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Keywords: Camu-camu; Myrciaria dubia; Phenolics; Ellagitannins; Ellagic acid conjugates; Proanthocyanidins; Antioxidant capacity; Vitamin C; Dehydrated powders.

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Cover Letter

MINISTERIO
DE ECONOMÍA
Y COMPETITIVIDAD

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

CENTRO DE EDAFOLOGÍA Y BIOLOGÍA
APLICADA DEL SEGURA (CEBAS)

Prof. G. Birch

Editor Food Chemistry

15th-September-2012

Dear Prof. Birch,

Please find with this message the article entitled 'Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics, vitamin C and antioxidant capacity of two powder products from camu-camu fruit (*Myrciaria dubia*)', for consideration to be published in *Food Chemistry*.

Although there are previous articles on camu-camu antioxidant activity and phenolic composition, this is the first study in depth of the phenolic compounds present in this berry. Particularly relevant are the proanthocyanidins that are the main constituents in the dried powder prepared from the skin residue of pulp preparation. Camu-camu is an emerging Amazonian berry that has a promising future in the field of functional foods and nutraceuticals.

Yours sincerely,

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Highlights.

- Although there are previous articles on camu-camu antioxidant activity and phenolic composition, this is the first study in depth of the phenolic compounds present in this berry.

- 53 phenolic compounds were characterized by HPLC-DAD-ESI-MS-MS and High-Resolution UPLC-Q-TOF MS-MS.

- Particularly relevant are the pranthocyanidins that are the main constituents in the dried powder prepared from the skin residue of pulp preparation (3.5 g/100 g powder). These had a mean degree of polypmerization of 3, and were oligomers of catechin, gallocatechin, catechin gallate and gallocatechin gallate.

- This is also a relevant source of ellagitannins, free ellagic acid, and glycosidic conjugates.

- Camu-camu is an emerging Amazonian berry that has a promising future in the field of functional foods and nutraceuticals. The preparation of powders is a convenient procedure for camu-camu ingredients for these industries.

*Manuscript
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1 **Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics,**
2 **vitamin C and antioxidant capacity of two powder products from camu-camu**
3 **fruit (*Myrciaria dubia*)**

4

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19
20

Abstract

The aims of this study were the evaluation of polyphenols and vitamin C content, and antioxidant capacity of dehydrated pulp powder and the dried flour obtained with the skin residue remaining after pulp preparation from camu-camu (*Myrciaria dudia*). Fifty three different phenolics were characterized by HPLC-DAD-ESI-MS-MS and UPLC-HR-QTOF-MS-MS and their amount in camu-camu flour was higher than the pulp powder (4007.95 mg/100 g *vs* 48.54 mg/100 g). In both powders the flavonol myricetin and conjugates, ellagic acid and conjugates and ellagitannins were detected. Cyanidin 3-glucoside, and quercetin and its glycosides were only found in the pulp powder, while proanthocyanidins were only present in the flour (3.5g/ 100g, mean degree of polymerization 3). The vitamin C content was smaller in pulp powder (3.5%) than in the flour (9.1%). The radical-scavenging capacity of both powders was determined by the DPPH, ABTS and ORAC assays, and was higher for camu-camu flour as could be expected for its higher phenolics and vitamin C content.

Keywords: Camu-camu; *Myrciaria dubia;* Phenolics; Ellagitannins; Ellagic acid conjugates; Proanthocyanidins; Antioxidant capacity; Vitamin C; Dehydrated powders.

1. Introduction

Consumption of fruits and vegetables is known to lower the risk of several diseases, including cardiovascular diseases, cancer and stroke (Willett, 2002) and such health benefits are mainly attributed to the content in antioxidant compounds and particularly vitamin C and phytochemicals such as polyphenols and carotenoids (Steinmetz & Potter, 1996).

Among the fruits, berries are reported to exhibit many beneficial effects in human health (Seeram, 2010; Seeram, 2012). This is well documented by several studies and has been the focus of much current research on chemoprevention of cardiovascular diseases (Basu, Rhone, & Lyons, 2010). As well as being a good source of vitamin C, dietary fiber, and minerals, berries contain high levels of natural polyphenol components that act as potent antioxidants.

Among the berries, those produced by the genus *Myrciaria* have received attention recently due to their high content in antioxidants including vitamin C and polyphenols. Thus *Myrciaria dubia* (camu-camu) (da Silva et al., 2012), *M. jaboticaba* (Leite, Malta, Riccio, Eberlin, & Pastore, 2011; Wu, Dastmalchi, Long, & Kennelly, 2012) and *M. vexator* (Dastmalchi et al., 2012) fruits have been recently studied. An increase of plasma antioxidant potential of rats after the intake of freeze-dried jaboticaba peel has recently been demonstrated (Leite et al., 2011). Camu-camu is a native Amazonian bush from the Myrtaceae family. Its fruits are round berries having an average diameter of 2.5 cm; its pulp is pink, while its skin is green when immature and changes during the ripening process from green to red-purple due to the presence of anthocyanins (Zanatta & Mercadante, 2005). Camu-camu is appreciated for its high content of ascorbic acid, which varies from 1.9-2.3 g / 100 g fresh matter depending on the maturity stage (Chirinos, Galarza, Betalleluz-Pallardel, Pedresch, & Campos, 2010). Compared to other fruits, camu-camu is considered one of the richest sources of vitamin C, with a higher content than acerola (Rufino, Alves, de Brito, Péere-Jiménez, Saura-Calixto, & Mancini-Filho, 2010).

64 Camu camu fruits have a significant use history as edible and as traditional medicines with
65 different ethnobotanical uses throughout the tropical and subtropical world (Flores, 1998).
66 In addition to vitamin C, several studies have reported that camu-camu is a good source of
67 bioactive phytochemicals. Different concentrations of flavan-3-ols, flavonols, flavanones, gallic
68 acid and ellagic acid were also detected (Chirinos et al., 2010; de Souza Schmidt Gonçalves, Lajolo,
69 & Genovese, 2010; Genovese, Da Silva Pinto, De Souza Schmidt Gonçalves, & Lajolo, 2008;
70 Reynertson, Yang, Jiang, Basile, & Kennelly, et al., 2008; Rufino et al., 2010), although no
71 complete study with a detailed characterization of the main phenolic compounds
72 (proanthocyanidins, ellagitannins and ellagic acid conjugates) has been reported to date.
73 Research on phenolic compounds and health has been a focus of interest in the last decade
74 due to their antioxidant activity and free radical-scavenging ability (Tomás-Barberán & Andres-
75 Lacueva, 2012). In particular, the polyphenols seem to be involved in several beneficial effects in
76 human health (Ross & Kasum, 2002; Tomás-Barberán & Andres-Lacueva, 2012; Traka & Mithen,
77 2011). The results obtained so far suggest the potential application and positive biological effects of
78 camu-camu berries and derived food products in human health (Inoue, Komoda, Uchida, & Node,
79 2008; Yazawa, Suga, Homna, Shirosaki, & Koyama, 2011), although human intervention studies
80 are necessary.
81 Camu-camu consumption as fresh fruit is rare due to its high acidity and bitterness (Flores,
82 1998). It is mainly consumed as juice, or as an ingredient for jellies, ice-creams, liquors, wines or
83 other foods (Cavalieri, 1993; Villachica, 1997; Franco & Janzantti, 2005). Its commercial interest
84 has particularly increased due to its high vitamin C content. However, due to the loss of this vitamin
85 during postharvest storage and processing, alternative technological processes need to be developed
86 to preserve the nutritional value of camu-camu berries. Water is the main component of the fruits
87 and has a direct implication on quality loss through its effect on many physicochemical and
88 biological attributes. Therefore, the dehydration process has been suggested as an alternative to

89 obtain camu-camu ingredients that in powdered form can be used to enhance vitamin C and
90 bioactive compounds of different food products.

91 Few studies have reported a thorough chemical characterization, particularly phenolic
92 compounds characterization, of camu-camu fruit. The phenolics content has been often evaluated as
93 total polyphenols in the fruits, and the main components previously identified were ellagic acid,
94 quercetin, rutin and gallic acid (Chirinos et al., 2010; De Souza et al., 2010; Reynertson et al., 2008;
95 Rufino et al., 2010). No information has previously been reported either on the proanthocyanidins
96 or ellagitannins composition of camu-camu fruit or the powder products produced from the berry
97 pulp and skins by a dehydration processes.

