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A comprehensive investigation of the behaviour of phenolic compounds in legumes during domestic cooking and *in vitro* digestion

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Running title: Fate of legume phenolic compounds during cooking and digestion

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Abstract

Legumes represent staple foods rich in phenolic compounds, which are often consumed after soaking and boiling. This study determines the fate of phenolic compounds from six legumes varieties belonging to the species *Lens culinaris* Medik., *Phaseolus vulgaris* L. and *Cicer arietinum* L. after soaking, boiling and digestion. To this purpose, a new HPLC-DAD method was developed and validated. Results show that the cooking process strongly reduces the content in free and bound phenolic compounds and that the processing water is a valuable source of phenolics. Bioaccessibility of phenolics from the legume matrix was investigated separately in the coat and the cotyledons of three chosen varieties (black beans, black lentils and pinto beans) by means of a standardized *in vitro* digestion protocol. Results showed that only a fraction of the phenolic compounds is bioaccessible, which may have implications for human health.

Keywords: Legumes; anthocyanins; phenolic compounds; in-vitro digestion; bioaccessibility.

14 **1. Introduction**

15 Legumes and pulses are commonly eaten around the world and represent a valuable replacement for
16 meat as a source of proteins. Legumes have unique nutritional and health-promoting properties
17 related to the content in essential nutrients like proteins, low GI carbohydrates, minerals, vitamins
18 as well as phytochemicals including polyphenols (Hall et al., 2017). Pulses are commonly eaten
19 cooked, and a soaking step prior to cooking is a common practice to shorten the cooking time.
20 Despite the anticipated strong effect of cooking, studies on the phenolic profile in cooked pulses are
21 scarce. Kalogeroupolous and coworkers analyzed the content in phenolic compounds in 14 cooked
22 legumes varieties and reported a significant decrease in their concentration after cooking
23 (Kalogeroupoulos et al., 2010). Accordingly, Zhang et al. (2014) showed a significant reduction of
24 total phenolic and condensed tannin both in the free and the bound form in boiled lentils. Xu and
25 coworkers found that soaking, boiling and steaming significantly affected the total phenolic content
26 in peas, chickpeas and lentils depending on the type of legume and processing conditions (Xu et al.,
27 2008). Another study showed a decrease in the phenolic content of faba beans after soaking and
28 boiling (Siah et al., 2014). When phenolic acids are considered, conflicting results on the effect of
29 cooking on them have been reported, likely reflecting the simultaneous occurrence of several
30 phenomena: (i) partial release of free phenolics from covalently bound ones, (ii) thermal
31 degradation and (iii) increase of the physically bound form caused by interactions of phenolic acids
32 with macromolecules of the food matrix (Zaupa et al., 2015).
33 To the best of author's knowledge, there are only few studies that have investigated the distribution
34 of phenolics in the legumes coat and cotyledon. Luo and coworkers investigated the distribution of
35 phytochemicals in cooked coats and cotyledons of mung beans and adzuki beans (Luo et al., 2016),
36 while Ranilla and coworkers investigate the phenolic profile of coats and cotyledons of 25 Brazilian
37 and 3 Peruvian bean cultivars (Ranilla et al., 2008). More recently, Peng and coworkers have
38 reported the extractable and non-extractable bound phenolic compositions and their antioxidant
39 properties in seed coat and cotyledon of black soybean (*Glycinemax* L. *merr*). Therefore, there is

40 demand for a comprehensive analysis of these phytochemicals in cooked pulses with a special
41 emphasis on their localization in the seeds coat and cotyledons. Furthermore, previous reports have
42 not considered the leaching of phenolic compounds into soaking and cooking water.
43 To exert their beneficial effects, polyphenols should be bioavailable. The bioavailability depends on
44 bioaccessibility, i.e. the fraction of a compound that is released from its food matrix in the
45 gastrointestinal tract and thus becomes available for intestinal absorption (Cilla et al., 2017).
46 However, information on the effect of food processing on the bioaccessibility of polyphenol in
47 legumes is very scarce and limited to few commonly eaten varieties (La Parra et al., 2008;
48 Stanisavljevic et al., 2013; Hithamani et al., 2014; Zhang et al., 2017). Information on the
49 bioavailability or bioaccessibility of nutrients and bioactive compounds in food products is of
50 utmost importance for a correct estimation of the actual intake which may be largely overestimated
51 when only the mere content in the food product is considered (Barba et al., 2017). Within plant
52 cells, phenolic compounds are deposited in vacuoles (Beckman et al., 2000). During heat treatments
53 and chewing, plant cells are broken open and phenolic compounds are released and interact with
54 cell wall material. Current evidence suggests that interactions of anthocyanins, phenolic acids and
55 procyanidins with cellulose and pectin greatly limits phenolics bioavailability (Padayachee et al.,
56 2013; Padayachee et al., 2012a; Padayachee et al., 2012b; Phan et al., 2015; 2017; Liu et al., 2017).
57 Legume cell walls mostly consist of cellulose, pectins and hemicelluloses but their composition
58 shows huge differences in coats and cotyledons, so that a differential effect may be expected in
59 terms of phenolics bioaccessibility from different seed tissues. Therefore, the aims of this study
60 were to determine the effect of soaking and boiling on the content and bioaccessibility of fourteen
61 phenolic compounds in six pulses varieties, namely black lentils (*Lens culinaris* Medik), black
62 beans (*Phaseolus vulgaris* L.), ruviotto beans (*Phaseolus vulgaris* L.), pinto beans (*Phaseolus*
63 *vulgaris* L.), desi chickpeas (*Cicer arietinum* L.) and black chickpeas (*Cicer arietinum* L.).
64 Moreover, the bioaccessibility of the selected phenolics has been investigated in black lentils (*Lens*
65 *culinaris* Medik), black beans (*Phaseolus vulgaris* L.) and pinto beans (*Phaseolus vulgaris* L.). To

66 this purpose, a new HPLC-DAD method has been developed and validated. To determine the effect
67 of plant tissue on polyphenols bioaccessibility, coats and cotyledons of three chosen varieties (black
68 beans, black lentils and pinto beans) were separately digested using a standardized *in vitro* protocol.

69 2. Material and Methods

70 2.1 Reagents and chemicals

71 Pure standard analytes, including gallic acid, catechin, chlorogenic acid, syringic acid, epicatechin,
72 ferulic acid, rutin, quercetin, hydrochloric acid (HCl) and formic acid were obtained from Sigma–
73 Aldrich (Zwijndrecht, The Netherlands). Kaempferol 3-glucoside and delphinidin 3-glucoside were
74 provided by Phytolab (PhytoLab GmbH & Co, Vestenbergsgreuth, Germany). 4-hydroxy-benzoic
75 acid and ethyl acetate were purchased from Merck (Merck GmbH, Darmstadt, Germany). Cyanidin
76 3,5-diglucoside, cyanidin 3-glucoside, pelargonidin 3,5-diglucode and cyanidin chloride were
77 obtained from Extrasynthese (Genay, France). Porcine pepsin (P6887, 3.200-4.500 U/mg protein),
78 pancreatin (P1750, 4X USP) and porcine bile extract (B8631) were purchased from Sigma-Aldrich
79 Ltd. (St. Louis, MO, USA). HPLC grade methanol, acetonitrile, diethyl ether was acquired from
80 Actu-All chemical (Oss, The Netherlands). Deionised water (< 8 MΩ cm resistivity) was obtained
81 from Milli-Q PureLab Ultra (Veolia Water Technologies, Ede, The Netherlands).

