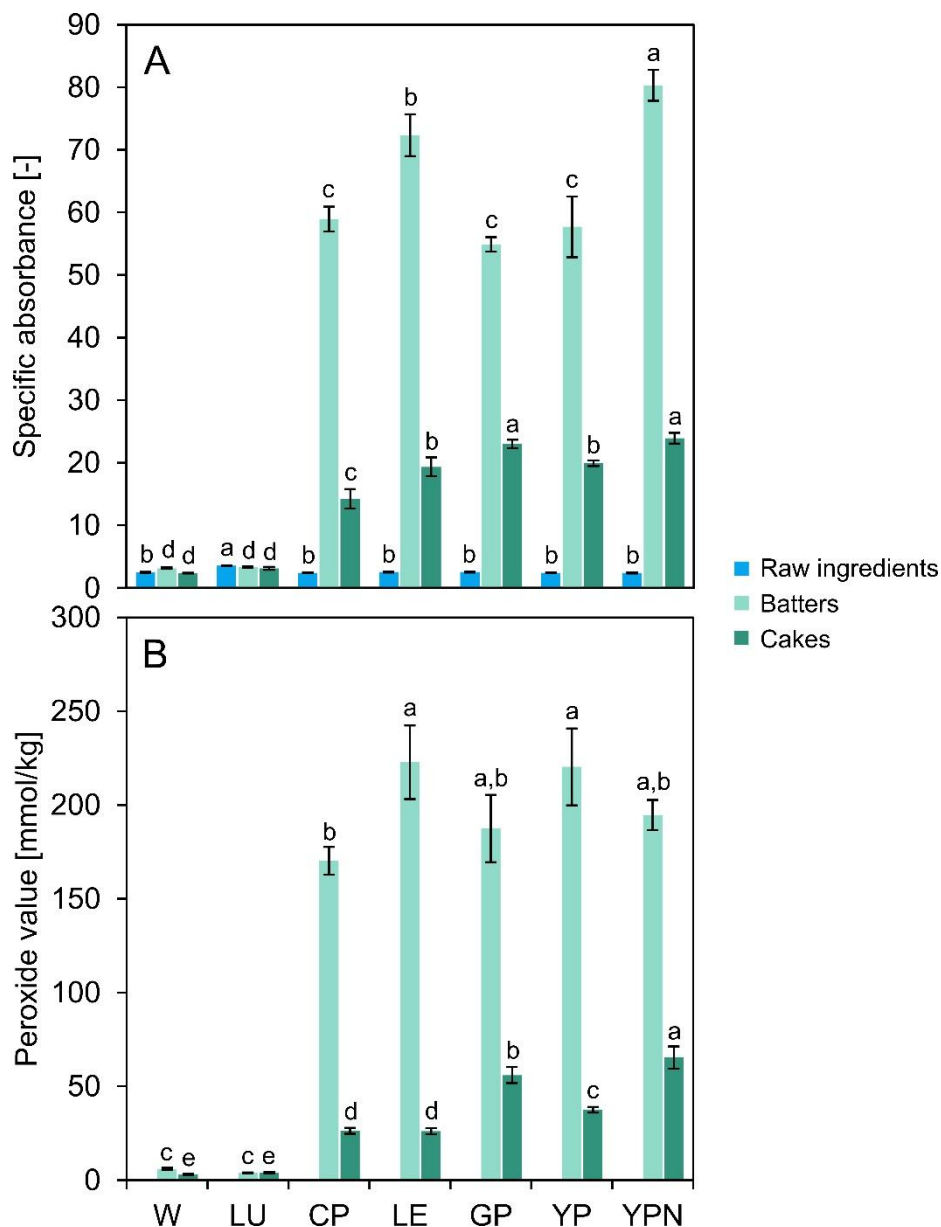
545 To verify that lipid oxidation was initiated during batter preparation and continued  
546 during baking, the concentrations of primary markers, namely CD and hydroperoxides, were  
547 monitored throughout the different steps of production (**Figure 3**). The starting values for CD  
548 were determined from the specific absorbance obtained for oil, egg and flour, taking the  
549 proportions used into account. This calculation was not possible for the hydroperoxides  
550 because of the limited fat contents in the flours.