98 Therefore, this study aims to the chemical characterization of two different powders
99 obtained from camu-camu fruit, one from the pulp and the second one from the remaining peel and
100 pulp (flour). Our main objective was to characterize the phenolic compounds, quantify the vitamin
101 C and determine the antioxidant capacity due to the nutritional potential which they might exhibit.

102

103 ## 2. Materials and methods

104

105 *2.1 Chemicals*

106 Standards of gallic acid, ellagic acid, quercetin, myricetin, rutin, catechin, and cyanidin 3-*O*-
107 glucoside (Cy-glc), sodium acetate, potassium phosphate, *o*-phenylene diamine (OPDA) manganese
108 dioxide, sodium fluoride, ethylenediaminetetraacetic acid (EDTA), 2,2'-azino-bis(3-
109 ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2'-azobis(2-amidino-propane)dihydrochloride
110 (AAPH), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich
111 (Darmstadt, Germany). Phloroglucinol, ascorbic acid, dehydro-ascorbic acid, potassium chloride,
112 hydrochloric acid, methanol, acetonitrile, acetic acid, and formic acid were from Merck (Darmstadt,
113 Germany). Vescalagin was used as external standard for ellagitannin quantification and was kindly

114 provided by Prof. Stephan Quideau (University of Bordeaux, France). Water was obtained from
115 Milli-Q apparatus (Millipore, Milford, MA, US).
116
117 *2.2 Samples*
118 Two powders from camu-camu fruit were provided by Agricola San Juan de la Amazonia
119 Europa (ASJAEU) (Valencia, Spain). Both powders were obtained from mature turning color
120 maturity state *Myrciaria dubia* fruits harvested during the summer 2010 in Pucallpa Ucayali (Peru).
121 One powder was produced from the pulp (pulp powder), dried in a spray drier at an inlet
122 temperature of 185 °C and an outlet temperature of 95 °C. The second powder (flour) was produced
123 from the remaining peel with adhered pulp after pulp extraction, and was dried in a fluid bed drier
124 at a temperature of 45-55 °C. Therefore two different drying processes were applied to obtain these
125 powders and this was due to the different water content of the raw materials which was about 90%
126 for the pulp fraction and about 20% for peel and pulp fraction. Both powders were packaged under
127 vacuum and stored at 4 °C until used.
128
129 *2.3 Determination of phenolic compounds*
130 The phenols extraction was performed as follows: 1 g pulp powder was dissolved in 25 mL
131 of 50% methanol in water acidified with1% formic acid; 1 g camu-camu flour was dissolved in 25
132 mL of 50% methanol in water. Different extractions were carried out in order to achieve the better
133 phenols recovery using variable ratio of water and methanol, with and without formic acid. The use
134 of 50% methanol in water acidified with 1% formic acid and methanol 50% for pulp powder and
135 camu-camu flour, respectively, allowed the better recovery of phenols. The powders were vortexed
136 for 2 minutes, sonicated for 15 minutes (Sonicator Branson 5510, Emerson, Danbury, CT, US) and
137 centrifuged at 3000 *rpm* for 15 min at 4 °C (Centrifuge 5804 R, Eppendorf, Hamburg, Germany).
138 The supernatants were recovered, freeze-dried under vacuum, suspended in 2 mL of the

139 corresponding extraction solvent, then filtered with a PVDF filter 0.22 μm (Millipore, Billerica,
140 MA, US) and injected in LC/MS.

141 The identification and quantification of phenols were performed using an Agilent 1100
142 Series equipment (Agilent, Santa Clara, CA, US) equipped with G1312A binary pump, G1313A
143 autosampler, G1315B photodiode array detector, and G1322A degasser controlled by the Agilent
144 software v. A08.03. HPLC was coupled with a detector MSD Trap 1100 Series (Agilent) with an
145 electrospray ionization system (ESI), with the following conditions: the heated capillary was 350 ºC
146 and 3-3.5 kV voltage, mass scan (MS) and MS/MS were measured from 100 to 1500 *m/z*. Collision-
147 induced fragmentation experiments were performed in the ion trap using helium as the collision gas,
148 and the collision energy was set at 75%. Mass spectrometry data were acquired in the negative
149 ionization modes. A column Pursuit XRs C18 250x40mm from Varian (Agilent, Santa Clara, CA,
150 US) was used and a flow rate of 0.8 mL min^{-1}. The used solvents were 1% formic acid in water (A)
151 and acetonitrile (B) which was in the following separation gradient: 1% B in A at 0 min, 9% B at 10
152 min, 35% B at 48 minutes, and 95% B at 52 minutes, following by washing and conditioning steps.
153 Data were registered from 250 nm to 700 nm and the phenolic compounds were quantified at 280
154 mn, 360 mn, and 520 nm, depending on the type of phenolic compound. Integrations were
155 performed by Agilent ChemStation for LC 3D, Rev. B.01.03 SR1. MS trap control was carried out
156 Bruker Daltonic version 5.2.

157 Quantification of gallic acid, ellagic acid, myricetin and their derivatives, and ellagitannins
158 was carried out with the calibration curves obtained for gallic acid (1-300 mg L^{-1}), ellagic acid (1-
159 300 mg L^{-1}), rutin (1-300 mg L^{-1}), and vescalagin (0.1-100 mg L^{-1}), respectively, at the appropriate
160 wavelengths. All the samples and standards were injected in triplicate.

161 Moreover, samples of pulp powder and flour were analyzed by UPLC-Q-TOF (Agilent) in
162 order to further confirm the phenolic compounds identified by MS Trap. The Q-TOF equipment had
163 the following conditions: ESI gas temperature 280 ºC, drying gas 9 l/min, nebulizer 35 psig, sheath

164 gas temp 400 ºC, sheath gas flow 12 l/min. MS TOF fragmentor 100V, mas range 100-1500,
165 negative mode. The column was Poroshell 120, EC-C18, 2.7 µm, 30 x 100 mm (Agilent); the
166 eluents were 0.1% formic acid in water (A) and acetonitrile acidified with 0.1% formic acid (B).
167 The separation gradient started with 1% B in A at 0 min, 9% at 3 min, 48% at 20 minutes, and 95%
168 at 23 minutes, following by washing and conditioning steps. The volume injected was 2 µL and the
169 flow rate was 0.4 mL/min. The determinations were carried out in triplicate.
170

171 *2.4 Analysis of proanthocyanidins*

172 Proanthocyanidins were quantified as previously reported by Kennedy and Jones (2001)
173 using an acid catalysis in the presence of phloroglucinol. Briefly, 50 mg sample were dissolved in
174 800 µL of phloroglucinol (50 mg mL^{-1}) added with ascorbic acid (10 mg mL^{-1}) dissolved in
175 methanol acidified with 0.1 N HCl. The reaction mix was vortexed and incubated at 50 °C for 20
176 minutes. The reaction tube was placed in ice and 1 mL 40 mM sodium acetate was added in order to
177 stop the reaction. The sample was centrifuged, filtered with a 0.22 µm PVDF filter, and injected in
178 LC/MS. The identification and quantification of catechin, epicatechin and their derivatives was
179 carried out by Agilent 1100 Series apparatus equipped with detector MSD Trap 1100 Series
180 (Agilent), as previously described (Buendía et al., 2010; Vallejo, Marín, & Tomás-Barberán, 2012).
181 Briefly, the column used was an Atlantis C18 (250 mm x 4.6 mm, 5 µm particle size; Water,
182 Milford, MA, US) operating at a flow rate of 1 mL min^{-1}; the injection volume was 10 µL. The
183 solvents were 2.5% acetic acid in water (A) and acetonitrile (B) with a separation gradient starting
184 with 3% B in A at 0 min, 9% at 5 min, 16% at 15 min, 50% at 45 min followed by washing and
185 conditioning steps. The phenolic compounds were quantified at 280 nm with a calibration curve of
186 catechin (1-300 mg L^{-1}). The MS detector operated in negative ion-mode. The Trap interface and
187 ion optics settings were the following: spray potential 65 psi; nebulization gas (nitrogen) relative

