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1 **Bioaccessibility and antioxidant activity of phenolic compounds in**
2 **cooked pulses**

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12

13 **Abbreviations:**

14 TPC: total phenolic content; DPPH: 2,2-diphenyl-1-picrylhydrazyl; TPTZ: tris(2-
15 carboxyethyl)phosphine hydrochloride; FRAP: ferric reducing antioxidant power; S.D.:
16 standard deviation; ANOVA: analysis of variance.

17 **Abstract**

18 The aim of this study was to evaluate the bioaccessibility of polyphenols and
19 antioxidant activity in cooked pulses and to study the effect of cooking on their total
20 phenolic content (TPC) and antioxidant capacity. Cooked faba beans showed the
21 highest TPC, followed by soybeans and lentils or peas. TPC ranged from 10.4 ± 0.2 to
22 52.9 ± 0.3 mg/100 g and was positively correlated with antioxidant activity. Cooking
23 resulted in increased TPC and antioxidant activity of the methanolic extracts, caused by
24 cell disruption and improved extraction of polyphenols. Although polyphenols were lost
25 in the cooking water, boiled legumes had more polyphenols than those resulting
26 cooking broths. *In vitro* gastrointestinal digestion resulted in increased TPC and
27 antioxidant capacity of the extracts. Soybeans showed the highest amount of
28 bioaccessible polyphenols. The release of phenolics from cooked legumes was mainly
29 achieved during the intestinal phase. Literature data may underestimate the TPC and
30 antioxidant capacity of pulses.

31 **Keywords:** bioaccessibility, polyphenols, pulses, in vitro digestion, antioxidant activity,
32 boiling

33 **1. Introduction**

34 Pulses, which include lentils, beans, chickpeas, and soybeans provide an important
35 source of proteins, fibres, minerals, vitamins, and bioactive compounds such as
36 polyphenols and other phytochemicals (Giusti *et al.*, 2017). A large amount of evidence
37 supports the cardioprotective, anticarcinogenic, anticholesterolemic, and antioxidant
38 properties of pulses (Singh *et al.*, 2017, López-Martínez *et al.*, 2017). To highlight their
39 present and future importance as nutritious protein-rich foods, the Food and Agriculture
40 Organization of the United Nations declared 2016 the International Year of Pulses
41 (Vollmann 2016).

42 Pulses are thermally processed in order to obtain desirable textures and flavours and are
43 not commonly eaten raw. Therefore, the effect of pressure, temperature, or pH
44 variations that occur during thermal processing of pulses and other foods is of key
45 importance. For example, glucosinolates found in cruciferous vegetables and seeds have
46 been proved to be heavily lost during thermal processing (Lafarga *et al.*, 2018a). In
47 addition, thermal processing of pulses including toasting, steaming, boiling, and
48 autoclaving usually degrade and reduce the content of phytic acid and other phenolic
49 compounds (López-Martínez *et al.*, 2017). Health benefits of foods depend not only on
50 their resistance to thermal processing or intake levels but also on their bioavailability.
51 Bioavailability can be defined as the fraction of ingested component available for
52 utilization in normal physiological functions (Cilla *et al.*, 2018). Bioavailability includes
53 another additional term, bioaccessibility, which is defined as the release of compounds
54 from their natural food matrix to be available for intestinal absorption and is one of the
55 main limiting factors for bioavailability (Stahl *et al.*, 2002).

56 Knowledge on resistance to cooking conditions and on bioaccessibility as the first step
57 of bioavailability is of great interest to ascertain the nutritional quality of a food

58 product. Therefore, the aim of this study was to evaluate the bioaccessibility of
59 polyphenols and antioxidant activity in a number of pulses namely lentils, cowpeas,
60 faba beans, chickpeas, soybeans, runner beans, common beans, and peas. A secondary
61 aim of this paper was to study the effect of cooking on the total phenolic content (TPC)
62 and antioxidant capacity of selected pulses. Assessment of the bioaccessibility and
63 nutritional quality in different varieties of cooked pulses may assist health programmes
64 and add economic and nutritional value to these foods.

65 **2. Materials and methods**

66 **2.1 Chemicals and reagents**

67 Methanol and ferric chloride were purchased from Panreac (Barcelona, Spain). Gallic
68 acid, ascorbic acid, hydrochloric acid, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-
69 diphenyl-1-picrylhydrazyl (DPPH), tris(2-carboxyethyl)phosphine hydrochloride
70 (TCEP), potassium phosphate monobasic, potassium phosphate dibasic, calcium
71 chloride, 1,2- benzenedithiol, α -amylase (EC 3.2.1.1), pepsin (EC 3.4.23.1), pancreatin,
72 fresh bile, sodium hydroxide, ammonium sulphate, and sodium carbonate were
73 purchased from Sigma-Aldrich (Steinheim, Germany). Folin-Ciocalteu's reagent was
74 purchased from VWR (Llinars del Vallès, Spain). All reagents used were of analytical
75 grade.

