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Phytochemicals in Japanese plums: impact of maturity and bioaccessibility

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Abstract

In recent years there has been increasing consumer interest in the potential health benefits of dietary derived phytochemicals such as polyphenols (including anthocyanins and flavonols) and carotenoids. A new variety of Japanese plum (*Prunus salicina* Lindl.), named Queen Garnet (QG), was developed as a high anthocyanin plum in a Queensland (Australia) Government breeding program and may be attractive to consumers but knowledge of other phytochemical content, and bioaccessibility, is currently limited. As a result, the present study examined (1) the impact of harvest date on anthocyanins, quercetin glycosides and carotenoids in Queen Garnet and another red fleshed commercial Japanese plum variety, Black Diamond (BD), (2) the content of bound phenolics in plum fruit and (3) the *in vitro* bioaccessibility of these phytochemicals as an initial measure to predict their potential bioavailability. For both QG and BD, the last harvest resulted in the highest anthocyanin content in peel, flesh and whole fruit, whereas no significant effects could be observed for quercetin glycosides, and total carotenoids decreased over time. The highest content of bound phenolics (30% of total amount) could be found in BD flesh. Between 53% and 59% of quercetin glycosides and anthocyanins was released from QG after the gastric and small intestinal digestion procedure, whereas the bioaccessible carotenoids ranged between 4-6%. A relative high release of anthocyanins and quercetin glycosides could be observed from QG which may result in a higher gastro-intestinal absorption rate of these compounds. However, follow-up studies (clinical trials) are warranted to investigate the *in vivo* bioavailability and subsequently biological activity of QG.

Keywords: Japanese plums; phytochemicals; maturity; in-vitro digestion; bioaccessibility

1. Introduction

In recent years there has been increasing consumer interest in the potential health benefits of dietary derived phytochemicals, such as polyphenols and carotenoids. Anthocyanins (e.g. cyanidin glycosides) and flavonols (e.g. quercetin glycosides), two main polyphenol subclasses, are one of the most abundant polyphenols in fruits and vegetables with an estimated daily intake of up to 64.9 mg anthocyanins and 54.9 mg flavonols, respectively (Zamora-Ros, Knaze, Lujan-Barroso, Slimani, Romieu, Fedirko, et al., 2011; Zamora-Ros, Knaze, Lujan-Barroso, Slimani, Romieu, Touillaud, et al., 2011). Recent publications indicate that the consumption of dietary anthocyanins as fresh food, juice, puree or powder may exert protection against cardiovascular risk factors, type 2 diabetes, oesophageal cancer and deterioration of bone tissues in humans (Cassidy et al., 2011; Chen et al., 2012; Hassellund et al., 2013; Jennings et al., 2012; Welch et al., 2012; Zhu et al., 2013)Jennings et al. 2014;.

Although afforded much less attention than some other phytochemical-rich fruits, certain varieties of Japanese plum (*Prunus salicina* Lindl.) are very significant sources of dietary anthocyanins (such as the variety Queen Garnet [QG]) and quercetin glycosides (Fanning, Topp, Russell, Stanley, & Netzel, 2014)Venter et al 2013). It has been previously shown in pilot trials that anthocyanin content increases with maturity in QG (Fanning et al., 2013) and other plums (Fanning et al., 2014) but content of quercetin glycosides and carotenoids are of interest but have not been studied. Furthermore the so-called bound phenolic fractions, including condensed tannins and hydrolysable polyphenols, which are generally not determined in fruits when the content is analysed by conventional extraction techniques have not been previously described in Japanese plum. A recent study with European plum (*Prunus domestica*) showed that >82% of the total antioxidant activity was due to the bound fractions (Kristl, Slekovec, Tojnko, & Unuk, 2011). It is anticipated that these “missing dietary polyphenols”, which often reach the colon and are then subject to microbial degradation, may mediate

92 significant health benefits (Saura-Calixto, 2012; Vizzotto, Cisneros-Zevallos, Byrne,
93 Ramming, & Okie, 2007).

