

# 1 **Nutritional and Phytochemical Content of High-Protein Crops**

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## 8 **ABSTRACT**

9 Sustainable sources of high-protein plants could help meet future protein requirements.  
10 Buckwheat, green pea, fava bean, hemp and lupin were analyzed by proximate analysis and  
11 ICP-MS to determine the macro- and micronutrient content and LC-MS to elucidate the  
12 phytochemical profiles. The protein content ranged from 20-43 % (w/w) and all were found  
13 to be rich in insoluble fibre; 9-25 % (w/w). The selected crops had a favourable micronutrient  
14 profile with phosphorous levels ranging from  $2.22 \pm 0.05 \text{ g kg}^{-1}$  to  $9.72 \pm 0.41 \text{ g kg}^{-1}$ , while  
15 iron levels ranged from  $20.23 \pm 0.86 \text{ mg kg}^{-1}$  to  $69.57 \pm 7.43 \text{ mg kg}^{-1}$ . The crops contained  
16 substantial amounts of phytochemical compounds. In particular, buckwheat was a rich source  
17 of pelargonidin ( $748.17 \pm 75.55 \text{ mg kg}^{-1}$ ), epicatechin ( $184.1 \pm 33.2 \text{ mg kg}^{-1}$ ), quercetin  
18 ( $35.66 \pm 2.22 \text{ mg kg}^{-1}$ ), caffeic acid ( $41.74 \pm 22.54 \text{ mg kg}^{-1}$ ) and 3-hydroxyphenylacetic acid  
19 ( $63.64 \pm 36.16 \text{ mg kg}^{-1}$ ), hemp contained p-coumaric acid ( $84.02 \pm 8.10 \text{ mg kg}^{-1}$ ), cyanidin  
20 ( $58.43 \pm 21.01 \text{ mg kg}^{-1}$ ), protocatechualdehyde ( $34.77 \pm 5.15 \text{ mg kg}^{-1}$ ) and gentisic acid  
21 ( $31.20 \pm 1.67 \text{ mg kg}^{-1}$ ) and fava bean was the richest source of ferulic acid ( $229.51 \pm 36.58$   
22  $\text{mg kg}^{-1}$ ) and its 5-5' ( $39.99 \pm 1.10 \text{ mg kg}^{-1}$ ) and 8-5 dimers ( $58.17 \pm 6.68 \text{ mg kg}^{-1}$ ).  
23 Demonstrating that these crops are rich sources of protein, fibre and phytochemicals could

24 encourage higher consumption and utilisation as healthy and sustainable ingredients by the  
25 food and drink industry.

26 **KEYWORDS:** *sustainable and healthy food ingredients; future protein supply; high-protein*  
27 *plants; legumes; food security*

## 28 INTRODUCTION

29 Sourcing sufficient amounts of protein to meet future dietary requirements is a critical issue,  
30 which scientists and policy makers are currently addressing worldwide. Indeed a recent report  
31 by the European Innovation Partnership 'Agricultural Productivity and Sustainability'<sup>1</sup>  
32 identified the need for greater plant based protein production, both for animal feed and  
33 increasingly for direct human consumption. There is an accruing body of evidence  
34 identifying the urgent need to shift towards a more plant based diet for both environmental  
35 and physiological reasons.<sup>2,3</sup> At the moment, the global population is rapidly growing and  
36 with it the request of dietary protein, mainly from animal origin, which is projected to  
37 increase by more than 50% by 2030 compared to that of 2000.<sup>4</sup> The traditional Western  
38 dietary pattern focuses predominantly on animal-based products to satisfy protein  
39 requirements, and is environmentally detrimental as it relies on intensive livestock farming  
40 which deeply deteriorates the natural resources.<sup>5</sup> It has been estimated that in European  
41 Union 27 the total greenhouse gas emissions attributable to the livestock sector are between  
42 12 and 61% of the total anthropogenic emissions.<sup>6</sup> Also, from the physiological standpoint,  
43 the high intake of meat, especially red and processed, is associated with higher incidence of  
44 coronary heart diseases, diabetes mellitus, and several forms of cancer.<sup>7-9</sup> Greater  
45 consumption of dietary protein from sustainable plant sources appears an immediate and  
46 effective way to both mitigate the environmental impact of our diet, and to reach and  
47 maintain a healthier diet.<sup>10</sup>

48 The production and consumption of local food, including high protein crops, may contribute  
49 towards achieving a sustainable diet, although in high latitude countries such as Scotland, the  
50 shift in the agricultural system towards novel protein crop production, first needs an  
51 assessment of the feasibility and ability of the agri-environment to deliver appropriate crops  
52 of suitable quality and yield. To this end, five high protein crops have been identified that can

53 be or have potential to be sustainably grown in Scotland. These are lupin (*Lupinus albus*),  
54 hemp (*Cannabis sativa*), buckwheat (*Fagopyrum esculentum*), green pea (*Pisum sativum*),  
55 fava bean (*Vicia faba*). These crops offer a valid alternative to importing protein rich crops  
56 such as soybean, and contribute to enhance the diversity, and hence the economic stability of  
57 local agricultural production. They represent a rich source of energy, provide complex  
58 carbohydrates and high quality protein,<sup>11</sup> and are considered important sources of bioactive  
59 non-nutrient plant compounds, generally known as phytochemicals.<sup>12,13</sup>

60 Among the large group of phytochemicals, phenolic compounds have been widely  
61 investigated due to their ubiquitous presence in plants, and their beneficial biological effects  
62 in humans.<sup>14</sup> Phenolic compounds comprise a varied family of molecules derived from the  
63 phenylpropanoid pathway, with phenolic acids and flavonoids being the main classes of  
64 dietary phenolics in the European population.<sup>15</sup> Phenolic compounds can occur in the free  
65 form or bound to other cell wall components.<sup>16</sup> This aspect significantly impacts upon their  
66 availability in the body and associated benefits. Flavonoids are a large polyphenolic  
67 subgroup, which are normally conjugated to sugar molecules and can be further classified  
68 into anthocyanins, flavones, isoflavones, flavanones, flavonols and flavan-3-ols; they are all  
69 common dietary components.<sup>17</sup> There are many studies demonstrating the positive effects of  
70 a regular consumption of food rich in phenolic compounds.<sup>18,19</sup> These include the effects of  
71 polyphenols such as proanthocyanidins and quercetin glycosides from azuki bean seed coats  
72 which attenuated blood pressure elevation and ameliorated diabetic nephropathy.<sup>20,21</sup>  
73 Nevertheless, proanthocyanidins from fava bean and bird's-foot trefoil have shown to  
74 precipitate protein and minerals in the gastrointestinal tract reducing their bioavailability.<sup>22,23</sup>  
75 The aim of the present work was to determine the macro- micro- and non- nutrient  
76 (predominantly phenolic) profiles of commercially available food grade flours from lupin,  
77 hemp, buckwheat, green pea, fava bean and compare them to wheat flour. Furthermore, the

78 potential dietary role of these crops, which can be considered sources of both sustainable  
79 plant protein and bioactive phytochemicals, and the health benefits of plant protein rich diets  
80 are discussed.

81

## 82 MATERIALS AND METHODS

83 **Standards, reagents and plant material.** Standards and general laboratory reagents were  
84 purchased from Sigma-Aldrich (Gillingham, England) and Fisher Scientific UK Ltd.  
85 (Loughborough, England). Chemicals used for Inductively-Coupled Mass Spectrometry  
86 (ICP-MS) analysis were nitric acid of TraceSelect Ultra grade from Fluka (Gillingham,  
87 England), hydrochloric acid (30%) of Ultrapur grade from Merck (Darmstadt, Germany), and  
88 deionized water from Millipore (Bedford, USA). Single elements standards were purchased  
89 from all Inorganic Ventures (Christiansburg, USA). Commercially available flours were  
90 obtained as follows: strong white flour was purchased from Tesco Stores Ltd. (Cheshunt,  
91 UK); buckwheat flour was purchased from Arrowhead Mills, Inc. (Melville, USA); hemp  
92 flour was purchased from Yorkshire Hemp Ltd. (Drifffield, UK); fava bean flour was  
93 purchased from The Barry Farm (Wapakoneta, USA); green pea flour was purchased from  
94 Bob's Red Mill Natural Foods (Milkwaukie, USA); lupin flour was purchased from Terrena  
95 Lup' Ingredients (Martigne Ferchaud, France). All the products were purchased dried and  
96 milled. Flours were stored at room temperature apart from green pea flour which has been  
97 kept at 4 °C as specified by the manufacturer.