76 **2.2 Thermal processing**

77 Dry legumes, shown in Figure 1, were sown in July 2017 in an open field in Northeast
78 Spain (41° 30' 08.2" N, 2° 01' 02.2" E) and were cultivated using the traditional
79 methods in the area including drip irrigation. Legumes were divided into two lots. The
80 first lot was milled to a thin powder using a MINIMOKA GR-020 grinder (Taurus
81 Group, Barcelona, Spain), passed through a sieve of 1 mm, and stored at -20 °C until
82 further use. The second lot was soaked in tap water at a sample:water ratio of 1:10 (w/v)
83 at room temperature for 24 h (as it is generally done in Spanish homes). After this
84 period, the soaking water was discarded and the soaked beans were boiled at a
85 sample:water ratio of 1:10 (w/v) as described by de Oliveira *et al.*, (2018). Boiling
86 times, shown in Figure 1, were optimised by preliminary experiments carried out for
87 each variety in which samples were considered cooked according to the judgement of a
88 group of semi-trained panellists. For all processing treatments, the minimum time

89 needed to reach tenderness for an adequate palatability and taste (according the Spanish
90 eating habits) was used. Immediately after processing, samples were chilled to
91 approximately 4 °C using an ABT 101L blast chiller (Infrico, Barcelona, Spain), milled
92 using a MINIMOKA GR-020 grinder (Taurus Group, Barcelona, Spain), and stored at -
93 20 °C until further use. Thermal processing was carried out in triplicate for each sample.

94 **2.3 Determination of total phenolic content**

95 The TPC was determined by the Folin Ciocalteu method. Briefly, for the extraction of
96 polyphenols from raw, soaked, and cooked legumes, the samples were homogenized
97 with methanol 70% (v/v) at a sample:methanol ratio of 1:4 (w/v) at 4 °C.
98 Homogenization was performed using a T-25 ULTRA-TURRAX[®] homogenizer (IKA,
99 Staufen, Germany) operating at 12,000 rpm for 30 s. Homogenized samples were
100 immediately place in a stirrer at room temperature for 2 h and centrifuged using a
101 Sigma-3-18 KS centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz,
102 Germany) at 10,000 × g for 20 min.

103 To determine the TPC of boiling water and gastric or intestinal digestive extracts,
104 proteins were first precipitated by saturation using ammonium sulphate as described by
105 García-Vaquero *et al.*, (2017). The assay was performed by adding 4.3 mL of MilliQ
106 water and 0.5 mL of Folin-Ciocalteu's reagent to 0.7 mL of methanolic or digestive
107 extract. After incubation for 5 min at room temperature in the dark, 2 mL of saturated
108 sodium carbonate solution was added. The mixture was shaken and further incubated
109 for 1 h at room temperature and in the dark. Absorbance was read at 760 nm using a
110 GENESYSTM 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, MA, USA).
111 TPC was determined in triplicate and results were expressed as mg of gallic acid
112 equivalents per 100 g of dry weight (DW). Standard curves were prepared daily.

113 **2.4 Antioxidant activity**

114 Antioxidant activity was assessed using two different methods: the ferric reducing
115 antioxidant power (FRAP) and the DPPH scavenging activity assays. Both
116 determinations were performed on the same extract used for the determination of TPC
117 and following the methodology described by Lafarga *et al.*, (2018b) with some
118 modifications.

119 **2.4.1 FRAP assay**

120 The FRAP reagent was freshly prepared by mixing 0.3 M acetate buffer (pH 3.6), 10
121 mM TPTZ in 40 mM hydrochloric acid, and 20 mM ferrous chloride in the proportion
122 10:1:1 (v/v/v). Determinations were carried out by mixing 1.4 mL of the FRAP reagent
123 and 0.1 mL of the methanolic extract obtained following the methodology described
124 above. After 20 min of incubation in the dark at 37 °C and constant shaking, the
125 absorbance was read at 593 nm using a GENESYS™ 10S UV-Vis spectrophotometer
126 (Thermo Fisher Scientific, MA, USA). Antioxidant activity assessed using the FRAP
127 assay was determined in triplicate and expressed as mg of ascorbic acid equivalents per
128 100 g of DW. Standard curves were prepared daily.

129 **2.4.2 DPPH assay**

130 The assay was performed by adding 1.4 mL of 0.1 mM DPPH· solution to 0.1 mL of the
131 methanolic extract obtained as described above. After 60 min of incubation at room
132 temperature and in the dark, the absorbance was read at 515 nm using a using a
133 GENESYS™ 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, MA, USA).
134 Antioxidant activity assessed using the DPPH assay was determined in triplicate and
135 expressed as mg of ascorbic acid equivalents per 100 g of DW. Standard curves were
136 prepared daily.

137 **2.5 Simulated gastrointestinal digestion**

138 A simulated gastrointestinal digestion was performed following the standardised static
139 *in vitro* method previously described by Minekus *et al.*, (2014). This method is an
140 international consensus which consists of three sequential states: (i) oral (pH 7.0, α -
141 amylase), (ii) gastric (pH 3.0, pepsin), and (iii) intestinal (pH 7.0, pancreatin and fresh
142 bile). The pancreatin used contained enzymatic components including trypsin, amylase
143 and lipase, ribonuclease, and protease, produced by the exocrine cells of the porcine
144 pancreas, which allow hydrolysing proteins, starch, and fats. The simulated digestion
145 was performed in triplicate for each sample and a blank was prepared using only
146 distilled water instead of sample and following the same procedure. Determinations of
147 TPC and antioxidant activity were carried out in triplicate after both gastric and
148 intestinal phase.