94 Polyphenols and carotenoids must be released from the fruit matrix and then absorbed
95 through the gut wall in some form to exert their systemic effects in the body. *In vitro*
96 models that mimic the gastric and small intestinal digestion process are a common
97 approach to determine the release/bioaccessibility of nutrients and phytochemicals as an
98 initial measure to predict their potential bioavailability (ref). Bioaccessibility is defined
99 as the fraction of a nutrient (or phytochemical) that is released from a food matrix and
100 potentially available for intestinal absorption (Parada & Aguilera, 2007). To date, no
101 bioaccessibility data of the major phytochemicals in Japanese plum are available.
102 However, although anthocyanin content is more concentrated in peel in QG (and other
103 Japanese plums) the high flesh content of dark red fleshed varieties such as QG is novel
104 compared with many other anthocyanin-rich fruits such as many berries and grapes,
105 where anthocyanin is almost exclusively in the peel, and may result in higher
106 bioaccessibility.

107 Therefore, the objectives of the present study were (1) to assess the impact of maturity
108 on anthocyanins, quercetin glycosides and carotenoids in QG and another red fleshed
109 Japanese plum variety, Black Diamond (BD), (2) to determine the content of
110 nonextractable phenolic compounds in peel, flesh and whole fruit and (3) to evaluate the
111 bioaccessibility of these phytochemicals as an initial measure to predict their potential
112 bioavailability using an *in vitro* digestion procedure.

113

114 **2. Material and methods**

115 *2.1. Chemicals*

116 Unless otherwise stated, all chemicals were from Merck (Darmstadt, Germany),
117 Scharlau Chemie S.A. (Barcelona, Spain) or Sigma-Aldrich (Sydney, NSW, Australia)
118 and were of HPLC or analytical grade. Throughout the experiments, deionized water

was used (MILLIPORE Australia Pty Ltd, Kilsyth, VIC, Australia). Cyanidin-3-glucoside and cyanidin-3-rutinoside were purchased from ChromaDex (Irvine, CA, USA). Lutein, zeaxanthin and *β*-cryptoxanthin were purchased from Extrasynthase (Genay, France). Quercetin-3-rutinoside (rutin), quercetin-3-glucoside, quercetin-3-galactoside and *β*-carotene were purchased from Sigma-Aldrich.

2.2. Sample Preparation

Fruit were harvested from trees grown at the Applethorpe Research Facility (Applethorpe, Queensland, Australia [latitude: -28.6217, longitude: 151.9533, elevation: 876m]) as described for Queen Garnet previously (Fanning et al 2013). QG fruit were harvested on 17 January, 24 January, 31 January and 6 February (144, 151, 158 and 164 days post full bloom), and BD (suplumeleven) fruit were harvested on 17 January, 24 January and 31 January (with no fruit left on trees on 6 February). On each date mature fruit were picked and transported to Brisbane and stored at 1˚C overnight. All plums, 12 for each harvest date of each variety, were cut into half and the flesh and peel separated. The samples were freeze-dried and cryomilled (Retsch MM301 mill, Haan, Germany) prior to extraction.

For the in vitro digestion procedure fresh plums were picked in January 2013 from Applethorpe Research Facility, transported to Brisbane, stored overnight at 1˚C and processed the next day as detailed below.

2.3. Extraction

Polyphenols (anthocyanins and quercetin glycosides) were extracted as described previously (Fanning et al., 2013).

Carotenoids were extracted following the method published by Fanning et al. (2009) with the following modifications. Approximately 0.2 g was weighed into 50 mL tubes,

10 mL acetone was added and then the tubes were vortexed for 20 s. Samples were then handled as previously described (Fanning et al 2009).

