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Simulated gastrointestinal digestion and invitro colonic fermentation of date (*Phoenix dactylifera* L.) seed polyphenols

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8 Simulated gastrointestinal digestion  
9 and *in-vitro* colonic fermentation of  
10 date (*Pheonix dactylifera* L.) seed  
11 polyphenols

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## 25 **Abstract**

26 Date seeds are a by-product of date fruit industry and a rich source of polyphenols. In this study, *in-*  
27 *vitro* bioaccessibility and colonic fermentation of major polyphenols from date seed powder (DSP)  
28 and DSP fortified yoghurt (DSPY) were investigated using HPLC. Catechin, epicatechin, and  
29 procyanidin A2, B1 and B2 were stable during simulated gastric and sequential intestinal digestion.  
30 Bioaccessibility was significantly ( $P<0.05$ ) higher for all compounds from DSPY compared with DSP.  
31 After *in-vitro* colonic fermentation of insoluble digestion materials, most of the target compounds  
32 were metabolised by faecal bacteria to ferulic acid, 3-hydroxyphenylacetic acid, 3-phenyl-propionic  
33 acid and 3-(4-hydroxyphenyl) propionic acid. DSPY contained significantly ( $P<0.05$ ) higher level of  
34 free polyphenols as indicated by higher bioaccessibility, however, the stability of the polyphenols  
35 and their fermentation products from DSPY were similar to that of DSP alone. These data would be  
36 useful in product developments incorporating DSP as a source of polyphenols in food products.

## 37 **Introduction**

38 Polyphenols are secondary plant metabolites and form an integral component of the human diet.  
39 The importance of polyphenols as bioactive compounds is widely appreciated. One mechanism of  
40 their bio-activity is related to their contribution to the body antioxidant defence and to modulating  
41 biochemical activities against oxidative stresses (Forman *et al.*, 2014; Mateen *et al.*, 2016).  
42 Nonetheless, bioaccessibility of polyphenols from food matrixes and their bioavailability, which is  
43 affected by chemical stability and absorption kinetics in the gastrointestinal tract, metabolic  
44 modification in the liver and pharmacokinetics of plasma clearance, are the major determinants of  
45 the health efficacy of polyphenols from foods or as supplements. For example, the interactions of  
46 polyphenols with food matrix components such as proteins, lipids and dietary fibre that influenced  
47 their accessibility and chemical stability for intestinal absorption and colonic fermentation have been  
48 widely documented (Mandalari *et al.*, 2016; Fu *et al.*, 2016; Oliveira and Pintado, 2015), and that  
49 polyphenol glycosylation and esterification also impact on their bioavailability (Scalbert *et al.*, 2002).

50 Polyphenols, if released from food matrix, can undergo substantial structural changes before and  
51 after absorption in the small intestine. These may include de-glycosylation and further structural  
52 changes at the intestinal cell membranes by various enzymes, including lactase-phlorizin hydrolases.  
53 Some polyphenols can be absorbed through the intestinal epithelial cells *via* active, passive, or  
54 facilitated transport (Bohn et al., 2015). Inside the epithelial cells, they are transformed into various  
55 metabolised forms by phase-II enzymes such as cytosolic  $\beta$ -glucosidase, glucuronosyltransferase,  
56 sulfotransferase, catechol-O-methyltransferase (Gleichenhagen & Schieber, 2016). These sulphated,  
57 methylated, or glucuronidated metabolites (referred to as phase-II metabolites) then enter the  
58 enterohepatic circulation, and may undergo further similar transformations in the hepatic cells.  
59 Finally they reach the systemic circulation, having access to blood, lymph, and various organs such as  
60 the brain, or enter kidneys leading to urinary excretion (Gleichenhagen & Schieber, 2016).  
61 Unabsorbed polyphenols such as large proanthocyanidins or those bound to food matrix  
62 components are inaccessible to digestive enzymes, and therefore reach the colon, where they are  
63 metabolised by the colonic microbiota. Subsequently, the health efficacy of polyphenols also  
64 depends on their intestinal solubility that affects bioavailability (Fu *et al.*, 2015), and from the  
65 generation of readily absorbable phenolic acids from undigested materials due to colonic bacterial  
66 fermentation (Aura, 2008).

67 Date palm is a unique crop as it grows in arid and semi-arid climates, thus seeds of the date fruits are  
68 able to survive these harsh conditions until germination (Chao, 2007). Secondary metabolites such as  
69 polyphenols are produced in response to such environmental stresses, and in addition serve as a  
70 major defence against microbial and herbivorous attacks. Date seeds as a by-product of date fruit  
71 industry are produced in large quantities and have been identified as a rich source of  
72 proanthocyanidins, glycosylated flavonoids (Habib *et al.*, 2014; Sirisena *et al.*, 2017) and dietary fibre  
73 (Al-Farsi and Lee, 2008). Therefore it is a potential low-cost ingredient which can be added to various  
74 food products to increase polyphenol and dietary fibre contents. The polyphenol contents in date  
75 seeds contained organic solvent extractable polyphenols (EPP) and non-extractable polyphenols  
76 (NEPP) (Sirisena *et al.*, 2017). The EPP included flavan-3-ol monomers, dimers and oligomers, as well  
77 as glycosylated flavones and flavonols. The NEPP contained insoluble tannin-like materials, which  
78 was partly made up of procyanidin monomeric constituents.

79 Commercially, date seed powder (DSP) is available as caffeine free, health promoting hot drink  
80 catering to niche markets (Brouk & Fishman, 2016). A roasting process for making a 'coffee-like'  
81 powder from date kernels has been patented by Cohen *et al.* (2011). Ghnimi *et al.* (2015) studied the  
82 sensory properties and chemical attributes of roasted date seed powder. The same authors as well

83 as Sekeroglu *et al.* (2012) reported the absence of caffeine in DSP. In addition, food products with  
84 enhanced level of polyphenols and dietary fibre have been developed in laboratory scale by  
85 incorporating DSP. Platat *et al.* (2015) reported that addition of DSP (Khalas variety) improved the  
86 dietary fibre and phenolic compounds contents, and lowered the acrylamide levels of regular pitta  
87 bread. Similarly, a previous study by Bouaziz *et al.* (2010) incorporated a defatted date seed fibre  
88 concentrate from Deglet nour variety seeds into pitta bread, and noticed improvement in cellulose  
89 and hemicellulose (50 and 20% dry weight basis, respectively). They reported that course-defatted  
90 DSP can improve dietary fibre contents without significant negative effects to the bread quality.

91 *In-vitro* and animal studies on the bioactivity of date seed polyphenols have provided valuable  
92 information on their potential health benefits (Dehghanian *et al.*, 2017; Saryono *et al.*, 2017).  
93 However, whether these bioactive polyphenols are readily accessible for absorption in the intestine  
94 (bioavailable), remains to be established. It is known that food matrix properties have a considerable  
95 effect on the release of polyphenols during digestion (Bohn *et al.*, 2015; Padayachee *et al.*, 2012). It  
96 is also known that the amount of polyphenols escaping absorption in the small intestine and  
97 reaching the colon is high, such that microbial fermentation products may significantly contribute to  
98 the dietary phytochemicals from polyphenol intake (Monagas *et al.*, 2010).