98

99 **Macronutrient Analysis.** Routine proximate analytical procedures were employed to  
100 determine the macronutrient composition of the flours. Protein was measured as total  
101 nitrogen by the Dumas combustion method using a Vario Max CN analyser and the nitrogen  
102 content multiplied by 6.25 to generate the protein concentration<sup>24</sup>. Resistant starch and

103 nonstarch polysaccharide (NSP) were measured according to the methods described by  
104 Englyst et al.<sup>25,26</sup> Total fat was determined by the Soxtec method (Soxtec™ 2050 Auto Fat  
105 Extraction System).<sup>27</sup>

106 **Micronutrient Analysis.** Samples (0.4 g) were suspended in distilled water (1 mL) and nitric  
107 acid (8 mL; 65% (v/v)) and placed in specialized acid digest tubes for microwave-assisted  
108 digestion (MARS 6, CEM, Matthews, USA). Samples were heated in two temperature  
109 gradient: (1) from 20 °C to 150 °C over 15 min and (2) 150 °C ramp to 165 °C over 10 min  
110 and then held at this temperature for 20 min. The measured isotopes analyzed by ICP-MS  
111 were: <sup>23</sup>Na, <sup>24</sup>Mg, <sup>31</sup>P, <sup>39</sup>K, <sup>44</sup>Ca, <sup>51</sup>V, <sup>52</sup>Cr, <sup>55</sup>Mn, <sup>56</sup>Fe, <sup>59</sup>Co, <sup>60</sup>Ni, <sup>63</sup>Cu, <sup>66</sup>Zn, <sup>78</sup>Se, <sup>95</sup>Mo,  
112 <sup>111</sup>Cd, <sup>202</sup>Hg, <sup>208</sup>Pb. All the element standards were used in stock solutions of 1000 mg L<sup>-1</sup>,  
113 which served for preparation of calibration solutions and internal standard solution. The  
114 decomposition matrix was nitric acid (2% (v/v)), hydrochloric acid (0.5% (v/v)) in distilled  
115 deionized water (Millipore, Bedford, MA) which was used for preparation of all the  
116 solutions. The ICP-MS measurements were done using Agilent 7700X spectrometer  
117 instrument (Agilent Technologies) equipped with a MicroMist nebulizer and nickel sampler  
118 and skimmer cones. The flow of standards and samples was joined together with a flow of  
119 Erbium internal standard solution (1 mg L<sup>-1</sup>). The mixed flow (approximately 500 µL min<sup>-1</sup>)  
120 was delivered by the peristaltic pump to the nebulizer of ICP-MS. Duration of ICP-MS  
121 analysis was 3.0 minutes. Data acquisition was one point, five replicates, 100 sweeps per  
122 replicate.

123

#### 124 **Phytochemical Analysis.**

125 The phytochemicals were extracted by four methods specifically optimized for flavan-3-ols,  
126 anthocyanins, other flavonoids/isoflavanoids and phenolic acids/other phenolic compounds.

127 **Flavanols.** Samples (approx. 0.25 g dry weight) were suspended in a solvent extraction  
128 mixture (acetone/water/acetic acid, 70/28/2 (v/v/v); 5 mL), placed in an ultrasound bath for  
129 10 min and the supernatant separated by centrifugation (5 min; 3220g; 4 °C). The extraction  
130 was repeated twice, the supernatants combined and the pellet freeze-dried for alkaline  
131 hydrolysis. The solvent was removed from the supernatants by rotary evaporation at  $T \leq 40$   
132 °C. The freeze-dried pellets were suspended in NaOH (3 mL;  $1 \text{ mol dm}^{-3}$ ) and stirred at room  
133 temperature for four hours under nitrogen. The pH was reduced to pH 2 with HCl ( $10 \text{ mol}$   
134  $\text{dm}^{-3}$ ) and the samples extracted into EtOAc (5 mL) by shaking for five minutes. This  
135 procedure was repeated twice and the EtOAc extracts combined and the solvent removed by  
136 rotary evaporation at  $T \leq 40$  °C. All extracts were dissolved in methanol/water (50% (v/v); 2  
137 mL) for Liquid Chromatography-Mass Spectrometry (LC-MS) analysis as detailed below.

138 **Anthocyanins.** Samples (0.1 g dry weight) were suspended in 1 mL of extraction mixture  
139 solvent (methanol/water/37%, HCl, 50/33/17 (v/v/v)) and placed in an ultrasound bath for 20  
140 min. The supernatants were separated by centrifugation (5 min; 3220 x g; 4 °C) and the  
141 remaining pellet freeze dried. The extraction was repeated twice and the supernatants  
142 combined and transferred to vials with a Teflon-lined screw cap. The vials were placed in a  
143 thermoblock and hydrolyzed at 100 °C for 60 min. Hydrolyzed samples were immediately  
144 cooled to room temperature, filtered on 0.2  $\mu\text{m}$  filter and analyzed by High-Performance  
145 Liquid Chromatography (HPLC), as detailed below. The freeze-dried pellets were suspended  
146 in 3 mL of extraction mixture solvent (methanol/water/37% HCl, 50/33/17 (v/v/v)) and  
147 placed in an ultrasound bath for 20 min. The samples were transferred to vials with a Teflon-  
148 lined screw cap and hydrolyzed as described above.

149 **Other flavonoids.** Samples (1 g dry weight) were suspended in methanol/water (60/40 (v/v))  
150 containing 0.1% acetic acid; 8 mL) and placed on ultrasound bath for 60 min then the  
151 supernatant was separated by centrifugation (5 min; 3220g; 4 °C) and the pellet freeze dried.

152 The extraction was repeated twice and the supernatants combined. The solvent was removed  
153 under reduced pressure at a temperature not exceeding 40 °C. The dried extracts were  
154 suspended in HCl (4 mL; 1 mol dm<sup>-3</sup>) and the samples incubated at 90 °C for one hour. After  
155 the acid hydrolysis the samples were extracted into EtOAc (10 mL) and the layers separated  
156 by centrifugation (5 mins; 3220g; 4 °C). The extraction was repeated three times and the  
157 EtOAc extracts combined. The solvent was removed under reduced pressure at a temperature  
158 not exceeding 40 °C. The residue was dissolved in methanol (1 mL) and analyzed by LC-MS  
159 as detailed below. The freeze dried pellet was suspended in HCl (7 mL; 1 mol dm<sup>-3</sup>),  
160 incubated at 90 °C for one hour and processed as described above.<sup>28</sup>

161 **Phenolic acids and other phenols.** Samples (approx. 0.1 g dry weight) were suspended in  
162 HCl (3 mL; 0.2 mol dm<sup>-3</sup>) and extracted into EtOAc (5 mL) and the layers separated by  
163 centrifugation (5 min; 1800g; 4 °C). The extraction was repeated twice and the EtOAc  
164 extracts combined. The organic layer was left to stand over sodium sulphate (anhydrous),  
165 filtered and the solvent removed under reduced pressure at a temperature not exceeding 40  
166 °C. The remaining aqueous fraction, obtained after EtOAc extraction, was neutralized and  
167 freeze dried. The freeze-dried pellets were suspended in NaOH (3 mL; 1 mol dm<sup>-3</sup>) and  
168 stirred at room temperature for four hours under nitrogen. The pH was reduced to pH 2 and  
169 samples extracted into EtOAc (5 mL). This was repeated twice and processed as described  
170 above. The pH of the aqueous fraction was then brought to pH 7 and the aqueous fraction was  
171 freeze dried. The freeze dried aqueous fractions were suspended in HCl (3 mL; 2 mol dm<sup>-3</sup>)  
172 and incubated at 95 °C for 30 min with intermittent mixing. The samples were cooled and  
173 extracted with EtOAc (5 mL x 3) and processed as described above. All extracts were  
174 dissolved in methanol (1 mL) for LC-MS analysis as described below.<sup>28</sup>

175



176 **Preparation of the extracts for LC-MS analysis.** An aliquot (20  $\mu\text{L}$ ) of the each type of  
177 extract prepared above was transferred to an eppendorf. Internal standard 1 for negative mode  
178 mass spectrometry (IS1;  $^{13}\text{C}$  benzoic acid; 2  $\text{ng } \mu\text{L}^{-1}$  in 75% methanol containing 0.02%  
179 acetic acid; 20  $\mu\text{L}$ ), internal standard 2 for positive mode mass spectrometry (IS2; 2-amino-  
180 3,4,7,8-tetramethylimidazo[4,5-f] quinoxaline; 0.5  $\text{ng } \mu\text{L}^{-1}$  in 75% methanol containing  
181 0.02% acetic acid; 20  $\mu\text{L}$ ) and acidified (HCl; 0.4  $\text{mol dm}^{-3}$ ) methanol (40  $\mu\text{L}$ ) were added.  
182 The samples were mixed well, centrifuged (12,500g; 5 min; 4  $^{\circ}\text{C}$ ) and the supernatants  
183 analyzed by LC-MS as detailed below.

184

185 **LC-MS Analysis.** Liquid chromatography separation of the metabolites was performed on an  
186 Agilent 1100 LC-MS system from using a Zorbax Eclipse 5 $\mu\text{m}$ , 150 mm x 4mm column  
187 from Agilent Technologies (Wokingham, UK) as described elsewhere.<sup>28,29</sup> Three gradients  
188 were used to separate the different categories of metabolites and the mobile phase solvents in  
189 each case were water containing 0.1% acetic acid (A) and acetonitrile containing 0.1% acetic  
190 acid (B). Method 1: 40–90% B (13 min), 90% B (1 min), 90–40% B (1 min), 40% B (9 min);  
191 method 2: 10–55% B (45 min), 55–80% B (15 min), 80% B (3 min), 80–10% B (0.2 min),  
192 10% B (4.8 min) and method 3: 50–80% B (10 min), 80% B (2 min), 80–50% B (1 min),  
193 50% B (4 min). In all cases the flow rate was 300  $\mu\text{L min}^{-1}$  with an injection volume of 5  $\mu\text{L}$ .  
194 The LC eluent was directed into, without splitting, an ABI 3200 triple quadrupole mass  
195 spectrometer (Applied Biosystems, Warrington, UK) fitted with a Turbo Ion Spray<sup>TM</sup> (TIS)  
196 source. For LC methods 1 and 2, the mass spectrometer was run in negative ion mode with  
197 the following source settings: ion spray voltage -4500 V, source temperature 400  $^{\circ}\text{C}$ , gases 1  
198 and 2 set at 15 and 40 respectively and the curtain gas set to 10 (units). For LC method 3, the  
199 mass spectrometer was run in positive ion mode with the following source settings; ion spray  
200 voltage 5500 V, source temperature 400  $^{\circ}\text{C}$ , gases 1 and 2 set at 14 (units) and 40 (units),

201 respectively, and the curtain gas set at 10 (units). All the metabolites were quantified using  
202 multiple reaction monitoring (MRM). Standard solutions (10 ng  $\mu\text{L}^{-1}$ ) for all analytes were  
203 prepared and pumped directly via a syringe pump. The ion transitions for each of the analytes  
204 were determined based upon their molecular ion and a strong fragment ion. For several  
205 categories of compounds, it was inevitable that their molecular ion and fragment ion would  
206 be the same, but this was overcome by their different elution times. Their voltage parameters;  
207 declustering potential, collision energy and cell entrance/exit potentials were optimized  
208 individually for each analyte.

