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Original article

Stability of the phenolic compounds and antioxidant capacity of five fruit (apple, orange, grape, pomelo and kiwi) juices during *in vitro*-simulated gastrointestinal digestion

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Summary The *in vitro* digestive stability of phenolic compounds and the antioxidant capacity of five kinds of commonly consumed fruit juices in the daily diet, including apple juice (AJ), orange juice (OJ), grape juice (GJ), pomelo juice (PJ) and kiwifruit juice (KJ), were studied. Following *in vitro* digestion, the total phenolic (TP) content of fruit juices decreased to different extents by 35%, 25.3%, 23.5%, 22.2% and 7.8% for KJ, OJ, PJ, GJ and AJ, respectively. The individual phenolic content showed similar changes to the TP content, showing reductions of naringenin-trisaccharide in OJ and PJ, epicatechin in GJ, and chlorogenic acid in AJ by 43.74%, 27.59%, 47.11% and 33.28%, respectively. Conversely, the antioxidant capacity of fruit juices during digestion measured by ABTS assay increased from 4.79% to 35.53%, except in KJ, which decreased by 19.34%. These results show the health benefits of fruit juices after processing and contribute towards establishing suitable dietary recommendations.

Keywords Antioxidant, fruit juice, *in vitro* digestion, phenolic compounds, stability.

Introduction

Fruit is a necessary component of our daily diet as it contains various nutrients, such as vitamin C, carotenoids and amino acids (Choi *et al.*, 2016). The World Health Organization (WHO) and Food and the Agriculture Organization (FAO) of the United Nations suggest a fruit intake of 400 g per day for a healthy diet. The function of vitamins and amino acids has been reported widely, and bioactive compounds such as polyphenols found in fruit have been a recent research focus. The modern medical study pointed out that excessive reactive oxygen species, including superoxide radical, hydrogen peroxide, hydroxyl-free radical, singlet oxygen and nitric oxide in the human body may directly leads to chronic metabolic diseases such as diabetes mellitus, cancer and hyperglycaemia (Osman *et al.*, 2006; Ferrazzano *et al.*, 2009; Pawlaczyk *et al.*, 2011; Ochiai *et al.*, 2014). Fruit polyphenols are able to quench radicals by inhibiting their initiation and changing chain propagation, which is reported to be important in preventing these metabolic diseases (Re *et al.*, 1999).

The principal fruit polyphenols are phenolic acids, flavonoids, anthocyanins and tannins, as reported by Fu's research on 62 fruits in China (Fu *et al.*, 2011). It is well known that all the bioactive compounds have beneficial effects at the systemic level and should be digested, absorbed and metabolised through the complex digestive system of the human body (Hanhineva *et al.*, 2010). When estimating the potential functionality of a compound, it is more important to focus on the amount after gastrointestinal digestion rather than the original quantity since the phenolics undergo several oxidation and polymerisation reactions with pH change, leading to the formation of other high molecular weight or low solubility phenolic derivatives that are not available for absorption (Mosele *et al.*, 2016). Furthermore, polyphenols may bind to some dietary

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constituents such as proteins, carbohydrates, fibre and iron, leading to a significant decrease after gastrointestinal digestion (Bermudez-Soto *et al*., 2007; He *et al*., 2015a,b, 2016a,b,c).

Though it is difficult to exactly simulate the complex physiological conditions of living organisms, *in vitro* digestion models have been widely used in recent investigations to mimic the situation *in vivo* (Mosele *et al*., 2015), since *in vivo* methods are more complicated and costly, and it is difficult to control differences between animals and humans (Jang *et al*., 2015).

Fruit juice is a popular way to consume fruit worldwide, and changes during storage to phenolics and other bioactive compounds have been studied (Dawes & Keene, 1999; de Lima *et al*., 2014; Lopes *et al*., 2016). An increased number of studies have focused on the stability and antioxidant behaviour of polyphenols in fruit juice using *in vitro*-simulated digestion methods. However, these have only studied a few types of fruit juice, and little attention has been paid to changes to individual phenolics (Ryan & Prescott, 2010; Rodriguez-Roque *et al*., 2013; Pereira-Caro *et al*., 2015; Stanisavljevic *et al*., 2015; Coelho *et al*., 2016). In addition, few studies have focused on changes to individual and total phenolics (TPs) during *in vitro* gastric and intestinal digestion. It is well known that changes to the pH environment affect the stability and antioxidant capacity of individual phenolics, and during the gastrointestinal digestion stage, digestive enzymes (proteins) are combined with polyphenols and thus have an impact on their characteristics (Mosele *et al*., 2015; Oliveira & Pintado, 2015).

Therefore, five kinds of the most common daily fruits consumed in China—apple, grape, orange, pomelo and kiwifruit were chosen as the raw materials to produce juice, and changes to the individual and TP compounds of these five fruit juices and their antioxidant ability during gastrointestinal digestion using an *in vitro* digestion model were studied. The results would permit a better understanding of the transit and stability of polyphenols in fruit juices during digestion and how to protect fruit polyphenols from gastrointestinal digestion and maintain their health benefits through modulating the food matrix and processing conditions.

Materials and methods

Chemicals

Polyphenol standards contain quercetin, naringenin, naringin, hesperidin, hesperetin, phloridzin, proanthocyanidin, gallic acid, luteolin, epigallocatechin-3-gallate (EGCG), quinic acid, ferulic acid, epigallocatechin (EGC), epicatechin (EC), chlorogenic acid, and caffeic acid, HPLC grade acetonitrile and 2,2-azinobis(3-ethylbenzthiazoline-6-sulphonate) (ABTS) were purchased from J&K Scientific Co. Ltd. (Beijing, China). Folin–Ciocalteu's phenol reagent, pepsin (porcine gastric mucosa, ≥400 units per mg protein), pancreatin (porcine pancreas, 4 USP specifications), lipase and bile salt and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carbonsaure (Trolox) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Sample preparation

Apple, grape, pomelo, kiwifruit and orange fruits were purchased at a local farmers market (Wuxi, China). Fresh and undamaged fruits were carefully washed under running tap water to remove dirt, then rinsed with distilled water and freshly squeezed to produce apple juice (AJ), grape juice (GJ), pomelo juice (PJ), kiwifruit juice (KJ) and orange juice (OJ) using a domestic squeezer (SJ3039; Supor, Zhejiang, China). The juices were filtrated by filter sieve (pore size is 0.15 mm) to remove the pulp and fibre, only the clear liquid was vacuum packed in aluminium foil bags and kept frozen at −80 °C until analysed.