The non-extractable phenolics were extracted using a method that was adopted from Kristl *et al*. (2011). To the residue left from the polyphenol extraction, 10 mL of acetone was added and then vortexed and centrifuged at 5000 rpm for 10 min. The supernatant was then removed and the residue was stored over night at -84 °C and then freeze-dried to remove any residual solvent. Two mL methanol and 0.2 mL concentrated sulphuric acid were then added to dried residue and incubated for 20 h in a shaking water bath (100 rev/min) at 85 °C. Following this the samples were centrifuged (10 min at 5000 rpm), and the supernatant (termed the 'hydrolysable tannins' fraction), was transferred to another 15 mL tube and diluted to 10 mL (with water). 0.5 mL acetone and 3 mL butanol/HCl (95/5, v/v) were then added to the residue, vortexed (1 min) and placed in a shaking water bath (100 rev/min) at 95 °C for 40 min. Following centrifugation (10 min at 5000 rpm), the supernatant (termed the 'proanthocyanidin' fraction), was transferred to another 15 mL tube and was diluted to 5 mL with butanol. These fractions were diluted with ethanol prior to analysis. All samples were then analyzed for total phenolic content (Folin-Ciocalteu method) as detailed below.

2.4. In-vitro digestion

Three large BD and three large QG fruits were separately cut into 12 pieces (without seed) and blended with a kitchen blender (Breville WIZZ stick, Breville, Sydney, NSW, Australia) to chop the plums. *In vitro* digestion procedure, adopted from Netzel et al. (2011) with slight modifications, was undertaken using this plum material. Briefly, ~5 g of either BD or QG was weighed into 10 (5 for gastric digestion and 5 for gastric and small intestinal digestion) individual 50 mL tubes (the remaining BD and QG material was stored separately as reference starting material at -84 °C until analysis). 6M HCl was added, with thorough mixing, until the samples reached pH 2. The pepsin (P7000,

172 Sigma-Aldrich) solution was then added and mixed. The tubes were incubated for 1h at
173 37 °C in a shaking water bath (85 rev/min). After 1h the gastric samples were removed,
174 centrifuged (10 min, 5000 rpm) and the supernatants were stored at -84 °C until
175 analysis. 0.1M $NaHCO_3$ with calcium (824 mg $CaCl_2*2H_2O$ in 500 mL 0.1M $NaHCO_3$)
176 was added until the pH reached 5.7. The samples were mixed well and incubated for a
177 further 30 min at 37 °C in a shaking water bath (85 rev/min). The pH was increased
178 from 2 to 5.7 to simulate the slow increase of the pH between the gastric and small
179 intestinal digestion. After removing the small intestinal samples from the water bath,
180 1M NaOH was added until pH of 7.0 was reached, with thorough mixing throughout.
181 One mL pancreatin-bile mixture (P1750, Sigma-Aldrich) was then added and mixed
182 well. Samples were incubated for another 2 h at 37°C in a shaking water bath
183 (85 rev/min). After removing from the water bath the samples were centrifuged (10 min,
184 5000 rpm) and supernatants were then transferred into 15 mL tubes. Samples which
185 were designated for polyphenol analysis were mixed with 500 µL formic acid (90%).
186 All samples were then stored at -84°C until extraction and analysis of anthocyanins,
187 quercetin glycosides and carotenoids.
188
189 *2.5. Analysis*
190 Total phenolic content was determined as previously described (Netzel et al., 2012) and
191 results were expressed as mg of gallic acid equivalents per 100g (mg GAE/100g).
192 Anthocyanins and carotenoids were both analyzed by HPLC as detailed previously
193 (Netzel et al., 2012) carotenoids - Fanning et al 2009).
194 Quercetin glycosides were analyzed with the same HPLC system as outlined in Fanning
195 et al 2009. The samples were diluted in water and filtered prior to injection onto a
196 Scientific Acclaim^{TM} PolarAdvantageII C18 3µm 120 Å (4.6 × 150 mm) column
197 (Thermo Scientific). The mobile phase A was 0.1% formic acid in water and B was
198 0.1% formic acid in acetonitrile. A gradient with a flow of 1.5ml/min was used: 0 min,

20% phase B; 12 min, 40% phase B; 21 min, 60% phase B; 23 min, 80% phase B; 27 min, 80% phase B; 29 min, 20% phase B; 35 min, 20% phase B. Identification and quantitation of quercetin glycosides was undertaken at 355 nm using the following standards, quercetin-3-rutinoside (rutin), quercetin-3-glucoside, quercetin-3-galactoside, and comparison with literature (Jaiswal et al 2013, Venter et al 2013).

