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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 N₂ 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 (Locminé, 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-d₄ (≥96%) and 2-methylpyrazine-d₆ (98.4%) were purchased from C/D/N
78 Isotopes Inc. (Pointe-Claire, Quebec, Canada), hexanal-d₁₂ (≥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 cm³) 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 SensIONTM+ 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-d₄,
 210 2-methylpyrazine-d₆ and hexanal-d₁₂ 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, β -myrcene, β -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).

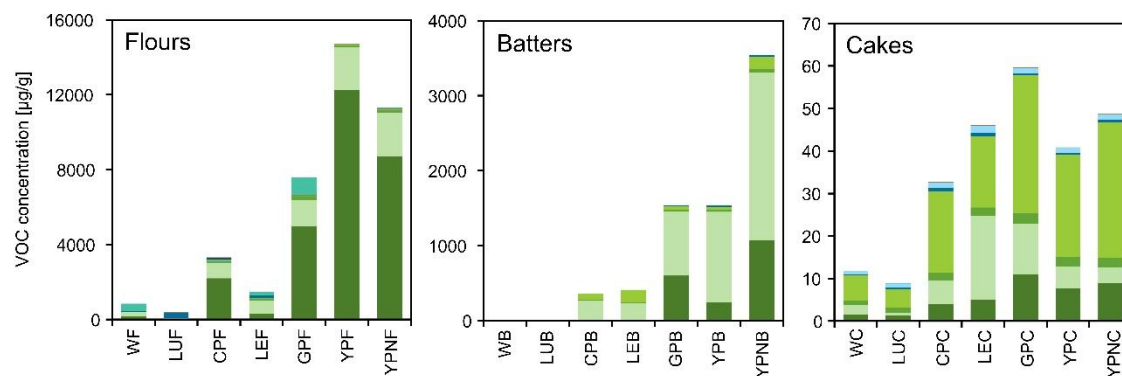
261 By contrast, the most abundant constituents of CPF, GPF and YPF/YPNF included a
262 complex mixture of alkanes (**Figure 1**). These compounds might have been produced by the
263 oxidation of lipids (Paucean *et al.*, 2018; Stevenson *et al.*, 1996) or they could have migrated
264 from the packaging (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 ^c	235 \pm 40 ^c	16.2 \pm 2.5 ^d	6.19 \pm 0.32 ^b	45 \pm 12 ^c	0.013 \pm 0.011 ^b	n.d.	n.d.	n.d.	381 \pm 14 ^b	843 \pm 54 ^d
	LU	23.1 \pm 2.1 ^c	30.9 \pm 1.3 ^c	29.6 \pm 1.4 ^d	2.054 \pm 0.014 ^c	291 \pm 11 ^a	0.0575 \pm 0.0038 ^a	11.45 \pm 0.66 ^c	n.d.	n.d.	0.54 \pm 0.14 ^d	389 \pm 11 ^d
	CP	2200 \pm 550 ^c	835 \pm 100 ^{b,c}	69.8 \pm 9.7 ^d	n.d.	27.4 \pm 1.3 ^{c,d}	0.0178 \pm 0.0067 ^b	n.d.	n.d.	126 \pm 17 ^a	57.8 \pm 9.2 ^d	3310 \pm 680 ^{c,d}
	LE	293 \pm 29 ^c	729 \pm 99 ^{b,c}	105.4 \pm 1.8 ^{b,c}	12.9 \pm 1.8 ^a	144.6 \pm 5.0 ^b	0.022 \pm 0.012 ^b	n.d.	n.d.	29.0 \pm 1.1 ^b	174 \pm 66 ^c	1490 \pm 180 ^d
	GP	4990 \pm 180 ^{b,c}	1390 \pm 320 ^b	218 \pm 39 ^a	6.871 \pm 0.048 ^b	5.02 \pm 0.44 ^e	0.0090 \pm 0.0037 ^b	42.95 \pm 0.86 ^a	n.d.	n.d.	922 \pm 21 ^a	7570 \pm 480 ^{b,c}
	YP	12200 \pm 2000 ^a	2280 \pm 480 ^a	124 \pm 20 ^{b,c}	14.54 \pm 0.36 ^a	14.66 \pm 0.43 ^{d,e}	0.0077 \pm 0.0046 ^b	30.6 \pm 8.6 ^b	n.d.	n.d.	21.7 \pm 4.9 ^d	14700 \pm 2400 ^a
	YPN	8700 \pm 4700 ^{a,b}	2320 \pm 480 ^a	152 \pm 35 ^b	13.4 \pm 1.3 ^a	32.6 \pm 3.4 ^{c,d}	0.0124 \pm 0.0055 ^b	41.2 \pm 3.5 ^{a,b}	n.d.	15.0 \pm 7.3 ^b	22.9 \pm 2.8 ^d	11300 \pm 4500 ^{a,b}
