1 **Bioaccessibility and antioxidant activity of phenolic compounds in**

2 **cooked pulses**

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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.

Abstract

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

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

1. Introduction

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

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

Knowledge on resistance to cooking conditions and on bioaccessibility as the first step 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.

2. Materials and methods

2.1 Chemicals and reagents

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

2.2 Thermal processing

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

98   Homogenization was performed using a T-25 ULTRA-TURRAX® homogenizer (IKA,

99   Staufem, 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 $10,000 \times g$ 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   GENESYS™ 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.

2.4 Antioxidant activity

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

2.4.1 FRAP assay

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

2.4.2 DPPH assay

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

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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 ± 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 $p < 0.05$.

3. Results and discussion

3.1 Total phenolic content and antioxidant capacity of dry legume seeds

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

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

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

4. Conclusions

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

283 **Conflict of interests**

284 The authors declare no conflict of interests

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383 **Legends to Figure**

384 **Figure 1. Studied legumes**

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]

Samples A-H are shown in Figure 1. Values represent the mean of three independent experiments ± S.D. Different capital letters indicate

significant differences between dry, soaked, and cooked samples. Different lower case letters indicate significant differences between samples.

The criterion for statistical significance was $p < 0.05$.

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]

Samples A-H are shown in Figure 1. Values represent the mean of three independent experiments ± S.D. Different capital letters indicate significant differences between measurements made before and after protein precipitation. Different lower case letters indicate differences 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 $\pm$ 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 $p<0.05$.

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 $p < 0.05$.