99 In this paper we reported on the stability and *in-vitro* bioaccessibility of the EPP in date seed during  
100 simulated gastric and sequential gastric-intestinal digestions, followed by identification of the  
101 polyphenol metabolites from *in-vitro* colonic fermentation using HPLC. We also reported on the  
102 influence of a food matrix on bioaccessibility using DSP incorporated into yoghurt. The data obtained  
103 gave valuable insight on polyphenol-food matrix interactions, as well as useful information for  
104 potential functional food development using date seed powder.

105

## 106 **Materials and Methods**

### 107 **Materials**

108 Calcium chloride dihydrate, hydrochloric acid (HCl), magnesium sulphate heptahydrate, sodium  
109 chloride, sodium hydroxide, potassium phosphate (mono basic), potassium phosphate (di-basic),  
110 sodium hydrogen carbonate were ACS reagent grade and purchased from Bio21 Stores, University of  
111 Melbourne, Victoria, Australia.

112 Nutrient Agar (NA) and MRS Agar (Oxoid®), AnaeroGen® sachets (AN 0010W, Oxoid®), Anaerobic  
113 indicator strips (BR55, Oxoid®), peptone (LP0034B) and tryptone (LP0042R) were purchased from  
114 Thermo Fisher scientific, Vic, Australia. Nitrogen gas (purity 4.0, O<sub>2</sub> free N<sub>2</sub>) was purchased from  
115 Coregas pty Ltd, NSW, Australia. BD Gas pak™ 3.5 L anaerobic jars were purchased from BD  
116 Australia, NSW, Australia.

117 Bile extract from porcine (B8631), casein (C3400), guar (G4129), L-cysteine HCl (C7880), mucin from  
118 porcine stomach (M1778), pancreatin from porcine pancreas (P6976), pectin from citrus peel  
119 (P9135), pepsin from porcine gastric mucosa (P6887), phosphate buffered saline (P3813), soluble  
120 potato starch (S2004), and yeast extract (09182) were purchased from Sigma-Aldrich, NSW,  
121 Australia.

### 122 **Preparation of date seed powder**

123 Date seed powder (DSP) for the preparation of DSP yoghurt (DSPY), and *in-vitro* digestion were  
124 prepared as previously described by Sirisena *et al.* (2016). Briefly, seeds were separated from date  
125 fruits (Deglet nour variety) harvested from date palms cultivated in Arid Zone Research Institute in  
126 Alice Springs, Australia. Date seeds were washed with warm water and air dried at room  
127 temperature in the laboratory for 5 days. Seeds were grounded into a powder fine enough to pass a  
128 1 mm sieve (cutting mill Retsch SM100, Germany).

### 129 **Preparation of yoghurt containing date seed powder**

130 Yoghurt fortified with 5% (w/w) date seed powder (DSPY) was prepared, adapting the methods of  
131 Mudannayake (2015). In this method, 13 g of skim milk powder (Devondale, Victoria, Australia) was  
132 dissolved in 100 mL of milliQ water to obtain 13% (w/v) reconstituted skim milk with a protein  
133 content of 35 mg/mL. The reconstituted skim milk was distributed into 50 mL sterile centrifuge tubes  
134 (20 ml each), and DSP was added at 5% (w:v). These milk samples were then heated to 90 °C for 10  
135 min in a water bath, cooled to 40 °C, and inoculated with pre-cultures La-5 and Bb-12 at a  
136 concentration of 1% (w/v) each. The mixture further incubated at 40 °C for 6 hr to set the yoghurt.  
137 The pH was monitored before and after incubation to track fermentation progress and yoghurt  
138 formation. The set yoghurt was stirred using a tube homogeniser to obtain uniform distribution of  
139 DSP throughout the system before subjecting to digestions. Controls of yoghurt without adding DSP  
140 and DSP in milk without heat treatments were also prepared and treated similarly for digestion.

### 141 **Effect of heat-treatment on polyphenol release**

142 In order to examine the effect of heat-treatment during yoghurt preparation on polyphenol release,  
143 DSP was added to the reconstituted skim milk (5%, w:w) and subjected to similar heat treatment but

144 without the fermentation step. The heat treated unfermented milk containing DSP (DPSM +heat)  
145 was then stirred vigorously to uniformly suspend the DSP, and 5 g sample was subjected to gastric  
146 digestion. DSP in milk without going through the heat treatment (DSPM -heat) was also prepared  
147 and samples subjected to gastric digestion as a control (no heat treatment).

## 148 **Gastric and intestinal digestion**

### 149 **Preparation of Simulated Gastric Fluid (SGF) and Intestinal Fluid (SIF)**

150 SGF and SIF were prepared following US pharmacopeia Convention (2009). For SGF, 2 g NaCl and 7  
151 mL HCl (37%, w/v) were mixed with 800 mL MilliQ water, followed by pH adjustment to 1.2 using 1M  
152 NaOH. For each experiment, SGF with pepsin was prepared fresh by adding pepsin at a  
153 concentration of 6.4 mg/mL. The solution was stirred 15 min before use. To prepare SIF, 5 mM  
154 phosphate buffered saline was prepared with NaCl and CaCl<sub>2</sub> to concentration of 0.4 M and 15 mM.  
155 pH was adjusted to 6.8 using 1 M HCl, and stirred using magnetic stirrer immediately prior to use in  
156 order to keep CaCl<sub>2</sub> in solution. Fasted and fed state bile salt concentrations for this study were  
157 selected based on published literature (Holm, Müllertz, & Mu, 2013). Two bile concentrations were  
158 selected to simulate the fasted and fed conditions. Fasted state SIF was prepared with 2.5 mg/mL  
159 and fed state SIF with 40 mg/mL bile concentrations (Fu *et al.*, 2015). To each fasted and fed state  
160 SIF, pancreatin was added immediately before experiment to a concentration of 10 mg/mL.  
161 Prepared solutions were incubated with constant shaking at 37 °C prior to experiment. Pancreatin  
162 was prepared freshly in 5 mM phosphate buffer on the same day of the experiment and was also  
163 kept at 37 °C with shaking.

### 164 **Digestions**

165 Gastric digestion and sequential gastric-intestinal (hereafter referred to as intestinal) digestion  
166 methods were adapted from (Fu *et al.*, 2015). All digestions were carried out in triplicates.

167 Gastric digestion was carried out by adding the SGF to DSP and DSPY in 50 mL sterile centrifuge  
168 tubes. The tubes were placed in a tube shaker inside an incubator set at 37 °C, and incubated with  
169 constant gentle shaking programme for 2 h. The tubes were then centrifuged at 8422 g for 10 min to  
170 separate soluble and insoluble fractions for HPLC analysis of the polyphenols. SGF were also added  
171 to DSPM +heat and DSPM -heat samples and similarly treated and separated into soluble and  
172 insoluble fractions for HPLC analysis.