149 **2.6 Statistical analysis**

150 Results are expressed as mean \pm standard deviation (S.D.). Difference between samples
151 were analysed using analysis of variance (ANOVA) with JMP 13 (SAS Institute Inc.,
152 Cary, USA). Where significant differences were present, a Tukey pairwise comparison
153 of the means was conducted to identify where the sample differences occurred. The
154 criterion for statistical significance was $p < 0.05$.

155 3. Results and discussion

156 3.1 Total phenolic content and antioxidant capacity of dry legume seeds

157 Dry soybeans showed the highest TPC, followed by faba beans, and lentils (Table 1;
158 $p < 0.05$). TPC ranged from 14.70 ± 0.13 to 37.14 ± 0.29 mg/100 g DW and was
159 positively correlated with FRAP ($r^2 = 0.680$; $p < 0.05$) and DPPH ($r^2 = 0.620$; $p < 0.05$)
160 values. Results were comparable to those reported previously for dry pulses such as
161 chickpeas, with TPC values ranging between 2.78 to 12.7 mg/100 g and field peas
162 (*Pisum sativum*) with TPC values ranging between 9.6 to 25.4 mg/100 g (Magalhaes *et*
163 *al.*, 2017). Results were also comparable to those reported by Parikh and Patel (2018),
164 who calculated the TPC of field beans and white cowpeas as 41.4 and 39.9 mg/100 g.
165 Antioxidant activity of selected pulses assessed using the FRAP and DPPH methods is
166 shown in Table 1. In the current study, dry faba beans showed the highest antioxidant
167 capacity when assessed using both the FRAP and DPPH assays ($p < 0.05$). Similar
168 antioxidant capacity values were reported for dry pulses previously (Parikh and Patel
169 2018). The highest antioxidant capacity of faba beans could be attributed to a higher
170 content of coloured polyphenols such as tannins, anthocyanins, or proanthocyanins. Xu
171 *et al.*, (2007) suggested that antioxidant capacity of pulses is mainly caused by the
172 coloured pigments found in their seed coats and positive correlations have been
173 associated with dark colours and antioxidant capacities of legume seeds previously
174 (Dueñas *et al.*, 2006). In addition, Gan *et al.*, (2016) recently reported a higher TPC and
175 antioxidant capacity of coloured sword bean varieties when compared to the white ones,
176 especially in their bean coats and Siah *et al.*, (2014) reported total proanthocyanin
177 contents ranging between 0.11 to 0.32 mg/g for different faba bean varieties.

178 **3.2 Effect of cooking on the total phenolic content and antioxidant activity of**
179 **selected pulses**

180 The cooking method used in the current study mimics common domestic cooking
181 methods, which generally include a rinsing and soaking step before boiling. Therefore,
182 TPC determinations made herein are likely to closely represent a usual intake of these
183 pulses when consumed at home. The effect of soaking and boiling on the TPC of
184 selected legumes is shown in Table 1. Soaking in tap water for 24 h resulted in a
185 significant decrease of the TPC ($p<0.05$). Soaking resulted in reduced phenolic content
186 previously (Hailelassie *et al.*, 2016). Percentage of TPC loss after soaking ranged
187 between 3.4 (soybeans) and 26.6% (common beans). When expressed on a wet weight
188 basis, boiling resulted in decreased TPC ($p<0.05$). However, when calculated on a DW
189 basis, boiling resulted in increased TPC ($p<0.05$), probably due to a higher extraction
190 yield caused by cell disruption during cooking. Similar results were observed previously
191 after thermal processing of legumes (Siah *et al.*, 2014). Results showed in next sections
192 also support this hypothesis. Cooking resulted in decreased TPC when calculated on a
193 wet weight basis previously (Garretson *et al.*, 2018). Decreased TPC values after
194 cooking were attributed to dissolution of polyphenols in water, degradation of phenolics
195 during processing, or chemical transformations previously (Bubelová *et al.*, 2018). In
196 the current study, the amount of polyphenols lost in the boiling water are shown in
197 Table 2. Similar results were reported by Siah *et al.*, (2014), who observed that a
198 significant amount of polyphenols was leaked to the cooking medium, but that boiled
199 beans had a higher TPC than those of resulting cooking broths. These results support the
200 hypothesis that the observe increase in the TPC was caused by an inefficient extraction
201 from raw legumes.

202 Boiling led to an overall increased antioxidant activity of the extracts when assessed
203 using both the FRAP and DPPH methods ($p<0.05$), caused by a higher TPC. Indeed, a
204 positive correlation was observed between TPC and FRAP ($r^2 = 0.816$; $p<0.001$) or
205 DPPH values ($r^2 = 0.639$; $p<0.05$) for cooked samples. Both the antioxidant capacity
206 and the TPC of the boiling water were calculated after protein precipitation. Protein
207 precipitation led to a significantly lower TPC and antioxidant activity ($p<0.05$). These
208 could be caused by protein interactions with the Folin-Ciocalteu reagent and to the
209 generation of antioxidant peptides during cooking.