82 2.2 Sampling and sample preparation

83 Black lentils, pinto beans, ruviotto beans, black beans, black chickpeas and desi chickpeas were
84 kindly provided by Fertitecnica Colfiorito (Fertitecnica Colfiorito S.r.l., Italy). An aliquot of the
85 dried seeds was directly powdered with a Miller mix (Miller mix MM 400, Retsch GmbH, Haan,
86 Germany), passed through sieve n. 40 (0.420 mm) and the flour obtained was stored in an airtight
87 container at -20 °C till extraction.
88 Soaked and cooked samples were obtained following the instructions for use reported on the
89 packaging of each legume. Soaking time for black lentils was 2 h and boiling time was 20 min;
90 soaking time for black and pinto beans was 12 h and boiling time was 40 min. Each seed variety

91 was soaked with a fourfold weight of tap water and boiled in water in a ratio 1:9 (w:v). The cooked
92 samples were manually de-hulled in order to obtain a coat fraction and a cotyledon fraction.
93 Boiled seeds, coats and cotyledons from each sample were freeze dried (freeze drying, SALM EN
94 KIPP, Breukelen, The Netherlands), milled (Miller mix MM 400, Retsch GmbH, Haan, Germany),
95 sieved (particle size less than 500 μm) (Analysette Pulverisette, Laborette, FRITSCH GmbH, Idar-
96 Oberstein, Germany) and stored in an airtight container at -20 °C till the extraction. Soaking water
97 and boiling water were also freeze dried. Prior of the HPLC analysis, they were reconstituted with
98 methanol and then filtered on PTFE 0.20 μm filters (Phenomenex, Utrecht, Netherlands).

99 **2.3 Extraction of polyphenols**

100 Free phenolics were extracted using the method reported by Giusti et al. (Giusti et al., 2017) with
101 few modifications. In brief, 1.5 g of the powdered sample was added with 7.5 mL of extraction
102 solvent (ethanol and redistilled water in the ratio of 70/30, v/v), added with HCl 2N until pH 4 and
103 extracted in an ultrasonic bath (Ultrasound bath, HBM Industriële, Moordrecht, The Netherlands)
104 for 1 h at 40 °C. This procedure was repeated twice. After centrifugation (Heraeus Multifuge X3R,
105 Thermo Scientific, Bleiswijk, The Netherlands) at 4415 *g* for 10 min at 3 °C, supernatants were
106 pooled. This extract solution was filtered (PTFE 0.20 μm, Phenomenex, Utrecht, Netherlands) and
107 then analyzed for the free phenolic fraction with HPLC-DAD. The obtained residues were blended
108 twice with 7.5 mL of 2 N NaOH added with 0.01% EDTA 10 mM and 0.1% ascorbic acid for 1 h at
109 room temperature on a multi reax shaking (Heidolph Instruments GmbH & Co., Schwabach,
110 Germany) to release ester- or ether-linked phenolics. Both supernatants were pooled and adjusted to
111 pH 2 by using 6 N HCl and extracted again using 10 mL ethyl-acetate:diethyl ether (50:50 v:v)
112 twice. After centrifugation at 4415 *g* for 10 min at 3 °C, the supernatants were concentrated by a
113 rotary evaporator at 20 °C (BÜCHI Labortechnik AG, Flawil, Switzerland). The residues were
114 reconstituted with 10 mL methanol and analyzed for the bound phenolic fraction through HPLC-
115 DAD after filtration on 0.20 μm PTFE filters (Phenomenex, Utrecht, Netherlands).

116 **2.4 *In vitro* bioaccessibility study**

117 Polyphenols bioaccessibility was studied in freeze dried cooked coat and cotyledon. After freeze-
118 drying, the material was reduced into fine powder (particle size <500 μm).
119 The *in vitro* digestion followed the procedure published by Minekus and coworkers (Minekus et al.,
120 2014) starting with the careful blending of 5 g of dried sample with the simulated oral fluids for 2
121 min at 37 °C.
122 To start the gastric phase, 10 mL of simulated gastric fluid (SGF) were added to the mixture. SGF
123 consisted of an electrolyte solutions (eSGF), a pepsin solution to achieve a 2000 U/mL activity in
124 the final mixture, and HCl 1 N to adjust the pH to 3. The samples were then incubated for 2 h at 37
125 °C. Finally, 20 mL of the gastric phase was mixed with 20 mL of simulated intestinal fluid (SIF),
126 which consisted of an electrolyte solutions (eSIF), bile extract solution (10 mM bile salts in the final
127 mixture), pancreatin (100 U/mL trypsin activity in the final mixture) and NaOH 1 N to adjust the
128 pH to 7. The samples were then incubated for 2 h at 37 °C. At the end of the intestinal digestion, the
129 digested samples were centrifuged and the collected supernatants were filtered and analyzed by
130 HPLC-DAD. Bioaccessibility % for each phenolic was calculated by the following equation:

131 $$Bioaccessibility\ \% = \frac{amount\ of\ phenolic\ in\ digestion\ supernatant}{amount\ of\ phenolic\ in\ digested\ sample} \times 100$$

132 **2.5 HPLC/DAD analyses of phenolic compounds**

133 HPLC/DAD analysis was performed using a Dionex Ultimate 3000 apparatus (Thermo
134 FisherScientific, Bleiswijk, The Netherlands) that includes a degasser, a quaternary pump, an auto
135 sampler, a column thermostat and a PDA detector. The separation of the analytes was achieved on a
136 RP-Polaris C18 column (150 x 4.6 mm I.D., 5 μm particle size) equipped with a Polar RP security
137 guard cartridge (4 x 3 mm I.D.). The column temperature was set at 30 °C. The mobile phase
138 consisted of 1% formic acid in water (v/v) (A) and methanol (B). Injection volume was 10 μL and
139 the flow rate was kept at 1 mL/min for a total run time of 35 min. The gradient program was: 0 min
140 90% (A), 17-22 min 40 % (A), 28 min 90% (A) 28-35 min 90% (A). HPLC/DAD analyses were
141 performed monitoring three different wavelengths: 275 nm for gallic acid, catechin, 4-

142 hydroxybenzoic acid, syringic acid, epicatechin, rutin and kaempferol 3-glucoside; 325 nm for
143 chlorogenic acid, ferulic acid and quercetin; 550 nm for cyanidin 3-glucoside, pelargonidin 3,5-
144 diglucoside, delphinidin 3-glucoside, cyanidin chloride and cyanidin 3,5-diglucoside. Phenolic
145 compounds were identified by comparing retention times and UV absorption spectra with available
146 standards. Quantification was performed with standard curves of external standards generated by
147 plotting HPLC peak areas against the concentrations (ppm) ($r^2 \geq 0.99$).