188 flow value 11; capillary temperature 325 °C. Full-scan mass spectra were acquired scanning the
189 range 100–800 *m/z*.
190
191 *2.5 Determination of ascorbic and dehydroascorbic acids*
192 Quantification of ascorbic acid and dehydroascorbic acid was carried out as previously
193 described by Zapata and Dufour (1992). Briefly, 50 mg sample were dissolved in 10 ml of 5%
194 methanol added with citric acid (21 g L^{-1}) and EDTA (0.5 g L^{-1}). The homogenate was filtered
195 through a 0.45 µm PVDF filter and purified on a C18 Sep-Pak cartridge (Waters, Mil-ford, MA,
196 US). The HPLC analysis was achieved after derivatization of DHAA into the fluorophore 3-(1,2-
197 dihydroxyethyl) furol [3,4-b]quinoxaline-1-one (DFQ), with 1,2-phenylenediamine dihydrochloride
198 (OPDA). Standard solutions, column conditioning, mobile phase, flow rate, wavelengths and
199 derivatization procedures used were previously reported by Gil, Ferreres, and Tomás-Barberán
200 (1999), and Martínez-Sánchez, Tudela, Luna, Allende, and Gil (2011). The results were expressed
201 as g ascorbic acid and dehydroascorbic acid per 100 g powder.
202
203 *2.6 Antioxidant capacity assays*
204 The antioxidant capacity of both powders was carried out through three different methods
205 measuring the free radical scavenging capacity, such as DPPH, ABTS and ORAC assays.
206 The free radical scavenging activity determining with DPPH was in accordance to Brand-
207 Williams, Cuvelier ,and Berset (1995) with some modifications (Espín, Soler-Rivas, Wichers, &
208 García-Viguera, 2000; Llorach, Tomás-Barberán, & Ferreres, 2004). The DPPH solution was
209 diluted with methanol to an absorbance of 1.00 (± 0.03) at 515 nm. In a 96-wells micro plate (Nunc,
210 Roskilde, Denmark), 250 µL of DPPH solution were placed in each well and 2 µL sample were
211 added. The sample was dissolved in 70% methanol (20 g L^{-1}) and, after centrifugation, it was
212 serially diluted.

213 The ABTS method was performed according with Mena et al. (2011). The ABTS solution
214 was diluted with water to an absorbance of 1.00 (± 0.03) at 414 nm. In a 96-wells micro plate
215 (Nunc, Roskilde), 250 µL of ABTS solution were put in each well and 2 µL sample were added.
216 The sample was dissolved in water (20 g L^{-1}) and, after centrifugation, it was serially diluted. For
217 both assays, the reaction kinetic was monitored for 50 minutes at 25 °C by micro plate reader
218 (Infinite® M200, Tecan, Grödig, Austria). A calibration curve was made by adding increasing
219 concentration of Trolox ranged from 50 µM to 1000 µM; each concentration was assayed in
220 quadruplicate, as well each sample.

221 The free radical scavenging activity determined by ORAC assay was in accordance to Prior
222 et al., (2003) with some modifications. In a 96-wells micro plate, 100 µL 14 µM fluoresceine
223 prepared in 75 mM phosphate buffer pH 7.4, 20 µL sample (or standard) and 30 µL 75 mM
224 phosphate buffer pH 7.4 were placed into each well. After 15 minutes incubation at 37 °C, 30 µL
225 AAPH (21.6 mg/mL) were added. Readings were carried out with a fluorescent microplate reader
226 (Multi-Detection Microplate Reader, Synergy ™ HT, Biotek Instruments USA) which was
227 programmed to read the fluorescence with an excitation wavelength of 485 nm and an emission
228 wavelength of 528 nm at 1 min interval for 90 minutes using software Gen 5™. Calibration curve
229 was obtained with increasing concentration of Trolox prepared in 75 mM phosphate buffer pH 7.4,
230 ranged from 5 µM to 50 µM.

231 The results were expressed as µM of Trolox per g of sample. Each concentration was
232 assayed in quadruplicate, as well as each sample.

233

234 ## 3. Results and discussion

235

236 *3.1 Phenolics characterization*

237 The phenolic compounds characterized in camu-camu powders are shown in Table 1 and
238 Figure 1A, 1B and 1C. The UV spectra of the different compounds recorded with a Diode Array
239 Detector (DAD) showed that flavonols, ellagic acid conjugates, ellagitannins and proanthocyanidins
240 were the main polyphenols present. One anthocyanin was detected in pulp powder, and at least two
241 hydroxycinnamic acid derivatives, which showed the characteristic UV/Vis spectrum of a caffeic
242 acid derivative (maximum at 332 nm, and shoulder at 298 nm and m/z 353) were detected, although
243 their molecular weights could not be clearly established from the HPLC-MS analyses. The different
244 compounds were characterized by their UV spectra, their molecular ion and fragments obtained
245 with an ESI-MS-MS detector (Table 1) and comparison, wherever possible, with authentic markers.
246 In addition, the different compounds were confirmed using a high resolution analysis on a UPLC-
247 Q-TOF equipment in which the molecular formulae were calculated (Table 2). All identified
248 compounds showed a low error (-3.30) and a high score (better than 92.15) that indicated the
249 accuracy of the exact mass and the molecular formulae obtained.

250 The flavonols myricetin (3,5,7,3',4',5'-hexahydroxyflavone) (**28**) and its 3-*O*-hexoside (**4**)
251 (probably glucoside) and its two 3-*O*-pentoside isomers (**6**) (probably arabinoside and xyloside),
252 were detected. They showed characteristic UV spectra of flavonols with a free hydroxyl at 3
253 position for myricetin (UV band I maximum at 374 nm), and a blocked hydroxyl at 3 for the *O*-
254 glycosides (UV band I maximum at 356 nm) (Table 1). The pseudomolecular ions recorded with the
255 HPLC-ESI MS and the fragments obtained confirmed these structures with the characteristic losses
256 of a hexosyl and a pentosyl residue respectively leading to the myricetin aglycone fragment at *m/z*
257 317. The High-resolution Mass Spectrometry Analysis confirmed these structures (Table 2). In
258 addition two quercetin (3,5,7,3',4'-pentahydroxyflavone) 3-O-glycosides were also detected,
259 quercetin 3-O-hexoside (**26**) and 3-O-pentoside (**27**) and these two were only detected in the pulp.
260 Rutin (quercetin 3-*O*-rutinoside) that was previously reported in camu-camu (Reynertson et al.,
261 2008), was not detected in the analyzed powders. In addition, the flavanones naringenin and

262 eriodictyol, that had previously been reported (Akter, Oh, Eun, & Ahmed, 2011) were not detected
263 here either.

264 The anthocyanins were easily detected with UV-Vis detector set at 520 nm. Only one
265 pigment was detected its UV-Vis spectrum with a maximum at 520 nm suggested a cyanidin
266 derivative, and this was confirmed by the MS-MS analysis that showed that this was a cyanidin
267 hexoside. This is most probably Cyanidin 3-*O*-glucoside (**29**) that was previously reported in fresh
268 camu-camu fruits (Zanatta & Mercadante, 2005). The structure was confirmed by high-resolution
269 Q-TOF MS-MS (Table 2). No delphinidin 3-*O*-glucoside was detected, although this anthocyanin
270 had been previously reported in camu-camu (Zanatta & Mercadante, 2005). This is not unexpected
271 as delphinidin is more susceptible to oxidative and thermal degradation than cyanidin.

272 A number of compounds with the characteristic UV spectrum ellagic acid were detected
273 (Table 1). The main one was free ellagic acid (**7**) that showed a pseudomolecular ion at *m/z* 301 and
274 coincided chromatographically with an authentic standard of this polyphenol. Nine other different
275 compounds showed a UV spectrum similar to that of ellagic acid and all of them but compound **1**
276 produced a fragment in the MS analysis at *m/z* 301 confirming that they were ellagic acid
277 conjugates. Compound **1** showed a pseudomeolecular ion at *m/z* 469 and a fragment at *m/z* 425 that
278 are characteristic of valoneic acid bilactone, a compound that often occurs in plants containing
279 ellagitannins. The conjugates included a *O*-hexoside (**2**) (most probably glucoside), a *O*-pentoside
280 (**3**) (most probably arabinoside or xyloside), and a *O*-deoxyhexoside (**5**) (most probably
281 rhamnoside). In addition, two isomeric compounds with pseudomolecular ion at *m/z* 489 (**8**, **9**) were
282 detected. The fragmentation and the high resolutions MS-MS coincided with ellagic acid acetyl
283 rhamnoside, previously reported in several Myrtaceae species, but that were not reported in camu-
284 camu. In addition other ellagic acid derivatives were detected with pseudomolecular ions at *m/z* 585
285 (**10**) and *m/z* 719 (**12**, **13**). All these compounds produced fragments at *m/z* 301 for ellagic acid, and
286 the molecular formulae were established with the high-resolution Q-TOF equipment, but these

287 compounds were not completely characterized as the fragmentation did not provide enough
288 information to suggest a chemical structure.