551 As expected, mixing the ingredients into a foam initiated lipid oxidation (**Figure 3A**).  
552 The significant increment in specific absorbance was more pronounced with pulses than with  
553 wheat, thus corroborating their higher susceptibility to FA breakdown. The extraordinary  
554 behavior of YPNB, which was already characterized by extremely high oxidation-derived  
555 VOC concentrations (**Section 3.2**), was underlined by its specific absorbance values. This  
556 implied that the air-free processing of the associated flour protected important precursors  
557 from oxidation and made them prone to immediate degradation when they came into contact  
558 with oxygen. Moreover, lupin displayed striking oxidative stability as could be noted from its

559 similar specific absorbance when passing from raw materials to batter (**Figure 3A**). This  
 560 affirmed that lipid oxidation was mainly enzyme-mediated.

561 Coinciding with the literature, baking led to a decline of both CD and hydroperoxide  
 562 levels (**Figure 3A**, **Figure 3B**), thereby emphasizing the instability of the markers and their  
 563 subsequent transformation into volatiles (Maire *et al.*, 2013). This trend was more pronounced  
 564 with pulses (except lupin) than wheat, thus indicating the pursuit of oxidation during baking  
 565 and explaining their higher VOC levels in the cakes.

566



567  
 568 **Figure 3.** Evolution of (A) hydroperoxides and (B) specific absorbance of conjugated dienes from raw ingredients (■) to  
 569 batters (■) to cakes (■). Different letters within one production step indicate significantly different means (p<0.05). W =  
 570 wheat, LU = lupin, CP = chickpea, LE = lentil, YP = yellow pea and YPN = yellow pea processed and stored under nitrogen.

571 **3.7 Impact of flour type on reactivity and sensory-related volatile markers of cakes**

572 As described in **Section 3.5**, complex profiles of VOCs deriving mainly from lipid  
573 oxidation, the Maillard reaction and caramelization during different cake making steps were  
574 identified in the various cakes. The majority of these molecules are known to exhibit intense  
575 odor activities, so are therefore likely to contribute to the flavor quality of the final food. A  
576 multivariate approach made it possible to visualize which VOCs were responsible for the  
577 main differences between pulse-based products and how reaction markers were correlated to  
578 each other. Closer inspection revealed that WC and LUC differed considerably from the other  
579 cakes by their distinctively lower potential to form volatiles through the aforementioned  
580 pathways. Based on this finding, it was of interest to gain deeper insight into the  
581 discrimination of LEC, CPC, GPC, YPC and YPNC in order to associate specific reaction  
582 markers with individual products, and hence deduce hypotheses regarding possible impacts on  
583 flavor perceptions and cake qualities. PCA was therefore performed on all the VOCs  
584 identified in the five selected cakes.