2.6. Statistics

The significance of differences between harvest dates or plum type were evaluated using one-way analysis of variance (ANOVA) and the Tukey HSD procedure using JMP software (Version 7; SAS, Cary, NY, USA).

3. Results

3.1. Anthocyanins and quercetin glycosides

The last harvest resulted in the highest anthocyanin content (sum of cyanidin-3-glucoside and cyanidin-3-rutinoside) in peel, flesh and whole fruit with QG (Fig 1A) having significantly higher ($p<0.05$) levels than BD (Fig. 1B). The relative anthocyanin content in QG flesh (% of total (whole fruit) content) was significantly higher ($p<0.05$) than in BD at all harvest dates with an observed maximum of 47% on 6 February (Table 1).

Quercetin-3-glucoside and quercetin-3-rutinoside were identified as the main quercetin glycosides in QG and BD. There was a significant ($p < 0.05$) increase in quercetin glycosides in peel of both plum cultivars, as well as the whole fruit of BD, between the first and the last harvest date (Fig. 2). Overall, QG had significantly ($p<0.05$) higher levels in peel, flesh and whole fruit than BD. On 31 January (last harvest date of BD), 24% and 27% of quercetin derivatives were located in the flesh of QG and BD, respectively. However, an increase to 41% could be observed in QG flesh on 6 February (Table 1).

226

227 *3.2. Bound phenolics*

228 The average concentration of bound phenolics in peel, flesh and whole fruit ranged
229 between 14.0 and 27.1 mg GAE/100 g for hydrolysable tannins and between 52.4 and
230 73.4 mg GAE/100 g for proanthocyanidins. For both plum cultivars, the relative content
231 (% of total (free and bound) content) of bound phenolics was higher ($p<0.05$) in the
232 flesh than in the peel, with BD having the highest content of 30 % (Fig. 3).

233

234 *3.3. Carotenoid content*

235 Five carotenoids could be quantified in QG, namely lutein, β-cryptoxanthin, β-carotene,
236 zeaxanthin and α-carotene (the latter was found only in the peel). The slight decrease in
237 total carotenoids in the peel, between the first and last harvest date, was not significant,
238 whereas it was significant ($p<0.05$) in the flesh and whole fruit (Fig. 4A). Lutein, β-
239 cryptoxanthin and β-carotene could be quantified in BD. There was a significant
240 decrease ($p<0.05$) in total carotenoids in the peel between the first and the last harvest
241 date, whereas the slight decrease in the flesh and whole fruit was not significant (Fig.
242 4B).

243

244 *3.4. In vitro digestion*

245 The anthocyanin release after the mimicked gastric and small intestinal digestion
246 process was similar in both plum cultivars with 56% for BD and 59% for QG,
247 respectively (Table 2). However, the release of quercetin glycosides was significantly (p
248 <0.05) higher for QG with 53% released in total compared to 45% from BD (Table 2).
249 The amount of bioaccessible (released) carotenoids was relatively low (and similar) in
250 both plum cultivars with 4% and 6% for QG and BD, respectively (Table 2).