289 Ten ellagitannins were characterized in camu-camu powders. Their UV spectra provided
290 information on the number of hexahydroxydiphenoyl (HHDP) and galloyl residues that every
291 compound had on the glucose nucleus (Salminen, Ossipov, Loponen, Haukioja, & Pihlaja, 1999).
292 The isomeric *C*-glucosides vescalagin (**16**) and castalagin (**15**) were characterized by the
293 pseudomolecular ion at *m/z* 933, and the characteristic fragments that did not include the ellagic
294 acid fragment at *m/z* 301, and were confirmed by chromatographic comparisons with authentic
295 standards. Two isomers of HHDP-galloyl-glucose (**19**, **48**), with a pseudomolecular ion at *m/z* 633
296 and fragments at *m/z* 463 (M-H-galloyl) and 301 (ellagic acid), were characterized. Two isomers of
297 di-HHDP-galloyl-glucose (causarictin/potentillin) (**23**, **47**), with a pseudomolecular ion at m/z 935
298 and fragments at *m/z* 917 (M-H-H_20), 633 (M-H-HHDP) and 301 (ellagic acid), were also detected.
299 Di-HHDP-glucose (pedunculagin) (**18**) with a pseudomolecular ion at *m/z* 783 and fragments at *m/z*
300 481 (M-H-HHDP) and 301 was also detected, as well as digalloyl-HHDP-glucose (**21**) with *m/z* 785
301 and fragments at *m/z* 483 (M-H-HHDP) and 301, and trigalloyl-HHDP-glucose (tellimagrandin II)
302 (**24**) with *m/z* 937 and fragments at *m/z* 767 (M-H-galloyl), 741, 465 (M-H-galloyl-HHDP) and 301
303 (ellagic acid).

304 The different ellagitannins were confirmed by High Resolution MS analyses with the Q-
305 TOF detector, with the calculation of the corresponding molecular formulae (Table 2).

306 Gallic acid (**14**) was also detected as well as an unidentified gallic acid derivative (**25**) with a
307 pseudomolecular ion at *m/z* 569.

308 Proanthocyanidins were also present in the camu-camu flour, although the UV spectra and
309 UV response was not relevant compared with those of the rest of phenolic compounds that had a
310 higher UV absorption coefficient. Three main proanthocyanidins were detected in the HPLC-DAD-
311 MS-MS chromatograms (Table 1), in which gallocatechin-gallate (**46**) with a pseudomolecular ion

312 at *m/z* 457, a dimer of gallocatechin-gallate (**22**) at *m/z* 915 and fragments at *m/z* 457 and *m/z* 169
313 (gallate), and a trimer of gallocatechin-gallocatechingallate-gallocatechingaltate (**17**) at *m/z* 1221
314 with a main fragment at *m/z* 915 (M-H-gallocatechin), were detected. These were confirmed by
315 High-resolution Q-TOF, with the determination of the structural formulae (Table 2). In addition,
316 gallocatechin (**49**), catechin (**50**), a B-type procyanidin dimer (**51**) at *m/z* 577, a gallocatechin-
317 gallate dimer (**22**), gallocatechin gallate (**46**), and catechin gallate (**53**) were detected. The
318 sensitivity of the Q-TOF equipment allowed the detection of these proanthocyanidins, although
319 some of them were not detected in the HPLC-ESI-MS-MS analyses (Table 1).
320
321 *3.2 Phenolics quantification*
322 The total phenolics content of both camu-camu powders was calculated as an addition of the
323 individual characterized compounds by their UV absorbance at the convenient wavelength, and
324 using the appropriate external standards for each type of compound (Table 3). In the case of
325 proanthocyanidins, they were quantified by HPLC-UV after the acid catalyzed degradation in the
326 presence of phloroglucinol (Kennedy & Jones, 2001) (Table 4).
327 The content in pulp powder was 48.54 mg/100 g (Table 3). Among the phenols the total
328 concentration of myricetin and its 3-*O*-glycosides (3 mg/100g) was higher than that reported in
329 fresh fruit (Reynertson et al., 2008). The content of ellagic acid and its conjugated derivatives was
330 9.75 mg/100 g powder, of which the 60% was for free ellagic acid. The amount of quercetin and its
331 glycosides (< 0.05 mg/100 g) was lower than that reported in literature related to either fresh fruit
332 (Reynertson et al., 2008) or dry matter (De Souza Schmidt Gonçalves et al., 2010). Neither gallic,
333 proanthocyanidins or kaempferol derivatives were detected in pulp powder.
334 Higher amounts of phenols were detected in the camu-camu flour (Table 3, Table 4, Figure
335 1). As for the pulp powder, only the identified peaks in the chromatograms are quantified. No quer-
336 cetin, rutin, kaempferol, and anthocyanins were detected in the camu-camu flour analysed although

337 these compounds were previously found in camu-camu fresh berries (Reynertson et al., 2008; De
338 Souza Schmidt Gonçalves et al., 2010). The total concentration of phenols in camu-camu flour,
339 quantified by addition of the individual compounds characterized, was 4007.95 mg/100 g (Table 3,
340 Table 4). The amount of proanthocyanidins, namely catechin, and gallocatechin, and their gallate
341 derivatives (and trace amounts of the epicatechin isomers) was up to 3423.5 mg/100 g (Table 4,
342 Figure 1D), which is more than twice the content of other fruits recognized as a good source of
343 proanthocyanidins, such as strawberry (1170 mg/100 g dry matter), apple (1460 mg/100 g dry mat-
344 ter), and grape (1420 mg/100 g dry matter) (Tarascou et al., 2010). Moreover, the content of these
345 compounds was higher than that found in cocoa powder (278 mg/100 g dry matter) (Lee, Kim, Lee,
346 & Lee, 2003) and Brazil nut (419 mg/100 g dry matter) (John, & Shahidi, 2010). Among the proan-
347 thocyanidins, the most abundant compound was epigallocatechin (845.5 mg/100 g), followed by
348 epigallocatechin gallate (767.5 mg/100 g) and catechin gallate (729.8 mg/100 g). The proanthocya-
349 nidin composition resulted to be similar to the phenolic profile of green tea which has been reported
350 to have a relevant nutritional potential (McKay & Blumberg, 2002). Catechin-gallates have recently
351 been described as potent inhibitors of α-amylase and α-glucosidase with implications in obesity
352 prevention (Yilmazer-Musa, Griffith, Schneider, & Frei, 2012). In addition, the polymerization de-
353 gree of the-proanthocyanidins present in this powder was around 3, suggesting a relatively high ab-
354 sorption in humans (Scalbert & Williamson, 2000) which can consequently have potential biologi-
355 cal activities. It has been reported that proanthocyanidins play an important role in several biologi-
356 cal processes resulting in health benefits. Several beneficial effects of proanthocyanidins have been
357 reported such as antioxidant, anti-inflammatory, antimicrobial, antiproliferative, cardioprotective,
358 hypolipidemic and antidiabetic properties (Nandakumar, Singh, & Katiyar, 2008; Serrano, Puuppo-
359 nen-Pimia, Dauer, Aura, & Saura-Calixto, 2009; Bladé, Arola, & Salvado, 2010).

360 Ellagitannins were also relevant constituents of camu-camu powders although the concentra-
361 tion found was lower than that of the proanthocyanidins (405.4 mg/100 g) (Table 3). However, the

362 level of ellagitannins was higher than that of strawberry (162 mg/100 g dry matter) (Buendía et al.,
363 2010) and similar to that of raspberry (415 mg/100 g dry matter) (Bobimaité, Viskles, & Venskuto-
364 nis, 2012). The most abundant ellagitannins were vescalagin (228.9 mg/100 g), followed by casta-
365 lagin (64.5 mg/100 g) and di-HHDP-galloyl glucose (38.4 mg/100 g). In addition, the content of
366 free ellagic acid and its glycosidic conjugates (129.80 mg/100g) was higher than those previously
367 reported in strawberry (8.8-20.6 mg/100 g dry matter) (Bobimaité et al., 2012), and Brazil nut (11.4
368 mg/100 g dry matter) (John & Shahidi, 2010). More than half of the ellagic acid was present as free
369 ellagic acid (Table 3, Figure 1). A small quantity of myricetin derivatives was also present (1.40
370 mg/100 g) although in lower concentration than that reported in camu-camu berries (24 mg/100 g
371 dry matter) (Reynertson et al., 2008).