209

210 **HPLC Analysis.** Liquid chromatography separation of the was performed on an Agilent  
211 1200 HPLC system (Agilent Technologies, Wokingham, UK) equipped with binary pumps,  
212 thermostated autosampler, column oven and diode-array detector. The column used was a  
213 Synergi Polar-RP (250 x 4.6 mm; 4  $\mu\text{m}$  i.d.; 80 $\text{\AA}$ ), the guard column (4 x 3 mm), both from  
214 Phenomenex (Macclesfield, UK). The mobile phase was 2.13% formic acid aqueous solution  
215 (A) and acetonitrile/methanol (B; 85/15 (v/v)) with an isocratic program of 45 min. Operating  
216 conditions were: column temperature (35  $^{\circ}\text{C}$ ), injection volume (20  $\mu\text{L}$ ), flow rate (1  $\text{mL min}^{-1}$ )  
217 with UV/VIS photo-diode array detection at 530 nm.

218

219 **Statistical Analysis.** All the analyses were performed in triplicate and are presented as the  
220 mean  $\pm$  standard deviation. The statistical analysis on macro- and micro-nutrient contents was  
221 performed using SPSS 23.0 for Windows. The Shapiro–Wilk test was applied to verify the  
222 normal distribution of the variables. When the statistical distribution was not normal, a  
223 logarithmic transformation of the variables was performed. The Levene’s test was applied to  
224 detect possible non-homogeneity of the variances. The data were analyzed using One-Way-

225 Analysis of Variance (ANOVA) to compare the groups and performed Tukey's test for  
226 multiple comparisons. Data on phytochemicals were analyzed by principal component  
227 analysis (PCA), unit variance (UV)-scaled using Sigma P<sup>+</sup> 12 (Umetrics, Cambridge).

## 228 RESULTS AND DISCUSSIONS

229

230 **Macronutrient Composition.** The macronutrient composition of the flours is shown in Table  
231 1. The protein content varied, with the highest values observed for lupin ( $43.0 \pm 0.17$  %  
232 (w/w)) and hemp ( $38.55 \pm 0.32$  % (w/w)). Green pea and fava bean had similar protein  
233 contents ( $24.60 \pm 0.09$  and  $22.12 \pm 0.04$  % (w/w) respectively), while buckwheat showed  
234 lower values ( $20.05 \pm 0.05$  % (w/w)). These observations, which are in line with previous  
235 reports,<sup>30,31</sup> were not unexpected, as the crops from the *Fabaceae* family such as lupin, green  
236 pea and fava bean, are known to be one of the best sources of plant protein. Conversely,  
237 buckwheat from the *Polygonaceae* family was expected to have lower protein content than  
238 legumes. Furthermore, the high protein content of hemp is well documented in the literature.<sup>32</sup>  
239 Also, previous research suggested that the crops had a good essential amino acids profile,  
240 even though some of them, mainly the legumes, may not provide the necessary amounts of  
241 sulphur-containing amino acids.<sup>33-35</sup> However, the amino acid deficiency, which is usually  
242 rare in Western diets, can be overcome by integrating legume-based dishes with cereal  
243 products, as the amino acid contents of the two kinds of crops are complementary with  
244 respect to human nutritional requirements.<sup>36</sup> Moreover, the protein content of the selected  
245 crops were all significantly higher ( $p < 0.001$ ) than wheat and are well suited to supplement  
246 carbohydrate-based diets.<sup>37</sup> Thus, the selected crops may have potential to be applied as  
247 affordable local sources of dietary protein. All the selected crops had significantly higher ( $p <$   
248  $0.001$ ) dietary fibre content (Table 1) compared to wheat ( $0.55 \pm 0.1$  % (w/w)). In particular,  
249 lupin and hemp were shown to be the richest sources of insoluble fibre ( $> 23$  % (w/w)),  
250 which due to its fermentative capacity can stimulate the growth of bifidobacteria, exerting  
251 prebiotic effects.<sup>38</sup> Moreover, since dietary fibre has been shown to be effective in lowering  
252 the blood cholesterol,<sup>39</sup> it is likely that the selected crops, particularly lupin and hemp, also in

253 the form of flour ingredients for bread, would merit consideration in the ongoing efforts to  
254 design healthy foods, with potential to increase the excretion of fat and cholesterol, and  
255 promote the production of short chain fatty acids with anti-inflammatory activities. The total  
256 fat content was generally low (<4 % (w/w)) for the flours studied, apart from hemp and lupin  
257 ( $12.46 \pm 0.32$  and  $6.78 \pm 0.34$  % (w/w), respectively, which were significantly higher ( $p <$   
258  $0.001$ ). From the nutritional standpoint, when below 37%, fat quality is more important than  
259 fat quantity, and it is acknowledged that lupin and hemp oils are characterized by a well-  
260 balanced fatty acid composition with amounts of total unsaturated fatty acids much higher  
261 than total saturated fatty acids.<sup>40,41</sup> Remarkably, lupin even though belonging to the *Fabaceae*  
262 family like fava bean and green pea, showed levels of total fats notably higher than the other  
263 crops. In this context, lupin and to some extent hemp could be potentially considered valuable  
264 industrial crops, as partial replacement of meat with these plant products could result in a  
265 reduction of less healthy saturated animal-derived fats. On the other hand, the higher level of  
266 unsaturated fats, although beneficial, do come with inherent problems of rancidity  
267 development. These values are in agreement with previous studies,<sup>42</sup> however, the  
268 macronutrient composition of plant foods is likely to be strongly influenced by numerous  
269 factors such as cultivar, environment and grade of processing.

270

271 **Micronutrient Composition.** The results of the micronutrient mineral analysis of the flours  
272 are presented in Table 2. All the flours generally contained a broad variety of minerals with  
273 high levels of sodium, magnesium, phosphorous, potassium, calcium, manganese. Hemp  
274 flour was significantly higher ( $p < 0.001$ ) in sodium, magnesium, phosphorous, potassium  
275 and calcium with values of  $260.62 \pm 21.00$ ,  $4340.91 \pm 184.80$ ,  $9721.65 \pm 413.39$ ,  $8572.49 \pm$   
276  $425.11$ ,  $1790.98 \pm 76.36$  mg kg<sup>-1</sup>, respectively. Iron content ranged from  $69.57 \pm 7.43$  mg kg<sup>-1</sup>  
277 <sup>1</sup> in green pea to  $11.62 \pm 0.89$  mg kg<sup>-1</sup> in wheat. Fava bean and buckwheat resulted minor

278 sources of calcium ( $269.26 \pm 7.57$  and  $267.77 \pm 27.01 \text{mg kg}^{-1}$ , respectively), as the RDI for  
279 calcium is 800 mg per day, while hemp and green pea resulted significant dietary sources of  
280 zinc ( $74.38 \pm 3.30$  and  $64.51 \pm 4.34 \text{mg kg}^{-1}$ , respectively), given its RDI of 15 mg per day.  
281 Manganese, and selenium were found in all the flours, even though at very low levels in some  
282 cases, and wheat had the lowest value for all the minerals except for calcium ( $1057.86 \pm$   
283  $20.53 \text{mg kg}^{-1}$ ). Clearly there are variations in the mineral content detected compared with  
284 previous research.<sup>43,44</sup> Again, these variations are likely to be due to the use of different  
285 varieties, varying environmental conditions, such as soil characteristics and fertilizer  
286 applications, and to a lesser degree, method of detection. However, some general  
287 commonalities were observed, such as the concentrations of phosphorous in hemp and  
288 potassium in buckwheat. The dietary deficiencies of mineral elements can have detrimental  
289 effects on health, and it has been estimated that 3.7 billion people worldwide are ferrous  
290 deficient, even though other common mineral elements deficiencies are zinc, iodine,  
291 magnesium, calcium and selenium.<sup>45</sup> Unfortunately, common foods do not always have  
292 adequate mineral concentrations to meet the dietary requirements, hence, mineral  
293 fortifications are necessary. Therefore, the use of the selected flours to enhance the mineral  
294 contents of nutritionally poor food could be a cost effective approach to control some mineral  
295 deficiencies, e.g. the use of hemp flour to manage zinc deficiency. However, some  
296 antinutrients found in plants, such as phytic acid and tannins, must be inactivated or removed  
297 prior to mineral fortification.<sup>44</sup> The flour samples also showed exhibited traces of some heavy  
298 metals. The contamination may be due to certain anthropogenic activities, such as mining,  
299 urban development, the application of fertilizers and pesticides, but also to the milling  
300 process. However, their concentrations are all below the safe limits for adults.<sup>46</sup>