173 Intestinal digestion was carried out by adding fasted or fed state SIF to separate sets of gastric digest  
174 after 2 h of incubation as described before, with pH adjustment to 6.8 using 1 M NaOH to carry the  
175 digestion forward. This was followed by addition of pancreatin (in buffer phosphate buffer 5 mM, pH

176 7.0) to a final concentration of 1.2 mg/mL in the digestion mixture. The tubes were then placed in a  
177 portable tube shaker and incubated for 3 hr at 37 °C with gentle shaking, followed by centrifugation  
178 at 8422 g (Jouan C3i Q5, California, U.S.A.) to separated soluble and insoluble fractions for HPLC  
179 analysis.

## 180 **Colonic fermentation**

### 181 **Basal media preparation**

182 For basal medium, 5 g soluble starch, 5 g peptone, 5 g tryptone, 4.5 g yeast extract, 4.5 g NaCl, 4.5 g  
183 KCl, 2 g pectin, 4 g mucin, 3 g casein, 1.5 g NaHCO<sub>3</sub>, 0.8 g L-Cysteine HCl, 1.23 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.0 g  
184 guar, 0.5 g KH<sub>2</sub>PO<sub>4</sub>, 0.5 g K<sub>2</sub>HPO<sub>4</sub>, 0.4 g bile salts, 0.11 g CaCl<sub>2</sub> and 1 mL Tween 80 were dissolved  
185 and made up to 1 L in MilliQ water. pH was and adjusted with HCl (1 M) or NaOH (1 M) at 25 °C, and  
186 sterilized at 121 °C for 20 min in the autoclave (Fu 2015).

### 187 **Faecal slurry preparation**

188 Freshly void faeces from a healthy female aged 30 yr, who did not have previous intestinal disease,  
189 maintained a diet free of any polyphenol supplements, and has not consumed probiotics or  
190 antibiotics for 3 months, was used to prepare the faecal slurry. Ethical approval was obtained from  
191 the Veterinary and Agricultural Sciences Human Ethics Advisory Group, University of Melbourne  
192 (Ethics Approval ID: 1545922). Although the limitation in using one donor is acknowledged, this  
193 preliminary report is focussed on biotransformation and the primary emphasis is on detection of  
194 biotransformation of the polyphenols. Briefly, 20 g faeces were weighed into a stomacher bag and  
195 80 g sterilized pre-N<sub>2</sub> flushed 0.1M phosphate buffer (pH = 7.0) was added to make 20 % w/w faecal  
196 slurry. It was then homogenized for 5 min in a stomacher mixer and filtered through sterile muslin  
197 cloth to remove particulate matter. Faecal slurry was then transferred to 50 mL sterile, pre-N<sub>2</sub>  
198 flushed tubes with 5 mL aliquots using sterile pipette. The tubes were used for experiment on the  
199 same day, or stored in -80 °C for analysis within a week. All work involving faecal samples were  
200 carried aseptically under bio-safety chamber (Fu 2015). As the aim of this study was to investigate if  
201 some of the date seed bound polyphenols reach the colon and undergo fermentation, identification  
202 was the bacterial species were not carried out. Also, the fermentation results are related to the  
203 microbiota activities from a single individual, since the experiment only aim to show fermentability  
204 DSP.

### 205 **Bacterial enumeration**

206 Aerobic and anaerobic bacteria were enumerated before the start of the fermentation and after 24  
207 hr of fermentation. For the initial enumeration, 5 mL faecal slurry was mixed with 5 mL of sterile

208 basal medium, and serially diluted to  $10^{-10}$  in sterile buffered peptone water (0.1%) containing 0.5g/L  
209 L-cysteine HCl. The same was done after 24 hr fermentation samples in triplicate.

210 From each dilution 100  $\mu$ L was transferred onto either MRS or NA plates in triplicate, using spread  
211 plate method. The NA plates were incubated aerobically at 37 °C for 48 h. MRS plates were  
212 incubated anaerobically using anaerobic chamber containing anaerobic gas generator and indicator,  
213 at 37 °C for 48 hrs. Selected dilutions were used for enumeration.

#### 214 ***In-vitro* colonic fermentation**

215 Colonic fermentation was carried on the insoluble fraction (8422 g precipitate) from intestinal  
216 digesta. Previously prepared tubes containing 5 mL faecal slurry was mixed with 5 mL of basal media,  
217 both pre-warmed to 37 °C. To this, 0.5 g sediments from intestinal digestion (DSP or DSPY) were  
218 added. All treatments were prepared in triplicate. All tubes were then flushed with O<sub>2</sub> free N<sub>2</sub> and  
219 then lids were closed with hand-tight. They were then placed in an anaerobic chamber with  
220 anaerobic gas generator and anaerobic condition indicator. The chambers were placed in a shaking  
221 incubator (ZWYR-240, Labwit, Shanghai, China) maintained at 100 rpm and 37 °C, and the  
222 fermentation was carried out for 24 hrs. Blanks were prepared without the sample, and also sample  
223 and basal media without any faecal slurry. After 24 hr fermentation the tubes were stored at -20 °C  
224 and processed for HPLC analysis.

#### 225 **HPLC analysis**

##### 226 **Preparation of samples for HPLC analysis**

227 The gastric and intestinal digestions as well as colonic fermentation samples were centrifuged at  
228 8422 g for 10 min at 20 °C and separated into soluble and precipitate fractions. Polyphenols were  
229 then extracted from the soluble (8422 g supernatant) and insoluble fractions (8422 g precipitate) of  
230 gastric and intestinal digestions using 20 mL acetone/methanol (1:1, v:v). Organic extracts from each  
231 fraction were evaporated to a small volume under vacuum at 40 °C (Buchi Vac V500 system, Flawil,  
232 Switzerland), reconstituted to 5 mL with the extraction solvents, and filtered through 0.45  $\mu$ M nylon  
233 filter disc for HPLC analysis. Additionally, gastric and intestinal digestion mixtures without separation  
234 into supernatant and precipitate by centrifugation were also extracted following the same  
235 procedures.

##### 236 **HPLC methods**

237 HPLC separation and quantitation of polyphenols and polyphenol fermentation products were  
238 performed using a Waters 2690 Alliance HPLC separation module (Waters, NSW, Australia),  
239 equipped with a Gemini C18 Silica 250  $\times$  4.6 mm, 5  $\mu$ M column (Phenomenex Inc., NSW, Australia),

240 and a Waters 2998 Photodiode Array (PDA) detector. The mobile phases used were; MilliQ water  
241 with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B).

242 HPLC analysis of gastric and intestinal digested samples was based on a method described by  
243 Sirisena *et al.* (2017) for analysis of date seed free polyphenols. A linear gradient of 5-30% of B in A  
244 over 60 min was used for the elution. PDA detection was set at  $\lambda$  280 nm. For colonic fermentation  
245 samples, a linear gradient of 2-100% B in A over 60 min was used, with the PDA detector set at  $\lambda$  270  
246 nm (Appeldoorn *et al.*, 2009a). HPLC analyses were carried out for 3 extracted samples per each  
247 treatment.