372 The phenolic compounds in camu-camu are characterized for a high number of hydroxyls, in
373 which groupings of three phenolic hydroxyls are the common feature as are the cases of gallic acid,
374 ellagitannins, myricetin, and gallocatechin-gallate (Figure 2), and this can be linked to a high anti-
375 oxidant activity.

376

377 *3.3 Ascorbic acid and dehydroascorbic acid quantification*

378 The ascorbic acid contents were 3.51 $\pm$ 0.97 g/100 g and 9.04 $\pm$ 0.95 g/100 g in camu-camu
379 pulp powder and camu-camu flour respectively. These values are higher than those reported in fresh
380 camu camu fruit (Rufino et al., 2010; Chirinos et al., 2010) due to the lower moisture content of the
381 powder (1-3%) (De Souza Schmidt Gonçalves et al., 2010). This content was very relevant although
382 the amount of ascorbic acid present in fresh berries could be partly degraded during the production
383 of these powders from the fresh fruit that needs thermal treatments (Shofian et al., 2011), and some
384 variations in the content could had happened as ascorbic acid concentration in berries depends on
385 the ripening stage (Marques, Ferreira, & Freire, 2007). The ascorbic acid content in these camu-
386 camu powders was much higher than those reported for freeze-dried fruits, such as red pummel

387 (0.02 g/100 g dry matter), strawberry (0.6 g/100 g dry matter), and tomato (0.6 g/100 g dry matter),
388 which are known to be a source of vitamin C (Tsai, Chang, & Chang., 2007; Asami, Hong, Barret,
389 & Mitchell, 2003; Chang, Lin, Chang, & Liu, 2006).

390 The dehydroascorbic acid values were of 0.69 ± 0.31 g/100 g and 0.578 ± 0.027 g/100 g for
391 camu-camu pulp powder and camu-camu flour, respectively. Dehydroascorbic acid usually
392 represents about 10-20% of the ascorbic acid present in fruit and vegetable products (Riemer &
393 Karel, 1978; Borowski, Szajdek, Borowska, Ciska, & Zieliński, 2008), and is considered to be the
394 first compound produced in the oxidative degradation of ascorbic acid (Chang et al., 2006). In the
395 presnt study, dehydroascorbic acid represented the 19.5% of the vitamin C (ascorbic +
396 dehydroascorbic acid) for pulp powder and 6.4% for camu-camu flour, confirming the data
397 previously reported in literature, and suggesting that the higher temperature used for the production
398 of the pulp powder led to a higher transformation of ascorbic acid into dehydroascorbic acid. In
399 addition, the higher content of antioxidant polyphenols in the skin than in the pulp could also help
400 preventing the transformation of ascorbic acid into dehydroascorbic acid.

401

402 *3.4 Antioxidant capacity*

403 The antioxidant capacity was determined in both pulp powder and flour using ABTS, DPPH
404 and ORAC assays. The ABTS assay showed the free-radical scavenging activity for pulp powder
405 (167.5 ± 54.4 µmol Trolox / g) which is about 4 fold lower than that of camu-camu flour (752.3 ±
406 41.0 µmol Trolox / g). These values are higher than those reported in foods well known for their
407 high antioxidant capacity, such as guava (98 µmol Trolox / g) (Corral-Aguayo, Yahia, Carrillo-
408 Lopez, & González-Aguilar, 2008), strawberry (about 250 µmol Trolox / g) (Proteggente et al.,
409 2002), and cocoa powder (617 µmol Trolox/g) (Lee et al., 2003). Similarly, the antioxidant capacity
410 determined as DPPH was higher in camu-camu flour (1036.4 ± 211.2 µmol Trolox /g) than in pulp
411 powder (510.5 ± 156.1 µmol Trolox/g). These values were again higher than those reported for

412 guava (300 µmol Trolox / g) (Corral-Aguayo et al., 2008), apple and kiwi (37 µmol Trolox / g)
413 (Wijngaard, Rößle, & Brunton, 2009), cocoa (458 µmol Trolox / g) (Lee et al., 2003), and broccoli
414 (3 µmol Trolox / g powder) (Borowski et al., 2008).

415 The camu-camu flour revealed an antioxidant capacity value of ORAC two fold higher (755.2 ±
416 77.9 µmol Trolox / g) than that of the pulp powder (337.1 ± 77.9 µmol Trolox / g). As this method
417 determines both lipophilic and hydrophilic antioxidants, the high value obtained in the camu-camu
418 flour was expected as this product revealed high contents of both vitamin C and phenolic
419 compounds. This antioxidant capacity was higher than those reported for strawberry (153.6 µmol
420 Trolox/g) (Wang, Cao, & Prior 1996), and blackberry (204 µmol Trolox/g) (Wang & Lin, 2000)
421 and were consistent with those previously reported for camu-camu (De Souza Schmidt Gonçalves et
422 al., 2010).

423 The antioxidant capacity determined by the ABTS assay was lower than that determined by the
424 DPPH assay for the camu-camu flour and pulp powder, and this differs from previous studies with
425 other plant extracts (Arnao, 2000). This could be explained by the higher content of phenolics,
426 particularly proanthocyanidins, in the flour, which are more soluble in methanol than in water.
427 Nevertheless, the high antioxidant capacities of the flour determined by the ABTS and the ORAC
428 assays can also be related to the high content of vitamin C and phenolic compounds present. The
429 ORAC values were also lower than those determined as DPPH for both powders. This could again
430 be due to the extraction of the camu-camu powders in water which can probably limit the extraction
431 of some phenolics, such as the proanthocyanidins.

432

433 ## 4. Conclusion

434 Camu-camu fruit is considered as a good source of bioactive compounds potentially beneficial
435 for human health. The consumption of this fruit is limited by its acidity and bitter taste, and
436 therefore the dehydration process is a good alternative for its use as a powdered food ingredient.

437 These results revealed that the two camu-camu powder products studied are excellent sources of
438 bioactive substances including vitamin C and phenolic compounds which resulted in high
439 antioxidant capacity values. Camu-camu flour showed a higher concentration of vitamin C than the
440 camu-camu pulp powder and high proanthocyanidins content. Therefore, camu-camu flour has a
441 good potential to be used as an ingredient for the formulation of functional foods. The results
442 provide further evidence that this flour is a rich source of bioactive compounds with potential
443 health-promoting properties such as antioxidant, anti-inflammatory, and hypocholesterolemic
444 activities which have been related to vitamin C and phenolic compounds such as flavonoids.
445 However, *in vivo* and intervention studies are needed to assess the nutritional and functional
446 potential of this camu-camu product.
447
448
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455

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614 Zapata, S. & Dufour, J. F. (1992). Ascorbic, dehydroascorbic and isoascorbic acid simultaneous 615 determinations by reverse phase ion interaction HPLC. *Journal of Food Science, 57*, 506–511.