301

302 **Phytochemical Composition.** The principal component analysis (PCA) (Figure 1) regarding  
303 the phytochemical profiles of the selected crops (Tables 3-6), as measured by LC-MS,  
304 showed that green pea, fava bean, and wheat clustered, suggesting similar metabolite profiles.  
305 Comparing the individual metabolites, these crops had lower levels of benzoic acid  
306 derivatives (Table 6). Green pea and fava bean are both from the *Fabaceae* family, however,  
307 wheat (*Poaceae*) also shares the lower left-handed quadrant of the diagram and had the  
308 lowest content of benzoic acid and derivatives among the flours studied ( $19.95 \pm 2.71 \text{ mg kg}^{-1}$ ).  
309 Even though lupin belongs to the *Fabaceae* family, unlike green pea and fava bean, it is  
310 not rich in benzoic acid and derivatives, and discriminates from the other two legume crops  
311 due to its distinctive flavonoid profile. It is the only crop to show sizeable amounts of  
312 genistein and tyrosol. The PCA plot also indicated that buckwheat and hemp discriminated  
313 from the other samples. Buckwheat presented a different metabolite profile due to the higher  
314 concentrations of flavonoids mainly quercetin ( $35.66 \pm 2.22 \text{ mg kg}^{-1}$ ), whereas hemp had an  
315 overall higher content of phenolic acids ( $348.61 \pm 20.28 \text{ mg kg}^{-1}$ ).

316 The cumulative sum of the phenolic compounds from the selected flours ranged between 0.1  
317 and  $1.3 \text{ g kg}^{-1}$ , in the following order: buckwheat > fava bean > hemp > lupin > wheat >  
318 green pea. It should be noted that some metabolites such as phenylpyruvic acid could be  
319 derived from protein degradation,<sup>47</sup> and this may explain why levels are higher in lupin  
320 compared to the other crops analyzed, as it had the highest protein content ( $43.0 \pm 0.17 \%$   
321 (w/w)).

322 Table 3 shows the content of flavanols in the flours. Among the flours analyzed, the highest  
323 content was found in buckwheat  $253.99 \pm 35.41 \text{ mg kg}^{-1}$  followed by fava bean  $41.21 \pm 1.74$   
324  $\text{mg kg}^{-1}$ . The concentrations of catechin and epicatechin in both samples are higher in the free  
325 than bound form. Very low levels of catechin and epicatechin were found in hemp flour ( $1.66$   
326  $\pm 0.26$  and  $0.46 \pm 0.14 \text{ mg kg}^{-1}$  in the bound form, respectively). With regard to individual

327 compounds, epicatechin was the flavanol most abundant in the flours analyzed, galliccatechin,  
328 epigallocatechin, epigallocatechin gallate were not identified in any of the flours studied and  
329 catechin and epicatechin were not detected in lupin, green pea or wheat. The presence of low  
330 molecular weight flavanols in fava bean is confirmed in literature,<sup>48</sup> even though some  
331 variations are likely due to the impact of food processing on seeds (drying, milling, etc.) as a  
332 number of studies reported reduction in monomeric flavanols when exposed to high  
333 temperatures.<sup>49,50</sup> High levels of epicatechin were already identified in buckwheat extracts,  
334 but former studies focused their attention on the vegetative parts of the plant, such as leaves  
335 and flowers, which in some countries are consumed as a vegetable<sup>51</sup>. As human intervention  
336 studies clearly suggests that flavanols exert numerous beneficial effects, particularly on  
337 cardiovascular health,<sup>52,53</sup> the detection of high levels of flavanols in buckwheat after the  
338 milling process is noteworthy, as it enhances its applicability as functional ingredients in  
339 processed food products.

340 Table 3 shows the content of bound anthocyanins from the flours released after the acid  
341 hydrolysis. Pelargonidin was present in substantial amounts ( $748.17 \pm 75.55 \text{ mg kg}^{-1}$ ) in  
342 buckwheat flour, even though cyanidin was the most common among the flours ( $29.72 \pm 3.71$   
343  $\text{mg kg}^{-1}$  in buckwheat,  $23.15 \pm 5.28 \text{ mg kg}^{-1}$  in fava bean,  $58.43 \pm 21.01 \text{ mg kg}^{-1}$  in hemp).  
344 Delphinidin was present at the lowest concentration amongst the anthocyanins detected  
345 ( $25.55 \pm 1.27 \text{ mg kg}^{-1}$  only in fava bean). As anthocyanins have shown to exert protective  
346 activities against cardiovascular disease and cancer when high concentrations were  
347 consumed,<sup>54,55</sup> there are still insufficient data to infer that the consumption of the selected  
348 crops could ameliorate some pathological conditions, while the role of anthocyanin rich fruits  
349 such as wild blackberries (anthocyanins content about  $2800 \text{ mg kg}^{-1}$ )<sup>56</sup> against the deleterious  
350 effects of chronic diseases seems more convincing. There are only very few studies on the  
351 identification and quantification of anthocyanins in foods with no distinctive red, blue and



352 purple colours.<sup>57,58</sup> Therefore, the present work identifies novel anthocyanin rich crops with  
353 potential for industrial utilization.

354 The content of the other individual flavonoids in the grain flours is reported in Table 3. The  
355 bound flavonoids were released after acid hydrolysis. Buckwheat had the highest content of  
356 flavonoids ( $43.92 \pm 2.24 \text{ mg kg}^{-1}$ ) followed by lupin ( $19.66 \pm 0.39 \text{ mg kg}^{-1}$ ), fava bean ( $10.62$   
357  $\pm 0.52 \text{ mg kg}^{-1}$ ), green pea ( $7.05 \pm 0.46 \text{ mg kg}^{-1}$ ) hemp ( $6.86 \pm 1.17 \text{ mg kg}^{-1}$ ) and wheat ( $0.65$   
358  $\pm 0.06 \text{ mg kg}^{-1}$ ). The main flavonoids in flours were quercetin for buckwheat ( $35.66 \pm 2.22$   
359  $\text{mg kg}^{-1}$ ), tyrosol for lupin and hemp ( $15.28 \pm 0.33$  and  $3.80 \pm 1.14 \text{ mg kg}^{-1}$ , respectively),  
360 quercetin and kaempferol for fava bean  $3.97 \pm 0.16$  and  $3.49 \pm 0.42 \text{ mg kg}^{-1}$ , respectively),  
361 kampferol and tyrosol for green pea ( $2.74 \pm 0.24$  and  $2.11 \pm 0.04 \text{ mg kg}^{-1}$ , respectively).  
362 Quercetin was detected in all the samples, although only in buckwheat, and slightly in hemp,  
363 at relevant concentrations. Lupin was the only crop to show a significant content of tyrosol.  
364 Over the last decades, flavonoids have earned great scientific attention due to their beneficial  
365 effects and their ubiquity in plant foods. Therefore, a wealth of research has been carried out  
366 on identification of dietary sources of flavonoids, and some products such as spinach,  
367 cauliflower and onions resulted particularly abundant in flavonoids.<sup>59</sup> However, up to date  
368 most of the studies have based their investigations on raw food, even though the human diet  
369 includes plenty of cooked and processed products. Contrary, the selected crops stand out as  
370 flavonoid rich new ingredients, as being in the form of flours, they are already available to be  
371 included in formulated products, and offer the opportunity to improve the nutritional  
372 characteristics of processed food delivering flavonoid compounds, mainly quercetin,  
373 kampferol and tyrosol.

374 The content of phenolic acids and derivatives from the selected flours are shown in Table 4,  
375 and ranged from 50 to 350  $\text{mg kg}^{-1}$  in the following order: hemp > fava bean > buckwheat >  
376 wheat > green pea > lupin. The 3-hydroxyphenylacetic acid in bound form was the main

377 phenolic acid found in buckwheat ( $63.64 \pm 36.16 \text{ mg kg}^{-1}$ ), bound ferulic acid was the main  
378 phenolic acid in green pea, fava bean and wheat flours ( $12.11 \pm 0.58$ ,  $229.51 \pm 36.58$ ,  $60.21 \pm$   
379  $3.64 \text{ mg kg}^{-1}$ , respectively), bound *p*-coumaric acid was the main phenolic acid in hemp flour  
380 ( $82.78 \pm 8.09 \text{ mg kg}^{-1}$ ), while bound 4-hydroxyphenylpyruvic acid was the main phenolic  
381 acid in lupin flour ( $25.15 \pm 4.16 \text{ mg kg}^{-1}$ ). Benzoic, salicylic, protocatechuic, vanillic and  
382 ferulic acids were reported in both free and bound forms in all the flours, whereas gallic acid  
383 was not detected in lupin and wheat and syringic and caffeic acids were not detected in lupin.  
384 As ferulic dimers are widely acknowledged as compounds with strong antioxidant activity,<sup>60</sup>  
385 noteworthy were the amounts of bound 5-5' and 8-5 linked ferulic acid in fava bean flour.  
386 Human intervention studies showed that high plasmatic concentration of lignans were  
387 associated with lower risk of colon cancer.<sup>61</sup> Therefore, noteworthy are the amounts of bound  
388 secoisolariciresinol and syringaresinol in hemp flour, the use of which might be efficacious to  
389 increase the intake of dietary lignans, which ranges between 0.15 and 1.1 mg per day in  
390 Western diets.<sup>61</sup> As the content and composition of phenolic acids is highly dependent on  
391 variables such as species and variety, growth conditions, extend of development, harvest  
392 time, type of soil and method of detection, it was not always possible to match the values  
393 found in the previous literature, however, some trends have been identified, such as high  
394 levels of *p*-coumaric and ferulic acids in fava bean,<sup>62</sup> and the significant concentrations of  
395 protocatechuic acid in buckwheat.<sup>63</sup>

396 The detailed analysis on the phenolic composition of selected crops in this study suggests that  
397 phenolic acids are mainly found attached to other plant components, most likely polymers of  
398 the cell wall and this is in agreement with previous studies.<sup>29,64</sup> It is very likely that the bound  
399 phenolic compounds, which constitute the major phenolic fraction in legumes and cereals,<sup>65</sup>  
400 are delivered to the colon, where they are available for metabolism by the gut microbiota.

