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1 **Reduction of pulse “antinutritional” content by optimizing pulse canning process is**
2 **insufficient to improve fat-soluble vitamin bioavailability**

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16

17 **Abbreviated running title:** Pulse canning process and fat-soluble vitamin bioavailability

18
19

20 Data described in the manuscript will be made available upon request pending application and
21 approval.

22

23

24 **Abstract**

25

26 Some bioactive compounds found in pulses (phytates, saponins, tannins) display
27 antinutritional properties and interfere with fat-soluble vitamin bioavailability (i.e.,
28 bioaccessibility and intestinal uptake). As canned chickpeas are consumed widely, our aim
29 was to optimize the chickpea canning process and assess whether this optimization influences
30 fat-soluble vitamin bioavailability. Different conditions during soaking and blanching were
31 studied, as was a step involving prior germination. Proteins, lipids, fibers, vitamin E, lutein, 5-
32 methyl-tetrahydro-folate, magnesium, iron, phytates, saponins and tannins were quantified.
33 Bioaccessibility and intestinal uptake of vitamin D and K were assessed using in vitro
34 digestion and Caco-2 cells, respectively. Significant reductions of phytate, saponin and tannin
35 contents (-16 to -44%), but also of folate content (up to -97%) were observed under
36 optimized canning conditions compared with the control. However, bioaccessibility and
37 cellular uptake of vitamin D and K remained unaffected after in vitro digestion of test meals
38 containing control or optimized canned chickpeas.

39

40 **Keywords:** Chickpeas; germination; phytates; tannins; saponins; vitamin D; vitamin K;
41 bioaccessibility; Caco-2 cells.

42 **1. Introduction**

43

44 Pulses display interesting nutritional properties, as they are good sources of proteins, fibers
45 and micronutrients (Margier et al., 2018). Pulses are also a significant source of bioactive
46 compounds such as phytates, saponins and tannins (Margier et al., 2018), the consumption of
47 which demonstrates some interesting lipid-lowering properties. For instance, dietary fibers
48 positively impact postprandial lipemia (Desmarchelier et al., 2019; Lee et al., 2020) and
49 cholesterolemia (Schoeneck & Iggman, 2021). Phytates at high concentrations and tannins
50 were shown to interfere with human lipase activity (Antoine et al., 2021). Finally, saponins
51 have often been described as anti-obesogenic compounds (Marrelli et al., 2016), partly
52 because they also interact with lipase activity (Antoine et al., 2021; Marrelli et al., 2016).
53 However, these compounds can also be considered “antinutritional”, as they can negatively
54 interact with micronutrients such as minerals. In particular, phytates and tannins are divalent
55 cation quenchers that can decrease iron and zinc bioavailability (Antoine et al., 2021;
56 Lestienne et al., 2005). Furthermore, we recently showed that fibers, phytates, tannins and
57 saponins from pulses can reduce fat-soluble vitamin bioavailability in vitro, and more
58 precisely that they can modulate the bioavailability of vitamin K (Margier et al., 2019) and
59 vitamin D (Antoine et al., 2021).

60 Different strategies can be set up to decrease pulses’ “antinutritional” content, one of the most
61 straightforward being the optimization of pulse cooking/transformation methods (Margier et
62 al., 2018). As canned pulses are consumed widely, we decided to investigate the optimization
63 of canning process. Chickpeas were selected as the pulse to be studied. The objective of this
64 study was thus i) to identify and optimize the parameters of the canning process that could
65 reduce chickpeas’ levels of bioactive compounds displaying antinutritional properties
66 (phytates, saponins and tannins); and ii) assess whether the decreases in phytates, saponins

67 and tannins resulting from canning process optimization were sufficient to improve the
68 bioavailability of vitamins D and K in test meals containing canned chickpeas.

69 **2. Material and methods**

70

71 **2.1. Chemicals**

72 Cholecalciferol (vitamin D₃), phylloquinone (vitamin K₁), γ -tocopherol, retinyl acetate (all
73 >95% pure), phytic acid sodium salt hydrate, soyasaponin I, 4,5-dimethylthiazol-2-yl)-2,5-
74 diphenyltetrazolium bromide (MTT), catechin 4-(dimethylamino)cinnamaldehyde (DMACA),
75 2-(N-cyclohexylamino) ethane sulfonic acid (CHES), 4-(2-hydroxyethyl)-1-piperazine
76 ethanesulfonic acid (HEPES), α -amylase from bacillus sp., protease from Streptomyces
77 griseus, and 5-methyltetrahydrofolate (5-CH₃-H₄ folate calcium salt) were purchased from
78 Sigma-Aldrich (Saint-Quentin-Fallavier, France). Lutein (>95% pure) was from
79 Extrasynthèse (Genay, France). Chicken pancreas conjugase was from R-Biopharm
80 (Darmstadt, Germany). Isio4 oil was from Lesieur (Asnières-sur-Seine, France). Iron (III)
81 chloride hexahydrate and vanillin (all 99% pure) were from Acros Organics (Noisy-le-Grand,
82 France). Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/L glucose and
83 trypsin-EDTA (500 and 200 mg/L, respectively), non-essential amino acids,
84 penicillin/streptomycin and phosphate-buffered saline (PBS) were purchased from Life
85 Technologies (Illkirch, France). Fetal bovine serum (FBS) came from PAA (Vélizy
86 Villacoublay, France). Chickpeas (*Cicer arietinum* 'Eldorado'), were produced locally and
87 provided by the GPS France cooperative (Manosque, France). All solvents were HPLC grade
88 and obtained from Carlo Erba (Peypin, France).

89

90 **2.2. Chickpea preparation**

91 *2.2.1. Control canning process*

92 The control canning protocol was devised by the Centre Technique de Conservation des
93 Produits Agricoles (CTCPA, Avignon, France) and implemented in their pilot plant. In brief,

94 chickpeas were soaked in low-ionized water (hardness titration [HT]=30 °f) at 15 °C at a ratio
95 of 1:3 (Chickpeas:water; w/w) for 14h. Afterwards, immersion blanching was done at 90 °C
96 for 5 min. Chickpeas were then conditioned in cans (200 g), and hot brine (0.5% salt, 70°C) at
97 a ratio of 86:114 (w:w) was added. The cans were then sterilized at 127 °C for 16 min, and
98 subsequently cooled to 30 °C for 10 min.

99 2.2.2. *Preliminary tests*

100 Preliminary tests were performed to address the effect of modifying single parameters.
101 Soaking and blanching parameters were thus modified independently (Table 1). An
102 “antinutritional” compound analysis was conducted after each step, but not on the final
103 canned products.