Batter	W	0.62 \pm 0.43 ^c	4.83 \pm 0.37 ^c	n.d.	0.531 \pm 0.019 ^d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.98 \pm 0.62 ^c
	LU	3.85 \pm 0.29 ^c	1.580 \pm 0.027 ^c	0.598 \pm 0.034 ^d	0.1292 \pm 0.0074 ^d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.20 \pm 0.24 ^c
	CP	9.5 \pm 4.8 ^c	255 \pm 19 ^c	9.28 \pm 0.64 ^{c,d}	89.2 \pm 4.9 ^b	n.d.	n.d.	89.2 \pm 4.9 ^b	n.d.	n.d.	n.d.	363 \pm 29 ^c
	LE	0.84 \pm 0.40 ^c	232 \pm 26 ^c	7.4 \pm 1.1 ^d	172 \pm 10 ^a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	413 \pm 31 ^c
	GP	600 \pm 100 ^{a,b}	857 \pm 74 ^b	20.81 \pm 0.76 ^{b,c}	48.5 \pm 2.9 ^c	12.9 \pm 1.9 ^a	n.d.	n.d.	n.d.	n.d.	n.d.	1540 \pm 140 ^b
	YP	247 \pm 69 ^{b,c}	1200 \pm 170 ^b	28.8 \pm 3.6 ^b	37.7 \pm 2.9 ^c	21.0 \pm 6.8 ^a	n.d.	n.d.	n.d.	n.d.	n.d.	1540 \pm 100 ^b
	YPN	1070 \pm 480 ^a	2240 \pm 520 ^a	50.4 \pm 8.2 ^a	159.1 \pm 9.4 ^a	21.4 \pm 3.7 ^a	n.d.	n.d.	n.d.	n.d.	n.d.	3540 \pm 930 ^a
Cake	W	1.456 \pm 0.014 ^d	2.299 \pm 0.071 ^e	1.143 \pm 0.032 ^c	5.84 \pm 0.34 ^a	0.197 \pm 0.011 ^f	0.790 \pm 0.025 ^d	n.d.	n.d.	n.d.	n.d.	11.72 \pm 0.46 ^e
	LU	1.35 \pm 0.10 ^d	0.593 \pm 0.026 ^f	1.168 \pm 0.034 ^c	4.37 \pm 0.14 ^f	0.472 \pm 0.013 ^d	0.8409 \pm 0.0069 ^d	n.d.	0.00542 \pm 0.00078 ^a	n.d.	n.d.	8.81 \pm 0.27 ^e
	CP	3.993 \pm 0.053 ^c	5.48 \pm 0.15 ^c	2.013 \pm 0.058 ^b	19.06 \pm 0.43 ^c	0.713 \pm 0.028 ^b	1.211 \pm 0.010 ^{b,c}	0.1579 \pm 0.0070 ^b	0.112 \pm 0.085 ^a	n.d.	n.d.	32.74 \pm 0.84 ^d
	LE	5.13 \pm 0.71 ^c	19.56 \pm 0.81 ^a	2.00 \pm 0.13 ^b	16.74 \pm 0.35 ^d	0.770 \pm 0.021 ^a	1.69 \pm 0.12 ^a	0.241 \pm 0.021 ^a	n.d.	n.d.	n.d.	46.1 \pm 1.9 ^b
	GP	10.98 \pm 0.61 ^a	11.68 \pm 0.11 ^b	2.527 \pm 0.042 ^a	32.47 \pm 0.33 ^a	0.4312 \pm 0.0029 ^{d,e}	1.261 \pm 0.039 ^{b,c}	0.1453 \pm 0.0091 ^b	n.d.	n.d.	n.d.	59.49 \pm 0.48 ^a
	YP	7.7 \pm 1.1 ^b	5.15 \pm 0.11 ^c	2.180 \pm 0.030 ^b	24.11 \pm 0.48 ^b	0.415 \pm 0.018 ^e	1.171 \pm 0.051 ^c	0.1136 \pm 0.0071 ^c	n.d.	n.d.	n.d.	40.9 \pm 1.6 ^c
	YPN	8.9 \pm 1.2 ^b	3.666 \pm 0.055 ^d	2.390 \pm 0.071 ^a	31.80 \pm 0.65 ^a	0.592 \pm 0.017 ^c	1.356 \pm 0.012 ^b	0.1419 \pm 0.0059 ^{b,c}	n.d.	n.d.	n.d.	48.8 \pm 1.6 ^b