In vitro gastrointestinal digestion

A simulation of the physiological situation of *in vitro* gastrointestinal digestion in the human stomach and intestine was carried out according to a previous publication (He *et al*., 2015a,b, 2016a,b,c) except for two different steps for digestion. In the gastric digestion, 20 mL of thawed fruit juice sample was mixed with 3 mL of 40 mg mL^{-1} pepsin solution in a 50-mL tube and adjusted to 30 mL by adding 0.9% NaCl. The pH of the mixtures was adjusted to 2.0 using 0.1 M HCl and then the mixtures were flushed with N_2 and incubated at 37 °C for 1 h in a Shz-82 shaking water bath (Xinhang Instrument Factory, Jintan, China) at 120 strokes per min. In the pancreatic digestion, 0.09 M $NaHCO_3$ was used to change the pH to 5.0, then mixed with 5 mL of 24 mg mL^{-1} bile solution and 4 mg mL^{-1} pancreatin–lipase solution. The pH of the samples was readjusted to 7.5 using 0.1 M NaOH, then flushed with N_2 and incubated at 37 °C for 2 h in a shaking water bath. Control samples were run in parallel, and an equivalent volume of purified water (20 mL) was processed using the above-mentioned gastrointestinal digestion. After digestion, 5 mL aliquots of gastric digesta and small intestinal digesta were placed into 10-mL tubes and flushed with N_2 then stored at −80 °C until analysed.

TP content measurement

The TP content of the fruit juices was determined using the Folin–Ciocalteu method with some

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modifications. In brief, gallic acid was dissolved in methanol and used as a standard. Fruit juice sample (5 mL) was ultrasonicated for 30 min at 25 °C with 5 mL of a mixture of 1% (v/v) formic acid in 80% methanol. Then the resulting slurries were centrifuged 10 min at 4500 ***g***. The residue was re-extracted under the same conditions. After centrifugation, the combined extracts were retained for determination of TP content. One millilitre of diluted extract fruit juice samples was mixed with 1 mL of Folin–Ciocalteu's phenol reagent and 3 mL of 0.1 M sodium carbonate. The mixture was made up to 10 mL with distilled water and kept in the dark for 2 h at 30 °C. Control samples consisted of an equivalent volume of distilled water using the same method. The absorbance was measured at 760 nm using a UV-2800H spectrophotometer (Unico Instrument Co. Ltd, Shanghai, China), and concentrations were determined using a calibration curve generated using gallic acid. All tests were carried out in triplicate. Results were expressed as milligrams of gallic acid equivalents (GAE) per litre of original fruit juice. The TP content of digested fruit juice during *in vitro* digestion was obtained by subtracting the TP value of the control digestion sample from that of the fruit juice digesta.

ABTS radical scavenging activity

The ABTS radical quenching activities of the fruit juice digesta after *in vitro* digestion were measured according to the method of Robards *et al.* (1999) with some modifications. Briefly, 10 mL of ABTS was mixed with 2.45 mM potassium persulfate and kept at room temperature in the dark for 12–16 h. The ABTS solution was diluted with 0.01 M phosphate buffer (pH 7.4) to obtain an absorbance of 0.700 (±0.02) at 734 nm before use. Next, 3.9 mL diluted ABTS solution was mixed with 0.1 mL of sample and incubated at 30 °C for 10 min. The absorbance was recorded at 734 nm on a UV-2800H spectrophotometer. An equivalent volume of purified water was subjected to the *in vitro* digestion and used as a blank, and 0–1000 μM Trolox was used as a standard. The ABTS scavenging capacity of the test samples was expressed as the Trolox equivalent antioxidant capacity (TEAC) in μM Trolox. The ABTS value of digested fruit juice during *in vitro* digestion was obtained by subtracting the ABTS value of the blank digestion sample from that of fruit juice digested at the gastric and intestinal phases.

UPLC-PAD-QTOF-MS analysis of the phenolic compounds

As per our previous study (He *et al.*, 2016a,b,c), 5 mL of fruit juice samples collected at the three phases of digestion was blended with 5 mL of 1% (v/v) formic acid in methanol. The mixture was sonicated for 20 min and centrifuged at 10 000 ***g*** for 10 min at 4 °C, and the supernatant was collected and filtered through a 0.22-μm PTFE filter for phenolic profile analysis. Ultra-performance liquid chromatography–photodiode array detector–quadrupole time of flight–mass spectroscopy (UPLC–PAD–QTOF–MS) analysis of the phenolics was performed on a Waters ACQUITY UPLC equipped with a Waters Acquity PDA and a Waters MALDI SYNAPT Q-TOF MS (Waters Corporation, Milford, MA, USA). Separation of the phenolic compounds was carried out on a BEH C-18 packed column (1.7 μm, 100 × 2.1 mm i.d.; Waters Corporation). The column temperature was 35 °C, and the injection volume of the samples was 10 μL. The solvent gradient consisted of acetonitrile (solvent A) and 0.1% (v/v) formic acid (solvent B) with the following proportions: 0–5 min, from 10% to 30% A; 5–7 min, from 30% to 80% A; 7–10 min, from 80% to 100% A; 10–12 min, 100% A; 12–16 min, from 100% to 10% A. The flow rate of gradient elution was 0.3 mL min^{-1}. The phenolic compounds were detected at 280 nm using UPLC–PAD. The mass spectrometer operated in V mode for high sensitivity, and mass spectra in the m/z range 100–2000 were obtained by electrospray ionisation in positive-ion mode. The typical tuning parameters were as follows: the capillary voltage was 3.0 kV, and the cone voltage was 30.0 V, the cone and desolvation gas (nitrogen, 99.9% purity) flow rates were 10 and 700 L h^{-1}, respectively, the collision gas (argon, 99.9% purity) flow rate was 0.15 mL min^{-1}, and the source block and desolvation temperatures were 100 and 400 °C, respectively. The main phenolic compounds in the fruit juices were identified by comparing their retention times and spectra to those of the standards or data reported in the literature. Quantification of individual phenolic compounds was carried out by integrating the peak areas and using the calibration curves. Caffeoyl-tartaric acid and caffeoyl-glucoside were quantified as caffeic acid; naringenin-trisaccharide and naringenin-rutinoside were quantified as naringenin; quercetin-trisaccharide and sinensetin were quantified as quercetin; protocatechuic-glucoside, hesperetin-rutinoside, luteolin-rutinoside, feruloyl-glucoside and procyanidin-glucoside were quantified as epicatechin, hesperetin, luteolin, ferulic acid and proanthocyanidin, respectively. The results were expressed as milligrams of each phenolic compound per litre of fruit juice.