- 104 • *Modification of the soaking step (Table 1)*

105 Hot water soaking (water at 80°C) was done in a Stephan® Combicut TC System (Stephan
106 Bvba, Nazareth, Belgium) for 2h. The “soft” water (HT=6 °f) was Volvic water (Société des
107 eaux de Volvic – Danone group, Volvic, France) and the “hard” water (HT=184 °f) was
108 Hépar water (Nestlé Waters Vosges, Vittel, France). Chickpeas were soaked at different
109 ratios: 1:3, or 2:3, or 1:10 (chickpeas:water; w:w). An agitation step could be added either
110 with a pump (Yamada, Arlington Heights, IL, USA) during soaking at 15 °C or with paddles
111 in a Stephan® Combicut TC during soaking at 80°C. Water could be replaced at the midpoint
112 of the soaking time. Citric acid (La Littorale - Erbslöh, Servian, France) and sodium
113 bicarbonate (Cérébos, Levallois-Perret, France) were used for a soaking at pH=5 and pH=9,
114 respectively. Finally, a test was done after the addition of a germination step performed before
115 soaking and blanching: chickpeas were immersed for 12 h in water (1:3, w:v), then kept
116 between thick layers of damp cotton cloth and allowed to germinate for 3 days at room
117 temperature (20-25°C).

- 118 • *Modification of the blanching step (Table 1)*

119 Tests on the blanching step were carried out on chickpeas previously soaked via the control
120 process. A longer blanching time was also tested (20 min). Moreover, the immersion
121 blanching was changed into either steaming or cascading. Steaming consisted of a 5-min
122 exposure to steam using a steam oven (Bourgeois, Faverge, France). Cascading was
123 performed using vertically flowing water in a steam-heated autoclave (Auriol, Marmande,
124 France).

125 *2.2.3. Optimization tests on the global process (Table 2)*

126 Once the preliminary tests were analyzed, different processes combining the most efficient
127 soaking and blanching parameters were tested. For reasons of technological practicality, hot
128 soaking was done with a high chickpea-to-water ratio (1:10) for a shorter time (2h).
129 Conditioning and sterilization parameters were not modified. Contrary to the preliminary
130 tests, bioactive compounds were only analyzed in the final products.

131

132 **2.3. Texture analysis**

133 The texture of the canned chickpeas was analyzed to check whether process modifications
134 induced changes in chickpea texture compared to chickpeas treated with the control process.
135 A multipurpose texture analyzer TA-plus (Lloyd Instruments, Farenham, UK) was used with
136 a method adapted from Chenoll and colleagues (Chenoll et al., 2009). The peak compression
137 force (N) was recorded as the hardness of the chickpeas.

138

139 **2.4. In vitro digestion experiments**

140 The test meals used for in vitro digestion experiments were made of 3.35 g of canned
141 chickpeas and 100 mg of Isio 4 oil. Isio 4 oil contributed minor amounts of fat-soluble
142 vitamins to the meal, without consequences to data interpretation, as oil was present in all
143 conditions. Each test meal was supplemented with 125 μ M of fat-soluble vitamin D or K

144 dissolved in the oil. The *in vitro* digestion experiments were conducted as previously
145 described (Malapert et al., 2018). Aliquots of digesta and mixed micelles were taken and
146 stored at $-80\text{ }^{\circ}\text{C}$ under a nitrogen atmosphere until analysis. Bioaccessibility was calculated
147 as the percentage of vitamin recovered in mixed micelles divided by the amount of vitamins
148 recovered in the digesta at the end of the digestion.

149

150 **2.5. Fat-soluble vitamin absorption by intestinal cells in culture**

151 *2.5.1. Preparation of micelles from *in vitro* digestion*

152 Preliminary cytotoxicity tests were conducted. Briefly, Caco-2 cells were seeded and grown
153 for 3 days in 96-well plates, prior to a 1 h-incubation with diluted micelles obtained from *in*
154 *vitro* digestion. The cell culture medium was then removed, and a MTT solution (1 mg/mL)
155 was added for 2 h at $37\text{ }^{\circ}\text{C}$. The supernatant was then replaced by DMSO (100 μL per well)
156 and the absorbance was measured at 540 nm. In accordance with these tests, fat-soluble
157 vitamin micelles obtained from *in vitro* digestions were diluted in DMEM at a 1:6 ratio (v:v)
158 for further cell experiments (data not shown).

159 *2.5.2. Cell experiments*

160 Caco-2 clone TC7 cells were cultured as previously described (Goncalves et al., 2016). For
161 each experiment, cells were seeded and grown for 2 weeks in 6-well plates (24 mm diameter,
162 1 μm -pore-size polycarbonate membrane; Becton Dickinson, Le Pont-de-Claix, France).
163 Twelve hours prior to each experiment, the complete medium (i.e., DMEM supplemented
164 with 16% FBS, 1% non-essential amino acids and 1% antibiotics) was replaced by serum-free
165 complete medium. At the beginning of the experiment, cell monolayers received diluted
166 micelles obtained from *in vitro* digestions. Cells were incubated for 1h at $37\text{ }^{\circ}\text{C}$ with the
167 micelles. Next, cells were washed with 0.5 mL of ice-cold PBS to eliminated adsorbed fat-
168 soluble vitamins, then scraped and collected in 0.5 mL of ice-cold PBS. Vitamin absorption
169 percentage was calculated by dividing the amount of vitamin recovered in cells by the total

170 amount of vitamin delivered to the cells (i.e., vitamin found in the medium plus vitamin
171 remaining in the cells at the end of the experiment). All samples were stored at $-80\text{ }^{\circ}\text{C}$ until
172 analysis.

173

174 **2.6. Chickpea lyophilization**

175 The canned chickpeas were first lyophilized for 48h in a Cosmos freeze dryer (Cryotec, Saint-
176 Gély-du-Fesc, France) and then ground into powder using a Proline 700W blender (Proline,
177 Paris, France).

178

179 **2.7. Bioactive compound assays**

180 *2.7.1. Phytate assay*

181 Phytates from chickpea powder were analyzed using a spectrophotometric method derived
182 from that published by Dost & Tokul (Dost & Tokul, 2006). Briefly, phytates were extracted
183 from 200 mg of powder with 10 mL of chloridric acid (0.5 M, Fisher Scientific, Saint-
184 Herblain, France) for 1 h at room temperature (20-25°C). The extract was then centrifuged 5
185 min at 800×g and the supernatant was recovered. One hundred μL of supernatant was added
186 to 0.9 mL of water and 2 mL of iron (III) chloride hexahydrate. This mixture was mixed for
187 2h and 30 min at 40 °C and centrifuged 5min at 800×g. The absorbance of the supernatant
188 was measured at 480 nm against water using a Shimadzu UV 8000 spectrophotometer
189 (Shimadzu, Marne-la-Vallée, France). The results were expressed as phytic acid equivalent.

190 *2.7.2. Saponin assay*

191 Saponins were analyzed by a spectrophotometric method inspired by the method previously
192 published by Li and colleagues (Li et al., 2010). The first step was the extraction of saponins
193 from the chickpea powder. One gram of flour was added to 5 mL of methanol (80% in water),
194 mixed for 24 h at room temperature (20-25°C) and centrifuged 5 min at 800×g. This step was

195 repeated twice. Supernatants (0.2 mL) were completed with 0.3 mL of methanol (80% in
196 water), 0.5 mL of vanillin (8% in water), and 5 mL of sulfuric acid (72%). The mixtures were
197 incubated at 60°C for 10 min and placed into an ice bath. The absorbance of the mixtures was
198 measured at 544 nm against methanol (80% in water). The results were expressed as
199 soyasaponin I equivalent.