### 248 **Quantification of polyphenols**

249 We focused on 5 major DSP polyphenols (catechin, epicatechin, procyanidin B1, procyanidin B2, and  
250 procyanidin A2) that were previously identified by HPLC-MS (Sirisena *et al.*, 2017). Their stability and  
251 *in-vitro* bioaccessibility were monitored in gastric and intestinal digestion stages. HPLC peaks  
252 corresponding to the polyphenols were confirmed by an internal standard spiking method: the  
253 putative peak was spiked with added standard and the increased in peak area of the target peak  
254 indicates spiking of peak. Quantification of individual polyphenol in the sample was based on its  
255 peak area and the peak area of standard curve generated with the standard compounds, and was  
256 expressed as  $\mu\text{g/g}$  DSP.

### 257 **Statistical analysis**

258 Statistical analyses were performed using SAS software (Version 9.2). The collected data were  
259 subjected to one-way analysis of variance (ANOVA), and the means were separated using Least  
260 Significant Difference (LSD) test ( $p < 0.05$ ). Final results were reported as mean  $\pm$  SD ( $n=3$ ) with 2  
261 significant figures according to EURACHEM guidelines (Ellison and Williams, 2012).

## 262 **Results and Discussion**

263 Bioaccessibility is define as the accessibility of a nutrient from food matrixes that are available for  
264 absorption during digestion, thus is a prerequisite for its bioavailability in the body. It is known that  
265 in addition to free polyphenols, significant amount are associated with or weakly bound to food  
266 matrix components such as protein and dietary fibre (Padayachee *et al.*, 2012). The release of bound  
267 polyphenols from the food matrix into intestinal fluid during digestion requires their solubilisation,  
268 which is also a prerequisite for their absorption and thus bioavailability (Fu *et al.*, 2015). Additionally,  
269 plant materials in general, including DSP, contain significant amount of tannin like high molecular  
270 weight (HMW) polymeric polyphenolic materials that are not soluble, thus are not accessible during

271 digestion (Arranz *et al.*, 2010). Free and bound low molecular weight (LMW) polyphenols that  
272 escaped absorption in the small intestine are passed next into the colon where bacterial  
273 fermentation may occur, generating secondary absorbable products (Bohn, 2014). Fermentation of  
274 HMW polyphenols may also occur but to a much lesser extent (Aura, 2008).

275 In this study, two monomeric and 3 dimeric polyphenols from the seeds of *Phoenix dactylifera* L.  
276 were monitored in gastric and intestinal digestion stages. These polyphenols were identified in a  
277 previous study using HPLC-MS as date seeds extractable polyphenols (EPP) and included, catechin,  
278 epicatechin, procyanidin B1, procyanidin B2, procyanidin A2 (Sirisena *et al.*, 2017). These were the  
279 most abundant date seed polyphenols, and those confirmed with HPLC-MS and internal standard  
280 spiking, therefore were selected to be monitored in this digestion study.

281 The reason for not comparing the initial quantities of polyphenols in the DSP to the polyphenol  
282 quantities in SGF is due to the effect of extraction process on the quantities. The quantities extracted  
283 by organic solvents from the raw DSP cannot be directly compared with the quantities released in  
284 the gastric juice, which acts somewhat as an extraction itself. Therefore it makes more sense to  
285 compare the quantities of DSP polyphenols released in the gastric stage and how much is available in  
286 the soluble intestinal stage (i.e. bioaccessible).

287 We determined the level of free (therefore accessible) and matrix bound (therefore not accessible)  
288 LMW polyphenols from DSP and DSPY after *in vitro* gastric or sequential gastric-intestinal digestion.  
289 Free polyphenols were defined by their availability in the supernatant fraction of the digesta as  
290 detected by HPLC after centrifugation of the digestion fluid at 8422 g for 10 min (Figure 1). Similarly,  
291 bound LMW polyphenols associated with the DSP matrixes were recovered from the 8422 g pellet by  
292 organic solvent extraction, and detected by HPLC. The recoveries of the target polyphenols from the  
293 combined free and bound fractions were high, indicating most of polyphenols in the digestion  
294 protocol can be accounted for. This good recovery conclusion was based on comparison with the  
295 polyphenol recoveries from DSP (84-99%) and DSPY (81-99%) in gastric or intestinal digesta without  
296 centrifugation (Supplementary Tables 1 and 2).

### 297 **Bioaccessibility and stability of polyphenols from DSP during gastric and intestinal** 298 **digestion**

299 Accessible polyphenols can be defined as polyphenols that are released from the food matrix into  
300 digestion fluids during digestion so that absorption can occur (Fu *et al.*, 2015). The accessibility of  
301 the target polyphenols from DSP ranged widely (Table 1). Accessibility of catechin, epicatechin,  
302 procyanidin B1 and procyanidin B2 ranged from 31.6-49.6% in gastric digest, 19.4-40.5% in fasted

303 and 27.1-47.2% in fed intestinal digests. By contrast, procyanidin A2 was not released during gastric  
304 or intestinal digestions of DSP, indicating it may not be accessible for absorption.

305 In terms of absolute quantities, procyanidin B1 was the most abundantly accessible polyphenol from  
306 DSP in gastric digest ( $191.6 \pm 2.4 \mu\text{g/g DSP}$ ), which was followed by procyanidin B2 ( $95.8 \mu\text{g/g DSP}$ ),  
307 catechin ( $45.43 \pm 0.81 \mu\text{g/g DSP}$ ) and epicatechin ( $18.25 \pm 0.56 \mu\text{g/g DSP}$ ). The levels in intestinal  
308 digest were smaller, suggesting that most of these polyphenols were released during gastric  
309 digestion. This indicated that the majority of these polyphenols were released in gastric fluid, which  
310 was then carried forward to the intestinal digestion state. The intestine is the main site of absorption  
311 for both polyphenolic monomers and dimers (Appeldoorn *et al.*, 2009a). The 4 polyphenols were  
312 most likely released from their associations with proteins and fibres matrix within the DSP due to the  
313 low pH conditions of the gastric fluid which loosen fibre matrixes (Oliveira and Pintado, 2015), and to  
314 the action of gastric pepsin in unravelling polypeptide structures in digestions. For example, Işık *et*  
315 *al.*, (2014) reported a 70% release of polyphenols embedded in pea protein isolate after 1 h  
316 simulated gastric digestion with pepsin treatment. Still, a significant amount of the polyphenols in  
317 DSP was associated with the DSP matrixes as indicated by their percentage accessibility. These  
318 bound polyphenols were released from the matrix by solvent extraction with acetone/methanol and  
319 quantified by HPLC, but covalently bonded polyphenols to matrix materials would not be extracted  
320 by the procedure.

321 Most of the simulated digestion studies on polyphenols have used extracts or pure compounds, and  
322 studies involving food matrices are limited. Properties on individual food matrices can have a  
323 substantial effect on bioaccessibility of polyphenols as shown by our results. A solid food matrix such  
324 as DSP has to be disrupted and the polyphenols solubilized into digestive fluids to be bio-accessible.  
325 Most polyphenols are located in the plant cell vacuole and apoplast where they are usually  
326 conjugated to monosaccharides, polysaccharides and proteins (Bohn *et al.*, 2015). Once they are  
327 released from the cell upon cell wall break-down due to processing or metabolism, free polyphenols  
328 may form interactive associations with proteins and dietary fibre in the food matrix (Padayachee *et*  
329 *al.*, 2012).