210 **3.3 Bioaccessibility of total phenols and antioxidant activity**

211 Cooked soybeans followed by faba beans and lentils showed the highest amount of
212 bioaccessible polyphenols ($p<0.05$; Table 3). The TPC was higher after the gastric and
213 intestinal phases of digestion of all samples when compared to the initial stage ($p<0.05$).
214 Similar results were obtained previously for other foods such as cereals (Pérez-Jiménez
215 and Saura-Calixto 2005). Enzymatic hydrolysis of mung bean and adzuki bean also
216 resulted in an increased release of polyphenols previously (Sangsukiam and Duangmal
217 2017). However, results contrast with those reported by Zhang *et al.*, (2017), who
218 calculated the TPC of cooked green lentils after a water:methanol extraction as 618
219 mg/100 g and the bioaccessibility after a simulated gastrointestinal digestion as 51%.
220 The lower extraction time used by Zhang *et al.*, (2017) could partially explain their
221 findings, as the methanolic extract was obtained in that study after extraction for over
222 15 h and the enzymatic digestive extracts were obtained after an approximately 3 h
223 digestion. Phenolic compounds exist in free, soluble conjugated, and insoluble bound
224 forms. Although free and some conjugated phenolic compounds are thought to be
225 available for absorption in the human small and large intestines, those bound covalently
226 to indigestive polysaccharides may be absorbed after being released from cell structures

227 by digestive enzymes or microorganisms in the intestinal lumen (Wang *et al.*, 2014).
228 Chemical structure and matrix interactions can affect the bioaccessibility. Indeed, Chen
229 *et al.*, (2015) observed an increase in TPC after the intestinal phase when compared to
230 the gastric phase of digestion, suggesting that the release of phenolics from cooked
231 lentils is mainly achieved during the intestinal phase. The same trend was observed by
232 Zhang *et al.*, (2017), who observed higher total phenolic, flavonoid, and tannin contents
233 after the intestinal phase when compared to gastric digestion. Finally, Pérez-Jiménez
234 and Saura-Calixto (2005) reported that TPC and antioxidant activity of the digestive
235 enzymatic extracts (of cereals) were significantly higher when compared to that of the
236 water-organic extracts and that literature data may underestimate the actual TPC and
237 antioxidant capacity of cereals. Results obtained herein support the hypothesis that the
238 release of phenolics from cooked legumes is mainly achieved during the intestinal
239 phase.
240 Moreover, the antioxidant capacity of the digestive enzymatic extracts obtained after
241 gastric and gastrointestinal digestion, was assessed using the FRAP and DPPH assays.
242 Results, listed in Table 4 and Table 5 respectively, correlate well to those obtained for
243 TPC, as the antioxidant capacity was higher after gastrointestinal digestion ($p < 0.05$).
244 Indeed, a positive correlation was observed between the TPC and the FRAP ($r^2 = 0.913$;
245 $p < 0.05$) and DPPH ($r^2 = 0.798$; $p < 0.05$) values after *in vitro* digestion of cooked
246 samples. Boiling facilitated the release of polyphenols from the different legume
247 varieties. This is clear from the data, as the TPC after the intestinal phase of digestion
248 was higher for cooked samples when compared to those digested raw ($p < 0.05$).
249 Previous studies also reported an increased bioaccessible polyphenolic content of
250 roasted pearl millet, green gram, and finger millet when compared to those in their
251 native state (Hithamani and Srinivasan, 2017). Cilla *et al.*, (2018) recently reviewed the

252 effect of processing on the bioaccessibility of bioactive compounds and concluded that
253 thermal processing can exert a positive or a negative effect, depending on the food
254 matrix and on the processing conditions. Antioxidant capacity of digestive enzymatic
255 extracts was also higher for cooked samples ($p<0.05$). Although the current study
256 included a protein precipitation step, in order to avoid the interference of proteins with
257 the TPC determination method, short peptides could still be soluble at high salt
258 concentrations. Thus, the observed increase in antioxidant capacity could be also
259 attributed to the generation of antioxidant peptides, as pulses are protein-rich foods
260 (Nosworthy *et al.*, 2017) and excellent sources of bioactive peptides (Sangsukiam and
261 Duangmal, 2017).

262 **4. Conclusions**

263 Legumes are rich sources of not only proteins but also polyphenols. Boiling increased
264 both the content of polyphenols and the *in vitro* antioxidant capacity of the methanolic
265 extracts obtained from selected pulses. This was caused by an inefficient extraction
266 from the raw samples, which was improved after the cell disruption caused during
267 cooking. Although the cooking water contained significant quantities of polyphenols,
268 boiled legumes had more polyphenols than those resulting cooking broths. The TPC and
269 antioxidant capacity of the digestive enzymatic extracts obtained after *in vitro* digestion
270 was higher when compared to that observed for water:methanol extracts. Protein
271 precipitation prior to assessment of TPC resulted in significantly lower values, as
272 proteins can interfere in the determination. Results suggest that literature data may
273 underestimate the actual TPC and antioxidant capacity of pulses and support the
274 hypothesis that the release of phenolics from cooked legumes is mainly achieved during
275 the intestinal phase.