200 2.7.3. Tannin assay

201 Tannins were extracted from 0.12 g of chickpea powder with 12 mL of water. Two milliliters
202 of a water/methanol mixture (1:1; v:v), 4 mL of tannin extract, and 1 mL of DMACA solution
203 were mixed together. After 20 min, samples were centrifuged 5 min at 800×g and the
204 absorbance of the supernatants was measured at 640 nm against a water/methanol mixture
205 (1:1). The results were expressed as catechin equivalent.

206

207 2.8. Lipid, protein, fiber and mineral assays

208 Lipids were extracted from the chickpea powder. Briefly, 0.5 g of powder, 2 mL of distilled
209 water, and 5 mL of ethyl acetate were mixed for 6 min. The extract was centrifuged 15 min at
210 1100 × g and the supernatant was recovered. This procedure was repeated once with the
211 addition of ethyl acetate. To wash the extract, 3 mL of water and 0.5 mL of ethanol were
212 added to the supernatant, and the mixture was vortexed for 5 min and centrifuged 10 min at
213 1100×g. Pooled supernatants were evaporated to dryness under nitrogen. Total lipids were
214 measured by weighing the dry extracts.

215 The analysis of protein, fibers, magnesium and iron analysis was subcontracted to
216 Phytocontrol Agrifood (Nîmes, France), which is accredited to conduct these specific analyses
217 (Cofrac #1-1904).

218

219 2.9. Vitamin assay

220 2.9.1. Vitamin E (γ -tocopherol) and lutein from chickpeas

221 Vitamin E (γ -tocopherol) and lutein were extracted from 250 mg of chickpea powder using
222 the following method: 1 mL of distilled water was added to the sample. The internal standard
223 (retinyl acetate) was added to the sample in 1 mL of ethanol. The mixture was extracted twice
224 with 4 mL of hexane. After centrifugation ($2500\times g$, 10 min at 4 °C), the hexane phase was
225 evaporated to dryness under nitrogen. The dried extract was dissolved in 200 μ L of methanol–
226 dichloromethane (65:35, v:v). A final volume of 180 μ L was used for HPLC analysis. Fat-
227 soluble vitamins and carotenoids were separated as previously described (Gleize et al., 2012).
228 Luteine and γ -tocopherol were detected at 445 and 298 nm, respectively, and identified by
229 retention time in comparison with pure standards. Quantification was performed using
230 Chromeleon 6.8 software (ThermoFisher Scientific, Villebon sur Yvette, France) to compare
231 the peak area with standard reference curves.

232 *2.9.2. Vitamin D and vitamin K assay from in vitro digestion and cell samples*

233 Vitamin D and vitamin K were extracted from 200 μ L of digesta and mixed micelles, and
234 from 500 μ L of apical medium and cells. The internal standard was retinyl acetate. Both
235 extraction and HPLC analysis were performed according to Antoine and al. (Antoine et al.,
236 2021) and Goncalves et al. (Goncalves et al., 2014), respectively. Vitamins were identified by
237 spectral analysis and/or retention time and co-injection in comparison with pure standards.
238 Quantification was performed using Chromeleon 6.8 software to compare the peak area with
239 standard reference curves.

240 *2.9.3. Folate assay*

241 Folates from chickpea powder were analyzed by a HPLC method derived from the method
242 published by Ruggeri and colleagues (Ruggeri et al., 1999). Powder (0.75g) was added to 10.5
243 mL of extraction buffer in ebullition (HEPES buffer, pH 7.85, containing 2% (w:v) sodium
244 ascorbate and 10 mM 2-mercaptoethanol). After flushing the samples with nitrogen, they
245 were heated for 10 min in a boiling water bath and homogenized with an Ultra Turrax device

246 for 30 s at a speed of 13500 rpm. Extraction buffer was then added (qsp 15 mL). The pH was
247 adjusted to 4.9 and 600 μ L of chicken pancreas conjugase, 120 μ L of rat serum and 600 μ L of
248 α -amylase were added. Samples were incubated under agitation at 37°C for 3 h. The pH was
249 adjusted to 7.0, then 1.2 mL of protease were added, and samples were incubated for 1h as
250 previously described. Enzymes were then inactivated by boiling for 10 min. Samples were
251 centrifuged for 30 min at 2000 \times g. Supernatants were transferred to a strong anion exchanger
252 (SAX) column (ThermoFisher Scientific) to purify folates (solid phase extraction). A final
253 volume of 20 μ L was used for HPLC analysis. The HPLC system comprised a Dionex
254 separation module (P680HPLC Pump and ASI-100 Automated Sample Injector, Dionex, Aix-
255 en-Provence, France) and a Jasco fluorimetric detector (Lisses, France). Analyses were
256 performed on a HypersilTM ODS C18 column (150 \times 4.6 mm; 5 μ m) coupled with a 10 \times 4 mm
257 C18-5 μ m HypersilTM ODS guard column (FisherScientific, Illkirch, France) kept at a
258 constant temperature (40 °C). The mobile phase consisted of a gradient of acetonitrile and
259 30mM phosphate potassium buffer at pH 2.6. The flow rate was 0.8 mL/min. The run time
260 was 35 min. The gradient profile of the mobile phase was set at 5-95% and changed linearly
261 to 17-83% from minute 9 to 30. The mobile phase then changed back to 5-95% from minute
262 30 to 35. 5-methyltetrahydrofolate, which is the main form of folate found in chickpeas
263 (Ruggeri et al., 1999), was detected at 356 nm after excitation at 290 nm, and identified by
264 retention time compared with a pure standard. Quantification was performed using
265 Chromeleon software to compare the peak area with standard reference curves.

266

267 **2.10. Statistics**

268 For all groups, data were expressed as mean \pm SEM. Statistical analyses were performed
269 using GraphPad Prism software, version 8.4.3 (GraphPad Software, San Diego, California,
270 U.S.A.). Prior to ANOVA (fixed-effects models), data were tested for equality of variances.
271 The distribution of all dependent variables was graphically checked and was considered

272 normal. Tukey's test was used as a post-hoc test for pairwise comparisons. Values of $p \leq 0.05$
273 were considered significant.

274 **3. Results**

275

276 **3.1. Chickpea contents in phytates, saponins and tannins can be decreased by** 277 **modifying canning parameters**

278 *3.1.1. Phytate and saponin contents*

279 Figures 1A and 1B show the results of preliminary tests. We observed a significant decrease
280 of phytate content after hot soaking (PT1), mixed soaking (PT7) and acid soaking (PT9) (-
281 $36.0 \pm 4.9\%$, $-25.0 \pm 7.0\%$, $-25.0 \pm 3.2\%$, $p \leq 0.05$, respectively), but also after soaking with
282 low-ionized water (PT4), with a low chickpeas-to-water ratio (PT6) or after water
283 replacement (PT8) ($-19.0 \pm 2.6\%$, $-18.0 \pm 6.3\%$, $-17.0 \pm 2.7\%$, $p \leq 0.05$, respectively) (Figure
284 1A). Moreover, Figure 1B shows that phytate content was significantly reduced when the
285 chickpeas were blanched by cascading (PT14, $-7.0 \pm 1.7\%$, $p \leq 0.05$).

