Food Chemistry 256 (2018) 252–258

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Changes in bioaccessibility, polyphenol profile and antioxidant potential of flours obtained from persimmon fruit (*Diospyros kaki*) co-products during *in vitro* gastrointestinal digestion

Raquel Lucas-González, Manuel Viuda-Martos, José A. Pérez Álvarez, Juana Fernández-López*

IPOA Research Group, Agro-Food Technology Department, Escuela Politécnica Superior de Orihuela, Miguel Hernández University, Orihuela, Alicante, Spain

ARTICLE INFO

Keywords:
In vitro digestion
Persimmon
Antioxidant
Polyphenol compounds
Bioaccessibility

ABSTRACT

The aim was to evaluate (i) the phenol and flavonoid recovery and bioaccessibility indexes, (ii) the stability of individual polyphenolic compounds and (iii) the antioxidant activity of persimmon flours (cultivars 'Rojo Brillante' and 'Triumph') during the *in vitro* digestion. The recovery index for phenolic and flavonoid content was dependent on flour type and digestion phase. After the dialysis phase, the bioaccessibility for phenolic compounds from both flours was similar; for flavonoids it was higher in 'Triumph' than 'Rojo Brillante' flour. After *in vitro* digestion, 13 polyphenolic compounds were detected in both flours, of which only six were detected in the intestinal phase. Their antioxidant activity (ABTS·+, FRAP and DPPH) decreased after intestinal phase, while their chelating activity (FIC assay) increased in both flours. So, persimmon flours could be included in the formulation of foods to improve either their scarcity of bioactive compounds or an unbalanced nutritional composition.

1. Introduction

The incidence of chronic diseases derived of an unbalanced dietary pattern, characterized by eating meals rich in saturated fats and refined carbohydrates and poor in fiber and bioactive compounds, is increased worldwide. This is one of the reasons why the past year the United Nations General Assembly proclaimed a Decade of Action on Nutrition that will run from 2016 to 2025, with the aim to reduce hunger and improving nutrition around the world (ONU, 2016). Taking it into consideration, the search of new based-vegetable ingredients rich in bioactive compounds and other dietary components like fiber, protein or complex carbohydrates is necessary for the development of new foods with high nutritional value and antioxidant properties. In this way, agro-industrial co-products could be used as potential functional ingredients for the development of different foods such as pâté, pasta, ice creams or biscuits, due to their important amounts of dietary fiber and antioxidant compounds (Viuda-Martos et al., 2012; López-Marcos, Bailina, Viuda-Martos, Pérez-Alvarez, & Fernández-López, 2015).

Nevertheless, the bioaccessibility and bioavailability of these bioactive compounds after human digestion determine their biological action in the body (Etcheverry, Grusak, & Fleige, 2012). In order to make a first screening of their behavior after human digestion, *in vitro* gastrointestinal digestion (GID) is being used. These tests simulate the physical (agitation, temperature and pH) and chemical (enzymatic and salinity) processes that occur during GID and provide information about the changes that occur in bioactive compounds, the release of food matrix, the interactions with other compounds and their bioaccesibility (Minekus et al., 2014); relevant information for designing and formulating foods with antioxidant potential.

Several researches have studied the bioaccesibility and recovery index of phytochemicals present in fruits, vegetables, seeds and agro-industrial co-products (Chandrasekara & Shahidi, 2011; Gawlik-Dzik, 2012; Neto et al., 2017), showing that dietary matrix, pH changes, enzymatic activity, interactions with dietary compounds, as well as the nature of each compound, are the factors that have the greatest impact on the stability and release of antioxidant compounds after GID (Kroll, Rawel, & Rohn, 2003; Alminger et al., 2014).