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283 **Conflict of interests**

284 The authors declare no conflict of interests

285 **References**

- 286 Bubelová, Z., Sumczynski, D. & Salek, R. N. (2018). Effect of cooking and germination
287 on antioxidant activity, total polyphenols and flavonoids, fiber content, and
288 digestibility of lentils (*Lens culinaris* L.). *Journal of Food Processing and*
289 *Preservation*, **42**, e13388.
- 290 Chen, P. X., Dupuis, J. H., Marcone, M. F., Pauls, P. K., Liu, R., Liu, Q., Tang, Y.,
291 Zhang, B. & Tsao, R. (2015). Physicochemical properties and in vitro
292 digestibility of cooked regular and nondarkening cranberry beans (*Phaseolus*
293 *vulgaris* L.) and their effects on bioaccessibility, phenolic composition, and
294 antioxidant activity. *Journal of Agricultural and Food Chemistry*, **63**, 10448-
295 10458.
- 296 Cilla, A., Bosch, L., Barberá, R. & Alegría, A. (2018). Effect of processing on the
297 bioaccessibility of bioactive compounds – A review focusing on carotenoids,
298 minerals, ascorbic acid, tocopherols and polyphenols. *Journal of Food*
299 *Composition and Analysis*, **68**, 3-15.
- 300 de Oliveira, A. P., Mateó, B. d. S. O., Fioroto, A. M., de Oliveira, P. V. & Naozuka, J.
301 (2018). Effect of cooking on the bioaccessibility of essential elements in
302 different varieties of beans (*Phaseolus vulgaris* L.). *Journal of Food*
303 *Composition and Analysis*, **67**, 135-140.
- 304 Dueñas, M., Hernandez, T. & Estrella, I. (2006). Assessment of *in vitro* antioxidant
305 capacity of the seed coat and the cotyledon of legumes in relation to their
306 phenolic contents. *Food Chemistry*, **98**, 95-103.
- 307 Gan, R.J., Lui, W.Y. & Corke, H. (2016). Sword bean (*Canavalia gladiata*) as a source
308 of antioxidant phenolics. *International Journal of Food Science & Technology*,
309 **51**, 156-162.
- 310 García-Vaquero, M., López-Alonso, M. & Hayes, M. (2017). Assessment of the
311 functional properties of protein extracted from the brown seaweed *Himantalia*
312 *elongata* (Linnaeus) S. F. Gray. *Food Research International*, **99**, 971-978.
- 313 Garretson, L., Tyl, C. & Marti, A. (2018). Effect of Processing on Antioxidant Activity,
314 Total Phenols, and Total Flavonoids of Pigmented Heirloom Beans. *Journal of*
315 *Food Quality*, **2018**, 786745.
- 316 Giusti, F., Caprioli, G., Ricciutelli, M., Vittori, S. & Sagratini, G. (2017). Determination
317 of fourteen polyphenols in pulses by high performance liquid chromatography-
318 diode array detection (HPLC-DAD) and correlation study with antioxidant
319 activity and colour. *Food Chemistry*, **221**, 689-697.
- 320 Gujral, H. S., Angurala, M., Sharma, P. & Singh, J. (2011). Phenolic content and
321 antioxidant activity of germinated and cooked pulses. *International Journal of*
322 *Food Properties*, **14**, 1366-1374.
- 323 Hailelassie, H.A., Henry, C.J. & Tyler, R.T. (2016). Impact of household food
324 processing strategies on antinutrient (phytate, tannin and polyphenol) contents of
325 chickpeas (*Cicer arietinum* L.) and beans (*Phaseolus vulgaris* L.): A review.
326 *International Journal of Food Science & Technology*, **51**, 1947-1957.

- 327 Hithamani, G. & Srinivasan, K. (2017) Bioaccessibility of polyphenols from selected
328 cereal grains and legumes as influenced by food acidulants. *Journal of the*
329 *Science of Food and Agriculture*, **97**, 621-628.
- 330 Lafarga, T., Bobo, G., Viñas, I., Collazo, C. & Aguiló-Aguayo, I. (2018a). Effects of
331 thermal and non-thermal processing of cruciferous vegetables on glucosinolates
332 and its derived forms. *Journal of Food Science and Technology*, **55**, 1973-1981.
- 333 Lafarga, T., Viñas, I., Bobo, G., Simó, J. & Aguiló-Aguayo, I. (2018b). Effect of
334 steaming and *sous vide* processing on the total phenolic content, vitamin C and
335 antioxidant potential of the genus *Brassica*. *Innovative Food Science &*
336 *Emerging Technologies*, **47**, 412-420.
- 337 López-Martínez, L. X., Leyva-López, N., Gutiérrez-Grijalva, E. P. & Heredia, J. B.
338 (2017). Effect of cooking and germination on bioactive compounds in pulses
339 and their health benefits. *Journal of Functional Foods*, **38**, 624-634.
- 340 Magalhaes, S. C., Taveira, M., Cabrita, A. R., Fonseca, A. J., Valentão, P. & Andrade,
341 P. B. (2017). European marketable grain legume seeds: further insight into
342 phenolic compounds profiles. *Food Chemistry*, **215**, 177-184.
- 343 Minekus, M., Alvinger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carriere,
344 F., Boutrou, R., Corredig, M. & Dupont, D. (2014). A standardised static in vitro
345 digestion method suitable for food—an international consensus. *Food &*
346 *Function*, **5**, 1113-1124.
- 347 Nosworthy, M. G., Neufeld, J., Frohlich, P., Young, G., Malcolmson, L. & House, J. D.
348 (2017). Determination of the protein quality of cooked Canadian pulses. *Food*
349 *Science & Nutrition*, **5**, 896-903.
- 350 Parikh, B. & Patel, V. (2018). Total phenolic content and total antioxidant capacity of
351 common Indian pulses and split pulses. *Journal of Food Science and*
352 *Technology*, **55**, 1499-1507.
- 353 Pérez-Jiménez, J. & Saura-Calixto, F. (2005). Literature Data May Underestimate the
354 Actual Antioxidant Capacity of Cereals. *Journal of Agricultural and Food*
355 *Chemistry*, **53**, 5036-5040.
- 356 Sangsukiam, T. & Dungmal, K. (2017). A comparative study of physico-chemical
357 properties and antioxidant activity of freeze-dried mung bean (*Vigna radiata*)
358 and adzuki bean (*Vigna angularis*) sprout hydrolysate powders. *International*
359 *Journal of Food Science & Technology*, **52**, 1971-1982.
- 360 Siah, S., Wood, J. A., Agboola, S., Konczak, I. & Blanchard, C. L. (2014). Effects of
361 soaking, boiling and autoclaving on the phenolic contents and antioxidant
362 activities of faba beans (*Vicia faba* L.) differing in seed coat colours. *Food*
363 *Chemistry*, **142**, 461-468.
- 364 Singh, B., Singh, J. P., Shevkani, K., Singh, N. & Kaur, A. (2017). Bioactive
365 constituents in pulses and their health benefits. *Journal of Food Science and*
366 *Technology*, **54**, 858-870.
- 367 Stahl, W., van den Berg, H., Arthur, J., Bast, A., Dainty, J., Faulks, R. M., Gärtner, C.,
368 Haenen, G., Hollman, P. & Holst, B. (2002). Bioavailability and metabolism.
369 *Molecular Aspects of Medicine*, **23**, 39-100.