286 Concerning saponins, there was no significant diminution of their concentrations when
287 soaking and blanching parameters were modified. Saponin content was slightly reduced when
288 soaking was done with low-ionized water (PT4) or with a low chickpeas-to-water ratio (PT6)
289 ($-13.0 \pm 3.1\%$, $-10.1 \pm 1.2\%$, respectively), but these values remained non-significantly
290 different (Figure 1 A and 1B).

291 We then explored the phytate and saponin content in canned and sterilized chickpeas after
292 these different parameters were combined. As shown in Figure 1C, phytate and saponin
293 concentrations were both significantly decreased when chickpeas were canned after i) hot
294 soaking with water replacement followed by immersion blanching (T3) ($-26.0 \pm 1.9\%$, $-29.0 \pm$
295 1.7% , $p \leq 0.05$, respectively), ii) hot mixed soaking followed by cascading blanching (T8) (-
296 $37.0 \pm 1.6\%$, $-35.0 \pm 2.2\%$, $p \leq 0.05$, respectively), and iii) hot soaking with water replacement
297 followed by cascading blanching (T9) ($-28.0 \pm 0.7\%$, $-39.0 \pm 2.4\%$, $p \leq 0.05$, respectively).
298 Moreover, we observed a significant reduction of saponin content after soaking at 15 °C with

299 i) low-ionized water and blanching by cascading (T10), ii) agitation blanching by immersion
300 (T5), iii) water replacement and cascading blanching (T12) and vi) germination (T13) before
301 soaking and blanching ($-21.0 \pm 2.1\%$, $-17.0 \pm 2.1\%$, $-16.0 \pm 1.7\%$, $-29.0 \pm 6.2\%$, $p \leq 0.05$,
302 respectively). These 7 conditions were chosen for further analysis.

303 3.1.2. Tannin content

304 Our results show that tannin content was reduced in 3 conditions among 7 (Figure 1D): i) hot
305 soaking with water replacement followed by immersion blanching (T3), ii) hot soaking with
306 water replacement followed by cascading blanching (T9), and iii) germinated chickpeas (T13)
307 ($-44.1 \pm 8.6\%$, $-40.0 \pm 7.6\%$, $-43.0 \pm 6.8\%$, $p \leq 0.05$, respectively). However, tannin content
308 increased significantly relative to the control when chickpeas were soaked in hot mixed water
309 and blanched by cascading (T8) ($+74.0 \pm 20.4\%$, $p \leq 0.05$).

310

311 **3.2. The texture of optimized canned chickpeas is different from that of control** 312 **chickpeas**

313 The texture of the different batches of canned chickpeas was analyzed to check if process
314 modifications changed the chickpea texture compared to the control condition. Table 3 shows
315 that there was a significant increase in chickpea hardness after hot soaking followed by
316 immersion blanching: $+43.9 \pm 3.8\%$ for hot soaking followed by immersion blanching (T1),
317 $+48.4 \pm 3.8\%$ for mixed and hot soaking followed by immersion blanching (T2) and $+41.9 \pm$
318 5.2% for hot soaking with water replacement followed by immersion blanching (T3).
319 Moreover, we observed a significant decrease in chickpea hardness after soaking at 15 °C and
320 blanching by cascading: $-33.1 \pm 3.1\%$ after soaking at 15 °C with low-ionized water followed
321 by cascading blanching (T10) and $-26.2 \pm 4.1\%$ after mixed soaking at 15 °C followed by
322 cascading blanching (T11).

323

324 **3.3. Protein, mineral and vitamin contents can be modified after optimized canning**
325 **processes compared with control canning.**

326 Table 4 shows the concentrations of proteins, fibers, iron and magnesium, lipids and vitamins
327 in the 7 optimized canned chickpeas and in control chickpeas. There was a reduction in
328 protein content after soaking at 15 °C with i) low-ionized water followed by cascading
329 blanching (T10), ii) agitation followed by an immersion blanching (T5) and iii) a germination
330 step (T13) ($-35.8 \pm 0.1\%$, $-19.5 \pm 0.1\%$, $-23.6 \pm 0.1\%$, $p \leq 0.05$, respectively). The magnesium
331 content decreased similarly ($-35.9 \pm 0.04\%$, $-21.5 \pm 0.05\%$, $-27.6 \pm 0.06\%$, $p \leq 0.05$,
332 respectively), but increased after hot soaking when water is replaced, followed by immersion
333 blanching (T3, $+12.8 \pm 0.05\%$, $p \leq 0.05$). Moreover, our results show that relative to the
334 control, iron content was increased after hot soaking with i) water replacement and immersion
335 blanching (T3), ii) agitation and cascading blanching (T8) and iii) water replacement and
336 cascading blanching (T9): $+30.7 \pm 1.1\%$, $+24.5 \pm 0.8\%$, $+40.0 \pm 1.6\%$, $p \leq 0.05$, respectively.
337 We also found a higher iron content in chickpeas soaked in water at 15 °C with either i) low-
338 ionized water (T10) or ii) water replacement (T12), both followed by a cascading blanching
339 ($+26.7 \pm 0.8\%$, $+67.3 \pm 1.1\%$, $p \leq 0.05$, respectively).

340 Additionally, we noticed that germination induced a decrease in lipid content ($-36.1 \pm 4.6\%$,
341 $p \leq 0.05$) (Table 4).

342 Vitamin E content significantly increased for 4 conditions relative to the control: $+94.2 \pm$
343 16.7% for hot soaking with water replacement and cascading blanching (T9), $+82.1 \pm 17.3\%$
344 for soaking at 15 °C with low-ionized water followed by cascading blanching (T10), $+130.2 \pm$
345 14.3% for mixed soaking at 15 °C and immersion blanching (T5), and $+102.4 \pm 11.2\%$ for
346 soaking at 15 °C with water replacement followed by cascading blanching (T12). Conversely,
347 a significant decrease in lutein content occurred in the germination condition relative to the
348 control condition ($-63.8 \pm 3.8\%$, $p \leq 0.05$). Finally, 5-methyltetrahydrofolate content decreased

349 significantly for all conditions (from $-12.1 \pm 4.7\%$ to $-97.1 \pm 0.1\%$), except for a mixed hot
350 soaking followed by a cascading blanching (T8).

351

352 **3.4. Vitamin D and K bioavailability is not improved in meals containing optimized**
353 **canned chickpeas**

354 As shown in Figure 2A, there was no difference in vitamin D and K bioaccessibility between
355 meals containing control chickpea and meals containing optimized canned chickpeas. Similar
356 results were obtained regarding vitamin D and K uptake by differentiated Caco-2 cells (Figure
357 2B).