148 **2.6 Method validation**

149 The applied method was validated based on the previous method published by Giusti et al. in order
150 to analyse a larger number of phenolic compounds simultaneously (Giusti et al., 2017). The method
151 validation was performed taking into account the parameters of linearity, intraday and interday
152 repeatability, limit of detection (LODs), limit of quantification (LOQs) and recovery. Method
153 validation parameters can be found in Table 1, whereas a chromatogram of unprocessed black beans
154 is shown in Figure 1. Repeatability of the chromatographic procedure was calculated injecting three
155 replicate of a standard solution on the same day and on three different days. Run-to-run precision
156 %RSDs ranged from 0.04% for rutin to 8.92% for ferulic acid; day-to-day precision ranged between
157 0.15% for rutin to 9.56% for *p*-hydroxy-benzoic acid. The LODs and LOQs for each phenolic
158 compound were obtained by the analysis of standard solutions at known concentrations of the
159 analyte. Acceptable signal to noise ratios was set as 3:1 for the estimation of the LODs, and 10:1 for
160 the LOQs. The LODs achieved with this method were 0.015 mg/kg for all the analytes with the
161 exception of quercetin (0.03 mg/kg) and cyanidin 3,5-diglucoside (0.15 mg/kg). LOQs for all the
162 analytes were 0.05 mg/kg with the exception of quercetin (0.1 mg/kg) and cyanidin 3-5-diglucoside
163 (0.5 mg/kg). For each compound, the linearity of the analytical response was tested on a 6 points
164 calibration curve in a concentration range from 0.5 to 50 mg/kg. The obtained coefficients of
165 correlation were in any case higher than 0.992. Spiking studies at two fortification levels (10 and 20
166 mg/kg) were performed on the pulse samples, and percentages of recovery ranged between 48.4%
167 and 107.5% (10 mg/kg). For three analytes (i.e., gallic acid, cyanidin 3-glucoside and cyanidin

chloride) a recovery < 70% was obtained, which is anyway acceptable in a multiresidue methodology. Finally, quantification data of polyphenols in legumes were corrected for the recovery values obtained. The results are expressed as the mean of three independent samples ± SD.

2.7 Statistical analysis

Statistical analysis was carried out with Graphpad Prism 6 Version 6.01 – © 1992-2012. The results are expressed as the mean of three independent samples. Mean differences were tested using one-way analysis of variance (ANOVA), a further Tukey's test was applied. Data were considered significant for $p<0.05$.

3. Results and Discussion

3.1 Quantification of phenolic compounds in the raw legumes, soaking and boiling water

The validated analytical method described in section 2.6 was used to study the phenolic profile of the raw material and to monitor any loss of phenolics in the soaking and boiling water (Table 2). Differences among legumes were observed in the total phenolic content (i.e., the sum of free and bound phenolics) of raw legumes and in the relative distribution of phenolics between the food matrix and the processing water (soaking and boiling water) (Table 2). Soaking water was coloured in all the trials indicating that some soluble coloured constituents, such as anthocyanins, flavonoids and tannins, had leached into the soaking water. Gallic acid was detected only in the soaking water of black beans. In black beans, 4-hydroxy-benzoic and syringic acids were detected only in the boiling water. These compounds may have been released from more complex phenolics like lignin (for syringic acid) or anthocyanins (for 4-hydroxy benzoic acid) following the hydrothermal process. Likely for the same reason, 4-hydroxy-benzoic acid was found in higher concentration in soaking water than in the raw material of chickpeas and ruviotto beans. The same trend was observed for catechin in chickpeas where soaking water was richer in catechin than the raw material, likely because of procyanidins depolymerisation. Among hydroxycinnamic acids, ferulic acid was present in high concentrations in beans and showed a significant loss during soaking and boiling in black, pinto and ruviotto beans, while the leaching of chlorogenic acid was observed only

194 in the soaking water of chickpeas. Some leaching in the soaking water was also observed for rutin
195 and quercetin in black chickpeas. Kaempferol 3-glucoside was found in several legumes and at the
196 highest concentration in ruviotto beans but was never detected in the soaking or cooking water.
197 Cyanidin 3,5-diglucoside and cyanidin 3-glucoside were detected in the soaking water of raw black
198 beans and pinto beans, respectively. Pelargonidin 3,5-diglucoside was not detected in the raw pinto
199 beans due to a possible interaction with other matrix compounds that prevented an exhaustive
200 extraction (Tarascou et al., 2010; Padayachee et al., 2013; Bordenave et al., 2014) but was detected
201 in its soaking water probably due to a good water solubility of this compound. The same may hold
202 true for delphinidin 3-glucoside in black chickpeas that was detected only in the soaking water. Raw
203 black lentils showed the highest concentration of delphinidin 3-glucoside that was not detected in
204 any of their processing water. Conversely, a loss of delphinidin 3-glucoside from raw black beans
205 was detected in the boiling water. During boiling, therefore, thermal degradation of anthocyanins
206 and other phenolics occurred as well as leaching in the boiling water.
207 The presence of phenolics into the soaking and cooking water is due to the diffusion of the phenolic
208 compounds from the food matrix. However, some phenolics (e.g., rutin, delphinidin-3-glucoside
209 and quercetin) were found in soaking water but were not present in raw materials. An explanation
210 could be that the hydrolysis of some glycosylated forms, which were present in the raw material,
211 occurred and led to the aglycone form such as quercetin generated from rutin. However for other
212 phenolics, such as rutin, chlorogenic acid and delphinidin-3-glucoside, the only reasonable
213 explanation is that these phenolics were strongly bound to the cell wall material and could not be
214 efficiently extracted but they could be released in the soaking water. Furthermore, the diffusion of
215 phenolics in cooking water is certainly facilitated by the elevated temperature and by the destruction
216 of the structural integrity of the vegetal tissue during cooking (Kebe et al., 2015). However,
217 micro(structural) and compositional differences in legume tissues (e.g. a different content in fibers
218 and starch, Xu et al., 2008) may determine differences in the molecular diffusivity of phenolics into
219 soaking and cooking water as those observed in our samples.

220 **3.2 Quantification in cooked legumes: localization of phenolic in coat and cotyledons**

221 The quantification of the free and bound phenolic fraction present in the coat and cotyledon of the
222 analyzed legumes is shown in Tables 3 and 4, respectively. In general, from Tables 3 and 4 it can be
223 noted that the cooking process strongly reduced the content in free and bound phenolic compounds
224 in all the legumes studied. This loss was likely caused by thermal degradation of parent compounds
225 or leaching in the cooking water (Zaupa et al., 2015). Interestingly, the phenolic content was much
226 higher in the cooked cotyledon compared to the coat in all the legumes. This can be explained by a
227 more substantial solubilization of phenolics from the coat but also to inward diffusion of the coat
228 phenolics towards the cotyledon during the soaking step.

229 Among hydroxycinnamic acids, ferulic acid was mostly present in bound form in coat and
230 cotyledon of cooked black, pinto and ruviotto beans (Table 4). Free catechin was detected in cooked
231 chickpeas coat and cotyledons (Table 3). The lack of this flavonol in the raw chickpeas might be
232 explained by the release of monomers from oligomeric complexes (procyanidins) present in the raw
233 material (Zaupa et al., 2015). As bound form, catechin in chickpeas showed a slight stability and
234 small amounts were detected in both coat and cotyledons in cooked matrices. Free kaempferol 3-
235 glucoside was detected at a high concentration in cooked ruviotto cotyledons probably due to the
236 high concentration found in the raw product (Table 3). Small amounts were found also in black
237 lentils and its absence in the uncooked legume suggests that the extraction of kaempferol 3-
238 glucoside during cooking process was more efficient than the solvent extraction done in the raw
239 seed. The highest concentration of free anthocyanins after cooking was found in black legumes.
240 After cooking, small amounts of delphinidin 3-glucoside were found in black beans and black
241 lentils cotyledons. It is interesting to note that the legume matrix might have played a protective
242 role on the relatively thermally labile anthocyanins, compared to the leached anthocyanins, favoring
243 its retention in the cooked product. However, the release of phenolics during extraction and
244 processing can lead to co-pigmentation reactions where anthocyanins react with other molecules
245 (co-pigments) like organic acids, metals, or anthocyanins themselves (self-association,

246 intermolecular copigmentation, intramolecular copigmentation, metal complexation), creating more
247 complex molecules (Castaneda-Ovando et al., 2009; Sun et al., 2010). This reaction may have
248 affected the identification and quantification of anthocyanins in the present study (Jakobek et al.,
249 2011).