330 The slightly lower polyphenol levels in intestinal fluids indicated loss of polyphenols had occurred  
331 transiting from gastric to intestinal digestion. The levels of free polyphenols in the intestinal digest  
332 compared to the gastric digest ranged from 64.3-92.3% for the fasted state to 91.9-96.5% for the fed  
333 state (Table 1). Polyphenols are known to undergo irreversible structural changes by auto-oxidation,  
334 isomerisation and conjugation in alkaline pH, but some species are more stable than others  
335 dependent on the structure (Friedman and Jürgens 2000). Losses of polyphenols between gastric

336 and intestinal digestion stages have been observed before; for example, Correa-Betanzo *et al.*,  
337 (2014) reported a loss of 44% in wild blue berry. The higher level of polyphenols in the fed state  
338 compared to the fasted state was also observed, and pointed to the enhanced solubility effect due  
339 to bile salts (Bohn, 2014). This effect has been attributed to the ability of bile salts to form micelles  
340 with the polyphenols enhancing their solubility and chemical stability (Silva *et al.*, 2014).

341

### 342 **Bioaccessibility and stability of polyphenols from DSP yoghurt (DSPY) during** 343 **gastric and intestinal digestion**

344 Yoghurt is a good model system for fibre and polyphenol enrichment. DSP itself is a complex food  
345 matrix, and incorporation of yogurt protein and protein gel increases the complexity of the system  
346 to digestion. In addition to the dietary fibres present in DSP, milk proteins in yoghurt are also known  
347 to interact with polyphenols. The non-covalent association between polyphenols with  $\alpha$  and  $\beta$   
348 caseins is known to involve hydrophobic interactions and H-bonding, and a positive correlation has  
349 been observed between binding affinities and number of OH groups in polyphenol (Hasni *et al.*,  
350 2011). Whey protein  $\beta$ -lactoglobulin, which exists as a dimer with each polypeptide containing a  
351 hydrophobic groove, is known to bind polyphenols strongly at that site (Jianbo *et al.*, 2011, von  
352 Staszewski *et al.*, 2012).

353 Yoghurt has a possible impact on the accessibility of polyphenols from DSP as a consequence of  
354 yoghurt culture fermentation and heat treatment during yoghurt manufacturing process.  
355 Accessibility for all 5 polyphenols in the gastric digestion of DSPY was higher than for DSP, and  
356 ranged from 44.2–95.8% (Table 2). The increase in accessibility of procyanidin B1, procyanidin B2,  
357 and epicatechin from DSPY, compared to DSP were 1.5, 1.7 and 2.8 folds, respectively. In particular,  
358 procyanidin A2, which was not accessible from DSP, was highly accessible from DSPY. Appeldoorn *et*  
359 *al.* (2009b) reported that procyanidin A2 is better absorbed compared to procyanidin B1 and B2 in  
360 the rat intestine, indicating its' potential contribution to procyanidin related health benefits.

361 Another notable effect of yoghurt on accessibility was the large increase in the absolute quantities of  
362 free catechin, epicatechin, procyanidin B2 and procyanidin A2 in gastric digest, which were  $76.0 \pm$   
363  $2.1$ ,  $150.1 \pm 1.1$ ,  $431.7 \pm 7.9$ , and  $327.8 \pm 7.8$   $\mu\text{g/g}$  DSP, respectively (Table 2). Level of procyanidin B1  
364 in DSPY gastric digest ( $71.9 \pm 1.8$   $\mu\text{g/g}$  DSP) was however lower than from DSP gastric digestion  
365 ( $95.8 \pm 1.2$   $\mu\text{g/g}$  DSP). This could be a result of the loss of procyanidin B1 in gastric digest due to its  
366 characteristic chemical instability at very low pH (Dallas *et al.*, 2003).

367 The higher absolute quantities of free polyphenols would suggest that yoghurt culture fermentation  
368 has increased the level of monomeric and dimeric polyphenols from DSP and also have a positive  
369 effect on polyphenol release from DSP matrix. These effects have been observed before in other  
370 studies on polyphenol enrichment with yoghurts. Sun-Waterhouse *et al.* (2013) reported that pre-  
371 fermentation affected the profile and quantity of accessible polyphenols by over 3 folds compared  
372 to post fermentation with the addition of polyphenols to yoghurt.

373 Since DSP was added prior to heat treatment in yoghurt production, it might be possible that heat  
374 treatment has contributed to the higher level of free polyphenols. Indeed, adding DSP to the same  
375 milk mixture used to make the yoghurt and subjected it to yoghurt making heat treatment similar to,  
376 but without the bacterial fermentation step, increased the released of the polyphenols by 10-25%  
377 compared to unheated control (data not shown). Thus, heat treatment in yoghurt making may  
378 increase the free polyphenol levels in gastric digest.

379 Similar to DSP, the levels of the free polyphenols in DSPY intestinal digestion did not exceed the  
380 levels in DSPY gastric digest, indicating that they were completely released during gastric digestion.  
381 However, the stability in DSPY intestinal digestion was significantly ( $p < 0.05$ ) lower than their stability  
382 in DSP intestinal digestion for some polyphenols, but was similar for others in this complex system. It  
383 ranged from 31.5-96.5% in fasted state, and 35.4-99.5% in fed state when compared to the levels in  
384 DSPY gastric digest (Table 2). Epicatechin was least stable with less than 35% remaining while  
385 procyanidin B2 and A2 were the most stable with >87% remaining in intestinal digest. These  
386 differences could be due to different affinities of polyphenols towards milk proteins, which affected  
387 their stability in the intestinal pH. For example, Arts *et al.* (2002) observed that  $\beta$ -casein strongly  
388 masked the antioxidant activity of epicatechin compared with catechin at pH 7.4, indirectly  
389 indicating differences in binding affinities.

390

### 391 **Colonic fermentation**

392 The insoluble materials from DSP and DSPY intestinal digestion were carried forward to the colonic  
393 fermentation phase. The soluble materials were not carried forward as they are commonly assumed  
394 to be absorbable from the intestine (Bohn, 2014). In addition to monitoring fermentation products  
395 from polyphenols, pH changes and bacterial counts were also recorded (Supplementary Tables 3 and  
396 4). The initial pH of faecal slurry was at pH 6.9, but after 24 h of fermentation the pH was decreased  
397 in all samples. As expected, the decrease in pH was more pronounced for DSP yoghurt, due to  
398 continuing activity of lactic acid bacteria from the yoghurt on the yoghurt contents (Fu *et al.*, 2016).

399 Aerobic and anaerobic bacterial counts in the original faecal slurry were  $4.92 \pm 0.05$  and  $8.61 \pm 0.10$   
400  $\log_{10}$  CFU/mL, respectively. After 24 h fermentation, anaerobic counts increased by approximately 1  
401 log CFU/mL, while aerobic counts showed a smaller increase, indicating that the fermentation  
402 conditions were more favourable towards anaerobic bacteria.