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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 (■), nitrogenous compounds (■), pyrones (■), acids/esters (■), terpenes (■) and sulfurous compounds (■). n.d.: not detected. Relative amounts were estimated based on the internal deuterated standards hexanal- d_{12} , furfural- d_4 and 2-methylpyrazine- d_6 . Different letters within a column of one food matrix 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.

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 ³]	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 ^b	<20 ^d	5.61 ± 0.25 ^d	24.1 ± 1.2 ^d	1.88 ± 0.18 ^d	23.22 ± 0.74 ^b
LU	0.645 ± 0.027 ^a	<1 ^d	37.0 ± 1.3 ^a	12.07 ± 0.31 ^e	5.29 ± 0.15 ^{b,c}	4.512 ± 0.089 ^d
CP	0.618 ± 0.013 ^{a,b}	2510 ± 140 ^c	18.25 ± 0.80 ^b	38.14 ± 0.35 ^a	1.35 ± 0.24 ^d	5.01 ± 0.27 ^d
LE	0.603 ± 0.013 ^b	3200 ± 250 ^b	15.26 ± 0.51 ^c	22.77 ± 0.86 ^d	4.50 ± 0.58 ^c	42.522 ± 0.038 ^a
GP	0.560 ± 0.023 ^c	3870 ± 110 ^a	15.550 ± 0.070 ^e	28.22 ± 0.18 ^c	5.63 ± 0.31 ^{a,b}	10.59 ± 0.49 ^e
YP	0.621 ± 0.017 ^{a,b}	4350 ± 300 ^a	19.80 ± 0.39 ^b	35.51 ± 0.82 ^b	6.050 ± 0.060 ^{a,b}	10.94 ± 0.52 ^c
YPN	0.533 ± 0.028 ^c	4340 ± 160 ^a	19.70 ± 0.69 ^b	34.54 ± 0.95 ^b	6.42 ± 0.36 ^a	10.31 ± 0.46 ^c

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.

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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 ≤ 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°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

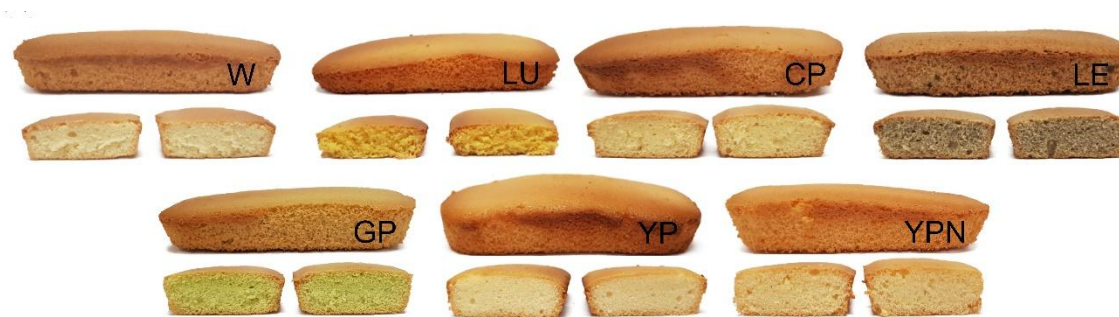
447 **Table 2.** Chemical composition [g/100 g] of different flours.

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

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.