250 **3.3 *In vitro* study of bioaccessibility**

251 An *in vitro* gastrointestinal model was used to mimic the *in vivo* physiological steps of digestion.
252 We decided to take in consideration only pinto beans, black beans and black lentils as these
253 legumes were the richest sources of phenolic compounds among those analyzed. For each sample,
254 we investigated the bioaccessibility of phenolic compounds from coats and cotyledons separately,
255 to study the potential effect of the plant tissue on the release of phenolic compounds.
256 The HPLC analysis of supernatants from gastric and intestinal digestion, however, highlighted a
257 poor selectivity of the HPLC method for gallic acid and catechin, likely because of interferences
258 from polar substances coming from the enzymes and/or the simulated digestion fluids used in the
259 simulated digestion model (Figure not shown). For this reason, gallic acid and catechin could not be
260 reliably monitored during digestion. Since phenolics are reported to variably interact with proteins,
261 parallel simulated digestions without the addition of digestive enzymes were carried out for free
262 phenolics extracts from pinto beans, black beans and black lentils to check any possible effect of
263 interaction with the digestive enzymes on phenolics bioaccessibility. No significant differences
264 were observed between the concentration of the phenolic compounds at the end of the intestinal
265 phase in the system with digestive enzymes compared to simulated digestion without enzymes (data
266 not shown). To investigate the bioaccessibility of phenolics from the legume matrix, finely freeze-
267 dried samples of coat and cotyledons have been used and the bioaccessibility % for each phenolic
268 reported in Figure 2. Delphinidin 3,5-diglucoside, cyanidin 3-glucoside and cyanidin 3,5-
269 diglucoside were absent in the intestinal phase, probably due to their instability at intestinal pH, as
270 repeatedly reported (Wahyuningsih et al., 2017; Loypimai et al., 2016).

271 Chlorogenic acid from coat and cotyledons of pinto and black beans was detected in the supernatant
272 at the end of the intestinal phase. The same was observed for ferulic acid in pinto and black beans.
273 Pelargonidin 3,5-diglucoside and delphinidin 3-glucoside were detected after intestinal digestion of
274 coat samples of black beans. In black lentils, delphinidin 3-glucoside was released from coat and
275 cotyledons. The release of anthocyanins from cotyledons could have been probably due to diffusion
276 of coat anthocyanins to the cotyledon during the cooking process, as already hypothesized to
277 explain the tannin inclusions in endosperm of colored varieties of rice after cooking (Zaupa et al.,
278 2016). Data from Figure 2 show that only a limited amount of legume phenolics may be released
279 from the legume matrix during digestion and are available for absorption in the small intestine. This
280 is mostly due to the strong physical interaction between free phenolics and cell wall material
281 (Padayachee et al., 2013; Padayachee et al., 2012a; Padayachee et al., 2012b) and to the covalent
282 bond between phenolic acid (mostly ferulic acid) and cell wall polysaccharides which cannot be
283 cleaved by human pancreatic enzymes. The limited bioavailability of phenolics in the small
284 intestine makes phenolics available for the microbial metabolism in large intestine where they can
285 possibly exert a range of beneficial effects (Tomás-Barberán et al., 2016; Ahne et al., 2015). The
286 loss of phenolics during digestion is also related to degradation of anthocyanins into smaller, still
287 partly unidentified, compounds. Cyanidin 3-glucoside has been reported to be degraded to
288 protocatechuic acid during gastrointestinal digestion (De Ferrars et al., 2014). Interestingly, no big
289 differences were found in the bioaccessibility of the same phenolic compounds from coat and
290 cotyledon, which suggests that the rate of release of phenolic compounds from different seed tissues
291 is comparable at least when the tissues are finely ground. Further investigation is needed to
292 elucidate whether the same holds true for a coarsely ground material, which is what would be
293 expected after chewing of legumes.

294 **4. Conclusions**

295 A simple, fast and cost-effective HPLC method was developed for the simultaneous determination
296 of 14 polyphenols including 5 anthocyanins in legumes. This method was validated and proved to
297 be specific, linear, precise, accurate and robust and can be used conveniently for routine phenolics'
298 analysis. The phenolic content during preparation of six varieties of legume has been monitored to
299 study the loss of phenolic compounds in the soaking and cooking water. Moreover, the content in
300 phenolic compounds has been quantified separately in the cotyledon and the coat of cooked
301 legumes. The results demonstrated a substantial loss of these substances in processing water and
302 that the loss was largely dependent on the type of processed legume. This study has highlighted that
303 the cooking process reduced the content in heat sensitive compounds such as anthocyanins, but, at
304 the same time, favors the formation of simpler phenolics from complex ones. Finally, the *in vitro*
305 simulated digestion of legume samples has revealed that only a small fraction of the phenolics
306 initially present in the raw legume matrix is actually available for absorption in the small intestine.
307 Whereas this may limit bioavailability of phenolic compounds, it may also contribute to the
308 beneficial effect of legumes on gut and systemic health through modulation of gut microbiota
309 metabolism.

310 **References**

311 Anhé, F. F., Varin, T. V., Le Barz, M., Desjardins, Y., Levy, E., Roy, D., & Marette, A. (2015). Gut
312 microbiota dysbiosis in obesity-linked metabolic diseases and prebiotic potential of polyphenol-rich
313 extracts. *Current Obesity Reports, 4(4),*389-400.

314 Barba, F.J., Mariutti, L. R. B., Bragagnolo, N., Mercadante, A. Z., Barbosa-Cánovas, G. V., &
315 Orlien, V. (2017) Bioaccessibility of bioactive compounds from fruits and vegetables after thermal
316 and nonthermal processing. *Trends in Food Science & Technology* in press
317 10.1016/j.tifs.2017.07.006

318 Beckman, C.H., (2000). Phenolic-storing cells: keys to programmed cell death and periderm
319 formation in wilt disease resistance and in general defence responses in plants? *Physiological and*
320 *Molecular Plant Pathology, 57,* 101-110.

321 Bordenave, N., Hamaker, B. R., & Ferruzzi, M. G. (2014). Nature and consequences of non-
322 covalent interactions between flavonoids and macronutrients in food. *Food & Function, 5,* 18-34.

323 Castañeda-Ovando, A., Pacheco-Hernández, M., Páez-Hernández, M. E., Rodríguez, J. A., &
324 Galán-Vidal, J. C. (2009). Chemical studies of anthocyanins: A review. *Food Chemistry, 113,* 859–
325 871.

326 Cilla, A., Bosch, L., Barberá, R., & Amparo, A. (2017). Effect of processing on the bioaccessibility
327 of bioactive compounds – a review focusing on carotenoids, minerals, ascorbic acid, tocopherols
328 and polyphenols. *Journal of Food Composition and Analysis* in press
329 http://dx.doi.org/10.1016/j.jfca.2017.01.009

330 De Ferrars, R. M., Czank, C., Zhang, Q., Botting, N. P., Kroon, P. A., Cassidy, A., & Kay, C. D.
331 (2014). The pharmacokinetics of anthocyanins and their metabolites in humans. *British Journal of*
332 *Pharmacology, 171(13),* 3268-3282.