Statistical analysis

The analysis of all components was conducted in triplicate, with values reported as the mean ± standard deviation of three independent experiments. Statistical

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analysis was carried out using the general linear model procedure of the statistics 9.0 software package (Analytical Software, Tallahassee, FL, USA). Analysis of variance of the results followed by the least significant difference test was performed to determine significant differences ($P < 0.05$) among the total phenolic and the individual polyphenol content of fruit juices subjected to *in vitro* gastrointestinal digestion, as well as their ABTS radical scavenging activities.

Results and discussion

TP content of fruit juices during *in vitro* GI digestion

The TP content of five different fruit juices was estimated using the Folin–Ciocalteu method, which relies on the transfer of electrons from phenolic compounds to the agent in an alkaline medium (Ballus *et al.*, 2015). As shown in Fig. 1, the TP content before digestion varied from 593.89 ± 11.08 to 1481.08 ± 24.45 mg GAE/100 g. OJ showed the highest TP content, followed by AJ and KJ, while GP and PJ were lowest. Despite juice containing some constituents that are not phenolic compounds, such as vitamins and minerals that react with the Folin–Ciocalteu reagent and may lead to overestimation (de Oliveira *et al.*, 2009), the TP content of fruit juices was similar to those previously reported, except GP which showed different values (Tagliazucchi *et al.*, 2010). This may have been caused by a different maturation stage and growing conditions of the grape (Bresciani *et al.* 2015).

As shown in Fig. 1, *in vitro* gastrointestinal digestion influenced the TP content of fruit juices to a different extent. After gastric digestion, there was a significant ($P < 0.05$) increase in the TP content of AJ and OJ, whereas a non-significant ($P > 0.05$) increase was found for PJ. The TP content of AJ was increased by 47.23%, which is the highest value among these five juices, followed by OJ (15.09%) and PJ (13.78%). Apple contains an abundance of fibres (de Lima *et al.*, 2014) which hinders the release of phenolic compounds from the fruit pulp, and after pepsin hydrolysation during the gastric digestion, most of the bound phenolic compounds became free and were detected by Folin–Ciocalteu method. This result is in accordance with previous research (Rodriguez-Roque *et al.*, 2013; Huang *et al.*, 2014; Celep *et al.*, 2015) showing that a number of polyphenols increased after the gastric phase of *in vitro* digestion, and the increase could be a synergistic effect between the original phenolic compounds released from the fruit and some new polyphenol generated by acidic conditions or oxidation. In addition, Folin–Ciocalteu reagent does not specifically react with phenolic compounds and can also be reduced by other substances including sugars, amines, small peptides hydrolysed by pepsin and organic acid, thus leading to an overestimation of the phenolic content in samples (Oliveira & Pintado, 2015; Mokrani *et al.*, 2016). TP in GJ and KJ decreased by 4.32% and 13.20%; however, this was not statistically significant ($P > 0.05$), and it is possible that a proportion of the phenolics was interaction with other macronutrients found in the matrix or digestive system such as digestive enzyme, polysaccharides, which can modify the stability of these molecules (Carbonell-Capella *et al.*, 2014; Barba & Orliena, 2017). Another explanation could be that a part of the polyphenol compounds were transformed into different structural forms when

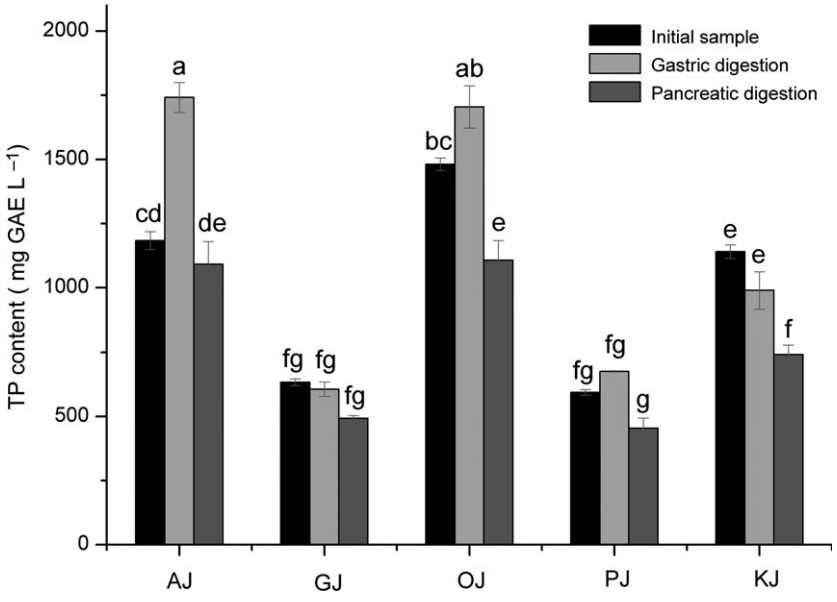

Figure 1 TP content of AJ, GJ, OJ, PJ and KJ during *in vitro* gastrointestinal digestion. Data are means ± SD ($n = 3$). Different lower case letters show significant differences ($P < 0.05$) in the TP content. TP, total phenolic; AJ, apple juice; GJ, grape juice; OJ, orange juice; PJ, pomelo juice; KJ, kiwi juice.

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exposed to acid conditions (Sengul *et al*., 2014), meaning the polyphenol compounds could not be detected using the Folin–Ciocalteu method.

After *in vitro* pancreatic digestion, a significant decline ($P < 0.05$) in TP content in AJ, OJ and KJ was observed (Fig. 1). Compared with gastric digestion, the TP content of AJ decreased by 37.34%, followed by OJ, PJ and KJ, with decreases of 35.09%, 32.79% and 25.14%, respectively. GJ showed the lowest decrease in 18.70%. The decreases in TP content of fruit juices during pancreatic digestion were attributed to an increase in pH. Phenolic instability under the alkaline environment of the intestinal tract indicated that the majority of polyphenols were highly sensitive to the neutral or near-alkaline conditions (Bouayed *et al*., 2011; Rodríguez-Roque *et al*., 2014), especially polyphenols in AJ and OJ which were released from cells and pectin at gastric digestion, and the anthocyanins and the chlorogenic acid became significantly lost after pancreatic digestion (Bouayed *et al*., 2011, 2012).