358

359 **4. Discussion**

360

361 Pulse components such as phytates, saponins and tannins have previously been shown to
362 modulate fat-soluble vitamin bioavailability (Antoine et al., 2021; Margier et al., 2019). The
363 objective of this study was to find technological solutions to address this negative effect. We
364 thus modified several parameters of the canning process to decrease phytate, saponin and
365 tannin content in chickpeas.

366 To this aim, we first modified some process parameters independently of each other. Our
367 results indicate that phytate content can be significantly decreased by modifying soaking or
368 blanching parameters. Under the conditions of our study, this is likely due to an improved
369 release of these compounds, caused by the agitation of chickpeas during soaking, the increase
370 of chickpea coat permeability at 80°C (Tabekhia & Luh, 1980), osmosis for a soaking with
371 low-ionized water (Vijayakumari et al., 1996), a low chickpeas-to-water ratio or with water
372 replacement/ cascading. Moreover, phytates can also be degraded by phytase during either hot
373 or acid soaking, as phytase activity is increased by heat (Khokhar & Chauhan, 1986) and
374 optimized at a pH of 4.6 (Wodzinski & Ullah, 1996).

375 According to our data, saponin content was not significantly reduced relative to the control
376 condition when a single parameter was changed. A mild reduction in saponin content was
377 observed after soaking with low-ionized water and with a low chickpeas-to-water ratio,
378 reflecting previously published results (Shi et al., 2009), but these differences remained non-
379 significant, likely because of the variability of the results. Yoshiki and colleagues showed that
380 saponin content was decreased after blanching for 30 min (Yoshiki et al., 1998). Our
381 blanching conditions (10 or 20 min) may thus not have been long enough.

382 After performing preliminary tests using single modifications, we then combined the most
383 effective processes (13 conditions compared with the control process). The decreases in

384 phytate and saponin content were greater than those in preliminary tests because of the
385 combinations of different parameters, followed by the use of hot brine and sterilization, in
386 accordance with previous data (Jood et al., 1986; Tabekhia & Luh, 1980).

387 It is noteworthy that no difference in phytate and saponin contents were observed after
388 germination. According to previous results (Kyriakidis et al., 1998)(El-Adawy, 2002),
389 phytates were expected to decrease due to a rise in phytase activity during germination. We
390 hypothesize that we failed to observe a decrease in phytates due to the method used - as we
391 did not perform a purification step, we may also have detected the product of phytic acid
392 hydrolysis (phosphoric acid) together with the remaining phytic acid. Saponin assay may also
393 detect the hydrolysis product of saponins: sapogenin (Hiai et al., 1976; Price et al., 1986)
394 together with remaining saponins. In addition, Ruiz and colleagues showed a reduction in
395 saponin content 6 days after germination (Ruiz et al., 1996), while our germination period was
396 of only 3 days. Ayet and colleagues observed a decrease in saponin content 3 days after
397 germination, but it was followed by an increase after 6 days (Ayet et al., 1997). Different
398 germination times should thus be tested to fully comprehend these phenomena, and other
399 analytical methods such as mass spectrometry detection should be set up for further
400 investigations.

401 Following these intermediate results, 7 conditions inducing the greatest decreases in both
402 phytate and saponin content were ultimately selected to be fully characterized. Changes in
403 texture were observed between these conditions, but further investigations including a sensory
404 analysis are needed to confirm whether consumers would perceive these changes.

405 The first additional compound evaluated were tannins. A decrease in tannin content was
406 observed for the 2 conditions incorporating water replacement during the soaking step. As
407 tannins are water-soluble, they are likely better released by osmosis during soaking when the
408 water is replaced (Deshpande et al., 1986). A decrease in tannin content was also observed

409 after germination. This is consistent with previous works (Khandelwal et al., 2010)(Rao &
410 Deosthale, 1982): polyphenol oxidase activity increases during germination and induces
411 tannin hydrolysis. Conversely, an increase in tannin content occurred after hot (80 °C) mixed
412 soaking and cascading blanching. We suggest this is due to an inactivation of polyphenol
413 oxidase (Buckow et al., 2009).

414 We then checked whether compounds of interest remained unaltered after modifying the
415 canning process. Fiber content was not modified. Although the presence of fibers can
416 decrease vitamin bioavailability (Margier et al., 2019), its preservation is important because
417 most people lack fiber in their diets (Lairon et al., 2003). In accordance with previous data
418 (El-Adawy, 2002), lipid content decreased after germination only, as sprouting chickpeas use
419 lipids for growth. Lutein content also decreased during germination, presumably for the same
420 reason.

421 Water-soluble compounds such as minerals or proteins were expected to decrease when
422 soaking or blanching were optimized. Protein content decreased moderately under 3
423 conditions (immersion blanching, cascading blanching and germination) that likely increased
424 chickpea moisture and softness. These increases may facilitate the release of water-soluble
425 compounds. Our results differ from previous data (Chitra et al., 1996)(El-Adawy, 2002), but
426 authors compared raw chickpeas with germinated chickpeas.

427 Surprisingly, iron content significantly increased under 4 conditions (as did magnesium
428 content in 1 condition). We suggest that this effect may be due either to i/ lower phytate
429 content, leading to fewer quenched cations, or to ii/ greater disruption of the chickpea matrix,
430 which may favor mineral extraction prior analysis. Vitamin E content also increased under 4
431 conditions. Much as with iron, we suggest that greater softening of the vegetal walls would
432 make vitamin E more extractable, as has been observed in kidney beans (Margier et al., 2018).
433 Finally, 5-methyltetrahydrofolate contents were reduced after most of the optimized processes

434 relative to the control process. Although heat does not appear to be the main factor in
435 inducing folate loss (Delchier et al., 2013), folates are known to be highly susceptible to
436 leaching during food transformation processes (Scott et al., 2000).

437 In a second set of experiments, we assessed vitamin bioavailability after in vitro digestion of
438 test meals made with the different batches of canned chickpeas. We focused especially on
439 vitamin D₃ (cholecalciferol) and vitamin K₁ (phylloquinone), as these vitamins have very
440 different structures and thus may behave differently, as previously observed (Antoine et al.,
441 2021; Margier et al., 2019).

442 No difference was observed between the different conditions in term of bioaccessibility (i.e.,
443 the vitamin amount transferred from the solid phase of the meal to the aqueous phase of the
444 digesta during in vitro digestion). No difference was observed between the different
445 conditions in term of cellular uptake by Caco-2 cells in culture, either. This means that the
446 decreases in phytate, saponin and/or tannins contents in optimized canned chickpeas were not
447 important enough to modulate micelle formation, vitamin incorporation in mixed micelles or
448 vitamin membrane transporter functioning. In a previous study, we observed significant
449 differences in terms of vitamin K bioaccessibility and bioavailability when we compared
450 household cooked chickpeas vs canned chickpeas (Margier et al., 2019). The decreases in
451 phytate, saponin, tannin and fiber content were of -24%, -4%, -17,5% and -22%, respectively.
452 We suggest that the difference between the two studies may be due partly to a decrease in
453 fiber content not observed in the present study.