1 Table 1. HPLC-DAD-ESI-MS-MS analysis of camu-camu powders phenolic compounds.

Number	Compound	Retention Time (min)	$[M-H]^-$	λ max (nm)	MS fragments
Flavonols					
4	Myricetin 3-O-hexoside	27.5	479	264, 358	**316**, 221, 179
6	Myricetin 3-O-pentoside	29.2	449	258, 356	**317**
6	Myricetin 3-O-pentoside	30.4	449	272, 356	**316**
26	Quercetin 3-*O*-hexoside	31.7	463	256, 362	417, **301**
27	Quercetin 3-*O*-pentoside	35.5	433	256, 352	**301**, 179, 151
28	Myricetin	38.3	317	256, 374	317, **179**, 151
Anthocyanins					
29	Cyanidin 3-*O*-glucoside	20.6	447	520	285
Ellagic acid derivatives					
1	Valoneic acid dilactone	15.4	469	255, 374	**425**
2	Ellagic acid hexoside	24.7	463	255, 362	**301**
3	Ellagic acid pentoside	28.9	433	254, 360	**301**
5	Ellagic acid desoxyhexoside	29.7	447	254, 364	**300**
7	Ellagic acid	30.9	301	256, 368	**229**
8	Ellagic acetyl rhamnoside	35.9	489	254, 362	**301**
9	Ellagic acetyl rhamnoside	36.5	489	254, 362	**301**
10	Ellagic acid derivative	37.2	585	254, 360	415, **301**
12	Ellagic acid derivative	40.8	719	254, 362	**301**
13	Ellagic acid derivative	41.5	719	254, 362	**301**
Ellagitannins					
15	Castalagin	11.5	933	246	**915**, 889, 631
16	Vescalagin	13.9	933	246	**915**, 889, 631
48	HHDP-galloyl-glucose	15.3	633	240, 270	463, **301**
18	Di-HHDP-glucose (Pedunculagin)	16.2	784	240	**481**, 301
47	Di-HHDP-galloyl-glucose (casuarictin/potentillin)	19.5	935	240, 270	917, **633**, 301
19	HHDP-galloyl-glucose	19.8	633	240, 270	463, **301**
20	Di-HHDP-galloyl-glucose (casuarictin/potentillin)	20.5	935	240, 270	917, **633**, 301
21	Digalloyl-HHDP-glucose	21.8	785	240, 272	484, **301**
23	Di-HHDP-galloyl-glucose (casuarictin)	25.9	935	240, 270	917, **633**, 301
24	Tri-galloyl-HHDP-glucose (tellimagrandin II)	27.9	937	240, 276	**767**,741,465,301
Gallic acid derivatives					
14	Gallic acid	9.0	169	274	**125**
25	Gallic acid derivative	37.8	569	238, 274	**551**, 523, 169
Proanthocyanidins					
17	Gallocatechin-gallocatechin gallate-gallocatechingallate	15.8	1221	240,274sh	**915**
46	Gallocatechin-gallate	24.0	457	240, 274	331,305,**169**
22	Gallocatechin-gallate-dimer	24.1	915	240, 274	**457**, 169

2

3 Table 2. High-Resolution UPLC-Q-TOF Characterization of phenolic compounds in camu-camu
4 powders.

Number	Compound	Exact mass	Exact mass theorical	Error	Score	Retention Time (min)	Formula
Flavonols							
4	Myricetin 3-O-hexoside	479.0842	479.0831	-2.85	91.42	8.2	$C_{21}H_{20}O_{13}$
6	Myricetin 3-O-pentoside	449.0734	449.0725	-2.32	96.00	8.6	$C_{20}H_{18}O_{12}$
6	Myricetin 3-O-pentoside	449.0734	449.0725	-2.37	95.17	9.0	$C_{20}H_{18}O_{12}$
6	Myricetin 3-O pentoside	449.0735	449.0725	0.20	94.43	9.1	$C_{20}H_{18}O_{12}$
26	Quercetin 3-O-hexoside	463.0890	463.0882	-2.18	96.46	9.2	$C_{21}H_{20}O_{12}$
27	Quercitin 3-O-pentoside	433.0787	433.0776	-2.35	97.02	10.3	$C_{20}H_{18}O_{11}$
28	Myricetin	317.0314	317.0303	-3.59	95.54	11.4	$C_{15}H_{10}O_8$
Anthocyanins							
29	Cyanidin 3-*O*-glucoside	447.0944	447.0933	-2.63	95.31	6.2	$C_{21}H_{21}O_{11}$
Ellagic acid derivatives							
1	Valoneic acid dilactone	469.0053	469.0049	-0.95	99.06	5.0	$C_{21}H_9O_{13}$
2	Ellagic acid hexoside	463.0533	463.0518	-0.42	98.59	7.3	$C_{20}H_{16}O_{13}$
3	Ellagic acid pentoside	433.0423	433.0412	-2.53	96.75	8.4	$C_{19}H_{14}O_{12}$
5	Ellagic acid desoxyhexoside	447.0593	447.0569	-4.62	85.05	8.7	$C_{20}H_{16}O_{12}$
7	Ellagic acid	301.0000	300.9990	-3.42	96.08	9.1	$C_{14}H_6O_8$
8	Ellagic acid acetyl-rhamnoside	489.0679	489.0675	-0.76	99.15	10.8	$C_{22}H_{18}O_{13}$
9	Ellagic acid acetyl-rhamnoside	489.0678	489.0675	-0.68	98.76	10.9	$C_{22}H_{18}O_{13}$
10	Ellagic acid derivative	585.0526	585.0522	-0.97	98.17	11.2	$C_{26}H_{18}O_{16}$
12	Ellagic acid derivative	719.2199	921.2193	-0.94	96.34	12.7	$C_{34}H_{39}O_{17}$
13	Ellagic acid derivative	719.2206	719.2193	-1.20	96.14	12.8	$C_{34}H_{40}O_{17}$
Ellagitannins							
15	Castalagin	933.0645	933.0640	-0.33	98.45	3.9	$C_{41}H_{26}O_{26}$
16	Vescalagin	933.0640	933.0640	-0.31	99.68	4.6	$C_{41}H_{26}O_{26}$
48	HHDP-galloyl-glucose	633.0746	633.0733	-3.35	93.54	4.8	$C_{27}H_{22}O_{18}$
18	Di-HHDP-glucose	783.0695	783.0686	0.22	98.40	5.0	$C_{34}H_{24}O_{22}$
47	Di-HHDP-galloyl-glucose	935.0794	935.0796	0.36	95.48	5.1	$C_{41}H_{28}O_{26}$
19	HHDP-galloyl-glucose	633.0747	633.0733	-3.10	85.20	5.2	$C_{27}H_{22}O_{18}$
20	Di-HHDP-galloyl-glucose	935.0801	935.0796	-0.96	97.97	6.4	$C_{41}H_{28}O_{26}$
21	HHDP-digalloyl-glucose	785.0857	785.0843	-2.06	95.92	6.7	$C_{34}H_{26}O_{22}$
23	Di-HHDP-galloyl-glucose	935.0797	935.0796	0.00	99.93	8.0	$C_{41}H_{28}O_{26}$
24	Tri-galloyl-HHDP-glucose	937.0945	937.0953	0.45	99.37	8.5	$C_{41}H_{30}O_{26}$
Proanthocyanidins							
49	Gallocatechin	305.0674	305.0667	-1.25	99.38	4.3	$C_{15}H_{14}O_7$
50	Catechin	289.0723	289.0718	-2.27	97.85	5.0	$C_{15}H_{14}O_6$
51	Procyanidin dimer B-type	577.1349	577.1351	-0.66	99.02	5.6	$C_{30}H_{26}O_{12}$
22	Gallocatechin gallate dimer	913.1461	913.1469	0.62	99.36	6.9	$C_{44}H_{34}O_{22}$
46	Gallocatechin gallate	457.0779	457.0776	-2.21	97.4	7.7	$C_{22}H_{18}O_{11}$
53	Catechin gallate	441.0828	441.0827	-1.49	98.27	9.7	$C_{22}H_{18}O_{10}$
Gallic acid and derivatives							
14	Gallic acid	169.0147	169.0142	-2.58	98.89	3.1	$C_7H_6O_5$
25	Gallic acid derivative	569.2246	569.2240	-0.75	97.97	10.9	$C_{27}H_{38}O_{13}$