401 However, this may not be the case of other phenolic compounds such as the flavanols, which  
402 were detected mainly in the free form.

403 Buckwheat, green pea, fava bean, hemp and lupin have a valuable macronutrient,  
404 micronutrient and non-nutrient (phytochemical) profile with significant potential to benefit  
405 human health. The favourable macro- and micronutrient profile of the flours suggests an  
406 important role in human nutrition as their use could range from being staples in the diet to  
407 new low-cost ingredients for healthier reformulations of processed foods. Indeed the shift  
408 towards a better diet by improving the basal raw materials in food and reformulation is a  
409 common message at global, national, and devolved government and industry levels.<sup>66</sup> The  
410 selected crops, being rich in protein, could also be considered alternative choices to soybean  
411 and meat, the production of which is responsible for a significant level of agri-food-related  
412 environmental pressure (e.g. GHG emissions). Their implementation or supplementation into  
413 the human diet is a possible approach to satisfy the global demand for protein in a sustainable  
414 manner. From an industrial standpoint, when used as food ingredients, they provide  
415 opportunities to enrich food considered nutritionally poor, through the development of  
416 functional and healthier products, the market share of which is quickly growing, as in  
417 Western Europe more than 50% of consumers embraces the fortification of food products  
418 with functional ingredients.<sup>67</sup> The health benefits, beyond the nutritional enhancement, are  
419 likely to be due to their favourable phytochemical profile, as a wide range of phenolic  
420 compounds was detected. Some of the phenolic compounds were found at significant  
421 concentrations, among them: p-hydroxybenzoic acid, protocatechuic acid, gallic acid, p-  
422 coumaric acid, catechin and epicatechin, which are known for their anti-inflammatory  
423 activities.<sup>68</sup> Also, the majority of the phytochemicals were detected in the bound form,  
424 particularly the phenolic acids. Their association to dietary fibre brings further beneficial  
425 opportunities, due to their beneficial impact on colonic bacteria. Furthermore, from a purely

426 industry processing perspective the recognized antioxidant activity of the phenolic  
427 compounds identifies the selected crops (and their associated products such as flour, etc) as  
428 being imbued with inherent natural food preservative ability, potentially allowing for a  
429 reduction in artificial antioxidant additives. However, their incorporation into foods will  
430 undoubtedly impact on sensorial features of the final products and further studies on their  
431 uses as food ingredient are required. Also, the prospect of sourcing plant protein from species  
432 (and varieties) adapted to the local climate, offers new opportunities for growers. In fact the  
433 diversification of the range of crops available to growers would also deliver into the  
434 “greening” requirements of the EU Common Agricultural Policy by crop diversification.<sup>69</sup>  
435 Therefore, the numerous health, environmental, and economic benefits, derived by their use,  
436 especially in high latitude countries where they offer resilience to the harder growing  
437 conditions, warrants further research initiatives.

438 **Acknowledgement**

439 The authors acknowledge support from the Scottish Government's Rural and Environment  
440 Science and Analytical Services Division (RESAS) via their strategic research and  
441 partnership programmes.

442

443 **Notes**

444 Authors declare no conflict of interests.

445

446 **ABBREVIATIONS USED**

447 CVD, cardiovascular disease

448 DAD, diode array detector

449 ESADI, estimated safe and adequate intake

450 EtOAc, ethyl acetate

451 GHGEs, greenhouse gas emissions

452 HPLC, high pressure liquid chromatography

453 ICP-MS, inductively coupled plasma-mass spectrometry

454 LC-MS, liquid chromatography-mass spectrometry

455 LDL, low-density lipoproteins

456 MRM, multiple reaction monitoring

457 NSP, non-starch polysaccharides

458 PCA, principal component analysis

459 RDI, recommended daily intake

460 UV, unit variance

461 UV/VIS, Ultraviolet–visible

462

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655

656 **FIGURE CAPTIONS**

657 **Figure 1.** Principal component analysis (unit variance-scaled) plot of the t(1) and t(2) axis showing discrimination of the crops based on all the  
658 phytochemicals measured.



659 **TABLES**660 **Table 1. Macronutrient composition of the flours**

661

Sample	Dry Matter % (w/w)	Ash % (w/w)	Protein % (w/w)	Fat % (w/w)	Resistant Starch % (w/w)	Fibre % (w/w)	
						soluble	insoluble
<b>green pea</b>	89.30±0.09 <sup>a</sup>	2.79±0.13 <sup>a</sup>	24.60±0.09 <sup>a</sup>	2.11±0.07 <sup>a</sup>	0.59±0.01 <sup>a</sup>	0.08±0.03 <sup>a</sup>	8.69±0.07 <sup>a</sup>
<b>buckwheat</b>	89.89±0.11 <sup>b</sup>	3.42±0.10 <sup>a,c</sup>	20.05±0.05 <sup>b</sup>	1.58±0.25 <sup>a</sup>	0.33±0.01 <sup>b,d</sup>	0.82±0.16 <sup>a,d</sup>	6.98±0.01 <sup>a</sup>
<b>hemp</b>	91.95±0.16 <sup>c</sup>	10.59±0.55 <sup>b</sup>	38.55±0.32 <sup>c</sup>	6.78±0.34 <sup>b</sup>	n/d	0.16±0.00 <sup>b,e</sup>	25.49±1.45 <sup>b</sup>
<b>lupin</b>	92.86±0.08 <sup>d</sup>	3.65±0.05 <sup>c,d</sup>	43.00±0.17 <sup>d</sup>	12.46±0.32 <sup>c</sup>	0.03±0.01 <sup>c</sup>	1.61±0.08 <sup>c</sup>	23.55±1.10 <sup>c</sup>
<b>fava bean</b>	91.19±0.07 <sup>e</sup>	2.76±0.00 <sup>a</sup>	22.12±0.04 <sup>e</sup>	3.98±0.32 <sup>d</sup>	0.34±0.05 <sup>d</sup>	0.56±0.10 <sup>d</sup>	9.39±0.30 <sup>a</sup>
<b>wheat</b>	88.02±0.09 <sup>f</sup>	4.84±1.19 <sup>d</sup>	13.57±0.09 <sup>f</sup>	1.59±0.25 <sup>a</sup>	0.25±0.00 <sup>e</sup>	0.25±0.03 <sup>e</sup>	0.30±0.07 <sup>d</sup>

662 Data are means of three replicates with standard deviations. n/d = not detected (i.e. below the detection level). <sup>a-f</sup>Data within the same column  
663 with different superscripts are significantly different (p < 0.05).