Individual phenolic compounds of fruit juices during *in vitro* GI digestion

As described in Table 1, the main individual polyphenols of the five fruit juices during *in vitro* digestion were identified by UPLC–MS and quantified by high-performance liquid chromatography (HPLC). Apple contains flavonol monomers, chlorogenic acid and small quantities of hydroxycinnamic acids (Contreras-Calderón *et al*., 2011; Fu *et al*., 2011), and phloridzin is a specific compound in AJ (Bouayed *et al*., 2011). In addition, anthocyanins and proanthocyanidin exist in grape (He *et al*., 2016a,b,c; Lingua *et al*., 2016), and hesperidin is commonly found in orange and pomelo (Pereira-Caro *et al*., 2015). The main polyphenols of kiwifruit were identified as derivatives of coumaric, caffeic acids containing chlorogenic acid, quinic acid and caffeoyl-glucoside, and flavonols were present as sinensetin (Dawes & Keene, 1999; Park *et al*., 2015; Izli *et al*., 2017). The flavonoids and their derivatives are the most abundant polyphenol in OJ, including the naringenin, naringenin-trisaccharide, luteolin-rutinoside, hesperetin-rutinoside and a type of flavanone (quercetin-trisaccharide). The chemical characteristics of phenolic compounds, such as solubility, hydrophobicity, molecular weight or even isomer configuration, are influencing their stability in the course of digestion (Barba *et al*., 2017), since after *in vitro* gastric digestion, a significant decrease ($P < 0.05$) was observed in the naringenin-trisaccharide, naringenin and hesperetin-rutinoside content of OJ (17.16%, 53.33%, and 19.93%, respectively). Chlorogenic acid in AJ also showed a

Table 1 Main individual phenolic compound content (mg L^{-1}) from five fruit juices subjected to different phase of *in vitro* digestion test*

Fruit juice name	Phenolic compounds	$[M + H]^+$	Fragment ions (*m/z*)	Initial sample	Gastric digestion	Pancreatic digestion
Orange juice	Caffeoyl-glucoside	683	342, 179	356.71 ± 76.8^{b}	538.33 ± 34.7^{a}	208.12 ± 41.1^{c}
	Naringenin-trisaccharide	777	579, 434, 271	273.44 ± 2.8^{a}	226.50 ± 5.4^{b}	153.84 ± 0.3^{c}
	Quercetin-trisaccharide	809	463, 301, 203	79.01 ± 9.6^{a}	63.49 ± 13.7^{a}	35.73 ± 3.3^{b}
	Naringenin	579	429, 271	431.71 ± 34.4^{a}	201.49 ± 54.7^{b}	106.12 ± 8.5^{c}
	Luteolin-rutinoside	639	593, 285	219.33 ± 7.1^{a}	217.01 ± 1.0^{a}	150.83 ± 3.9^{b}
	Hesperetin-rutinoside	609	301	74.21 ± 9.6^{a}	59.42 ± 2.1^{b}	54.89 ± 3.0^{b}
Apple juice	EGCG	458	337, 293, 193	5.83 ± 0.1^{a}	5.84 ± 0.5^{a}	3.57 ± 0.2^{b}
	Chlorogenic acid	354	191, 173, 135	132.22 ± 3.7^{a}	108.12 ± 14.4^{b}	88.21 ± 3.2^{b}
	Hesperidin	609	301, 205	5.90 ± 0.3^{a}	5.93 ± 2.0^{a}	4.37 ± 0.3^{b}
	Phloridzin	472	435, 273, 167	12.56 ± 0.1^{a}	13.07 ± 0.6^{a}	9.14 ± 0.5^{b}
Grape juice	Protocatechuic-glucoside	272	153	7.78 ± 0.2^{a}	8.02 ± 0.0^{a}	6.26 ± 0.0^{b}
	Caffeoyl-tartaric acid	312	179, 149, 133	41.71 ± 0.7^{b}	57.97 ± 0.2^{a}	39.11 ± 0.2^{c}
	Proanthocyanidin	577	289	3.38 ± 0.2^{b}	5.75 ± 0.0^{a}	3.34 ± 0.1^{b}
	Epicatechin	290	163, 119	9.87 ± 0.3^{a}	10.47 ± 0.1^{a}	5.22 ± 0.5^{b}
Pomelo juice	Feruloyl-glucoside	492	357, 195	12.87 ± 0.2^{a}	13.66 ± 0.2^{a}	9.94 ± 0.3^{b}
	Naringenin-trisaccharide	777	579, 434, 271	9.17 ± 0.0^{a}	9.19 ± 0.3^{a}	6.64 ± 0.3^{b}
	Proanthocyanidin-glucoside	785	577, 206	8.92 ± 1.6^{a}	8.99 ± 0.2^{a}	6.75 ± 0.0^{b}
	Naringenin-rutinoside	839	741, 578, 509	56.10 ± 0.2^{ab}	59.87 ± 0.3^{a}	43.28 ± 0.2^{b}
	Proanthocyanidin	577	289	9.44 ± 1.6^{a}	8.32 ± 0.2^{ab}	6.62 ± 0.0^{b}
Kiwi juice	Sinensetin	395	191, 173	32.68 ± 2.0^{b}	39.98 ± 1.6^{a}	33.89 ± 3.1^{ab}
	Quinic acid	767	383, 191, 111	182.05 ± 16.8^{a}	169.94 ± 9.0^{a}	120.63 ± 15.8^{b}
	EGC	483	305, 203	6.01 ± 0.5^{a}	5.39 ± 0.9^{a}	4.33 ± 0.3^{b}
	Caffeoyl-glucoside	683	342, 179	6.06 ± 0.8^{a}	5.05 ± 0.1^{a}	4.04 ± 0.1^{b}
	Chlorogenic acid	354	191, 173, 135	8.94 ± 0.3^{ab}	10.52 ± 0.4^{a}	7.23 ± 0.4^{b}

Different superscripts in the same row show significant differences ($P < 0.05$) among the same phenolic content at different phase of digestion.
*Data are expressed as the mean ± SD ($n = 3$).