5

6 Table 3. Content of phenolic compounds in camu-camu powders. Mean values (n=3) with standard deviation.

Number	Compound	Pulp powder (mg/100g powder)	Flour (mg/100g powder)
Flavonols			
4	Myricetin 3-O-hexoside	0.55 ±0.11	1.40 ± 0.05
6	Myricetin 3-O-pentoside	trace	Nd
6	Myricetin 3-O-pentoside	1.47 ±0.07	trace
26	Quercetin 3-*O*-hexoside	trace	Nd
27	Quercetin 3-*O*-pentoside	0.05±0.01	Nd
28	Myricetin	0.98±0.04	5.28 ± 0.10
	Total	**3.05 ±0.07**	**6.68 ± 0.16**
Anthocyanins			
29	Cyanidin 3-*O*-glucoside	19.63 ± 0.60	Nd
Ellagic acid derivatives			
1	Valoneic acid dilactone	Nd	5.46 ± 0.10
2	Ellagic acid hexoside	Nd	7.52 ± 0.08
3	Ellagic acid pentoside	1.22 ± 0.07	20.42 ± 0.16
5	Ellagic acid desoxyhexoside	2.84 ± 0.11	12.94 ± 0.28
7	Ellagic acid	5.60 ± 0.11	76.49 ± 0.49
8	Ellagic acetyl rhamnoside	Nd	3.90 ± 0.06
9	Ellagic acetyl rhamnoside	Nd	3.13 ± 0.06
10	Ellagic acid derivative	Nd	2.27 ± 0.14
12	Ellagic acid derivative	Nd	1.51 ± 0.03
13	Ellagic acid derivative	Nd	1.61 ± 0.01
	Total	**9.75 ±0.10**	**129.80 ± 0.15**
Ellagitannins			
15	Castalagin	Nd	64.51 ± 1.11
16	Vescalagin	12.71 ± 0.51	228.88 ± 1.89
18	Di-HHDP-glucose (Pedunculagin)	3.39 ± 0.16	17.93 ± 0.17
47	Galloyl-bis-HHDP-glucose (casuarinin(potentillin)	Nd	29.89 ± 0.21
19	HHDP-galloyl-glucose	Nd	37.80 ± 0.17
20	Di-HHDP-galloyl-glucose (casuarictin/potentillin)	Nd	38.37 ± 2.78
21	HHDP-galloyl-glucose	Nd	7.89 ± 0.41
23	Di-HHDP-galloyl-glucose (casuarictin)	Nd	5.71 ± 0.35
24	Tri-galloyl-HHDP-glucose (tellimagrandin II)	Nd	4.89 ± 0.17
	Total	**16.10 ±0.33**	**435.86 ± 0.98**
Gallic acid derivatives			
14	Gallic acid	Nd	29.56 ± 0.71
25	Gallic acid derivative	Nd	6.40 ± 0.50
	Total		**35.96 ± 0.54**
Proanthocyanidins			
17	Gallocatechin-gallocatechin gallate-gallocatechingallate	Nd	19.38 ± 0.78
46	Gallocatechin-gallate	Nd	27.35 ± 0.89
22	Gallocatechin-gallate-dimer	Nd	17.46 ± 0.39
	Total		**64.19 ± 0.54**
	TOTAL	**48.54±0.28**	**672.49±0.55**

7

8 Table 4. Proanthocyanidin analysis of camu-camu flour by phloroglucinolysis. HPLC Retention
9 times, UV/Vis spectra, HPLC-ESI-MS-MS fragmentations.

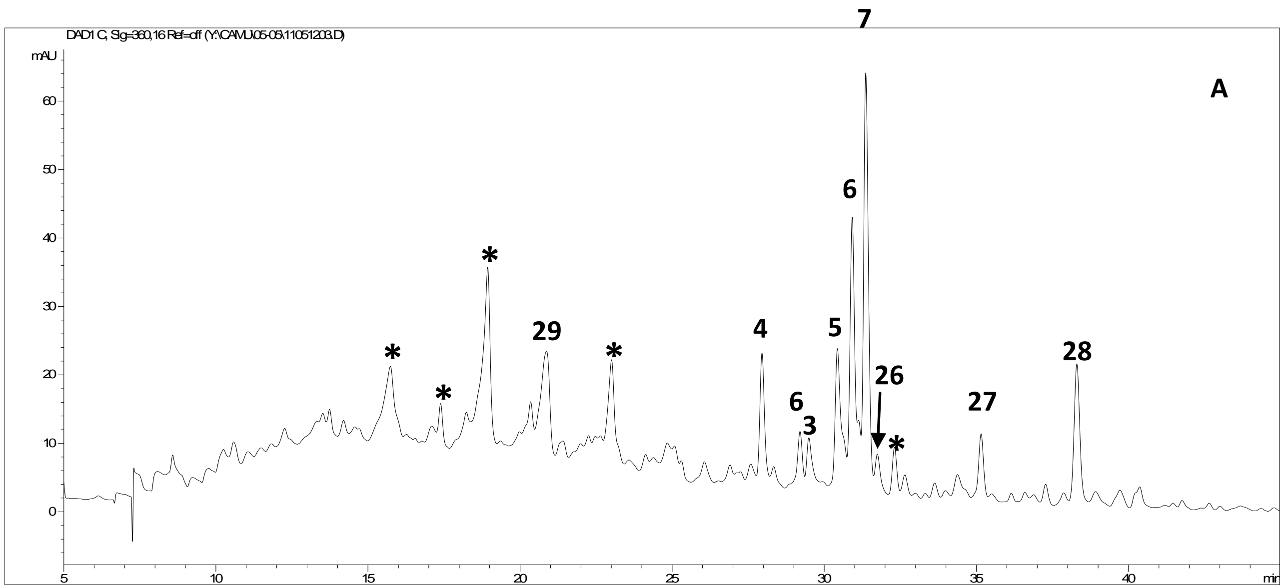
Number	**Compound**	**Retention Time (min)**	**[M-H]$^-$**	**λ max (nm)**	**MS fragments**	**mg/100 mg powder (average±sd)**
30	Catechin	15.3	289	275	**245**, 205, 179	213.53±3.27
31	Epicatechin	19.1	289			trace
32	Catechin aduct	11.5	413			trace
33	Epicatechin aduct	12.0	413	277	**287**, 261, 175	845.48±1.89
34	Catechin gallate	24.9	441	277	331, **289**, 169	52.02±0.40
35	Epicatechin gallate	27.4	441			trace
36	Gallocatechin aduct	17.4	565	277	439, **413**, 395	677.73±11.99
37	Epigallocatechin aduct	23.6	565			trace
38	Gallocatechin	10.7	305	277	287, 219, **178**	45.28±4.72
39	Epigallocatechin	14.4	305	277	287, 219, **178**	60.10±0.67
40	Gallocatechin aduct	6.1	429			trace
41	Epigallocatechin aduct	8.6	429	275	**303**, 261, 177	409.20±6.47
42	Gallocatechin gallate	19.3	457	275	331, 305, 287	352.75±0.13
43	Epigallocatechin gallate	22.2	457			trace
44	Gallocatechin gallate aduct	10.2	581			trace
45	Epigallocatechin gallate aduct	12.4	581	275	455, **429**, 319	767.46±0.13
					Total	**3525.54±3.30**

10

Figure Captions

Figure 1: HPLC analyses of phenolic compounds from camu-camu pulp powder at 360 nm (A), camu-camu flour at 360 nm (B) and at 280 nm (C). *: unidentified compound. For compound characterization see Table 1. HPLC-DAD-MS-MS chromatogram of camu-camu flour proanthocyanidins (D) analyzed using the acid catalyzed degradation in the presence of phloroglucinol (280 nm). For compound identification see Table 4.

Figure 2. Characteristic phenolic compounds of camu-camu powders.



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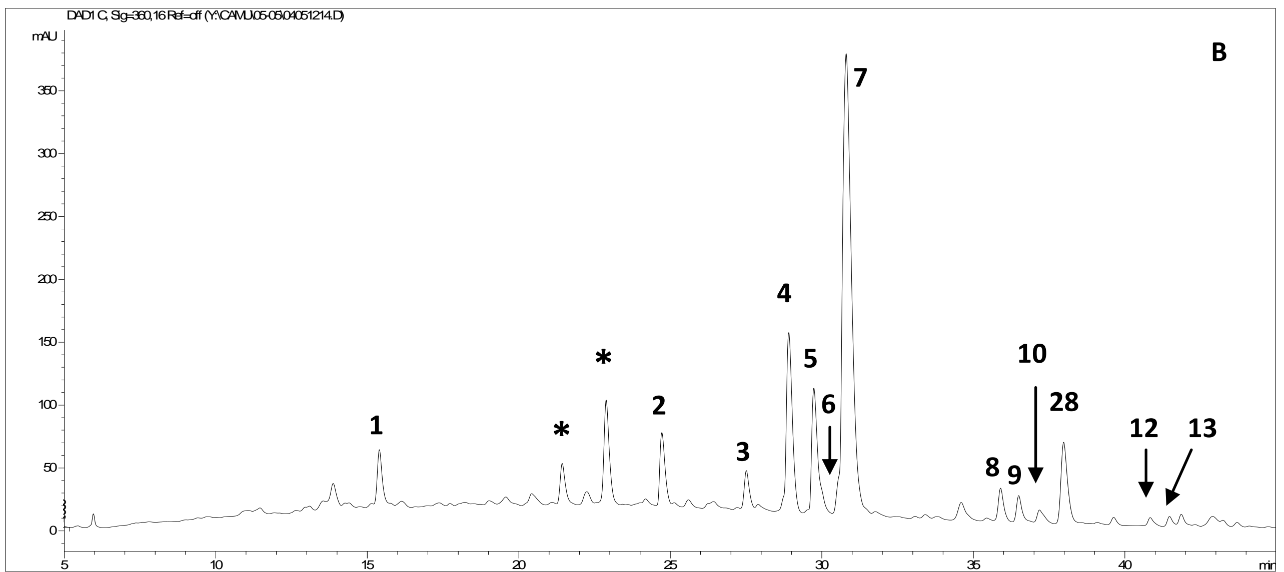