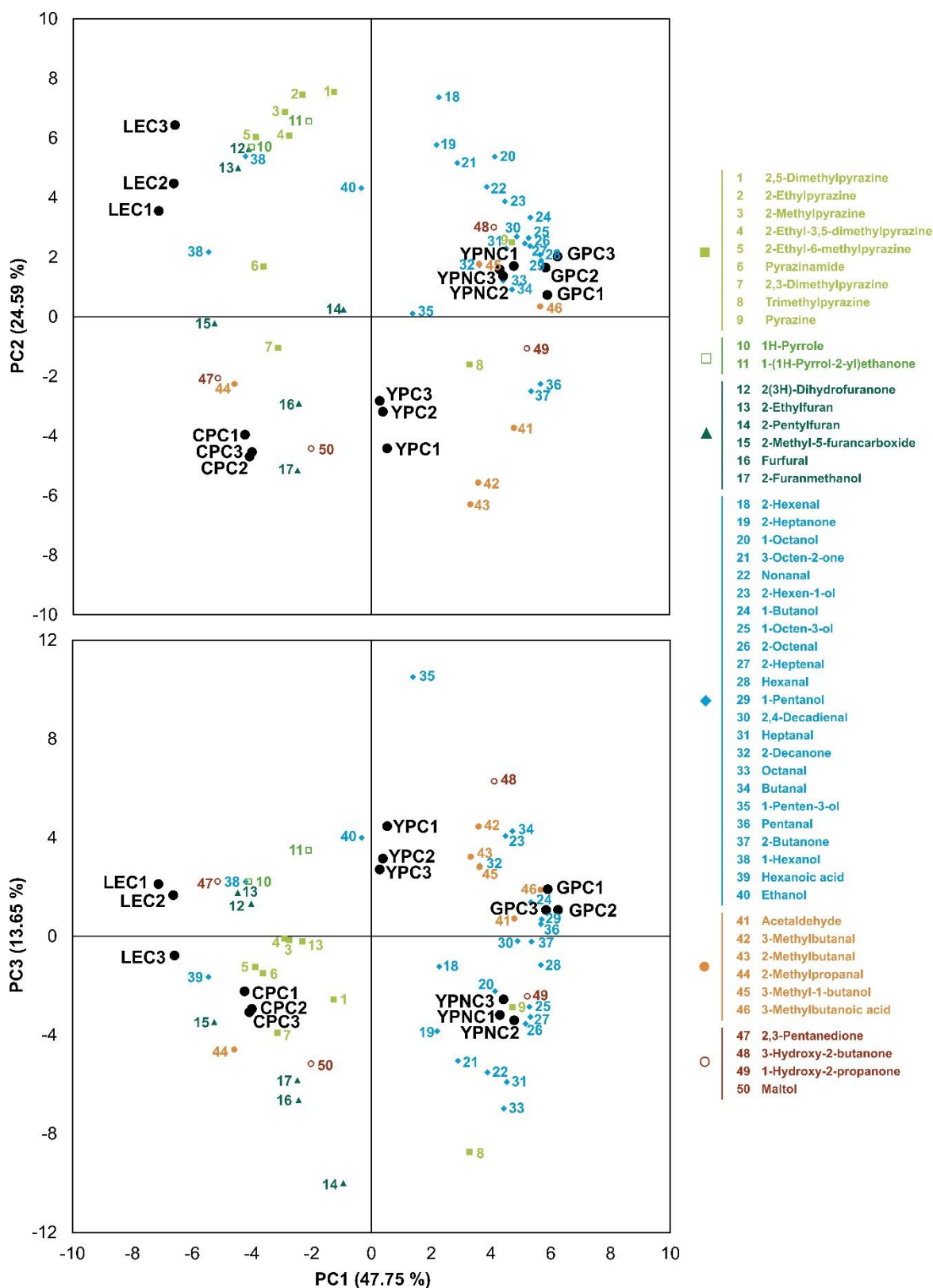
585 As depicted in **Figure 4**, PC1, PC2 and PC3 were responsible for 47.75%, 24.59% and  
586 13.65% of variability, respectively. Two clusters of cakes were opposed on PC1: (1) LEC and  
587 CPC, and (2) YPC, YPNC and GPC. This differentiation was primarily driven by lipid  
588 oxidation markers (alcohols, aldehydes, ketones) which were associated with positive  
589 loadings and thus with the pea products. This indicated that fatty acid breakdown played a  
590 major role during cake development using pea flours, probably attributable to their increased  
591 LOX activity compared to LEF and CPF (**Table 1**). Additionally, the literature has reported  
592 high levels of polyphenols (e.g. chlorogenic acid, sinapic acid, catechins, epicatechins) in  
593 chickpeas and lentils, suggesting that their antioxidant effects may have decelerated oxidative  
594 reactions in both products (Hall *et al.*, 2017). Due to the significantly lower concentrations of  
595 lipid oxidation markers in these cakes compared to their respective batters, it was assumed  
596 that the majority of molecules tended to be residues of batter beating rather than formed  
597 during baking. It could therefore be imagined that the particular assignment of 1-hexanol and  
598 hexanoic acid to LEC rather than the pea cakes may have been due to matrix effects markedly  
599 affecting the reactivity of the products, for instance by retarding volatilization of the named  
600 components during baking. According to the literature, the lipid oxidation markers detected  
601 are typically linked to green, beany and grassy odors (Xu *et al.*, 2019), implying that the pea  
602 products were probably marked by such off-flavors.