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decreasing trend (18.23%) during gastric digestion, which was different from the report of Bermudez-Soto *et al.* (2007) that showed chlorogenic acid did not change during gastric digestion. Meanwhile, Gil-Izquierdo *et al.* (2001) and Laurent *et al.* (2007) reported that the gastric environment had no negative influence on the stability of flavonoids in grape and OJ. These differences could be explained by a synergistic or antagonistic interaction between the phenolic compounds with other substances according to the food matrix (D'Archivio *et al.*, 2010). In addition, the differences in structure affected the stability of the flavonoids; for instance, Aschoff *et al.* (2015) reported that some flavonoids have a closed-loop precipitation under acidic conditions. By contrast, the caffeoyl-glucoside content of OJ and the caffeoyl-tartaric acid and proanthocyanidin content of GJ were significantly increased (50.91%, 38.98% and 70.12%, respectively; $P < 0.05$) after gastric digestion. The other phenolic compounds of these five fruit juices showed no statistically significant ($P > 0.05$) decrease or increase during gastric digestion (Table 1). These results indicate that the stability of polyphenols in gastric acid conditions might depend on some factors, for instance, the physicochemical characteristics of phenolic compounds and their interactions with gastric and dietary constituents. In addition, pepsin action and the pH environment of gastric digestion could release some phenolics that are bound to proteins and carbohydrates present in the food matrix, thus increasing the content of the polyphenol compounds (Tagliazucchi *et al.*, 2010; Pinacho *et al.*, 2015; Ti *et al.*, 2015).

During small intestinal digestion, a significant decrease ($P < 0.05$) of phenolic concentration among the major polyphenols in these five fruit juices was observed, ranging from 15.23% to 75.41%. In AJ, chlorogenic acid, the most abundant hydroxycinnamic acid, was completely unstable during intestinal digestion, similar to a previous report (Bouayed *et al.*, 2012), and was reduced by 33.17%. Chlorogenic acid from the KJ also showed a significant decrease in 31.27%. It was reported that there was a 30% decrease in chlorogenic acid content from black chokeberry during the intestinal digestion period due to the alkaline environment and the formation of neochlorogenic acid (Bermudez-Soto *et al.*, 2007). Flavonoids in the five fruit juices showed a significant decrease ($P < 0.05$), ranging from 26.31% to 47.33%, especially in OJ which contains a large amount of flavonoids. While some researchers found many pure flavonoid aglycones were stable in the mild alkaline environment (Tagliazucchi *et al.*, 2010), the flavonoids in the fruit juice were their derivatives. In fact, under alkaline conditions, 55–65% of flavanones transform into another compound with different chemical and physical properties, such as chalcones. The interaction between the flavonoids and digestive enzymes could make detection using HPLC analysis difficult (Rodriguez-Roque *et al.*, 2013; Pereira-Caro *et al.*, 2014). Proanthocyanidin and derivatives in the GJ decreased by 29.87% and 24.33% ($P < 0.05$), respectively. During the transition from the acidic gastric to the alkaline environment, since the proanthocyanidin which is compounds of high molecular weight has to be degraded into smaller molecules at the intestinal phase (Barba *et al.*, 2017), these results are in agreement with the previous report of Liang *et al.* (2012). In addition, proanthocyanidin from GJ seemed stable compared with the initial conditions. While proanthocyanidin is degraded at the alkaline pH, it could be that the proanthocyanidin linked closely to the cell wall in the grape skin cells and was released during the digestive gastro–pancreatic digestion phase in the acidic condition of the stomach and the alkaline environment of the intestine (Mosele *et al.*, 2015).

The ABTS radical scavenging activity of fruit juices during *in vitro* GI digestion

The ABTS radical scavenging activity is a common indicator in scientific study that aimed at assessing some functional properties (Granato *et al.*, 2017), and the result is shown in Fig. 2. There was a significant variation ($P < 0.05$) in the TEAC value for GJ and KJ after gastrointestinal digestion, whereas little change ($P > 0.05$) was observed for AJ, OJ and PJ. The antioxidant capacities of the AJ increased by 58.16% after gastric digestion, which showed the highest value (15,481.59 ± 421.79 μM Trolox) among these fruit juices, while a significant decrease ($P < 0.05$) was observed when the pH changed to mildly alkaline. Though the TEAC values of GJ and KJ slightly decreased after the gastric digestion, the antioxidant ability of GJ increased by 35.53% after the intestinal digestion while the antioxidant ability of KJ decreased by 19.34%. The antioxidant ability of OJ and PJ showed no significant change ($P > 0.05$) whether after gastric or intestinal digestion. The TEAC value change of KJ and PJ was similar to their TP content change after the gastric intestinal digestion. However, there was a different change in trend between the TP content and the TEAC values for the AJ, GJ and OJ. It is well known from the literature that the ABTS method is based on reducing the radical derived from ABTS back to the parent substrate (Parsa & Salout, 2016). In addition to the polyphenols having quenching activity, these radical cations can be scavenged by digestive enzymatic (Granato *et al.*, 2017), chemicals and electrochemical means, such as the vitamin C and E originally found in the fruit juices, the pepsin added for digestion (Zheng *et al.*, 2016), and some antioxidant peptides produced by digestion enzyme. Meanwhile, the radical scavenging ability of polyphenols is

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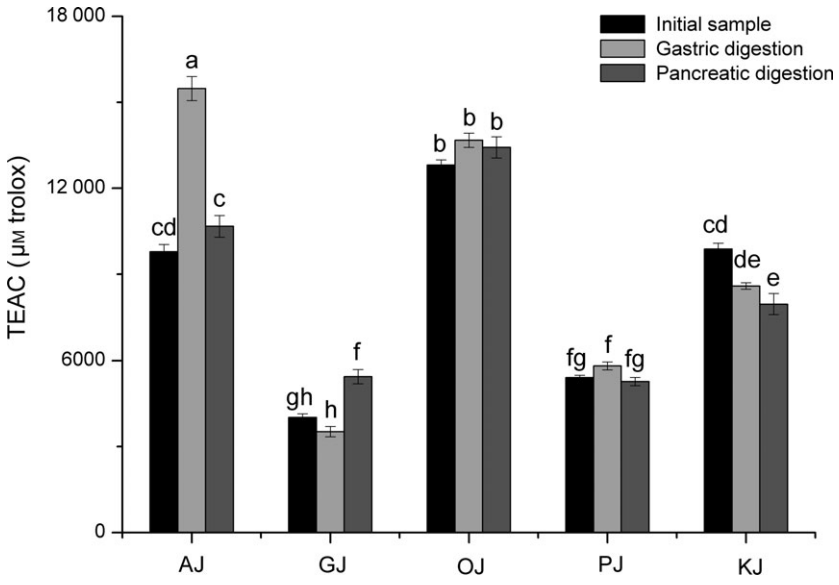

Figure 2 ABTS radical scavenging activity of AJ, GJ, OJ, PJ and KJ during *in vitro* digestion. Data are means ±SD ($n = 3$). Different lower case letters show significant differences ($P < 0.05$) in the ABTS radical scavenging activity. TEAC, Trolox equivalent antioxidant capacities; AJ, apple juice; GJ, grape juice; OJ, orange juice; PJ, pomelo juice; KJ, kiwi juice.