664 **Table 2. Micronutrient composition of the flours**

Sample	buckwheat	green pea	fava bean	hemp	lupin	wheat	RDI, ESADI & PTI
<b>Na</b>	138.72± 11.73 <sup>a</sup>	181.31± 8.32 <sup>a</sup>	224.29± 11.08 <sup>b</sup>	260.62± 21.00 <sup>c</sup>	142.38± 14.22 <sup>a</sup>	73.35d± 21.76 <sup>d</sup>	1100-3300 <sup>1</sup>
<b>Mg</b>	1608.41± 96.66 <sup>a</sup>	3813.58± 206.01 <sup>b</sup>	545.86± 13.25 <sup>c</sup>	4340.91± 184.80 <sup>d</sup>	947.82± 80.75 <sup>e</sup>	173.76± 6.68 <sup>f</sup>	300-350 <sup>2</sup>
<b>P</b>	2770.98± 171.18 <sup>a</sup>	8617.55± 545.99 <sup>b</sup>	2219.86± 48.62 <sup>a</sup>	9721.65± 413.39 <sup>c</sup>	2667.65± 300.89 <sup>a</sup>	721.62± 23.00 <sup>d</sup>	800 <sup>2</sup>
<b>K</b>	3384.02± 208.93 <sup>a</sup>	7450.66± 443.42 <sup>b</sup>	5496.49± 141.26 <sup>c</sup>	8572.49± 425.11 <sup>d</sup>	7008.94± 621.54 <sup>b</sup>	1045.23± 30.42 <sup>e</sup>	1875-5625 <sup>1</sup>
<b>Ca</b>	267.27± 27.01 <sup>a</sup>	1540.40± 83.87 <sup>b,c</sup>	269.26± 7.57 <sup>a</sup>	1790.98± 76.36 <sup>c</sup>	1062.74± 109.14 <sup>d,e</sup>	1057.86± 20.53 <sup>e</sup>	800 <sup>2</sup>
<b>Mn</b>	13.08± 0.73 <sup>a</sup>	108.30± 8.12 <sup>b,c</sup>	5.28± 0.12 <sup>a</sup>	128.00± 7.72 <sup>c</sup>	627.11± 50.17 <sup>d</sup>	5.44± 0.19 <sup>a</sup>	2.5-5.0 <sup>1</sup>
<b>Fe</b>	20.23± 0.86 <sup>a</sup>	69.57± 7.43 <sup>b,c</sup>	16.36± 0.47 <sup>a</sup>	51.91± 10.22 <sup>c</sup>	32.15± 22.48 <sup>a,c</sup>	11.62± 0.89 <sup>a</sup>	10-18 <sup>2</sup>
<b>Co</b>	n/d	0.08± 0.04	0.05± 0.01	0.16± 0.09	0.20± 0.18	n/d	0.003 <sup>3</sup>
<b>Cu</b>	3.81± 0.21 <sup>a</sup>	13.61± 0.91 <sup>b</sup>	3.97± 0.18 <sup>a</sup>	15.58± 1.36 <sup>c</sup>	5.99± 0.08 <sup>d</sup>	1.11± 0.12 <sup>e</sup>	2-3 <sup>1</sup>
<b>Zn</b>	18.50± 0.43 <sup>a</sup>	64.51± 4.34 <sup>b</sup>	20.49± 0.99 <sup>a</sup>	74.38± 3.30 <sup>c</sup>	36.54± 1.00 <sup>d</sup>	5.76± 0.16 <sup>e</sup>	15 <sup>2</sup>
<b>Se</b>	0.09± 0.03 <sup>a</sup>	0.10± 0.00 <sup>a</sup>	0.07± 0.00 <sup>a</sup>	0.11± 0.02 <sup>a</sup>	0.60± 0.12 <sup>b</sup>	0.04± 0.02 <sup>a</sup>	0.05-0.2 <sup>1</sup>
<b>Mo</b>	0.25± 0.01 <sup>a</sup>	0.44± 0.04 <sup>b,d</sup>	1.45± 0.04 <sup>c</sup>	0.47± 0.00 <sup>d,e</sup>	0.61± 0.14 <sup>e</sup>	0.08± 0.00 <sup>f</sup>	0.15-0.5 <sup>1</sup>
<b>Cd</b>	0.03± 0.00 <sup>a</sup>	0.03± 0.00 <sup>a,c</sup>	0.01± 0.00 <sup>b,d</sup>	0.04± 0.00 <sup>c</sup>	0.01± 0.01 <sup>d,e</sup>	0.01± 0.00 <sup>e</sup>	0.001 <sup>4</sup>
<b>Hg</b>	n/d	n/d	n/d	n/d	0.02± 0.00	0.01± 0.00	0.0007 <sup>4</sup>
<b>Pb</b>	0.05± 0.02	0.06± 0.04	0.04± 0.00	0.09± 0.07	0.12± 0.09	0.03± 0.01	0.007 <sup>4</sup>

665 Data are means of three replicates with standard deviations and is expressed as  $\text{mg kg}^{-1}$  dry weight. n/d = not detected (i.e. below the detection  
666 level). <sup>a-f</sup>Data within the same row with different superscripts are significantly different ( $p < 0.05$ ). <sup>1</sup>Estimated Safe and Adequate Dietary Intake  
667 (ESADI); <sup>2</sup>reference daily Intake (RDI); <sup>3</sup>expressed as weight of vitamin B<sub>12</sub> and <sup>4</sup>provisional tolerable intakes (PTI), expressed as  $\text{mg kg}^{-1}$   
668 body weight.

669 **Table 3. Flavonoids (A), coumarins (B) and isoflavonoid (C) flavanol (D) and anthocyanin (E) content of the flours**

	<b>buckwheat</b>	<b>green pea</b>	<b>fava bean</b>	<b>hemp</b>	<b>lupin</b>	<b>wheat</b>
A						
bergapten	0.03± 0.00	0.03± 0.00	0.03± 0.00	0.01± 0.00	0.02± 0.01	0.05± 0.00
coumestrol	n/d	0.02± 0.00	n/d	n/d	n/d	n/d
isoliquiritigenin	0.01± 0.00	0.01± 0.00	0.01± 0.00	n/d	n/d	n/d
phloretin	0.07± 0.02	n/d	n/d	n/d	n/d	n/d
naringenin	0.49± 0.06	0.09± 0.05	0.11± 0.01	0.01± 0.00	0.16± 0.02	n/d
hesperitin	0.04± 0.01	n/d	0.05± 0.00	0.01± 0.01	0.06± 0.05	0.03± 0.01
kaempferol	0.65± 0.11	2.74± 0.24	3.49± 0.42	0.04± 0.01	0.09± 0.02	0.07± 0.01
morin	n/d	n/d	n/d	0.08± 0.01	0.03± 0.05	n/d
quercetin	35.66± 2.22	1.08± 0.36	3.97± 0.16	0.73± 0.14	0.02± 0.01	0.11± 0.02
myricetin	0.05± 0.00	0.04± 0.04	1.31± 0.24	n/d	n/d	0.01± 0.00
quercetin-3-glucoside	0.66± 0.04	0.03± 0.00	0.17± 0.02	n/d	n/d	0.01± 0.00
taxifolin	0.28± 0.02	0.48± 0.11	0.18± 0.03	0.08± 0.03	n/d	n/d
scopoletin	0.06± 0.00	0.04± 0.01	0.01± 0.00	0.26± 0.06	n/d	n/d
hesperidin	0.02± 0.00	0.02± 0.00	0.01± 0.00	0.02± 0.00	0.01± 0.00	0.02± 0.01

quercitrin	n/d	0.04± 0.06	n/d	n/d	n/d	n/d
poncirin	n/d	0.01± 0.00	0.02± 0.00	n/d	n/d	n/d
didymin	0.01± 0.00	n/d	n/d	n/d	n/d	n/d
phloridzin	n/d	n/d	n/d	n/d	0.01± 0.01	n/d
galangin	n/d	n/d	0.01± 0.00	n/d	0.05± 0.01	n/d
luteolin	1.10± 0.03	n/d	0.10± 0.01	0.63± 0.16	0.44± 0.06	0.03± 0.00
fisetin	n/d	0.01± 0.01	0.01± 0.00	n/d	n/d	n/d
luteolinidin	0.21± 0.01	n/d	n/d	n/d	n/d	n/d
isorhamnetin	0.57± 0.05	0.09± 0.01	0.43± 0.03	0.19± 0.04	0.02± 0.00	0.24± 0.05
apigenin	0.08± 0.01	0.01± 0.00	0.06± 0.01	0.17± 0.03	0.69± 0.03	0.02± 0.00
tyrosol	1.73± 0.25	2.11± 0.04	0.06± 0.01	3.80± 1.14	15.28± 0.33	n/d
hydroxytyrosol	0.02± 0.00	n/d	0.15± 0.02	0.08± 0.05	0.01± 0.01	n/d
<b>B</b>						
coumarin	n/d	0.03± 0.03	0.01± 0.01	0.39± 0.03	0.02± 0.00	n/d
7,8-dihydroxy-6-methylcoumarin	n/d	n/d	0.02± 0.02	n/d	n/d	n/d
umbelliferone	0.24± 0.01	n/d	0.01± 0.00	0.05± 0.01	n/d	n/d

psoralen	0.04± 0.00	n/d	n/d	n/d	n/d	n/d
C						
biochanin A	n/d	n/d	0.15± 0.01	0.12± 0.03	0.02± 0.02	0.01± 0.00
daidzein	n/d	n/d	n/d	n/d	0.04± 0.02	n/d
genistein	0.01 0.00	n/d	n/d	n/d	2.37± 0.16	0.01± 0.01
formononetin	0.03± 0.01	0.05± 0.01	0.05± 0.02	0.03± 0.00	0.05± 0.05	0.02± 0.00
D						
catechin	68.93± 13.02	n/d	13.18± 0.45	1.66± 0.26	n/d	n/d
epicatechin	185.06± 33.42	n/d	28.03 ± 1.71	0.46± 0.14	n/d	n/d
E						
delphinidin	n/d	n/d	25.55± 1.27	n/d	n/d	n/d
cyanidin	29.72± 3.71	n/d	23.15± 5.28	58.43± 21.01	n/d	n/d
pelargonidin	748.17± 75.55	n/d	n/d	n/d	n/d	n/d

670 Data are means of three replicates with standard deviations and is expressed mg kg<sup>-1</sup> dry weight. n/d = not detected (i.e. below the detection  
671 level). 8-methylpsoralen, tangeretin, eriocitrin, naringin, neoeriocitrin, neohesperidin, gossypin, gallicocatechin, epigallocatechin, epigallocatechin  
672 gallate, peonidin, malvinidin, and petunidin were not detected in any of the samples.