333 Giusti, F., Caprioli, G., Ricciutelli, M., Vittori, S., & Sagratini, G. (2017). Determination of
334 fourteen polyphenols in pulses by high performance liquid chromatography-diode array detection
335 (HPLC-DAD) and correlation study with antioxidant activity and colour. *Food Chemistry, 221,*
336 689–697.

337 Hall, C., Hillen, C., & Robinson, J.G. (2017) Composition, nutritional value and health benefits of
338 pulses. *Cereal Chemistry Journal, 94(1)*, 11-31.

339 Hithamani, G., & Srinivasan, K. (2014). Bioaccessibility of polyphenols from wheat (*Triticum*

340 *aestivum*), sorghum (*Sorghum bicolor*), green gram (*Vigna radiata*), and chickpea (*Cicer arietinum*)
341 as influenced by domestic food processing. *Journal of Agricultural and Food Chemistry*, *62,*
342 11170−11179.

343 Jakobek, L., Seruga, M., & Krivak, P. (2011). The influence of interactions among phenolic
344 compounds on the antiradical activity of chokeberries (*Aronia melanocarpa*). *International Journal*
345 *of Food Sciences and Nutrition, 62(4),* 345–352.

346 Kalogeropoulos, N., Chiou, A., Ioannou, M., Karathanos, V.T. Hassapidou, M., & Andrikopoulos
347 N. K. (2010). Nutritional evaluation and bioactive microconstituents (phytosterols, tocopherols,
348 polyphenols, triterpenic acids) in cooked dry legumes usually consumed in the Mediterranean
349 countries. *Food Chemistry, 121(3)*, 682-690.

350 Kebe, M., Renard, C. M. C. G., Maataoui, M. E., Amani, G. N. G., & Maingonnat, J.F. (2015).
351 Leaching of polyphenols from apple parenchyma tissue as influenced by thermal treatments.
352 *Journal of Food Engineering, 166,* 237-246.

353 La Parra, J.M., Glahn, R.P., & Miller, D. (2008) Bioaccessibility of phenols in common beans
354 (*Phaseolus vulgaris* L.) and iron (Fe) availability to Caco-2 cells. *Journal of Agricultural and Food*
355 *Chemistry*, *56,* 10999–11005.

356 Liu, D., Martinez-Sanz, M., Lopez-Sanchez, P., Gilbert, E. P., & Gidley, M. J. (2017). Adsorption
357 behaviour of polyphenols on cellulose is affected by processing history. *Food Hydrocolloids, 63,*
358 496-507.

359 Loypimai, P., Moongngarm, A., & Chottanom, P. (2016). Thermal and pH degradation kinetics of
360 anthocyanins in natural food colorant prepared from black rice bran. *Journal of Food Science and*
361 *Technology, 53(1),* 461–470.

362 Luo, J., Cai, W., Wu, T., & Xu, B. (2016). Phytochemical distribution in hull and cotyledon of
363 adzuki bean (*Vigna angularis* L.) and mung bean (*Vigna radiate* L.), and their contribution to
364 antioxidant, anti-inflammatory and anti-diabetic activities. *Food Chemistry, 201,* 350–360.

365 Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, N. C., Carrière, F.,
366 Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus,
367 B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J.,
368 Ménard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. S., Weitschies, W.,

369 & Brodkorb, A. (2014). A standardised static *in vitro* digestion method suitable for food-An
370 international consensus. *Food & Function, 5(6),* 1113–1124.

371 Padayachee, A., Netzel, G. Netzel, M., Day, L., Mikkelsen, D., & Gidley, M.J. (2013). Lack of
372 release of bound anthocyanins and phenolic acids from carrot plant cell walls and model composites
373 during simulated gastric and small intestinal digestion. *Food and Function, 4,* 906-916

374 Padayachee, A., Netzel, G. Netzel, M., Day, L., Zabaras, D., Mikkelsen, D., & Gidley, M. J.
375 (2012a). Binding of polyphenols to plant cell wall analogues – Part 1: Anthocyanins. *Food*
376 *Chemistry, 134(1),* 155-161.

377 Padayachee, A., Netzel, G., Netzel, M., Day, L., Zabaras, D., & Mikkelsen, D. (2012b). Binding of
378 polyphenols to plant cell wall analogues – Part 2: Phenolic acids. *Food Chemistry, 135(4),* 2287-
379 2292.

380 Peng, H., Li, W., Li, H., Deng, Z., & Zhang, Z. (2017). Extractable and non-extractable bound
381 phenolic compositions and their antioxidant properties in seed coat and cotyledon of black soybean
382 *(Glycinemax (L.) merr). Journal of Functional Food*, 32, 296-312.

383 Phan, A. D. T., Netzel, G., Wanga, D., Flanagan, B. M., D'Arcy, B. R., & Gidley, M. J. (2015).
384 Binding of dietary polyphenols to cellulose: Structural and nutritional aspects. *Food Chemistry,*
385 *171,* 388-396.

386 Phan, A. D. T., Flanagan, B. M., D'Arcy, B. R., & Gidley, M. J. (2017). Binding selectivity of
387 dietary polyphenols to different plant cell wall components: Quantification and mechanism. *Food*
388 *Chemistry, 233,* 216-227

389 Ranilla, L.G., Genovese, M.I., & Lajolo, F.M. (2007). Polyphenols and antioxidant capacity of seed
390 coat and cotyledon from brazilian and peruvian bean cultivars (*Phaseolus vulgaris* L.). *Journal of*
391 *Agricultural and Food Chemistry, 55,* 90−98.

392 Siah, S., Wood, J.A., Agboola, S., Konczak, I., & Blanchard, C.L. (2014). Effects of soaking,
393 boiling and autoclaving on the phenolic contents and antioxidant activities of faba beans (*Vicia faba*
394 L.) differing in seed coat colours. *Food Chemistry, 142*, 461–468.

395 Sun, J., Cao, X., Bai, W., Liao, X., & Hu, X. (2010). Comparative analyses of copigmentation of
396 cyanidin 3-glucoside and cyanidin 3-sophoroside from red raspberry fruits. *Food Chemistry, 120(4),*
397 1131-1137.

398 Stanisavljevic, N., Jovanovi, Z., Tihomir, C., Luki, J., Milju, J., Adovi, D. S., & Miki, A. (2013).
399 Extractability of antioxidants from legume seed flour after cooking and *in vitro* gastrointestinal
400 digestion in comparison with methanolic extraction of the unprocessed flour. *International Journal*
401 *of Food Science and Technology, 48,* 2096–2104.

402 Tarascou, I., Souquet, J. M., Mazauric, J. P., Carrillo, S., Coq, S., Canon F., Fulcrand, H., &
403 Cheynier, V. (2010). The hidden face of food phenolic composition. *Archives of Biochemistry and*
404 *Biophysics, 501,* 16–22.

405 Tomas-Barberàn, F. A., Selma, M. V., & Espin, J. C. (2016). Interactions of gut microbiota with
406 dietary polyphenols and consequences to human health. *Current Opinion in Clinical Nutrition and*
407 *Metabolic Care, 19(6),* 471-476.

408 Wahyuningsih, S., Wulandari, L., Wartono, M. W., Munawaroh, H., & Ramelan, A. H. (2017). The
409 effect of pH and color stability of anthocyanin on food colorant. *International Conference on Food*
410 *Science and Engineering* doi:10.1088/1757-899X/193/1/012047.