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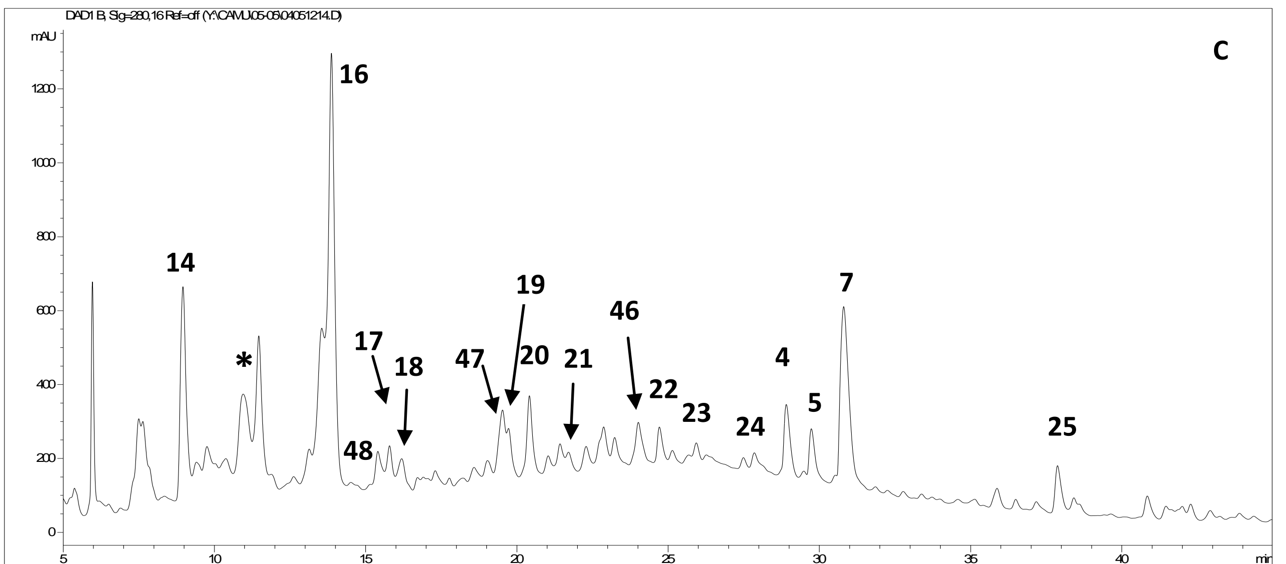

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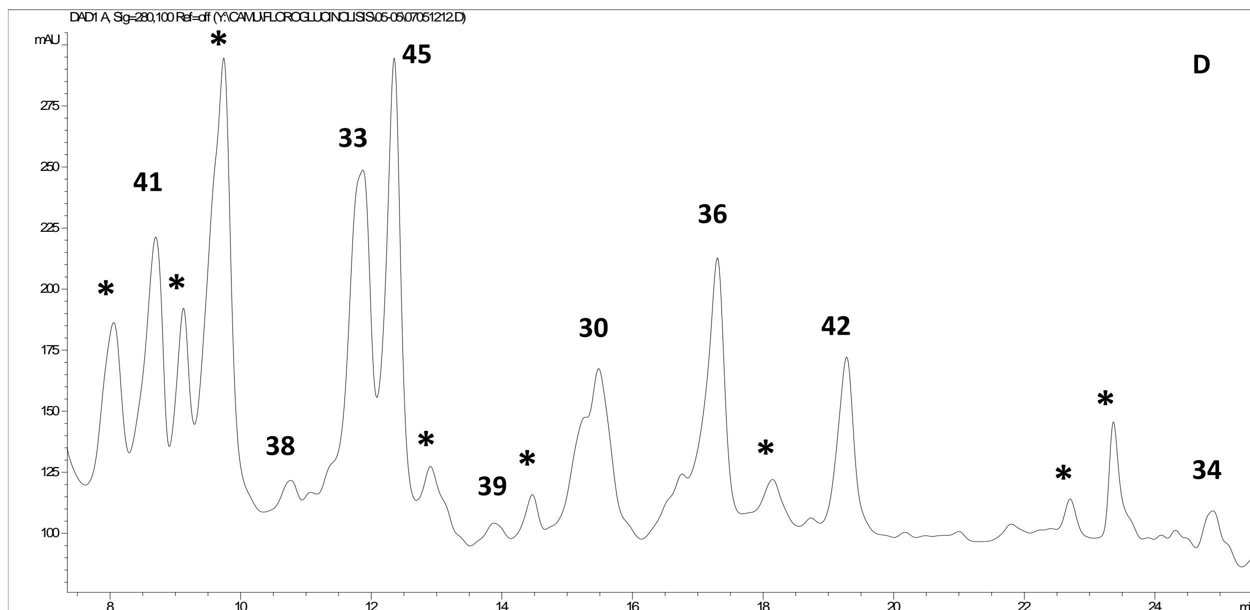
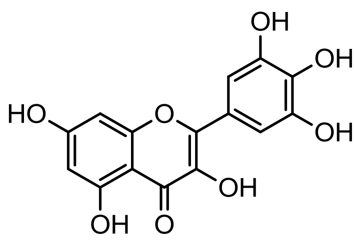
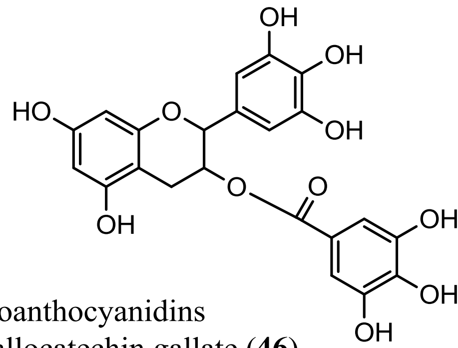


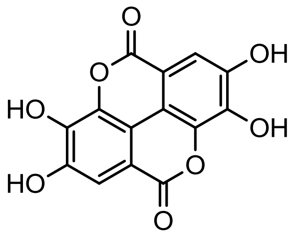
Figure 1



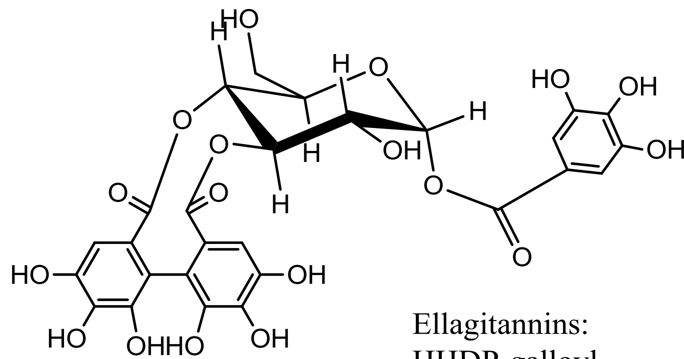
Flavonols: Myricetin (**28**)



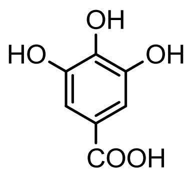
Proanthocyanidins
Gallocatechin gallate (**46**)



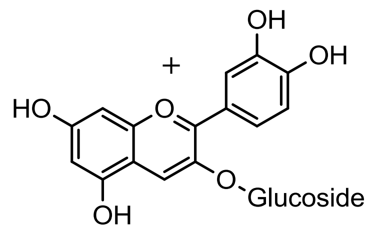
Ellagic acid (**7**)



Ellagitannins:
HHDP-galloyl-
glucose (**19**)



Gallic acid (**14**)



Anthocyanins: Cyanidin 3-glucoside (**29**)

Figure 2