673 **Table 4. Phenolic acids and other related compounds:** Benzoic acids, aldehydes and acetophenones (A), cinnamic acids and alcohols (B),  
 674 phenylacetic, phenylpyruvic and phenyllactic and phenylpropionic acid (C) simple phenols and nitrogen containing compounds (D) and phenolic  
 675 dimers and lignans (E)

	buckwheat		green pea		fava bean		hemp		lupin		wheat	
	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound
A												
benzoic acid	1.41± 0.12	10.78± 1.44	1.27± 0.13	2.48± 0.13	0.82± 0.19	2.94± 0.67	2.79± 0.16	3.57± 0.22	1.39± 0.15	6.53± 0.70	1.41± 0.28	4.37± 0.61
salicylic acid	0.88± 0.11	4.02± 1.95	0.13± 0.02	0.27± 0.03	0.63± 0.06	0.44± 0.05	13.56± 0.26	4.33± 0.27	0.20± 0.01	2.22± 0.66	0.12± 0.01	0.61± 0.14
m-hydroxybenzoic acid	5.75± 0.16	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
p-hydroxybenzoic acid	1.55± 0.12	21.41± 7.17	1.03± 0.07	8.25± 0.65	0.72± 0.06	7.54± 0.67	5.20± 0.13	13.31± 0.76	0.89± 0.28	10.18± 1.92	0.14± 0.01	0.99± 0.28
2,3-dihydroxybenzoic acid	0.15± 0.01	6.11± 3.55	0.01± 0.02	0.05± 0.06	0.08± 0.02	0.15± 0.03	0.12± 0.01	0.22± 0.05	0.04± 0.01	n/d	n/d	0.04± 0.01
2,4-dihydroxybenzoic acid	n/d	0.18± 0.04	n/d	n/d	n/d	n/d	n/d	0.10± 0.09	n/d	n/d	0.19± 0.01	n/d
gentisic acid	0.25± 0.05	9.98± 5.91	0.10± 0.10	0.82± 0.17	0.19± 0.01	2.18± 0.28	0.48± 0.08	31.20± 1.67	n/d	0.54± 0.11	n/d	0.17± 0.03
2,6-dihydroxybenzoic acid	n/d	0.14± 0.04	n/d	n/d	0.30± 0.02	0.04± 0.01	0.26± 0.01	0.03± 0.00	n/d	n/d	n/d	n/d
protocatechuic acid	6.61± 0.75	24.56± 7.80	1.26± 0.02	11.38± 1.18	0.61± 0.12	2.42± 0.26	5.63± 0.10	22.06± 2.13	0.15± 0.02	n/d	0.07± 0.01	0.11± 0.04
3,5-dihydroxybenzoic acid	n/d	n/d	n/d	n/d	n/d	0.79± 0.36	n/d	n/d	n/d	n/d	n/d	n/d
o-anisic acid	6.00± 0.10	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
m-anisic acid	n/d	0.18± 0.04	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d

	buckwheat		green pea		fava bean		hemp		lupin		wheat	
	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound
p-anisic acid	0.14± 0.05	0.76± 0.23	n/d	0.10± 0.04	0.16± 0.02	0.16± 0.07	0.04± 0.07	0.37± 0.02	n/d	0.28± 0.04	0.08± 0.07	0.46± 0.03
gallic acid	3.87± 0.43	30.88± 9.27	n/d	0.17± 0.02	0.44± 0.13	0.85± 0.19	0.14± 0.03	0.30± 0.16	n/d	n/d	n/d	n/d
vanillic acid	0.38± 0.02	5.61± 2.54	0.62± 0.03	4.14± 0.51	0.55± 0.07	4.72± 0.38	2.33± 0.35	13.74± 1.31	0.55± 0.17	9.09± 1.93	0.34± 0.05	2.64± 0.19
syringic acid	0.12± 0.03	1.40± 0.56	0.13± 0.01	0.45± 0.01	0.35± 0.01	3.06± 0.47	0.62± 0.03	6.41± 0.70	n/d	n/d	0.06± 0.02	1.68± 0.24
3,4-dimethoxybenzoic acid	n/d	0.42± 0.11	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
p-hydroxybenzaldehyde	0.59± 0.08	8.14± 3.60	0.10± 0.03	0.40± 0.06	0.45± 0.04	1.11± 0.24	7.16± 0.35	12.10± 0.96	0.42± 0.17	4.43± 0.43	0.12± 0.02	1.02± 0.12
protocatechualdehyde	3.65± 0.36	19.74± 6.05	0.12± 0.12	0.07± 0.03	0.68± 0.09	5.63± 0.47	6.41± 0.24	34.77± 5.15	n/d	n/d	n/d	0.19± 0.04
3,4,5-trihydroxybenzaldehyde	n/d	0.09± 0.08	n/d	n/d	1.09± 0.29	5.97± 1.64	0.05± 0.04	0.40± 0.24	n/d	n/d	n/d	n/d
vanillin	0.32± 0.05	2.73± 1.30	0.14± 0.03	0.47± 0.11	1.41± 0.16	2.05± 0.33	3.07± 0.16	24.56± 1.41	0.29± 0.06	5.28± 0.58	0.86± 0.09	2.66± 0.34
syringin	0.07± 0.01	0.80± 0.36	n/d	0.46± 0.06	0.16± 0.02	0.40± 0.07	1.44± 0.10	13.17± 0.78	n/d	n/d	0.05± 0.00	0.57± 0.07
4-hydroxyacetophenone	n/d	0.18± 0.07	n/d	0.04± 0.00	0.03± 0.01	0.09± 0.02	0.04± 0.00	0.40± 0.02	0.10± 0.02	0.73± 0.14	n/d	0.19± 0.03
4-hydroxy-3-methoxyacetophenone	n/d	0.17± 0.08	n/d	0.09± 0.02	0.08± 0.02	0.28± 0.07	0.06± 0.02	0.67± 0.08	0.03± 0.01	0.34± 0.02	0.03± 0.00	0.38± 0.03
4-hydroxy-3,5-dimethoxyacetophenone	n/d	n/d	n/d	0.10± 0.01	n/d	0.20± 0.06	n/d	0.43± 0.04	n/d	n/d	n/d	1.02± 0.13
3,4,5-trimethoxyacetophenone	n/d	0.01± 0.00	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.01± 0.00	n/d	0.01± 0.00

B



	buckwheat		green pea		fava bean		hemp		lupin		wheat	
	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound
cinnamic acid	0.69± 0.07	1.69± 0.07	0.50± 0.03	1.02± 0.07	0.60± 0.02	1.12± 0.07	0.78± 0.05	1.04± 0.07	0.93± 0.05	2.14± 0.40	0.09± 0.00	0.20± 0.02
o-coumaric acid	n/d	n/d	n/d	n/d	n/d	0.61± 0.53	n/d	n/d	n/d	2.86± 0.65	n/d	2.23± 0.27
m-coumaric acid	1.26± 0.06	4.36± 3.22	n/d	n/d	n/d	n/d	n/d	5.06± 1.00	n/d		n/d	n/d
p-coumaric acid	n/d	8.85± 1.51	1.10± 0.03	n/d	1.84± 0.35	20.32± 3.31	1.24± 0.32	82.78± 8.09	n/d	n/d	n/d	1.44± 0.11
caffeic acid	0.31± 0.02	41.74± 22.54	n/d	0.22± 0.01	0.14± 0.01	2.24± 0.34	0.15± 0.02	2.39± 0.81	n/d	n/d	n/d	0.42± 0.06
chlorogenic acid	1.19± 0.11	n/d	n/d	n/d	0.05± 0.01	n/d	1.70± 0.02	n/d	0.03± 0.06	n/d	n/d	n/d
ferulic acid	0.16± 0.00	5.38± 0.58	1.07± 0.09	12.11± 0.58	1.74± 0.31	229.51± 36.58	1.24± 0.06	20.65± 1.23	0.35± 0.11	3.34± 1.20	0.63± 0.34	60.21± 3.64
sinapic acid	0.26± 0.03	8.93± 1.08	0.26± 0.05	11.02± 0.82	0.25± 0.06	16.41± 2.83	0.08± 0.08	3.27± 0.40	n/d	n/d	n/d	14.70± 1.68
3,4-dimethoxycinnamic acid	n/d	0.17± 0.03	n/d	n/d	n/d	0.45± 0.11	n/d	n/d	n/d	n/d	n/d	n/d
3,4,5-trimethoxycinnamic acid	0.09± 0.01	1.15± 0.13	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.01± 0.00
coniferyl alcohol	n/d	n/d	n/d	0.03± 0.01	n/d	0.03± 0.01	n/d	n/d	n/d	n/d	n/d	n/d
C												
phenylacetic acid	0.49± 0.01	2.25± 0.42	0.47± 0.02	0.25± 0.02	0.40± 0.03	0.76± 0.10	0.39± 0.05	0.42± 0.05	0.55± 0.04	1.42± 0.27	0.30± 0.05	0.48± 0.13
3-hydroxyphenylacetic acid	0.86± 0.13	63.64± 36.16	n/d	0.47± 0.03	0.36± 0.05	4.29± 0.49	0.47± 0.07	3.44± 0.32	n/d	1.41± 0.21	n/d	0.30± 0.26
3,4-dihydroxyphenylacetic acid	n/d	0.71± 0.26	n/d	n/d	n/d	0.52± 0.05	n/d	n/d	n/d	n/d	n/d	n/d