403 A number of bacterial fermentation peaks, not present in control faeces, were tentatively identified  
404 in DSPY based on spiking of the chromatographic peaks with commercial standards, which showed  
405 that polyphenol fermentation metabolites were produced (Supplementary Fig. 1). These included  
406 simple phenolic compounds such as 4-hydroxyphenyl acetic acid, 3-hydroxyphenyl acetic acid, 3-(4-  
407 hydroxyphenyl) propionic acid, ferulic acid, 3-(2-hydroxyphenyl)propionic acid and 3-propionic acid  
408 (Table 3). All these compounds have been previously identified in *in-vitro* human faecal fermentation  
409 of polyphenols (Aura, 2008).

410 3-Phenylpropionic acid was the most abundant phenolic acid, detected at  $306.8 \pm 9.6 - 488 \pm 12 \mu\text{g/g}$   
411 and  $313.53 \pm 7.6 - 371.3 \pm 8.2 \mu\text{g/g}$  from DSP and DSPY, respectively after colonic fermentation. It is  
412 a known major metabolite from human gut microbial fermentation of flavanols, flavanones, flavones  
413 and polymeric flavan-3-ols (proanthocyanidin) and a final product of ring fission metabolic pathway  
414 of catechins (Aura, 2008). In addition, 3-(4-hydroxyphenyl)propionic acid was detected at  $117.0 \pm 5.8$   
415  $- 123.9 \pm 1.8 \mu\text{g/g}$  and  $96.9 \pm 1.0 - 107.8 \pm 4.1 \mu\text{g/g}$  DSP from DSP and DSPY, respectively. Rechner *et*  
416 *al.* (2004) reported this propionic acid derivative to be a fermentation metabolite of catechin and  
417 naringin.

418 B-type procyanidin dimers which are present in date seeds are known to produce 2-(3,4-  
419 dihydroxyphenyl)acetic acid and 5-(3,4-dihydroxyphenyl)- $\gamma$ -valerolactone as the main fermentation  
420 metabolites (Appeldoorn *et al.*, 2009a). The same authors reported also 3-hydroxyphenyl acetic acid,  
421 4-hydroxyphenylacetic acid, 3-hydroxyphenylpropionic acid, phenylvaleric acids, monohydroxylated  
422 phenylvalerolactone, and 1-(3',4'-dihydroxyphenyl)-3-(2'',4'',6''-trihydroxyphenyl) propan-2-ol in the  
423 B-type procyanidin dimer fermentation products. In this study, 3-hydroxyphenyl acetic acid was a  
424 major metabolite detected from both DSP ( $189.5 \pm 3.5 - 213 \pm 13$ ) and from DSPY ( $114.9 \pm 5.3 - 188.6$   
425  $\pm 3.1$ ), while 4-hydroxyphenyl acetic was detected at much lower levels ( $1.60 \pm 0.10 - 45.2 \pm 4.2$  and  
426  $48.95 \pm 0.54 - 59.0 \pm 2.8$  for DSP and DSPY, respectively).

427 Ferulic acid was detected at fairly high levels in DSP ( $140 \pm 9.1 - 216 \pm 6.3 \mu\text{g/g}$  DSP) and DSPY ( $372 \pm$   
428  $8.2 - 456 \pm 10 \mu\text{g/g}$  DSP). Free ferulic acid has previously been detected in some date seed varieties  
429 (Al-Farsi and Lee, 2008), however not in the Deglet nour (Habib *et al.*, 2014; Sirisena *et al.*, 2017).  
430 Ferulic acid is usually found esterified with hemicelluloses (Aura, 2008), and it has been reported

431 that fibre-bound ferulic acid is released in human colon by the action of human colonic microbiota  
432 (Kroon *et al.*, 1997; Rondini *et al.*, 2004). Furthermore, ferulic acid was found in human urine as a  
433 colonic metabolite of condensed tannin (proanthocyanidin) (Aura, 2008). We can therefore  
434 speculate that ferulic acid found in the colonic fermentation samples were microbial metabolites of  
435 the high molecular weight proanthocyanidins, or those bound to hemicelluloses.

436 According to our results, none of the five major polyphenols we were monitoring in this study were  
437 detected in the colonic fermentation samples. Therefore remaining amounts were not reported. It  
438 can be assumed that apart from some amount of loss in extraction, majority was available in the  
439 supernatant intestinal fluid, and the remaining were transformed/fermented to other compounds  
440 during colonic fermentation.

441

## 442 **Conclusions**

443 This study provides good preliminary evidence that validates the claim of DSP as a functional  
444 ingredient. The major date seeds polyphenols, catechin, epicatechin, and procyanidin A2, B1 and B2,  
445 were stable during *in-vitro* gastric digestion. While some losses occur with follow up intestinal  
446 digestion, substantial amounts were bio-accessible in the intestinal stage as soluble materials.  
447 Yoghurt culture, heating, and fermentation significantly increased the bioaccessibility of the  
448 polyphenols during gastric and intestinal digestion. Procyanidin A2, which was not accessible from  
449 DSP, was highly accessible from DSPY during digestion. Major phenolic metabolites from faecal  
450 bacteria fermentation of the digestion insoluble polyphenols were detected, and included 3-  
451 phenylpropionic, 4-hydroxyphenyl acetic, 3-(2-hydroxyphenyl) propionic acid, 3-(4-hydroxyphenyl)-  
452 propionic, 3-hydroxyphenyl acetic acid and ferulic acid. Thus, DSP can release substantial amounts of  
453 polyphenols upon gastro-intestinal digestion, and therefore can potentially be added to food  
454 products without further extraction. DSP may provide further health benefits by the production of  
455 phenyl derivatives of short chain fatty acid as a result of colonic fermentation of the insoluble  
456 polyphenols.

457

458

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460

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462 **References**

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580

## Legend to Figures

581

582 Figure 1: HPLC chromatograms of soluble fraction of digesta; (A) Date seed powder (DSP) gastric  
583 digest, (B) DSP intestinal fasted states digest, (C) DSP intestinal fed states digest, (D) Date seed  
584 powder yoghurt (DSPY) gastric digest, (E) DSPY intestinal fasted states digest, (F) DSPY intestinal fed  
585 states digest. Compounds were identified by a HPLC internal standard spiking method which was  
586 correlated by UHPLC-MS analysis in a separate study (Sameera et al. 2017): (1) procyanidin B1, (2)  
587 catechin, (3) procyanidin B2, (4) epicatechin (5) procyanidin A2.

588

## Legend for supplementary files

589 Supplementary Figure 1: HPLC chromatograms colonic fermentation products of date seed powder  
590 (DSP) (A) fasted state (B) fed state and date seed powder yoghurt (DSPY) fasted (C) and fed (D)  
591 states. Compounds were tentatively identified by a HPLC internal standard spiking method: (1) 4-  
592 hydroxyphenyl acetic, (2) 3-hydroxyphenyl acetic, (3) 3-(4 hydroxyphenyl)propionic acid, (4) ferulic  
593 acid, (5) 3-(2-hydroxyphenyl) propionic acid, (6) 3-phenylpropionic acid. Insert shows a blow up  
594 region of the chromatogram.