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

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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 α -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 α -aminocarbonyl compounds, which themselves are
534 generated next to Strecker aldehydes through Strecker degradation from the reaction between
535 α -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**

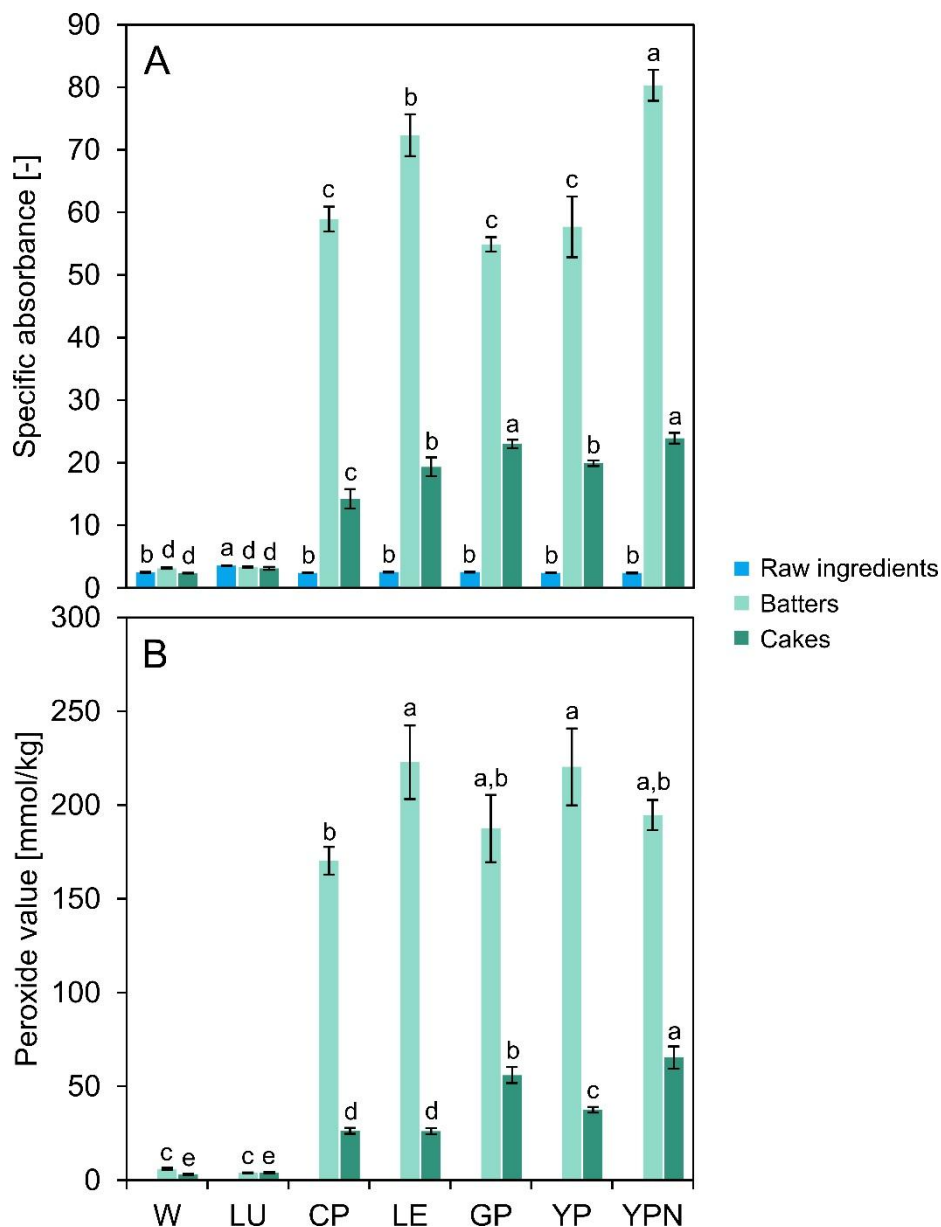
545 To verify that lipid oxidation was initiated during batter preparation and continued
546 during baking, the concentrations of primary markers, namely CD and hydroperoxides, were
547 monitored throughout the different steps of production (**Figure 3**). The starting values for CD
548 were determined 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**