411 Xu, B., & Chang, S. K. C. (2008). Effect of soaking and steaming on total phenolic content and
412 antioxidant activities of cool season food legumes. *Food Chemistry, 110*, 1-13.

413 Zaupa, M., Calani, L., Del Rio, D. Brighenti, F., & Pellegrini, N. (2015). Characterization of total
414 antioxidant capacity and (poly)phenolic compounds of differently pigmented rice varieties and their
415 changes during domestic cooking. *Food Chemistry, 187,* 338–347.

416 Zaupa, M., Ganino, T., Dramis, L., & Pellegrini, N. (2016). Anatomical study of the effect of
417 cooking on differently pigmented rice varieties. *Food & Structure, 7,* 6-12.

418 Zhang, B., Deng, Y., Tang, T., Chen, P.X., Liu, R., Ramdath, D.D., Liu, Q., Hernandez, M., &
419 Tsao, R. (2014). Effect of domestic cooking on carotenoids, tocopherols, fatty acids, phenolics, and
420 antioxidant activities of lentils (*Lens culinaris*). *Journal of Agricultural and Food Chemistry,*
421 *62(52),* 12585-12594.

422 Zhang, B., Deng, Z., Tang, Y., Chen, P.X., Liu, R., Ramdath, D.D., Liu, Q., Hernandez, M., &
423 Tsao, R. (2017). Bioaccessibility, in vitro antioxidant and anti-inflammatory activities of phenolics
424 in cooked green lentil (*Lens culinaris*). *Journal of Functional Food, 32,* 248-255.

425 **Table 1**: Retention times (Rt), absorbance wavelengths (λ), regression equations, linearity (R^2), limits of detection (LODs), limits of quantification
426 (LOQs), intraday and interday repeatability (expressed as relative % standard deviation) and extraction recoveries. Results are the mean of three
427 independent experiments.

	Analytes	**Rt (min)**	**λ (nm)**	**Regression equations**	R^2	**LODs mg/kg**	**LOQs mg/kg**	**Repeatibility**		**Recoveries (%)**
								Intraday	**Interday**	
1	Gallic acid	3.8	275	y = 0.2706x + 0.0019	0.997	0.015	0.05	0.38	0.42	62.5
2	Catechin	7.9	275	y = 0.0618x - 0.0215	0.998	0.015	0.05	0.28	0.61	107.2
3	Cyanidin 3,5-diglucoside	8.3	550	y = 0.1792x - 0.1209	0.997	0.15	0.5	2.85	4.45	73.1
4	*p*-Hydroxy-benzoic acid	8.3	275	y = 0.2913x + 0.0329	0.992	0.015	0.05	8.35	9.56	93.6
5	Chlorogenic acid	8.8	325	y = 0.361x - 0.6526	0.993	0.015	0.05	0.07	4.05	107.5
6	Delfinidin 3-glucoside	9.6	550	y = 0.3344x - 0.2419	0.997	0.015	0.05	2.24	3.27	103.0
7	Syringic acid	9.8	275	y = 0.3604x - 0.5795	0.988	0.015	0.05	0.76	1.35	88.6
8	Cyanidin 3-glucoside	11.1	550	y = 0.2249x + 0.2527	0.993	0.015	0.05	0.21	0.26	65.0
9	Pelargonidin 3,5-diglucoside	11.9	550	y = 0.3569x - 0.1701	0.997	0.015	0.05	0.67	0.95	71.2
10	Ferulic acid	12.9	325	y = 0.0725x + 0.0597	0.996	0.015	0.05	8.92	9.21	80.0
11	Cyanidin chloride	15.5	550	y = 0.5605x + 0.77	0.999	0.015	0.05	1.08	3.74	48.4
12	Rutin	15.5	275	y = 0.2836x + 0.0944	0.993	0.015	0.05	0.04	0.15	91.4
13	Kampferol 3-glucoside	16.4	275	y = 0.2733x - 0.2056	0.998	0.015	0.05	0.56	1.88	80.9
14	Quercetin	18.7	325	y = 0.8786x - 0.0163	0.995	0.03	0.10	1.22	1.41	84.4

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Table 2: Phenolic content (mg/100 g dry weight) in unprocessed raw dry legumes (UP), soaking water (SW) and boiling water (BW). Phenolics identified in the unprocessed material are the sum of free phenolics and bound phenolics. Results are expressed as the mean ± standard deviation of three independent samples. Different letters indicate significant differences ($p<0.05$) in the content of each single phenolic compound in UP or SW or BW of different legumes.

		GA	4 HBA	SA	FA	ChA	Cat	Rut	K 3 gluc	Querc	Cy 3,5 digluc	Cy 3 gluc	Pel 3,5 digluc	Delp 3 gluc
Black beans	UP	21.3± 0.01^{a}	nd	nd	206.1± 0.58^{b}	8.2± 0.00^{c}	nd	0.2± 0.01	0.1± 0.00^{c}	0.3± 0.00	0.4± 0.01	20.2± 0.09^{a}	nd	20.8± 0.27^{b}
	SW	10.0± 0.04	nd	nd	94.5± 0.47^{b}	nd	nd	0.7± 0.19^{b}	nd	nd	0.7± 0.04	nd	nd	nd
	BW	nd	4.4± 0.00	12.2± 0.03^{b}	137.0± 0.03^{a}	nd	nd	nd	nd	nd	nd	nd	nd	2.1± 0.03
Black lentils	UP	10.0± 0.00^{b}	nd	4.3± 0.06^{a}	nd	17.3± 0.83^{a}	nd	nd	nd	nd	nd	nd	nd	207.2± 6.50^{a}
	SW	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	BW	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pinto beans	UP	nd	4.6± 0.20^{a}	2.3± 0.01^{b}	413.6± 6.62^{a}	10.0± 0.01^{b}	nd	nd	nd	nd	nd	0.3± 0.01^{b}	nd	nd
	SW	nd	nd	nd	113.7± 0.21^{a}	nd	nd	nd	nd	nd	nd	0.1± 0.00	4.5± 0.01	nd
	BW	nd	nd	nd	90.5± 0.06^{c}	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ruviotto beans	UP	nd	0.5± 0.02^{c}	nd	85.9± 0.13^{c}	7.3± 0.16^{d}	2.3± 0.03^{b}	nd	65.1± 1.8^{a}	nd	nd	nd	nd	nd
	SW	nd	6.7± 0.01^{b}	nd	49.6± 0.01^{c}	nd	nd	nd	nd	nd	nd	nd	nd	nd
	BW	nd	nd	nd	110.6± 1.87^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd
Black chickpeas	UP	10.6± 0.10^{b}	0.5± 0.21^{c}	2.2± 0.14^{b}	nd	nd	nd	nd	1.6± 0.01^{b}	nd	nd	nd	nd	nd
	SW	nd	1.4± 0.26^{c}	nd	nd	nd	nd	13.4± 0.58^{a}	nd	0.5± 0.59	nd	nd	nd	2.9± 0.02
	BW	nd	nd	33.5± 0.14^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Chickpeas	UP	nd	2.1± 0.01^{b}	2.0± 0.00^{c}	nd	nd	2.6± 0.04^{a}	nd	nd	nd	nd	nd	nd	nd
	SW	nd	7.3±	nd	nd	5.2±	115.5±	nd	nd	nd	nd	nd	nd	nd

		0.08[a]			0.00	0.04							
BW	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

GA: Gallic acid; 4 HBA: 4-hydroxybenzoic acid; SA: Syringic acid; FA: Ferulic acid; ChA: Chlorogenic acid; Cat: Catechin; Rut: Rutin; K 3 gluc: Kaempferol 3-glucoside; Querc: Quercetin; Cy 3,5 digluc: Cyanidin 3,5-diglucoside; Cy 3 gluc: Cyanidin 3-glucoside; Pel 3,5 digluc: Pelargonidin 3,5-diglucoside; Delp 3 gluc: Delphinidin 3-glucoside.

nd: not detected.