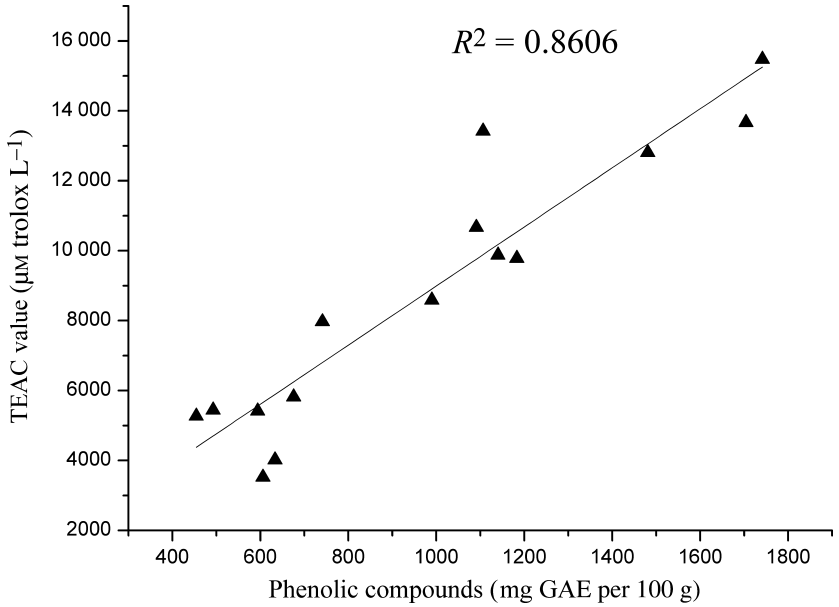

Figure 3 Correlation between the 2,2-azinobis(3-ethylbenzthiazoline-6-sulphonate) (ABTS) radical scavenging activity and total phenolic (TP) content of five fruit juices during *in vitro* digestion.

strongly pH-dependent due to the transition from the gastric to the intestinal conditions that may bring about structural changes in the phenolic compounds (Kahle *et al.*, 2011). For instance, polyphenols in apple showed higher antioxidant ability at lower pH conditions, whereas the apple polyphenols oxidised into quinones at higher pH conditions which performed lower ABTS radical scavenging activity. In addition, protein or peptides can bond with polyphenols at the antioxidant group sites through hydrogen bonding, covalent bonding, hydrophobic interactions and affect the antioxidant ability of the polyphenol (Wootton-Beard *et al.*, 2011).

Although the antioxidant capacities measured by the ABTS assay might be affected by these conditions, there was a correlation between the TEAC value and the TP content of the five fruit juices (Fig. 3). A highly positive correlation ($R^2 = 0.8606$) showed the polyphenols in the fruit juices could be the main components responsible for the antioxidant ability of the fruit juice.

Conclusion

In this study, an *in vitro* gastrointestinal digestion model was used to study the change of phenolic compounds and antioxidant capacity of five fruit juices

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during digestion. The results showed that the TP content of the five fruit juices was generally stable or increased after gastric digestion, but the TP content of AJ, OJ and KJ significantly decreased during intestinal digestion due to the transition from lower pH conditions to a mildly alkaline intestinal environment. From the viewpoint of individual phenolics, the content of naringenin-trisaccharide and naringenin in OJ and chlorogenic acid in AJ decreased significantly after gastric digestion, while the others remained stable or increased. After intestinal digestion, most individual phenolic content decreased significantly except for proanthocyanidin in GJ and sinensetin in KJ. Antioxidant ability estimated by the ABTS assay showed a diverse trend compared with the TP content. Although the TP content was reduced, AJ and OJ still showed a relatively high antioxidant activity, and *in vitro* digestion did not induce a significant effect, only KJ decreased significantly. The aim of this study was to mimic the *in vivo* process using *in vitro* gastrointestinal digestion. Our findings contribute to the knowledge of changes to fruit juice polyphenol during digestion and provide a scientific basis for further study of suitable processing and a matrix to protect phenolics from gastrointestinal digestion.

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References

Aschoff, J.K., Kaufmann, S., Kalkan, O., Neidhart, S., Carle, R. & Schweiggert, R.M. (2015). In vitro bioaccessibility of carotenoids, flavonoids, and vitamin C from differently processed oranges and orange juices *Citrus sinensis* (L.) Osbeck. *Journal of Agricultural and Food Chemistry*, **63**, 578–587.

Ballus, C.A., Meinhart, A.D., Campos, F.A.D. & Godoy, H.T. (2015). Total phenolics of virgin olive oils highly correlate with the hydrogen atom transfer mechanism of antioxidant capacity. *Journal of the American Oil Chemists Society*, **92**, 843–851.

Barba, F.J. & Orliena, V. (2017). Processing, bioaccessibility and bioavailability of bioactive sulfur compounds: facts and gaps. *Journal of Food Composition and Analysis*, **61**, 1–3.

Barba, F.J., Mariutti, L.R.B., Bragagnolo, N., Mercadante, A.Z., Barbosa-Canovas, G.V. & Orlien, V. (2017). Bioaccessibility of bioactive compounds from fruits and vegetables after thermal and nonthermal processing. *Trends in Food Science & Technology*, **67**, 195–206.

Bermudez-Soto, M.J., Tomas-Barberan, F.A. & Garcia-Conesa, M.T. (2007). Stability of polyphenols in chokeberry (*Aronia melanocarpa*) subjected to in vitro gastric and pancreatic digestion. *Food Chemistry*, **102**, 865–874.

Bouayed, J., Hoffmann, L. & Bohn, T. (2011). Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: bioaccessibility and potential uptake. *Food Chemistry*, **128**, 14–21.