595 Supplementary Table 1: Recovery of polyphenols from date seed powder (DSP) after gastric and  
596 intestinal digestion

597 Supplementary Table 2: Recovery of polyphenols from date seed powder yoghurt (DSPY) after  
598 gastric and intestinal digestion

599 Supplementary Table 3: pH changes in date seed powder (DSP) and date seed powder yoghurt  
600 (DSPY) colonic fermentations and control faecal slurry fermentation

601 Supplementary Table 4: Aerobic and anaerobic bacterial counts before and after colonic  
602 fermentation of date seed powder (DSP), date seed powder yoghurt (DSPY) and control samples

**Table 1: Quantification of free and bound polyphenols from DSP after SGF and SIF-Fasted and SIF-Fed digestion**

	Free	Matrix Bound	Total	Accessibility
	Polyphenols*	Polyphenols**		
	FPP (µg/g DSP)	BPP (µg/g DSP)		
	FPP + BPP (µg/g DSP)	FPP/(FPP+BPP) (%)		
<b>SGF</b>				
<b>Catechin</b>	45.43 ± 0.81 <sup>b</sup>	50.75 ± 0.87 <sup>b</sup>	96.18	47.2
<b>Epicatechin</b>	18.25 ± 0.56 <sup>a</sup>	39.3 ± 1.5 <sup>a</sup>	57.55	31.6
<b>Procyanidin B1</b>	191.6 ± 2.4 <sup>d</sup>	473.8 ± 5.3 <sup>d</sup>	665.4	28.7
<b>Procyanidin B2</b>	95.8 ± 1.2 <sup>e</sup>	97.0 ± 1.2 <sup>e</sup>	192.8	49.6
<b>Procyanidin A2</b>	ND	25.51 ± 0.28 <sup>f</sup>	25.51	0
<b>SIF-Fasted</b>				
<b>Catechin</b>	40.3 ± 1.1 <sup>g</sup> (88.8%) <sup>***</sup>	59.2 ± 1.0 <sup>h</sup>	99.5	40.5
<b>Epicatechin</b>	11.75 ± 0.33 <sup>f</sup> (64.3%)	48.6 ± 1.0 <sup>g</sup>	60.35	19.4
<b>Procyanidin B1</b>	177.0 ± 1.5 <sup>i</sup> (92.3%)	506.9 ± 3.7 <sup>j</sup>	683.9	25.8
<b>Procyanidin B2</b>	69.6 ± 3.0 <sup>j</sup> (72.6%)	112.5 ± 2.0 <sup>k</sup>	182.1	38.2
<b>Procyanidin A2</b>	ND	23.5 ± 1.3 <sup>l</sup>	23.5	0
<b>SIF-Fed</b>				
<b>Catechin</b>	43.88 ± 0.95 <sup>l</sup> (96.5%)	49.00 ± 0.59 <sup>b</sup>	92.88	47.2
<b>Epicatechin</b>	16.93 ± 0.85 <sup>k</sup> (92.7%)	41.4 ± 1.1 <sup>a</sup>	58.33	28.9
<b>Procyanidin B1</b>	181.6 ± 1.7 <sup>o</sup> (94.7%)	488.2 ± 5.6 <sup>n</sup>	669.8	27.1
<b>Procyanidin B2</b>	88.15 ± 0.62 <sup>p</sup> (91.9%)	98.83 ± 0.45 <sup>e</sup>	186.98	47.1
<b>Procyanidin A2</b>	ND	23.62 ± 0.56 <sup>l</sup>	23.63	0.00

\*Solubilised polyphenols associate with the 10 000g supernatant of the digesta, which was extracted from the supernatant materials into methanol/acetone and analysed by HPLC

\*\* Insoluble polyphenols associate with the 10 000g precipitate of the digesta, which was extracted from the precipitated materials into methanol/acetone and analysed by HPLC

\*\*\*Stability of polyphenol expressed as %: (free polyphenol in SIF)/(free polyphenol in SGF)

<sup>a,b,c...</sup> Means followed by different superscript letters within each column indicates statistically significant values (P<0.05)

**Table 2: Quantification of free and bound polyphenols from DSPY after SGF and SIF Fasted and SIF-Fed digestion**

	<b>Free Polyphenols*</b> FPP ( $\mu\text{g/g DSP}$ )	<b>Matrix Bound Polyphenols**</b> BPP ( $\mu\text{g/g DSP}$ )	<b>Total</b> FPP + BPP ( $\mu\text{g/g DSP}$ )	<b>Accessibility (%)</b> FPP/(FPP+BPP) (%)
<b>SGF</b>				
<b>Catechin</b>	76.0 $\pm$ 2.1 <sup>b</sup>	61.4 $\pm$ 1.2 <sup>b</sup>	137.4	55.3
<b>Epicatechin</b>	150.1 $\pm$ 1.1 <sup>a</sup>	14.67 $\pm$ 0.56 <sup>a</sup>	164.77	91.1
<b>Procyanidin B1</b>	71.9 $\pm$ 1.8 <sup>d</sup>	90.7 $\pm$ 2.8 <sup>d</sup>	162.7	44.2
<b>Procyanidin B2</b>	431.7 $\pm$ 7.9 <sup>e</sup>	56.5 $\pm$ 1.0 <sup>e</sup>	488.2	88.4
<b>Procyanidin A2</b>	327.8 $\pm$ 7.8 <sup>f</sup>	42.48 $\pm$ 0.44 <sup>a</sup>	1026.08	95.8
<b>SIF-Fasted</b>				
<b>Catechin</b>	48.2 $\pm$ 1.8 <sup>g</sup> (63.2%) <sup>***</sup>	80.8 $\pm$ 2.8 <sup>f</sup>	129.0	37.3
<b>Epicatechin</b>	47.4 $\pm$ 0.25 <sup>g</sup> (31.5%)	80.2 $\pm$ 1.4 <sup>f</sup>	127.6	37.1
<b>Procyanidin B1</b>	69.5 $\pm$ 2.1 <sup>d</sup> (96.5%)	71.9 $\pm$ 1.4 <sup>h</sup>	141.4	49.1
<b>Procyanidin B2</b>	309.5 $\pm$ 3.4 <sup>i</sup> (71.6%)	162.0 $\pm$ 2.2 <sup>i</sup>	471.5	65.6
<b>Procyanidin A2</b>	285.53 $\pm$ 1.3 <sup>j</sup> (87.0%)	44.07 $\pm$ 1.6 <sup>j</sup>	329.6	86.6
<b>SIF-Fed</b>				
<b>Catechin</b>	55.7 $\pm$ 2.5 <sup>k</sup> (73.2%)	88.1 $\pm$ 1.3 <sup>k</sup>	143.8	38.7
<b>Epicatechin</b>	53.2 $\pm$ 0.95 <sup>k</sup> (35.4%)	93.8 $\pm$ 5.5 <sup>d</sup>	147.0	36.1
<b>Procyanidin B1</b>	71.6 $\pm$ 3.0 <sup>bd</sup> (99.5%)	97.13 $\pm$ 1.4 <sup>m</sup>	168.73	42.4
<b>Procyanidin B2</b>	229.03 $\pm$ 4.2 <sup>f</sup> (53.0%)	149.06 $\pm$ 3.1 <sup>n</sup>	378.09	60.5
<b>Procyanidin A2</b>	298.8 $\pm$ 3.9 <sup>l</sup> (91.1%)	31.13 $\pm$ 0.85 <sup>g</sup>	329.93	90.5