Table 3.: Free phenolics content of unprocessed (UP) pulses, coats and cotyledons (Coty) of cooked pulses quantified by HPLC-DAD and expressed as mg/100 g of dry weight sample. Results are expressed as the mean ± standard deviation of three independent samples. Different letters indicate significant differences ($p<0.05$) in the content of each single phenolic compound in raw material or coat or cotyledon of different legumes.

	Black Beans			**Black lentils**			**Pinto beans**			**Ruviotto beans**			**Chickpeas**		
	UP	**Coat**	**Coty**	**UP**	**Coat**	**Coty**	**UP**	**Coat**	**Coty**	**UP**	**Coat**	**Coty**	**UP**	**Coat**	**Coty**
GA	11.60±0.01^{a}	1.29±0.01	8.75±0.00^{a}	9.99±0.00^{b}	nd	nd	nd	nd	nd	nd	nd	10.37±0.02^{b}	nd	nd	nd
4 HBA	nd	nd	nd	nd	nd	nd	1.50±0.03^{b}	nd	nd	0.45±0.02^{c}	nd	nd	1.63±0.00^{a}	nd	nd
SA	nd	nd	0.79±0.00^{b}	4.32±0.06^{a}	nd	1.15±0.01^{a}	2.26±0.01^{b}	nd	nd	nd	nd	nd	1.95±0.01^{c}	nd	nd
FA	15.35±0.01^{b}	0.10±0.01^{a}	5.42±0.00^{a}	nd	nd	nd	36.90±0.05^{a}	0.06±0.12^{b}	4.93±0.02^{b}	14.86±0.10^{c}	0.02±0.00^{c}	0.27±0.00^{c}	nd	nd	nd
ChA	8.22±0.00^{c}	0.02±0.01	1.36±0.05^{b}	17.30±0.83^{a}	0.02±0.05	1.72±0.03^{a}	9.98±0.01^{b}	0.02±0.03	1.34±0.00^{b}	7.24±0.16^{d}	0.02±0.00	1.23±0.05^{c}	nd	nd	nd
Cat	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.52±0.02	20.75±0.23
Rut	0.22±0.01^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.01±0.00^{b}	nd	nd
K 3 gluc	1.35±0.00^{b}	nd	nd	nd	0.03±0.00^{b}	nd	nd	nd	nd	62.52±1.79^{a}	0.13±0.00^{a}	3.64±0.01	nd	nd	nd
Querc	0.33±0.00	nd	0.05±0.00	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cy	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cy 3,5 digluc	0.39±0.02	0.01±0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cy 3 gluc	20.16±0.09^{a}	nd	nd	nd	nd	nd	0.31±0.01^{b}	nd	nd	nd	nd	nd	nd	nd	nd
Pel 3,5 digluc	nd	0.01±0.02	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Delp 3 gluc	20.77±0.27^{b}	0.01±0.00	0.33±0.00^{a}	206.42±6.50^{a}	0.02±0.02	1.77±0.06^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd

GA: Gallic acid; 4 HBA: 4-hydroxybenzoic acid; SA: Syringic acid; FA: Ferulic acid; ChA: Chlorogenic acid; Cat: Catechin; Rut: Rutin; K 3 gluc: Kaempferol 3-glucoside; Querc: Quercetin; Cy 3,5 digluc: Cyanidin 3,5-diglucoside; Cy 3 gluc: Cyanidin 3-glucoside; Pel 3,5 digluc: Pelargonidin 3,5-diglucoside; Delp 3 gluc: Delphinidin 3-glucoside.

nd: not detected.

Table 4: Bound phenolics content of unprocessed (UP) pulses, coats and cotyledons (Coty) of cooked pulses quantified HPLC-DAD and expressed as mg/100 g of dry weight sample. Results are expressed as the mean ± standard deviation of three independent samples. Different letters indicate significant differences ($p<0.05$) in the content of each single phenolic compound in raw material or coat or cotyledon of different legumes.

	Black Beans			**Black lentils**			**Pinto beans**			**Ruviotto beans**			**Chickpeas**		
	UP	**Coat**	**Coty**	**UP**	**Coat**	**Coty**	**UP**	**Coat**	**Coty**	**UP**	**Coat**	**Coty**	**UP**	**Coat**	**Coty**
GA	9.68± 0.01	nd	nd	nd	0.80± 0.00	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4 HBA	nd	nd	nd	nd	nd	nd	3.08± 0.23^{a}	0.01± 0.00	nd	nd	nd	nd	0.48± 0.00^{b}	nd	nd
SA	nd	nd	nd	nd	0.01± 0.00^{b}	1.10± 0.02	nd	0.01± 0.00^{b}	nd	nd	nd	nd	nd	1.44± 0.02^{a}	nd
FA	190.78± 0.58^{b}	0.67± 0.02^{a}	10.36± 0.03^{a}	nd	0.02± 0.00^{c}	nd	376.73± 6.57^{a}	0.57± 0.01^{b}	16.94± 0.98^{b}	71.03± 0.03^{c}	0.67± 0.00^{a}	81.25± 1.45^{a}	nd	nd	nd
ChA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cat	nd	nd	0.30± 0.00^{d}	nd	0.09± 0.00^{a}	5.09± 0.01^{a}	nd	0.08± 0.01^{a}	0.46± 0.01^{b}	2.30± 0.04	0.05± 0.00^{b}	0.33± 0.00^{c}	2.65± 0.04	0.01± 0.00^{c}	0.22± 0.00^{e}
Rut	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
K 3 gluc	nd	nd	nd	nd	0.01± 0.00	0.67± 0.01^{a}	nd	nd	nd	2.60± 0.03	nd	0.51± 0.00^{b}	nd	nd	nd
Querc	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cy 3,5 digluc	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cy 3 gluc	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cy	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pel 3,5 digluc	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Delp 3 gluc	nd	nd	nd	0.70± 0.00	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

GA: Gallic acid; 4 HBA: 4-hydroxybenzoic acid; SA: Syringic acid; FA: Ferulic acid; ChA: Chlorogenic acid; Cat: Catechin; Rut: Rutin; K 3 gluc: Kaempferol 3-glucoside; Querc: Quercetin; Cy 3,5 digluc: Cyanidin 3,5-diglucoside; Cy 3 gluc: Cyanidin 3-glucoside; Pel 3,5 digluc: Pelargonidin 3,5-diglucoside; Delp 3 gluc: Delphinidin 3-glucoside.