454 In conclusion, we identified different soaking and blanching parameters in the present study
455 that promoted decreases in phytate, tannin and saponin contents of canned chickpeas (-16 to -
456 44%). Further experiments are required to validate these data in other chickpea varieties and/
457 or in other pulse species. Additionally, when using these chickpeas in tests meals, we did not
458 observe any improvement in the bioaccessibility and the cellular uptake of vitamins D and K

459 as compared with the control conditions. Although further modification of the canning
460 process could lead to greater decreases in bioactive amounts, these modifications may be
461 more expensive and less environmentally friendly. We thus suggest that upstream solutions,
462 such as improved cultivar choice, should also be considered.

463

464

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480 **References:**

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613

614

615 **Figure legends**

616

617 **Figure 1: Bioactive compound contents in canned chickpeas.**

618 **A:** *Phytates and saponin content under different soaking conditions after preliminary tests.* **B:**

619 *Phytate and saponin content under different blanching conditions after preliminary tests.*

620 During preliminary tests (PT), canning process parameters were modified independently.

621 Phytate (white bars) and saponin (black bars) contents were analyzed in chickpea powder

622 after each step, but not in the final product. Soaking was done with hot (80°C), acidic (pH 5),

623 alkaline (pH 9), mixed, low- (HT = 6 °f) and high-ionized (HT = 184 °f) water, but also in

624 different chickpeas-to-water ratios (2:3 or 1:10), with water replacement and for a longer time

625 (24h). Blanching was tested for a longer time (20 min) and with different methods (cascading

626 or steaming). Data are expressed as mean ± SEM (n = 3).

627 **C:** *Phytate and saponin content in canned chickpeas.* Different processes combining the most

628 efficient soaking and blanching parameters were tested (T). Contrary to preliminary tests,

629 phytate (white bars) and saponin (black bars) contents were analyzed on the final product

630 powders, after conditioning and sterilization. Data are expressed as mean ± SEM (n = 9).

631 **D:** *Tannin content in canned chickpeas.* Once optimized conditions (low phytate and saponin

632 content) were chosen, tannins were analyzed in chickpea powders. Data are expressed as

633 mean ± SEM (n = 9).

634 A difference of $p \leq 0.05$ was considered significant. Cp: Control Process; Germ: Germination.

635 dm = dry matter.

636

637

638 **Figure 2. Vitamin D and K bioavailability in the presence of optimized chickpeas**

639 **A:** Vitamin D and K bioaccessibility. Test meals were made with the different canned
640 chickpeas and supplemented with vitamin D or vitamin K. Bioaccessibility was calculated as
641 the percentage of the vitamins recovered in mixed micelles divided by the vitamin amount
642 recovered from the digesta at the end of the digestion.

643 **B:** Vitamin D and K intestinal absorption. Mixed micelles obtained from in vitro digestions
644 were delivered to Caco2-TC7 cells after appropriate dilution. The apical side and the well
645 monolayers were collected after 1h of incubation. The vitamin absorbed was calculated as the
646 percentage of the vitamins recovered from the cells divided by the total vitamin amount
647 delivered to the cells .

648 Vitamin D: white bars; vitamin K: black bars; CP: Control Process; Germ: Germination. Data
649 are expressed as mean \pm SEM (n = 6). A difference of $p \leq 0.05$ was considered significant.

650 **Tables**

651

652 **Table 1: Chickpea soaking and blanching parameters for preliminary experiments**

	SOAKING												BLANCHING					
Parameters	CP	PT 1	PT 2	PT 3	PT 4	PT 5	PT 6	PT 7	PT 8	PT 9	PT 10	PT 11	Parameters	CP	PT 12	PT 13	PT 14	
Temperature (°C)	15	80	15	15	15	15	15	15	15	15	15	15	Temperature (°C)	90	90	90	90	
Time (h)	14	14	24	14	14	14	14	14	14	14	14	14	Time (min)	5	20	5	5	
Water hardness (°f)	30	30	30	184	6	30	30	30	30	30	30	30	Mode	Immersion		Steaming	Cascading	
Chickpeas-to-water ratio	1:3	1:3	1:3	1:3	1:3	2:3	1:10	1:3	1:3	1:3	1:3	1:3	-	-	-	-	-	
Agitation	-	-	-	-	-	-	-	Yes	-	-	-	-	-	-	-	-	-	-
Water replacement	-	-	-	-	-	-	-	-	Yes	-	-	-	-	-	-	-	-	-
Additive	-	-	-	-	-	-	-	-	-	Acid	Base	-	-	-	-	-	-	-
Germination	-	-	-	-	-	-	-	-	-	-	-	Yes	-	-	-	-	-	/

653

654 Different preliminary tests (PT) were done by modifying single soaking and blanching parameters of the control process (CP).

655

656

657 **Table 2: Optimization tests on global process**

		Control	T 1	T 2	T 3	T 4	T 5	T 6	T 7	T 8	T 9	T 10	T 11	T 12	T 13
Soaking	Temperature (°C)	15	80	80	80	15	15	15	80	80	80	15	15	15	15
	Time (h)	14	2	2	2	14	14	14	2	2	2	14	14	14	14
	Water hardness (°f)	30	30	30	30	6	30	30	30	30	30	6	30	30	30
	Chickpeas-to-water ratio	1/3	1/10	1/10	1/10	1/3	1/3	1/3	1/10	1/10	1/10	1/3	1/3	1/3	1/3
	Mixing	No	No	Yes	No	No	Yes	No	No	Yes	No	No	Yes	No	No
	Water replacement	No	No	No	Yes	No	No	Yes	No	No	Yes	No	No	Yes	No
	Additive	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Germination	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes	
Blanching	Temperature (°C)	90													
	Time (min)	5													
	Mode	Immersion							Cascading						Immersion
Conditioning in a can (200 g), and hot brine (0.5% salt, 70 °C) at a ratio of 86:114 (w:w)															
Autoclave sterilization at 10 rpm and 127 °C for 6 min															

658

659 Different tests (T) based on the results of preliminary tests and combining the most efficient
 660 soaking and blanching parameters were performed. Six soaking conditions were defined,
 661 followed either by immersion blanching or cascading blanching. Conditioning and
 662 sterilization parameters remained unchanged.

663

664 Table 3. Canned chickpea texture

665

	Texture Maximal applied force (N)
Control	9.58 ± 0.5
T1	13.77 ± 0.4 *
T2	14.02 ± 0.4*
T3	13.60 ± 0.5*
T4	8.33 ± 0.4
T5	8.60 ± 0.6
T6	12.10 ± 0.9
T7	10.14 ± 0.3
T8	10.00 ± 0.4
T9	9.50 ± 0.4
T10	6.53 ± 0.3*
T11	7.07 ± 0.4*
T12	8.50 ± 0.55

666

667 A compression force was applied on the pulses using an Uniaxial Microsystem TA XT texture
668 analyzer to determine the texture (compression forces are positively correlated with seed
669 hardness). Data are expressed as mean ± SEM (n = 30). A difference of $p \leq 0.05$ was
670 considered significant.