- 370 Vollmann, J. (2016). Soybean versus other food grain legumes: a critical appraisal of
371 the United Nations International Year of Pulses 2016. *Die Bodenkultur: Journal*
372 *of Land Management, Food and Environment*, **67**, 17-24.
- 373 Wang, T., He, F. & Chen, G. (2014). Improving bioaccessibility and bioavailability of
374 phenolic compounds in cereal grains through processing technologies: A concise
375 review. *Journal of Functional Foods*, **7**, 101-111.
- 376 Xu, B., Yuan, S. & Chang, S. (2007). Comparative analyses of phenolic composition,
377 antioxidant capacity, and color of cool season legumes and other selected food
378 legumes. *Journal of Food Science*, **72**, S167-S177.
- 379 Zhang, B., Deng, Z., Tang, Y., Chen, P. X., Liu, R., Dan Ramdath, D., Liu, Q.,
380 Hernandez, M. & Tsao, R. (2017). Bioaccessibility, in vitro antioxidant and anti-
381 inflammatory activities of phenolics in cooked green lentil (*Lens culinaris*).
382 *Journal of Functional Foods*, **32**, 248-255.

383 **Legends to Figure**

384 **Figure 1. Studied legumes**

385 **Table 1. Total phenolic content and antioxidant activity of raw, soaked, and cooked legumes**

Sample	TPC (mg/ 100 g DW)			FRAP value (mg/ 100 g DW)			DPPH value (mg/ 100 g DW)		
	Dry (raw)	Soaked (raw)	Cooked	Dry (raw)	Soaked (raw)	Cooked	Dry (raw)	Soaked (raw)	Cooked
A	19.06 ± 0.49 ^{Bc}	17.52 ± 0.23 ^{Cc}	35.95 ± 0.34 ^{Ac}	18.28 ± 0.16 ^{Bb}	17.77 ± 0.35 ^{Cb}	42.77 ± 0.35 ^{Aa}	18.88 ± 0.17 ^{Bb}	17.54 ± 0.18 ^C	38.49 ± 0.72 ^A
B	12.50 ± 0.27 ^{Bf}	11.45 ± 0.41 ^{Ce}	15.69 ± 0.12 ^{Af}	10.22 ± 0.08 ^{Be}	11.05 ± 0.87 ^{Be}	12.01 ± 0.17 ^{Ad}	8.59 ± 0.13 ^B	8.45 ± 0.14 ^B	9.22 ± 0.52 ^A
C	32.08 ± 0.41 ^{Bb}	28.22 ± 0.98 ^{Cb}	52.86 ± 0.31 ^{Aa}	33.88 ± 0.84 ^{Ba}	30.25 ± 0.79 ^{Ca}	41.32 ± 1.60 ^{Aa}	21.08 ± 0.39 ^{Ba}	17.46 ± 0.91 ^C	28.62 ± 0.89 ^A
D	14.24 ± 0.19 ^{Be}	12.12 ± 0.52 ^{Ce}	18.94 ± 0.36 ^{Ae}	5.47 ± 0.19 ^{Bf}	4.21 ± 0.83 ^{Cg}	8.00 ± 0.14 ^{Ae}	3.51 ± 0.18 ^A	3.12 ± 0.32 ^A	3.07 ± 0.19 ^A
E	37.14 ± 0.29 ^{Ba}	35.86 ± 0.15 ^{Ca}	45.78 ± 0.87 ^{Ab}	16.85 ± 0.59 ^{Bc}	14.97 ± 0.23 ^{Cc}	22.54 ± 0.18 ^{Ac}	12.20 ± 0.18 ^{Ac}	11.15 ± 0.08 ^B	3.51 ± 0.09 ^C
F	13.18 ± 0.30 ^{Bf}	11.84 ± 1.06 ^{Be}	15.98 ± 0.19 ^{Af}	10.69 ± 0.40 ^{Be}	8.26 ± 0.18 ^{Cf}	11.74 ± 0.29 ^{Ad}	7.38 ± 0.17 ^A	6.87 ± 0.11 ^B	6.76 ± 1.10 ^{AB}
G	14.70 ± 0.13 ^{Ad}	10.78 ± 0.18 ^{Be}	10.41 ± 0.19 ^{Bg}	14.87 ± 0.45 ^{Ad}	9.97 ± 1.05 ^{Bef}	8.14 ± 0.27 ^{Be}	11.13 ± 0.31 ^A	9.74 ± 0.25 ^B	5.38 ± 0.14 ^C
H	17.93 ± 0.24 ^{Bc}	14.89 ± 0.74 ^{Cd}	33.82 ± 0.34 ^{Ad}	15.98 ± 0.21 ^{Bc}	13.45 ± 0.08 ^{Cd}	36.26 ± 0.58 ^{Ab}	17.01 ± 0.49 ^B	15.59 ± 0.99 ^B	32.29 ± 0.66 ^A