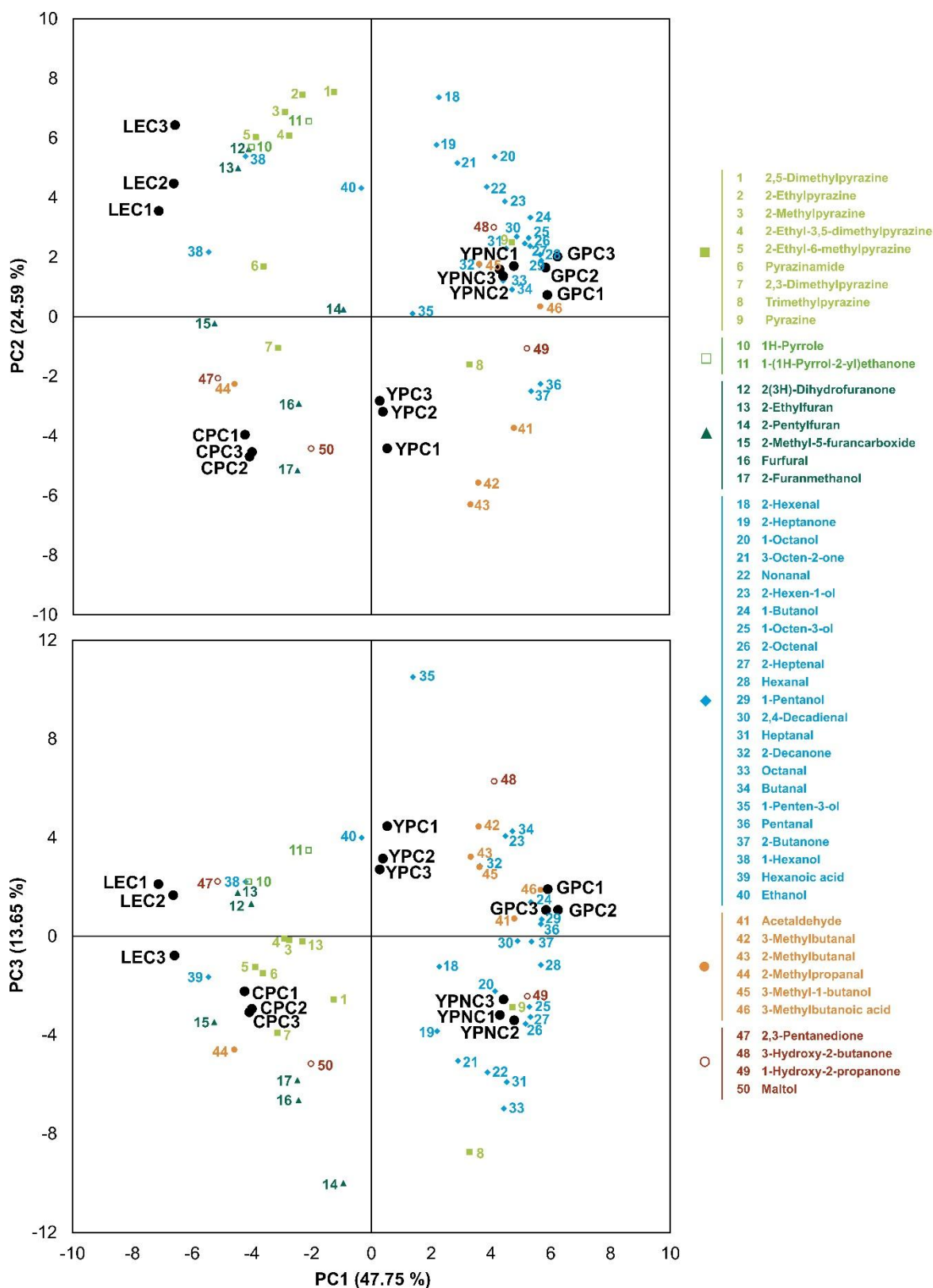
572 As described in **Section 3.5**, complex profiles of VOCs deriving mainly from lipid
573 oxidation, the Maillard reaction and caramelization during different cake making steps were
574 identified in the various cakes. The majority of these molecules are known to exhibit intense
575 odor activities, 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 α -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 α -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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