251

252 **4. Discussion**

253 *4.1. Anthocyanins and quercetin glycosides*

254 The anthocyanin content has been seen to increase in whole fruit, flesh and peel of
255 Japanese plums with increasing maturity on tree (Diaz-Mula et al., 2008; Fanning et al.,
256 2013; Netzel et al., 2012). Literature values for flesh and peel of commercial varieties
257 have ranged from 0.5-17.7 mg/100g and 12.9-916 mg/100g (Diaz-Mula et al., 2008;
258 Netzel et al., 2012; Tomas-Barberan et al., 2001). Whereas the peel content of QG and
259 BD was in the range of these reported levels, the flesh content of QG and to a lesser
260 extent BD, was outstanding being 6 and 1.6 times greater than the top of this range. The
261 flesh levels of QG, at the most mature stage, were ~20% higher than other high
262 anthocyanin selections (85 - 87 mg/100g, Cevallos-Casals, Byrne, Okie, and Cisneros-
263 Zevallos (2006)). Whole fruit anthocyanin content for red-dark peeled and red fleshed
264 commercial varieties often range from 10-80 mg/100g(Chun, Kim, Moon, Kang, & Lee,
265 2003; Diaz-Mula et al., 2008; Netzel et al., 2012). QG and BD had levels above this
266 range, with QG content (Fanning et al., 2013) comparable to or higher than breeding
267 selections with dark peel and red-dark flesh (Cevallos-Casals et al., 2006). Dark red
268 fleshed plums are a relatively unusual matrix given the fact that many anthocyanin-rich
269 fruits have these pigments heavily concentrated in the peel/skin, including grapes
270 (>99% in skin, Rebello et al 2013) and many berries. QG had an average of 45% of
271 anthocyanin content of the whole fruit in the flesh and BD had 28%. Whilst many
272 commercial varieties of Japanese plum also have only a small fraction of total
273 anthocyanin content in flesh (Fanning et al., 2014), high anthocyanin selections in other
274 breeding programs have also shown high flesh contributions (up to 71%) to total
275 anthocyanin content (Cevallos-Casals et al., 2006).

276 The content of quercetin glycosides in the peel of selected cultivars has been shown to
277 range from 16.6 to 35.2 mg/100 g (Black Beaut) (Tomas-Barberan et al., 2001). Both
278 plums had higher peel content, being 2.3 (BD) and 5.4 (QG) times higher than Black
279 Beaut. Mubarak *et al*. (2012) analyzed 29 prevarietal selections and saw flavonol

glycoside content of 0.9-27.9 mg/100 g fruit. Other commercial varieties have had levels of 2.3-27.0 mg/100 g fresh weight (Ozturk et al, Harnly et al, (Chun et al., 2003; Proteggente et al., 2002), Venter et al 2013) and the content of BD was in this range. However, the levels in QG were higher being 2-fold that of the highest literature values for commercial Japanese plum varieties and comparable or greater than levels seen for significant dietary sources of quercetin glycosides including onions (Proteggente et al., 2002).

4.2. Bound phenolics

Standard extraction methods have been appraised as underestimating the actual total amount of phenolic compounds in certain food products (Saura-Calixto, 2012). Saura-Calixto (2012) reported, that the "extractable [free] polyphenols may be only the tip of the iceberg". The work of Kristl *et al*. (2011) in four European plum cultivars showed that the free antioxidants (extracted using acidified methanol followed by acetone:water) accounted for less than 18% of the total antioxidant activity. However it is important to note that fresh, not freeze-dried plums were used for extraction in that study. Several possible reasons for the relatively low % (~20%) of bound phenolics, in QG and BD, include the high content of anthocyanins relative to the European plum cultivars, which are often nearly completely extractable in acidified methanol following homogenization (Yousef et al 2013), and the fact that QG/BD samples were freeze dried prior to extraction. The use of freeze-drying has been shown to significantly enhance the extractability of several phenolic acids in blueberries (Yousef et al 2013). However, even this relatively low content of bound phenolics in QG and BD may be of physiological significance. A meal of two large QG plums (~200 g) would easily provide up to 160 g bound phenolics according to our present findings. If transported to the colon without further degradation, these plant matrix/plant cell wall bound phenolics may either be fermented by the colonic microbiota forming absorbable metabolites such

as phenylacetic and phenylbutyric acids or could contribute to the maintenance of a healthy gut environment via prebiotic-like effects (Cardona, Andres-Lacueva, Tulipani, Tinahones, & Queipo-Ortuno, 2013; Padayachee et al., 2013). However, these compounds may also remain bound to the plant matrix and therefore be eliminated in faecal waste without any further interactions with the colonic microbial population.

4.3. Carotenoid content

The carotenoid content of flesh, peel and whole fruit of QG and BD were in the range of previous values for commercial Japanese plum varieties, flesh: 0.056-1.1 µg/g, peel: 0.27-9.9 µg/g, whole fruit: 0.09-1.9 µg/g (Diaz-Mula et al., 2008; Gil, Tomas-Barberan, Hess-Pierce, & Kader, 2002). In contrast to a decrease in carotenoid content with harvest date, in the present study, a previous study showed increases in both peel and flesh with longer time on tree in other varieties (Diaz-Mula et al., 2008). Varietal differences are most likely the reason for this observation and have been evidenced for changes in carotenoid content during postharvest storage (Diaz-Mula 2012).