386 Samples A-H are shown in Figure 1. Values represent the mean of three independent experiments ± S.D. Different capital letters indicate
 387 significant differences between dry, soaked, and cooked samples. Different lower case letters indicate significant differences between samples.
 388 The criterion for statistical significance was $p < 0.05$.

389 **Table 2. Total phenolic content and antioxidant activity of the boiling water (after protein precipitation)**

Sample	Before protein precipitation			After protein precipitation		
	TPC (mg/100 g DW)	FRAP (mg/100 g DW)	DPPH (mg/100 g DW)	TPC (mg/100 g DW)	FRAP (mg/100 g DW)	DPPH (mg/100 g DW)
A	17.38 ± 0.06 ^{Ab}	16.50 ± 2.10 ^{Ab}	13.45 ± 0.45 ^{Ab}	5.13 ± 0.11 ^{Bb}	5.17 ± 0.05 ^{Bb}	4.28 ± 0.26 ^{Ba}
B	13.04 ± 0.16 ^{Ad}	8.51 ± 0.14 ^{Ac}	5.95 ± 0.63 ^{Ad}	4.17 ± 0.08 ^{Bc}	3.31 ± 0.11 ^{Bd}	1.76 ± 0.06 ^{Bc}
C	15.12 ± 0.06 ^{Ac}	13.01 ± 1.58 ^{Ab}	4.04 ± 0.17 ^{Ae}	5.58 ± 0.08 ^{Ba}	6.61 ± 0.05 ^{Ba}	1.91 ± 0.12 ^{Bc}
D	4.43 ± 0.06 ^{Ag}	0.87 ± 0.19 ^{Ag}	7.08 ± 0.63 ^{Ac}	2.58 ± 0.06 ^{Bf}	0.85 ± 0.04 ^{Ag}	0.28 ± 0.01 ^{Be}
E	10.56 ± 0.43 ^{Ae}	4.97 ± 0.02 ^{Ad}	13.27 ± 0.53 ^{Ab}	5.47 ± 0.11 ^{Ba}	3.18 ± 0.09 ^{Bd}	1.15 ± 0.67 ^{Bcd}
F	5.49 ± 0.03 ^{Af}	2.89 ± 0.09 ^{Ae}	7.14 ± 0.29 ^{Ac}	2.90 ± 0.13 ^{Be}	1.94 ± 0.02 ^{Bf}	0.67 ± 0.20 ^{Bd}
G	4.60 ± 0.24 ^{Ag}	2.39 ± 0.09 ^{Af}	3.88 ± 0.20 ^{Ae}	3.82 ± 0.13 ^{Bd}	2.17 ± 0.02 ^{Be}	0.75 ± 0.02 ^{Bd}
H	28.56 ± 0.06 ^{Aa}	25.59 ± 0.45 ^{Aa}	18.97 ± 0.12 ^{Aa}	4.73 ± 0.09 ^{Bc}	4.12 ± 0.07 ^{Bc}	2.86 ± 0.06 ^{Bb}

390 Samples A-H are shown in Figure 1. Values represent the mean of three independent experiments ± S.D. Different capital letters indicate
 391 significant differences between measurements made before and after protein precipitation. Different lower case letters indicate differences
 392 between samples. The criterion for statistical significance was $p < 0.05$.

393 **Table 3. Bioaccessibility of phenolic compounds from raw and cooked pulses**

Sample	Gastric phase		Intestinal phase	
	Raw (mg/100 g DW)	Cooked (mg/100 g DW)	Raw (mg/100 g DW)	Cooked (mg/100 g DW)
A	28.61 ± 0.88 ^{Bb}	43.93 ± 1.56 ^{Ab}	36.44 ± 1.03 ^{Ba}	50.06 ± 2.54 ^{Aa}
B	19.90 ± 0.25 ^{Bb}	35.26 ± 1.11 ^{Ab}	22.22 ± 0.46 ^{Ba}	40.02 ± 1.08 ^{Aa}
C	46.51 ± 1.28 ^{Bb}	55.40 ± 0.71 ^{Ab}	50.16 ± 0.78 ^{Ba}	62.87 ± 1.36 ^{Aa}
D	19.96 ± 0.80 ^{Bb}	39.52 ± 2.03 ^{Ab}	22.47 ± 0.38 ^{Ba}	46.65 ± 1.40 ^{Aa}
E	57.29 ± 1.43 ^{Bb}	83.53 ± 1.67 ^{Ab}	65.57 ± 1.13 ^{Ba}	90.27 ± 1.24 ^{Aa}
F	20.23 ± 0.52 ^{Bb}	34.02 ± 2.09 ^{Ab}	23.00 ± 0.39 ^{Ba}	40.86 ± 1.12 ^{Aa}
G	20.63 ± 0.70 ^{Bb}	28.30 ± 0.79 ^{Ab}	23.25 ± 0.38 ^{Ba}	33.49 ± 0.93 ^{Aa}
H	25.35 ± 0.54 ^{Bb}	40.71 ± 1.46 ^{Ab}	30.39 ± 0.26 ^{Ba}	45.38 ± 0.83 ^{Aa}