603

604 Further differentiation was achieved when considering PC2 and PC3. On PC1 vs PC2  
605 and PC1 vs PC3 it emerged that pyrazines were mainly assigned to LEC and CPC (**Figure 4**).  
606 Chemical characterization revealed that both LEF and CPF were distinctively enriched in free  
607 amino acids (7  $\mu\text{g/g}$  each) compared to the pea flours (3-4  $\mu\text{g/g}$ ) (data not shown). According  
608 to Adams *et al.* (2008), alanine, serine and arginine are important precursors for the  
609 generation of 2-ethyl-3,5-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-methylpyrazine and  
610 2,5-dimethylpyrazine. The former two amino acids were identified at elevated concentrations  
611 in LEF, thus possibly explaining the greater presence of the aforementioned pyrazines in  
612 LEC. Amino acid analysis further revealed high arginine contents in all the flours (1-4  $\mu\text{g/g}$ ),  
613 which might have been responsible for the high levels of 2,5-dimethylpyrazine in all cakes  
614 (**Section 3.5**). In addition, other studies have discussed the ability of polyphenols to accelerate  
615 hexose degradation into  $\alpha$ -dicarbonyls. This hypothesis is based on the potential for  
616 polyphenols to reduce metals, and particularly iron, which may participate in the Fenton  
617 reaction (García-Lomillo *et al.*, 2016). The hydroxy radicals thus generated may promote  
618 sugar transformation into  $\alpha$ -dicarbonyls, which can undergo Strecker degradation,  
619 subsequently leading to pyrazines (García-Lomillo *et al.*, 2016). As indicated above, LEF and  
620 CPF are known to contain high levels of phenolic acids, which in combination with their high  
621 ash contents (36-38  $\mu\text{g/g}$  vs. 3  $\mu\text{g/g}$  in YPF/GPF; data not shown) might have stimulated  
622 pyrazine formation. In light of these results, it appeared that the VOC profiles of CPC, and  
623 particularly LEC, were influenced to a major extent by pyrazines, thus possibly contributing  
624 to a roasted odor in the cakes typically associated with pyrazines (Pico *et al.*, 2015).

625 Another result worth mentioning is the central location of 2-pentylfuran on PC1 vs  
626 PC2, from which it was concluded that this molecule might not only be produced through  
627 lipid oxidation but concurrently through alternative pathways involving amino group  
628 interactions, thus coinciding with findings in the literature (Cepeda-Vázquez *et al.*, 2018).





629  
 630 **Figure 4.** PCA biplots on volatile markers, categorized into pyrazines (■), pyrroles (□), furanic compounds (▲) lipid  
 631 oxidation markers (◆) Strecker aldehydes and derivatives (●) and further Maillard and/or caramelization markers (○). LEC =  
 632 lentil cake, CPC = chickpea cake, GPC = green pea cake, YPC = yellow pea cake and YPNC = cake made with yellow pea  
 633 flour stored and processed under nitrogen.

#### 634 **4 Conclusion**

635 The complete replacement of wheat with pulse flours yielded sponge cakes with  
636 attractive nutritional and structural properties. At the same time, these products were  
637 characterized by a more complex profile of volatile compounds with probable effects on  
638 sensory quality. The majority of molecules derived from the oxidative processes, which was  
639 initiated during batter. Lipoxygenase was identified as the main catalyst of lipid oxidation due  
640 to its significantly higher activity in the pulse flours, particularly in yellow and green pea  
641 flours, compared to wheat. One exception was lupin, in which the enzyme was practically  
642 inactive (probably due to heat treatment), thus explaining its elevated stability versus lipid  
643 degradation. By contrast, yellow pea flour produced and stored under nitrogen stood out for  
644 its oxidation lability, which might be linked to the greater susceptibility of protected  
645 precursors to oxidation.

646 Baking not only perpetuated lipid oxidation but also triggered the decomposition of  
647 sugars and amino acids *via* caramelization and the Maillard reaction, being accompanied by  
648 the release of pyrazines, Strecker aldehydes and furanic compounds. Larger quantities of both  
649 lipid oxidation and thermal reaction markers were detected in the pulse-containing products  
650 than in those made using wheat, most likely due to compositional and matrix effects. Principle  
651 component analysis revealed that lipid oxidation markers were mainly associated with green  
652 and yellow pea cakes, while pyrazines were assigned to lentil and chickpea cakes. Based on  
653 odor descriptors reported in the literature, it was assumed that the global flavor of the pea  
654 products might be impacted by a green-beany odor imparted by the lipid oxidation markers,  
655 whereas chickpea, and especially lentil, cakes tended to be characterized by pleasant aroma  
656 compounds arising from the Maillard reaction. Nonetheless, further studies need to be carried  
657 out in order to gain more insight into the most odor-active compounds in pulse-based  
658 applications such as cakes.

659 This outcome indicates that flour type and pre-processing exert a considerable impact  
660 on the reactivity potential throughout cake making, thus enabling products to be tailored to the  
661 desired odor profile.

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