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673

674 **Table 4. Protein, lipid, fiber, mineral, fat-soluble vitamin and folate content in canned**
 675 **chickpeas**

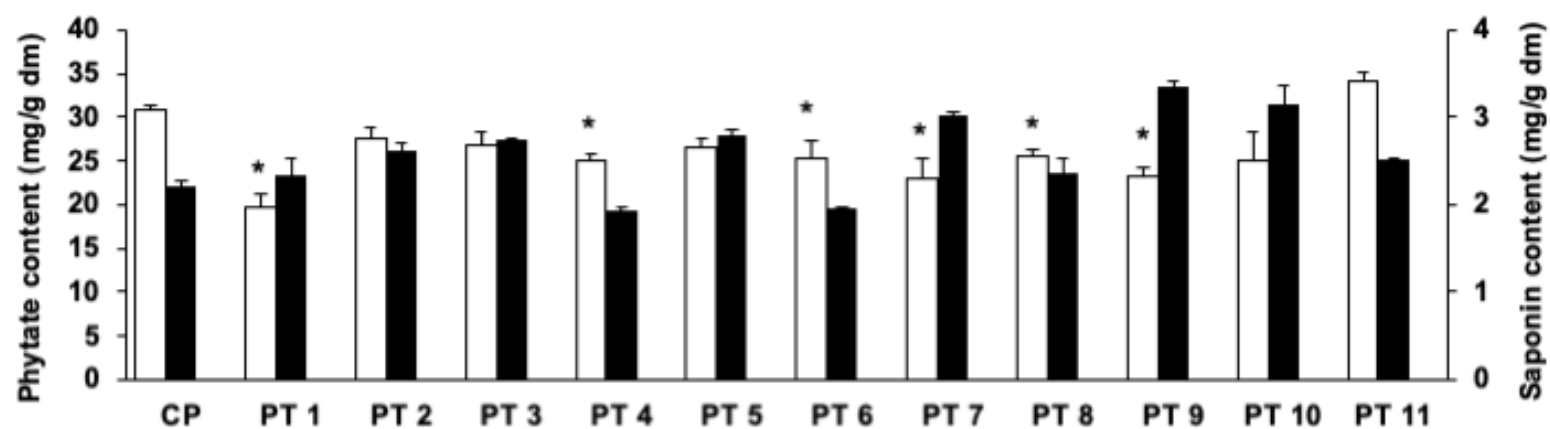
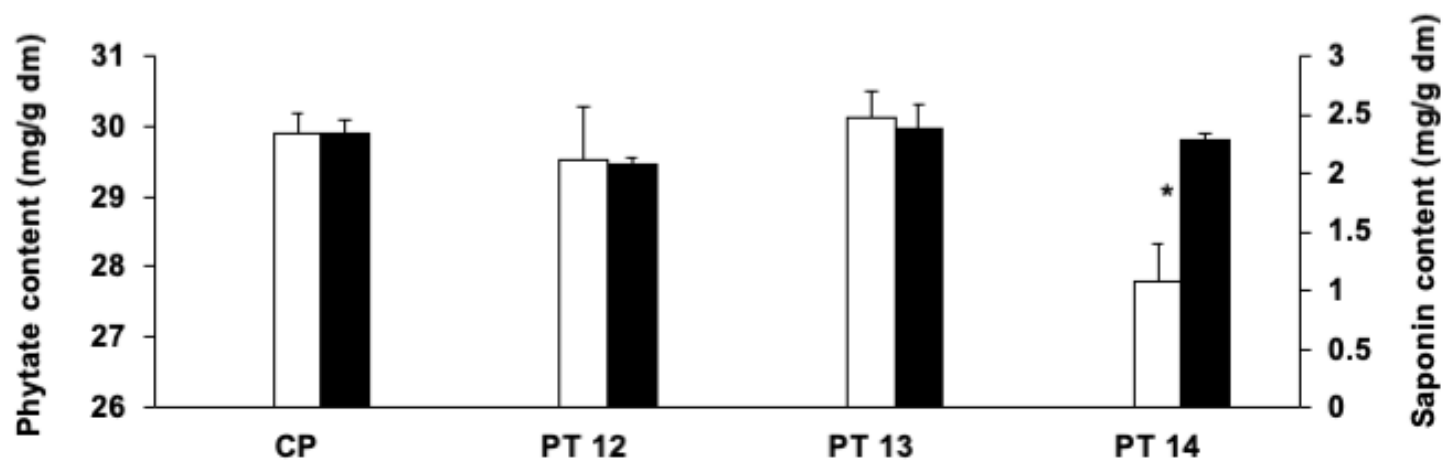
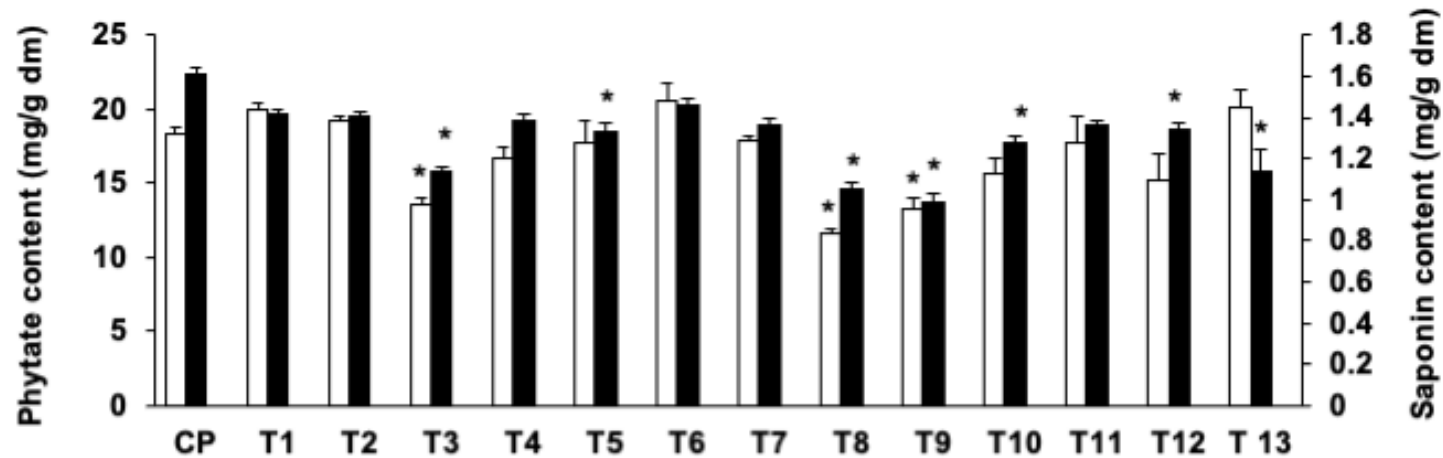
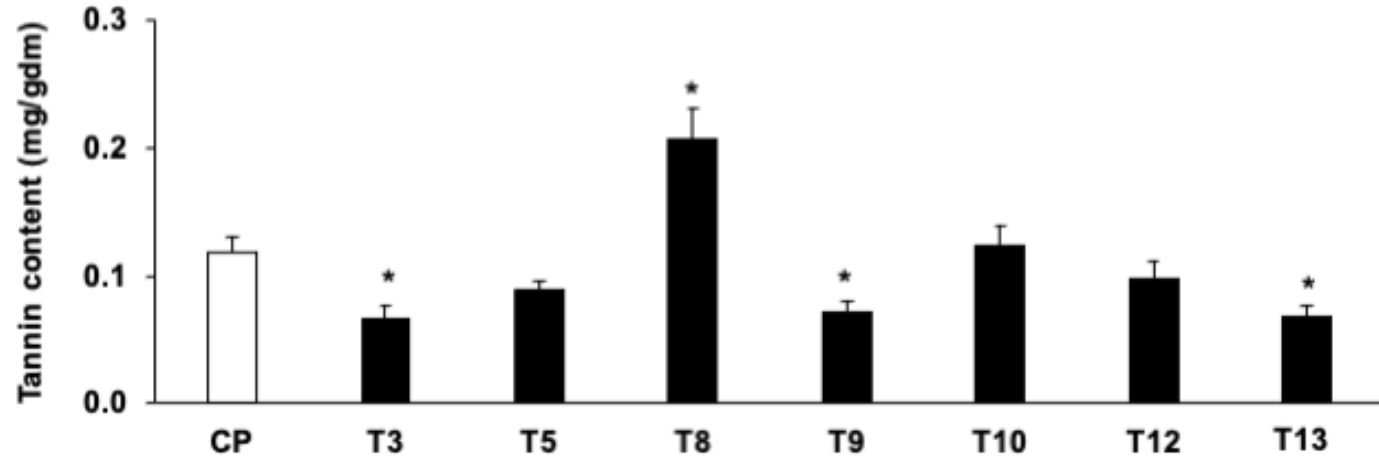
	Control process	T3	T5	T8	T9	T10	T12	T13
Proteins (g/100g dm)	23.1 ± 1.0	23.5 ± 1.1	18.6 ± 0.9*	21.4 ± 0.1	22.2 ± 1.0	14.8 ± 0.7*	22.1 ± 1.0	17.6 ± 0.8*
Total lipids (g/100g dm)	5.0 ± 0.1	4.8 ± 0.2	6.4 ± 1.6	6.0 ± 0.6	5.40 ± 0.05	5.1 ± 0.1	4.0 ± 0.3	3.2 ± 0.2*
Total fibers (g/100g dm)	14.9 ± 2.0	15.7 ± 1.9	14.8 ± 2.1	16.6 ± 2.0	14.1 ± 1.9	16.6 ± 2.0	14.9 ± 2.01	14.6 ± 1.9
Vitamin E (mg/100g dm)	2.2 ± 0.4	3.0 ± 0.3	5.2 ± 0.3*	3.1 ± 0.1	4.3 ± 0.4*	4.1 ± 0.4*	4.6 ± 0.3*	2.3 ± 0.3
Lutein (µg/100g dm)	226.7 ± 36.0	198.1 ± 20.8	321.4 ± 34.1	158.7 ± 5.1	140.7 ± 18.8	255.3 ± 38.5	220.0 ± 17.1	82.1 ± 8.6*
5-methyl-tetrahydro-folate (µg/100g dm)	32.1 ± 9.6	6.4 ± 1.4*	0.9 ± 0.1*	28.2 ± 1.5	1.8 ± 0.3*	3.5 ± 0.8*	1.8 ± 0.4*	4.9 ± 1.3*
Magnesium (mg/100g dm)	131.6 ± 9.5	148.5 ± 10.7*	103.3 ± 7.3*	122.9 ± 8.9	140.7 ± 10.1	84.4 ± 6.0*	116.6 ± 8.3	95.2 ± 6.7*
Iron (mg/100g dm)	32.2 ± 1.7	42.0 ± 1.6*	32.5 ± 1.7	40.0 ± 1.7*	45.0 ± 3.2*	40.7 ± 1.7*	53.8 ± 3.5*	27.2 ± 1.9
Dry matter (g/100g)	34.2	35.7	33.9	34.9	35.5	34.4	33.4	35.7