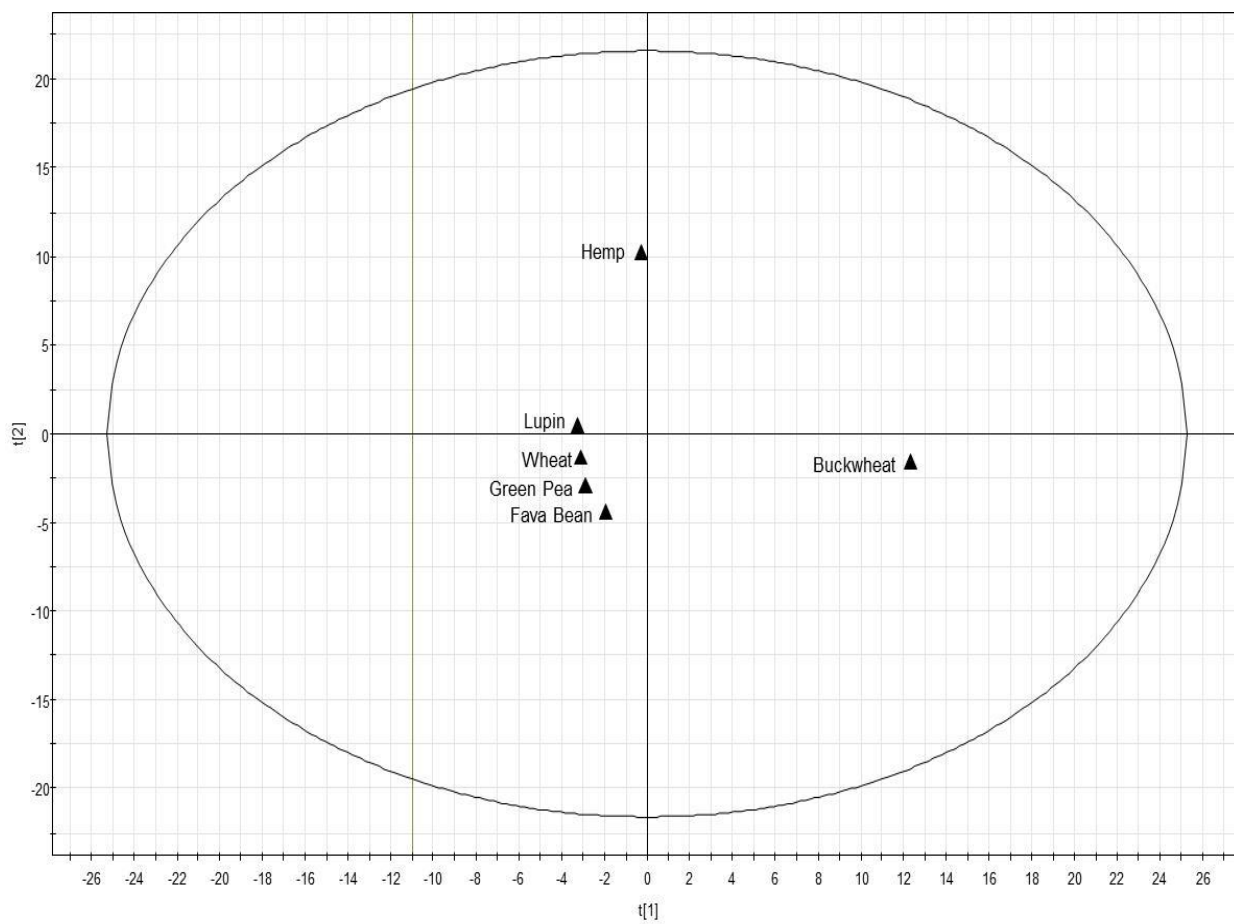
	buckwheat		green pea		fava bean		hemp		lupin		wheat	
	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound
4-hydroxy-3-methoxyphenylacetic acid	n/d	9.63± 5.84	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
4-methoxyphenylacetic acid	n/d	0.05± 0.00	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
3,4-dihydroxymandelic acid	0.19± 0.02	0.73± 0.17	n/d	n/d	0.15± 0.14	0.13± 0.12	0.62± 0.09	0.79± 0.22	0.04± 0.08	n/d	0.06± 0.00	0.04± 0.01
4-hydroxy-3-methoxymandelic acid	n/d	n/d	n/d	n/d	n/d	n/d	n/d	1.51± 0.08	n/d	n/d	n/d	n/d
phenylpyruvic acid	0.14± 0.02	0.11± 0.03	0.06± 0.01	0.12± 0.06	0.20± 0.02	0.23± 0.05	0.22± 0.02	0.22± 0.03	0.09± 0.01	0.43± 0.05	0.37± 0.05	0.86± 0.07
4-hydroxyphenylpyruvic acid	10.30± 1.50	10.37± 5.37	11.59± 3.42	11.83± 2.76	12.22± 1.49	11.45± 3.46	8.61± 0.72	11.87± 6.54	7.85± 1.40	25.15± 4.16	1.23± 0.24	5.96± 2.55
phenyllactic acid	0.18± 0.02	3.38± 1.55	0.74± 0.04	0.24± 0.03	0.67± 0.05	0.30± 0.03	0.23± 0.02	0.17± 0.08	0.19± 0.03	0.08± 0.01	0.05± 0.01	0.16± 0.03
4-hydroxyphenyllactic acid	n/d	6.65± 1.24	0.51± 0.18	n/d	0.80± 0.04	1.06± 0.44	0.26± 0.23	1.81± 0.21	n/d	n/d	n/d	0.30± 0.11
3-hydroxyphenylpropionic acid	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.24± 0.05	n/d	n/d	n/d	n/d
4-hydroxyphenylpropionic acid	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
3,4-dihydroxyphenyl propionic acid	n/d	n/d	n/d	n/d	n/d	0.11± 0.09	n/d	0.46± 0.07	n/d	n/d	n/d	n/d
4-hydroxy-3-methoxy phenylpropionic acid	n/d	0.17± 0.11	n/d	0.19± 0.02	n/d	0.64± 0.05	0.14± 0.03	1.02± 0.10	n/d	1.63± 0.37	n/d	0.27± 0.04
3-methoxyphenylpropionic acid	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
D												
phenol	4.14± 1.47	24.89± 13.13	1.69± 0.04	2.93± 0.54	4.03± 0.61	3.12± 0.54	56.42± 6.88	23.95± 2.06	n/d	n/d	n/d	1.29± 1.12

	buckwheat		green pea		fava bean		hemp		lupin		wheat	
	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound
1,2-hydroxybenzene	n/d	4.16± 2.46	n/d	n/d	0.05± 0.09	n/d	n/d	n/d	n/d	n/d	n/d	n/d
1,3-hydroxybenzene	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
1,2,3-trihydroxybenzene	n/d	n/d	n/d	n/d	0.28± 0.09	n/d	n/d	n/d	n/d	n/d	n/d	n/d
4-ethylphenol	n/d	n/d	n/d	n/d	n/d	0.01± 0.00	n/d	n/d	n/d	n/d	n/d	n/d
4-methylcatechol	0.01± 0.00	0.08± 0.03	n/d	0.11± 0.01	0.01± 0.00	0.09± 0.02	n/d	0.50± 0.08	n/d	0.37± 0.07	n/d	0.88± 0.05
anthranilic acid	0.05± 0.01	0.68± 0.19	0.03± 0.01	0.17± 0.05	0.04± 0.01	0.14± 0.01	0.16± 0.01	0.88± 0.27	n/d	0.51± 0.10	n/d	0.10± 0.02
quinaldic acid	0.14± 0.01	0.17± 0.06	n/d	0.02± 0.00	0.03± 0.00	0.17± 0.04	0.01± 0.00	0.01± 0.00	0.03± 0.00	0.03± 0.01	n/d	0.02± 0.00
E												
ferulic dimer (5-5 linked)	n/d	0.09± 0.02	n/d	0.07± 0.02	n/d	39.99± 1.10	n/d	0.02± 0.02	n/d	n/d	n/d	3.32± 0.46
ferulic dimer (8-8 linked)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
ferulic dimer (8-5 linked)	n/d	0.39± 0.11	n/d	0.19± 0.08	0.04± 0.03	58.17± 6.68	n/d	1.20± 0.22	n/d	n/d	n/d	n/d
secoisolariciresinol	0.11± 0.01	0.14± 0.08	n/d	n/d	0.06± 0.00	0.02± 0.00	0.04± 0.01	3.71± 0.18	n/d	n/d	n/d	0.22± 0.01
matairesinol	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.03± 0.01	n/d	n/d	n/d	n/d
enterodiol	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.04± 0.01	0.01± 0.00	n/d	n/d
enterolactone	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.16± 0.01	0.09± 0.01	n/d	n/d
syringaresinol	1.40± 0.11	2.12± 0.70	n/d	n/d	0.86± 0.05	0.52± 0.07	0.50 ± 0.44	24.86± 2.16	n/d	n/d	n/d	n/d

	buckwheat		green pea		fava bean		hemp		lupin		wheat	
	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound
pinoresinol	0.26± 0.03	0.07± 0.06	n/d	n/d	0.14± 0.01	n/d	0.35± 0.03	1.64± 0.11	n/d	n/d	n/d	n/d
lariciresinol	0.29± 0.04	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.30± 0.07	n/d	n/d	n/d

676 Data are means of three replicates with standard deviations and is expressed mg kg<sup>-1</sup> dry weight. n/d = not detected (i.e. below the detection  
677 level). The following compounds were not detected in any of the samples: 3-methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, 3,4,5-  
678 trimethoxybenzaldehyde, 2-hydroxycinnamyl alcohol, isovanillin, ellagic acid, 3-methoxycinnamic acid, 4-methoxycinnamic acid, 4-hydroxy-3-  
679 methoxycinnamyl alcohol, phenylpropionic acid, 2-hydroxyphenylpropionic acid, 3,4-dimethoxyacetophenone, 4-hydroxyphenylacetic acid,  
680 mandelic acid, 3-hydroxymandelic acid, 4-hydroxymandelic acid, 4-hydroxyphenylpropionic acid, ethylferulate, 5-hydroxymatairesinol.

681 **Figure 1**



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