Bouayed, J., Deusser, H., Hoffmann, L. & Bohn, T. (2012). Bioaccessible and dialysable polyphenols in selected apple varieties following in vitro digestion vs. their native patterns. *Food Chemistry*, **131**, 1466–1472.

Bresciani, L., Calani, L. & Cossu, M. (2015). (Poly)phenolic characterization of three food supplements containing 36 different fruits, vegetables and berries. *PharmaNutrition*, **3**, 11–19.

Carbonell-Capella, J.M., Buniowska, M., Barba, F.J., Esteve, M.J. & Frigola, A. (2014). Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: a review. *Comprehensive Reviews in Food Science and Food Safety*, **13**, 155–171.

Celep, E., Charehsaz, M., Akyüz, S., Acar, E.T. & Yesilada, E. (2015). Effect of in vitro gastrointestinal digestion on the bioavailability of phenolic components and the antioxidant potentials of some Turkish fruit wines. *Food Research International*, **78**, 209–215.

Choi, S.H., Kozukue, N., Kim, H.J. & Friedman, M. (2016). Analysis of protein amino acids, non-protein amino acids and metabolites, dietary protein, glucose, fructose, sucrose, phenolic, and flavonoid content and antioxidative properties of potato tubers, peels, and cortexes (pulps). *Journal of Food Composition and Analysis*, **50**, 77–87.

Coelho, E.M., De Azevedo, L.C., Correa, L.C., Bordignon-Luiz, M.T. & Lima, M.D. (2016). Phenolic profile, organic acids and antioxidant activity of frozen pulp and juice of the jambolan (*Syzygium cumini*). *Journal of Food Biochemistry*, **40**, 211–219.

Contreras-Calderón, J., Calderón-Jaimes, L., Guerra-Hernández, E. & García-Villanova, B. (2011). Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. *Food Research International*, **44**, 2047–2053.

D'Archivio, M., Filesi, C., Vari, R., Scazzocchio, B. & Masella, R. (2010). Bioavailability of the polyphenols: status and controversies. *International Journal of Molecular Sciences*, **11**, 1321–1342.

Dawes, H.M. & Keene, J.B. (1999). Phenolic composition of kiwifruit juice. *Journal of Agricultural and Food Chemistry*, **47**, 2398–2403.

Ferrazzano, G.F., Amato, I., Ingenito, A., De Natale, A. & Pollio, A. (2009). Anti-cariogenic effects of polyphenols from plant stimulant beverages (cocoa, coffee, tea). *Fitoterapia*, **80**, 255–262.

Fu, L., Xu, B.T., Xu, X.R. *et al.* (2011). Antioxidant capacities and total phenolic contents of 62 fruits. *Food Chemistry*, **129**, 345–350.

Gil-Izquierdo, A., Gil, M.I., Ferreres, F. & Tomas-Barberan, F.A. (2001). In vitro availability of flavonoids and other phenolics in orange juice. *Journal of Agricultural and Food Chemistry*, **49**, 1035–1041.

Granato, D., Nunes, D.S. & Barba, F.J. (2017). An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of functional foods: a proposal. *Trends in Food Science & Technology*, **62**, 13–22.

Hanhineva, K., Törrönen, R., Bondia-Pons, I. *et al.* (2010). Impact of dietary polyphenols on carbohydrate metabolism. *International Journal of Molecular Sciences*, **11**, 1365–1402.

He, Z., Yuan, B., Zeng, M., Tao, G. & Chen, J. (2015a). Effect of simulated processing on the antioxidant capacity and in vitro protein digestion of fruit juice-milk beverage model systems. *Food Chemistry*, **175**, 457–464.

He, Z., Yuan, B., Zeng, M., Tao, G. & Chen, J. (2015b). Effect of simulated processing on the antioxidant capacity and in vitro protein digestion of fruit juice-milk beverage model systems. *Food Chemistry*, **175**, 457–464.

He, Z., Chen, J., Moser, S.E., Jones, O.G. & Ferruzzi, M.G. (2016a). Interaction of beta-casein with (–)-epigallocatechin-3-gallate assayed by fluorescence quenching: effect of thermal processing temperature. *International Journal of Food Science and Technology*, **51**, 342–348.

He, Z., Tao, Y., Zeng, M. *et al.* (2016b). High pressure homogenization processing, thermal treatment and milk matrix affect in vitro bioaccessibility of phenolics in apple, grape and orange juice to different extents. *Food Chemistry*, **200**, 107–116.

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He, Z., Xu, M., Zeng, M., Qin, F. & Chen, J. (2016c). Preheated milk proteins improve the stability of grape skin anthocyanins extracts. *Food Chemistry*, **210**, 221–227.

Huang, H., Sun, Y., Lou, S., Li, H. & Ye, X. (2014). In vitro digestion combined with cellular assay to determine the antioxidant activity in Chinese bayberry (*Myrica rubra* Sieb. et Zucc.) fruits: a comparison with traditional methods. *Food Chemistry*, **146**, 363–370.

Izli, N., Izli, G. & Taskin, O. (2017). Drying kinetics, colour, total phenolic content and antioxidant capacity properties of kiwi dried by different methods. *Journal of Food Measurement and Characterization*, **11**, 64–74.

Jang, H., Choi, Y., Ahn, H.R., Jung, S.H. & Lee, C.Y. (2015). Effects of phenolic acid metabolites formed after chlorogenic acid consumption on retinal degeneration in vivo. *Molecular Nutrition & Food Research*, **59**, 1918–1929.

Kahle, K., Kempf, M., Schreier, P. *et al.* (2011). Intestinal transit and systemic metabolism of apple polyphenols. *European Journal of Nutrition*, **50**, 507–522.

Laurent, C., Besancon, P. & Caporiccio, B. (2007). Flavonoids from a grape seed extract interact with digestive secretions and intestinal cells as assessed in an in vitro digestion/Caco-2 cell culture model. *Food Chemistry*, **100**, 1704–1712.

Liang, L., Wu, X., Zhao, T. *et al.* (2012). In vitro bioaccessibility and antioxidant activity of anthocyanins from mulberry (*Morus atropurpurea* Roxb.) following simulated gastro-intestinal digestion. *Food Research International*, **46**, 76–82.

de Lima, A.C.S., Soares, D.J., da Silva, L.M.R., de Figueiredo, R.W., de Sousa, P.H.M. & Menezes, E.D. (2014). In vitro bioaccessibility of copper, iron, zinc and antioxidant compounds of whole cashew apple juice and cashew apple fibre (*Anacardium occidentale* L.) following simulated gastro-intestinal digestion. *Food Chemistry*, **161**, 142–147.