4.4. In-vitro digestion

The release of anthocyanins from QG and BD after gastric and small intestinal digestion was relatively high, with QG having a 3% higher release than BD (59 vs. 56%, $p > 0.05$). These findings are in line with results of *in vitro* release of anthocyanins reported by others. For example, Bermudez-Soto et al. (2007) recovered ~57% of cyanidin-3-glucoside and ~58% of total anthocyanins in the digesta after *in vitro* pancreatic digestion of chokeberry juice. However, "*in vitro* recoveries" as low as 20% for pomegranate anthocyanins were also reported in the literature (Perez-Vincente et al. 2002). The chemical structure of dietary anthocyanins, the food/fruit matrix and subsequently the exposure to the alkaline conditions of the pancreatic digestion process are regarded as the main reasons for these inconsistent results (Bermudez-Soto et al.

2007). The food matrix can play a particularly significant role in the stability of polyphenols/anthocyanins with reports that polyphenols transiently bind to food matrices (e.g. plant cell wall components) during the gastro-intestinal digestion, which may protect them (and especially the more labile anthocyanins) from degradation processes (Padayachee et al. 2013; Parada and Aguilera 2007). The observed higher release of anthocyanins (trend, $p>0.05$) from whole QG fruit compared to BD as a potential result of the higher flesh:total anthocyanin ratio needs to be investigated in a follow-up study. Finally, it should be also noted that the relative amount of anthocyanins released after gastric digestion was in the same range as reported for anthocyanins from purple figs, with up to 35% cyanidin-3-rutinoside in the gastric digesta (Kamiloglu and Capanoglu 2013).

The relative release (released amount vs. total amount) of quercetin-glycosides from QG and BD fruit after *in vitro* digestion was lower than it was for the anthocyanins. However, the difference between QG and BD (53% vs. 45% of released quercetin glycosides) was significant ($p < 0.05$) and may be due to the higher flesh:total quercetin glycoside ratio as already suggested for the anthocyanins. The observed amount of bioaccessible quercetin-glycoside in the present study is comparable with the results reported by Bermudez-Soto et al. (2007): recoveries of ~81%, ~70% and ~73% for quercetin-3-glucoside, quercetin-3-rutinoside and total flavonols, respectively, from chokeberry juice were found at the end of the *in vitro* digestion process. Boyer et al. (2005) reported a recovery of almost 90% of quercetin-3-glucoside after the *in vitro* digestion of onions. Kamiloglu and Capanoglu (2013) also saw a significant difference in the bioaccessible fraction of quercetin-3-rutinoside after gastric digestion of whole-fresh yellow and purple figs, ranging from 40% (yellow) to 107% (purple). The food/fruit matrix and potential interactions with matrix components and compounds influencing the stability and/or accessibility of quercetin glycosides (and also

anthocyanins) during the *in vitro* digestion process are most likely responsible for the observed differences.

A relatively low release of lipophilic carotenoids from the plum matrix was expected since the sample material wasn't thermally treated and oil or lipids weren't added (thermal (pre)treatment and the addition of oil/lipids to fruits and vegetables can significantly increase the bioaccessible fraction of carotenoids (Lemmens et al., 2009; Netzel et al., 2011; Parada & Aguilera, 2007)). Plums are commonly consumed raw and these findings are in line with the results reported by others (Hedren, Diaz, & Svanberg, 2002; Lemmens, Van Buggenhout, Oey, Van Loey, & Hendrickx, 2009; Veda, Kamath, Platel, Begum, & Srinivasan, 2006): the relative bioaccessibility of β-carotene (which is also the main carotene in QG and BD) from raw carrots, amaranth and fenugreek leaves ranged between 3% and 11%.