\* Solubilised polyphenols associate with the 10 000g supernatant of the digesta, which was extracted from the supernatant materials into methanol/acetone and analysed by HPLC

\*\* Insoluble polyphenols associate with the 10 000g precipitate of the digesta, which was extracted from the precipitated materials into methanol/acetone and analysed by HPLC

\*\*\*Stability of polyphenol expressed as %: (free polyphenol in SIF)/(free polyphenol in SGF)

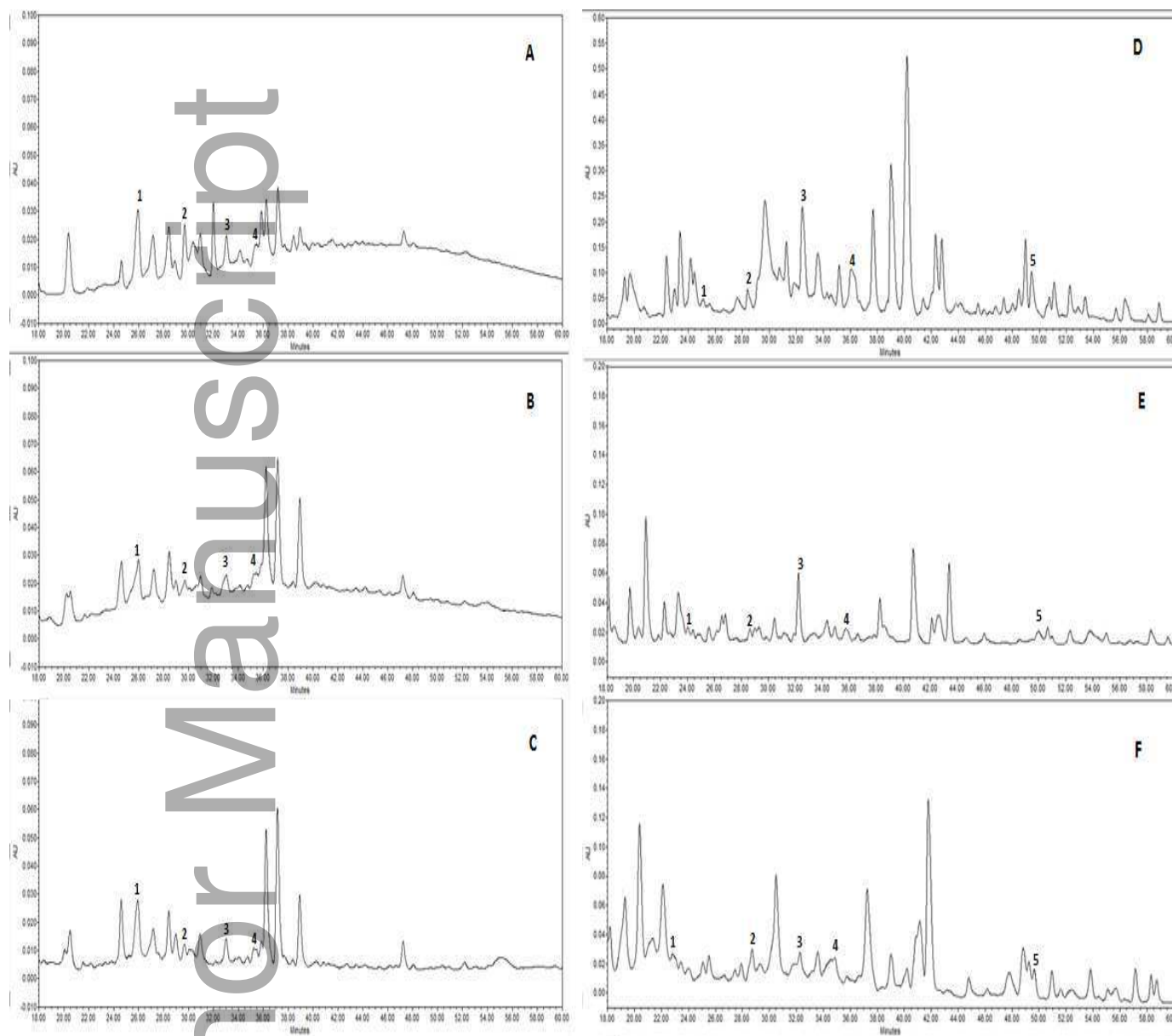
<sup>a,b,c...</sup> Means followed by different superscript letters within each column indicates statistically significant values (P<0.05).

**Table 3 Quantification of polyphenol metabolites from colonic fermentation of bound polyphenols in DSP and DSPY after intestinal digestion**

Peak no.	fermentation product	DSP colonic fasted ( $\mu\text{g/g DSP}$ )	DSP colonic fed ( $\mu\text{g/g DSP}$ )	DSPY colonic fasted ( $\mu\text{g/g DSP}$ )	DSPY colonic fed ( $\mu\text{g/g DSP}$ )
3	4-hydroxyphenyl acetic	$1.60 \pm 0.10^a$	$45.2 \pm 4.2^b$	$48.95 \pm 0.54^b$	$59.0 \pm 2.8^c$
4	3-hydroxyphenyl acetic	$213 \pm 13^a$	$189.5 \pm 3.5^b$	$114.9 \pm 5.3^c$	$188.6 \pm 3.1^d$
5	3-(4-hydroxyphenyl)-propionic acid	$117.0 \pm 5.8^a$	$123.9 \pm 1.8^a$	$107.8 \pm 4.1^b$	$96.9 \pm 1.0^b$
6	Ferulic acid	$216.2 \pm 6.3^a$	$140.8 \pm 9.1^b$	$372.4 \pm 8.2^c$	$456 \pm 10^d$
7	3-(2-hydroxyphenyl)propionic acid	$5.25 \pm 0.58^a$	$4.89 \pm 0.31^a$	$0.850 \pm 0.020^b$	$1.8600 \pm 0.0010^c$
8	3-phenylpropionic	$306.8 \pm 9.6^a$	$488 \pm 12^b$	$313.53 \pm 7.6^a$	$371.3 \pm 8.2^c$

<sup>a,b,c...</sup> Means followed by different superscript letters within each row indicates statistically significant values ( $P < 0.05$ ).

1



**Figure 1: HPLC chromatograms of soluble fraction of gastric and intestinal digesta. (A) DSP gastric digest, (B) DSP intestinal fasted states digest, (C) DSP intestinal fed states digest, (D) DSPY gastric digest, (E) DSPY intestinal fasted states digest, (F) DSPY intestinal fed states digest. Compounds were identified by a HPLC internal standard spiking method which was correlated by UHPLC-MS analysis in a separate study (Sameera et al. 2017): (1) procyanidin B1, (2) catechin, (3) procyanidin B2, (4) epicatechin (5) Procyanidin A2.**