394 Samples A-H are shown in Figure 1. Values represent the mean of three independent experiments ± S.D. Different capital letters indicate
 395 significant differences between raw and cooked samples after the same digestive phase. Different lower case letters indicate significant
 396 differences between digestive phases for raw or cooked samples. The criterion for statistical significance was $p < 0.05$.

397 **Table 4. FRAP values of enzymatic digestive extracts from raw and cooked pulses**

Sample	Gastric phase		Intestinal phase	
	Raw (mg/100 g DW)	Cooked (mg/100 g DW)	Raw (mg/100 g DW)	Cooked (mg/100 g DW)
A	21.73 ± 0.08 ^{Bb}	60.09 ± 1.51 ^{Aa}	24.83 ± 0.99 ^{Ba}	64.64 ± 2.97 ^{Aa}
B	12.18 ± 0.18 ^{Ba}	24.86 ± 0.54 ^{Aa}	12.49 ± 0.15 ^{Ba}	25.77 ± 1.07 ^{Aa}
C	42.73 ± 0.46 ^{Ba}	92.17 ± 4.13 ^{Aa}	45.08 ± 2.26 ^{Ba}	96.97 ± 5.22 ^{Aa}
D	7.51 ± 0.25 ^{Bb}	16.24 ± 0.72 ^{Ab}	8.89 ± 0.32 ^{Ba}	20.61 ± 0.88 ^{Aa}
E	19.91 ± 0.18 ^{Bb}	34.12 ± 0.51 ^{Ab}	21.11 ± 0.32 ^{Ba}	40.16 ± 1.35 ^{Aa}
F	12.41 ± 0.09 ^{Bb}	38.20 ± 0.80 ^{Ab}	14.39 ± 0.36 ^{Ba}	47.40 ± 3.01 ^{Aa}
G	17.98 ± 0.20 ^{Bb}	21.85 ± 2.21 ^{Ab}	22.18 ± 0.24 ^{Ba}	28.62 ± 0.74 ^{Aa}
H	19.74 ± 0.21 ^{Ba}	39.97 ± 0.91 ^{Ab}	15.15 ± 0.71 ^{Bb}	50.49 ± 0.64 ^{Aa}

398 Samples A-H are shown in Figure 1. Values represent the mean of three independent experiments ± S.D. Different capital letters indicate
 399 significant differences between raw and cooked samples after the same digestive phase. Different lower case letters indicate significant
 400 differences between digestive phases for raw or cooked samples. The criterion for statistical significance was $p < 0.05$.

401 **Table 5. DPPH values of enzymatic digestive extracts from raw and cooked pulses**

Sample	Gastric phase		Intestinal phase	
	Raw (mg/100 g DW)	Cooked (mg/100 g DW)	Raw (mg/100 g DW)	Cooked (mg/100 g DW)
A	20.89 ± 0.48 ^{Bb}	59.03 ± 0.52 ^{Ab}	22.24 ± 0.48 ^{Ba}	69.61 ± 1.05 ^{Aa}
B	10.87 ± 0.49 ^{Bb}	26.89 ± 0.37 ^{Ab}	15.23 ± 0.56 ^{Ba}	30.31 ± 1.19 ^{Aa}
C	24.82 ± 0.64 ^{Ba}	63.95 ± 1.16 ^{Ab}	25.87 ± 0.31 ^{Ba}	71.69 ± 2.41 ^{Aa}
D	5.50 ± 0.94 ^{Bb}	11.76 ± 0.33 ^{Ab}	9.16 ± 0.93 ^{Ba}	24.91 ± 2.16 ^{Aa}
E	15.34 ± 0.38 ^{Bb}	34.21 ± 0.48 ^{Ab}	17.34 ± 0.52 ^{Ba}	38.10 ± 0.84 ^{Aa}
F	10.49 ± 1.47 ^{Ba}	26.26 ± 0.59 ^{Ab}	10.39 ± 0.75 ^{Ba}	32.40 ± 1.87 ^{Aa}
G	14.01 ± 0.52 ^{Ba}	32.76 ± 0.86 ^{Ab}	14.59 ± 1.09 ^{Ba}	37.68 ± 1.00 ^{Aa}
H	21.39 ± 0.75 ^{Bb}	51.69 ± 1.43 ^{Ab}	24.50 ± 0.68 ^{Ba}	59.15 ± 1.95 ^{Aa}

402 Samples A-H are shown in Figure 1. Values represent the mean of three independent experiments ± S.D. Different capital letters indicate
 403 significant differences between raw and cooked samples after the same digestive phase. Different lower case letters indicate significant
 404 differences between digestive phases for raw or cooked samples. The criterion for statistical significance was $p < 0.05$.