However, several drawbacks and limitations of these *in vitro* digestion methods should be critically considered when interpreting the results: e.g. to date, no *in vitro* model is capable of covering all aspects of *in vivo* digestion and absorption, distribution, metabolism (including the metabolic activity of the gut microbiota) and elimination. Polyphenol metabolites generated *in vivo* are of particular interest in terms of their potential biological significance in the prevention of chronic diseases as well as the maintenance of a "healthy gut" (Tomas-Barberan and Andres-Lacueva, 2012; Williamson and Clifford, 2010). Therefore, human studies/clinical trials are still the "Gold Standard" to assess the bioavailability and metabolism (and subsequently bioactivity) of dietary polyphenols and carotenoids and yet cannot be replaced by any *in vitro* models.

5. Conclusions

QG has outstanding anthocyanin content and should be harvested as late as possible to maximise anthocyanins. The relative low content of bound phenolics in both plum

varieties needs further investigation to elucidate if this is a plum/cultivar specific feature or due to other reasons such as the handling procedure of the sample material prior to the extraction process. A relative high release of anthocyanins and quercetin glycosides could be observed from QG which may result in a higher gastro-intestinal absorption rate of these compounds. However, follow-up studies (clinical trials) are warranted to investigate the actual *in vivo* bioavailability and subsequently biological activity of QG.

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Tables

Table 1

Relative[a] phytochemical content in flesh of BD and QG at different harvest dates.

Plum cultivar	BD			QG			
Harvest date	17 Jan	24 Jan	31 Jan	17 Jan	24 Jan	31 Jan	6 Feb
Phytochemicals				% in flesh			
Anthocyanins	22 ± 1	33 ± 8	29 ± 3	43 ± 4*	46 ± 6*	42 ± 3*	47 ± 4*
Quercetin glycosides	19 ± 5	20 ± 3	27 ± 7	29 ± 5*	30 ± 5*	24 ± 2	41 ± 4*
Carotenoids	61 ± 6	57 ± 6	75 ± 16	73 ± 14	55 ± 12	57 ± 12	57 ± 13

[a]content in the flesh as % of total (whole fruit) content; values are means ± standard deviation, n=12 fruits; anthocyanins: sum of cyanidin-3-glucoside and cyanidin-3-rutinoside; quercetin glycosides: sum including quercetin-3-glucoside, quercetin-3-rutinoside and quercetin-3-galactoside; carotenoids: lutein, β-cryptoxanthin, zeaxanthin, α- and β-carotene for QG and lutein, β-cryptoxanthin and β-carotene for BD; *$p < 0.05$, QG vs. BD.

Table 2

Release (bioaccessibility) of phytochemicals from QG and BD

Plum cultivar	Phytochemical content in plum puree		Released phytochemicals	
			Gastric digestion (60 min)	Gastric and small intestinal digestion (180 min)
Queen Garnet	anthocyanins (mg/5 g):	10.0 ± 0.08	3.4 ± 0.39 (34%)[a]	5.8 ± 0.40 (59%)
	quercetin glycosides (mg/5g):	3.2 ± 0.03	0.6 ± 0.08 (20%)	1.7 ± 0.15 (53%)*
	carotenoids (µg/5 g):	19.2 ± 0.24	ND	0.8 ± 0.08 (4%)
Black Diamond	Anthocyanins (mg/5 g):	4.5 ± 0.07	1.7 ± 0.09 (38%)	2.5 ± 0.19 (56%)
	quercetin glycosides (mg/5 g):	1.2 ± 0.02	0.2 ± 0.01 (16%)	0.5 ± 0.05 (45%)
	carotenoids (µg/5 g):	15.6 ± 0.26	0.5 ± 0.05 (3%)	0.9 ± 0.09 (6%)

Values are means ± standard deviation, n=5 independent trials; anthocyanins: sum of cyanidin-3-glucoside and cyanidin-3-rutinoside; quercetin glycosides: sum including quercetin-3-glucoside, quercetin-3-rutinoside and quercetin-3-galactoside; carotenoids: lutein, β-cryptoxanthin, zeaxanthin, α- and β-carotene for QG and lutein, β-cryptoxanthin and β-carotene for BD; [a]percent release of phytochemicals (released amount vs. applied dose); %-data are rounded; *p < 0.05, QG vs. BD; ND, non-detectable.

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