Lingua, M.S., Fabani, M.P., Wunderlin, D.A. & Baroni, M.V. (2016). From grape to wine: changes in phenolic composition and its influence on antioxidant activity. *Food Chemistry*, **208**, 228–238.

Lopes, M.L.M., Miguel, M.A.L., Fialho, E. & Valente-Mesquita, V.L. (2016). Grape juice obtained using steam extraction and other small-scale extraction methods: phenolic content, antioxidant capacity and stability during storage. *International Journal of Food Science and Technology*, **51**, 1696–1702.

Mokrani, A., Krisa, S., Cluzet, S. *et al.* (2016). Phenolic contents and bioactive potential of peach fruit extracts. *Food Chemistry*, **202**, 212–220.

Mosele, J.I., Macià, A., Romero, M.P., Motilva, M.J. & Rubió, L. (2015). Application of in vitro gastrointestinal digestion and colonic fermentation models to pomegranate products (juice, pulp and peel extract) to study the stability and catabolism of phenolic compounds. *Journal of Functional Foods*, **14**, 529–540.

Mosele, J.I., Macia, A., Romero, M.P. & Motilva, M.J. (2016). Stability and metabolism of *Arbutus unedo* bioactive compounds (phenolics and antioxidants) under in vitro digestion and colonic fermentation. *Food Chemistry*, **201**, 120–130.

Ochiai, R., Sugiura, Y., Shioya, Y. *et al.* (2014). Coffee polyphenols improve peripheral endothelial function after glucose loading in healthy male adults[J]. *Nutrition Research*, **34**, 155–159.

Oliveira, A. & Pintado, M. (2015). Stability of polyphenols and carotenoids in strawberry and peach yoghurt throughout in vitro gastrointestinal digestion. *Food & Function*, **6**, 1611–1619.

de Oliveira, A.C., Valentim, I.B., Silva, C.A. *et al.* (2009). Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues. *Food Chemistry*, **115**, 469–475.

Osman, A.M., Wong, K.K.Y. & Fernyhough, A. (2006). ABTS radical-driven oxidation of polyphenols: isolation and structural elucidation of covalent adducts. *Biochemical and Biophysical Research Communications*, **346**, 321–329.

Park, Y.S., Leontowicz, M., Leontowicz, H. *et al.* (2015). Fluorescence and ultraviolet spectroscopic evaluation of phenolic compounds, antioxidant and binding activities in some kiwi fruit cultivars. *Spectroscopy Letters*, **48**, 586–592.

Parsa, A. & Salout, S.A. (2016). Investigation of the antioxidant activity of electrosynthesized polyaniline/reduced graphene oxide nanocomposite in a binary electrolyte system on ABTS and DPPH free radicals. *Journal of Electroanalytical Chemistry*, **760**, 113–118.

Pawlaczyk, I., Czerchawski, L., Kuliczkowski, W. *et al.* (2011). Anticoagulant and anti-platelet activity of polyphenolic-polysaccharide preparation isolated from the medicinal plant *Erigeron canadensis* L. *Thrombosis Research*, **127**, 328–340.

Pereira-Caro, G., Borges, G., van der Hooft, J.D. *et al.* (2014). Orange juice (poly)phenols are highly bioavailable in humans. *American Journal of Clinical Nutrition*, **100**, 1378–1384.

Pereira-Caro, G., Borges, G., Ky, I. *et al.* (2015). In vitro colonic catabolism of orange juice (poly)phenols. *Molecular Nutrition & Food Research*, **59**, 465–475.

Pinacho, R., Cavero, R.Y., Astiasarán, I., Ansorena, D. & Calvo, M.I. (2015). Phenolic compounds of blackthorn (*Prunus spinosa* L.) and influence of in vitro digestion on their antioxidant capacity. *Journal of Functional Foods*, **19**, 49–62.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, **26**, 1231–1237.

Robards, K., Prenzler, P.D., Tucker, G., Swatsitang, P. & Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry*, **66**, 401–436.

Rodriguez-Roque, M.J., Rojas-Grau, M.A., Elez-Martinez, P. & Martin-Belloso, O. (2013). Changes in vitamin C, phenolic, and carotenoid profiles throughout in vitro gastrointestinal digestion of a blended fruit juice. *Journal of Agricultural and Food Chemistry*, **61**, 1859–1867.

Rodríguez-Roque, M.J., Rojas-Graü, M.A., Elez-Martínez, P. & Martín-Belloso, O. (2014). In vitro bioaccessibility of health-related compounds from a blended fruit juice–soymilk beverage: influence of the food matrix. *Journal of Functional Foods*, **7**, 161–169.

Ryan, L. & Prescott, S.L. (2010). Stability of the antioxidant capacity of twenty-five commercially available fruit juices subjected to an in vitro digestion. *International Journal of Food Science and Technology*, **45**, 1191–1197.

Sengul, H., Surek, E. & Nilufer-Erdil, D. (2014). Investigating the effects of food matrix and food components on bioaccessibility of pomegranate (*Punica granatum*) phenolics and anthocyanins using an in-vitro gastrointestinal digestion model. *Food Research International*, **62**, 1069–1079.

Stanisavljevic, N., Samardzic, J., Jankovic, T. et al. (2015). Antioxidant and antiproliferative activity of chokeberry juice phenolics during in vitro simulated digestion in the presence of food matrix. *Food Chemistry*, **175**, 516–522.

Tagliazucchi, D., Verzelloni, E., Bertolini, D. & Conte, A. (2010). In vitro bio-accessibility and antioxidant activity of grape polyphenols. *Food Chemistry*, **120**, 599–606.

Ti, H., Zhang, R., Li, Q., Wei, Z. & Zhang, M. (2015). Effects of cooking and in vitro digestion of rice on phenolic profiles and antioxidant activity. *Food Research International*, **76**, 813–820.

Wootton-Beard, P.C., Moran, A. & Ryan, L. (2011). Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after in vitro digestion measured by FRAP, DPPH, ABTS and Folin-Ciocalteu methods. *Food Research International*, **44**, 217–224.

Zheng, L., Zhao, M., Xiao, C., Zhao, Q. & Su, G. (2016). Practical problems when using ABTS assay to assess the radical-scavenging activity of peptides: importance of controlling reaction pH and time. *Food Chemistry*, **192**, 288–294.

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