nd: not detected

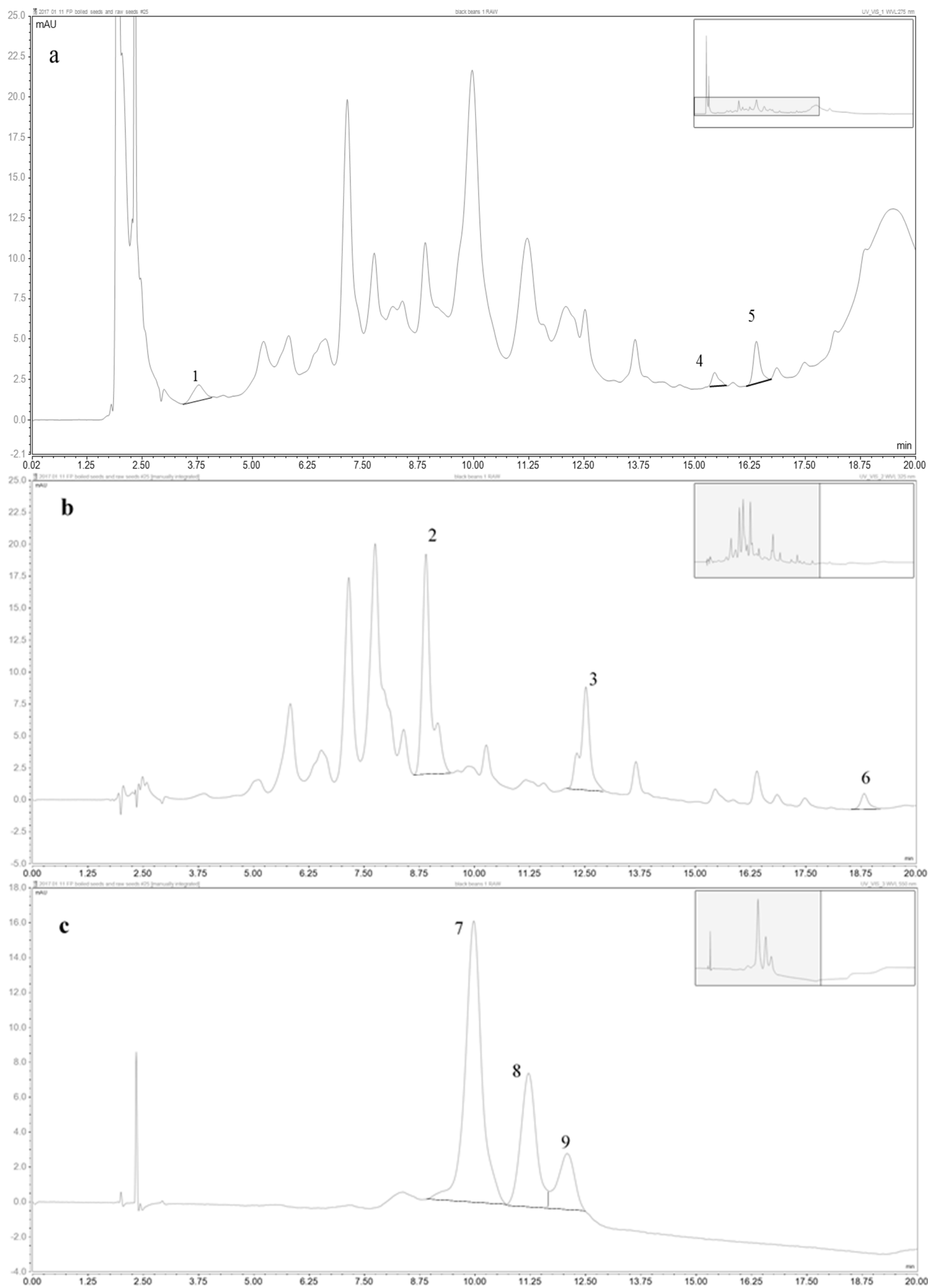

Figure 1: HPLC-DAD chromatogram of unprocessed black beans. The peak identification of the different polyphenols was observed at 275 nm (panel a), at 375 nm (panel b), and at 550 nm (panel c). (1) gallic acid, (2) chlorogenic acid, (3) ferulic acid, (4) rutin, (5) kaempferol 3 glucoside, (6) quercetin, (7) cyanidin 3-5 diglucoside, (8) cyanidin 3 glucoside, (9) delphinidin 3 glucoside.

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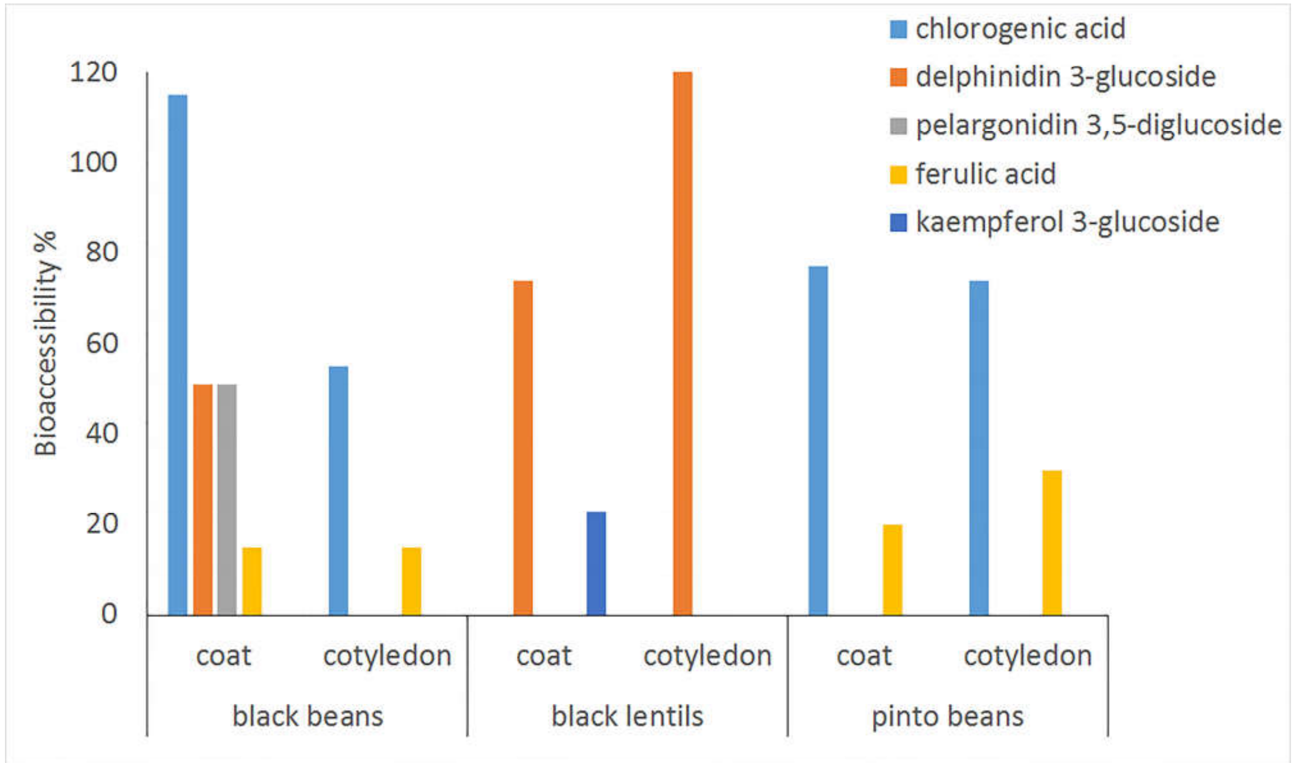

470 Figure 2. Bioaccessibility (%) of polyphenols from coat and cotyledon of cooked black beans, black
471 lentils and pinto beans at the end of an in vitro simulated gastro-intestinal digestion. Each data point
472 is the reported as average of 2 independent digestions ± SD.