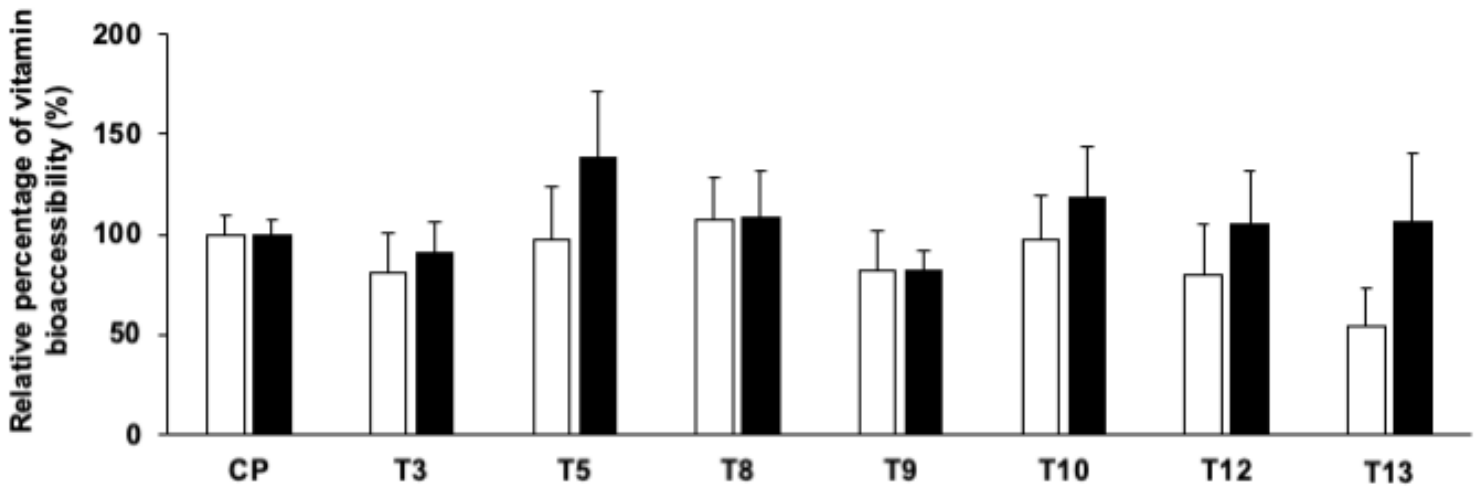
676

677 The nutritional characterization of canned chickpeas was performed on chickpeas processed
 678 according to either the control process (CP) or our 7 optimized conditions (See Table 2). dm =
 679 dry matter. Data are expressed as mean ± SEM (n = 3). A difference of p ≤ 0.05 was
 680 considered significant.

681

682

A**B****C****D**

A**B**