Persimmon fruit is rich in fiber and has important amounts of minerals, carotenes and polyphenols (De Ancos, González, & Cano, 2000; Park et al., 2006; Lucas-González, Viuda-Martos, Pérez-Álvarez, & Fernández-López, 2017). Additionally, several studied have showed antidiabetics, antiaetrogenic and antiobesity effects of persimmon

Abbreviations: RB, Rojo Brillante; TH, Triumph; GID, gastrointestinal digestion; TFC, total flavonoid content; TPC, total phenolic content; ABTS+, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical; DPPH, 2,2-diphenyl, 1-picryl hydrazyl; FIC, ferrous ion chelating; FRAP, ferric reducing antioxidant property; PF, pellet fraction SCF, soluble chime fraction; IN, intestinal absorbed fraction; OUT, intestinal unabsorbed fraction

* Corresponding author.
E-mail address: j.fernandez@umh.es (J. Fernández-López).

https://doi.org/10.1016/j.foodchem.2018.02.128
Received 13 November 2017; Received in revised form 22 February 2018; Accepted 25 February 2018
Available online 26 February 2018

leaves and fruits (Lee, Chung, & Lee, 2006; Son et al., 2013; Kim et al., 2016). These evidences make very attractive the use of persimmon co-products as potential functional ingredients.

For these reasons, the aim of the current work was to evaluate (i) the phenol and flavonoid recovery and bioaccessibility indexes, (ii) the stability of individual polyphenolic compounds and (iii) the antioxidant activity of both persimmon flours (obtained from juice co-products of cultivars 'Rojo Brillante' and 'Triumph') during the *in vitro* gastrointestinal digestion.

2. Material and methods

2.1. Plant material

Persimmon flours co-products of cultivars 'Rojo Brillante' and 'Triumph' were obtained as described by Lucas-González et al. (2017). The particle size of both persimmon flours were < 0.210 mm.

2.2. Simulated in vitro gastrointestinal digestion (GID) & extraction method

In vitro GID of three independent samples were made following the methodology described by Gullón, Pintado, Fernández-López, Pérez-Álvarez, and Viuda-Martos (2015a) (Fig. 1). Three phases were simulated: oral, gastric and intestinal. The intestinal phase was divided in two steps: agitation step and dialysis process. At the end of the dialysis process, the solution left inside the dialysis tubing was taken as the IN sample, representing the material that remained in the gastrointestinal tract (colon-available), and the solution that managed to diffuse into the dialysis tubing was taken as the OUT sample (serum-available). Finally, all digestion mixtures were lyophilized and stored at −20 °C until further use.

Polyphenol compounds of undigested sample and digested samples were extracted following the methodology described by Pellegrini et al. (2017).

2.3. Total phenol and total flavonoid content

To determine the total phenol content (TPC) of digested samples, the Folin-Ciocalteu's reagent (Singleton & Rossi, 1965) was used. The method described by Blasa et al. (2005) was used for determining total flavonoid content (TFC). Both methods are based on spectrophotometric measures (760 nm for TPC and 510 nm for TFC) which were made with a HP 8451 spectrophotometer (Hewleck Packard, Cambridge, UK). The results of TPC were expressed as mg gallic acid equivalents (GAE)/g sample, while in the case of TFC the results were expressed as mg rutin equivalents (RE)/g of sample.

2.4. Recovery and bioaccessibility index

To analyze the effect of *in vitro* GID on TPC and TFC two different indexes were applied, following the indications of Ortega, Macia, Romero, Reguant, and Motilva (2011): the recovery percentage and bioaccessibility percentage. The recovery percentage allows to know the amount of phenolic and flavonoid compounds recuperated after the oral, gastric and intestinal digestion, by comparison with the amount in the undigested sample. The recovery index was measured according to the equation *i*. Bioaccesibility index was calculated comparing the total amount of bioactive compounds in the intestinal phase (IN + OUT) with the amount in the OUT fraction (serum-available), following the equation *ii*.

$$Recovery\ index\ (\%) = \frac{PC_{DF}}{PC_{TM}} \times 100 \quad (1)$$

Where PC_{DF} is the TPC or TFC (mg) in the digested fraction (CSF + PF) after each digestion phase (oral, gastric and intestinal) and PC_{TM} is the TPC or TFC (mg) quantified in the test matrix.

$$Bioaccessibility\ index\ (\%) = \frac{PC_S}{PC_{DF}} \times 100 \quad (2)$$

Where PC_S is the TPC or TFC (mg) in the OUT sample after the dialysis phase and PC_{DF} is the TPC or TFC (mg) in the total digested

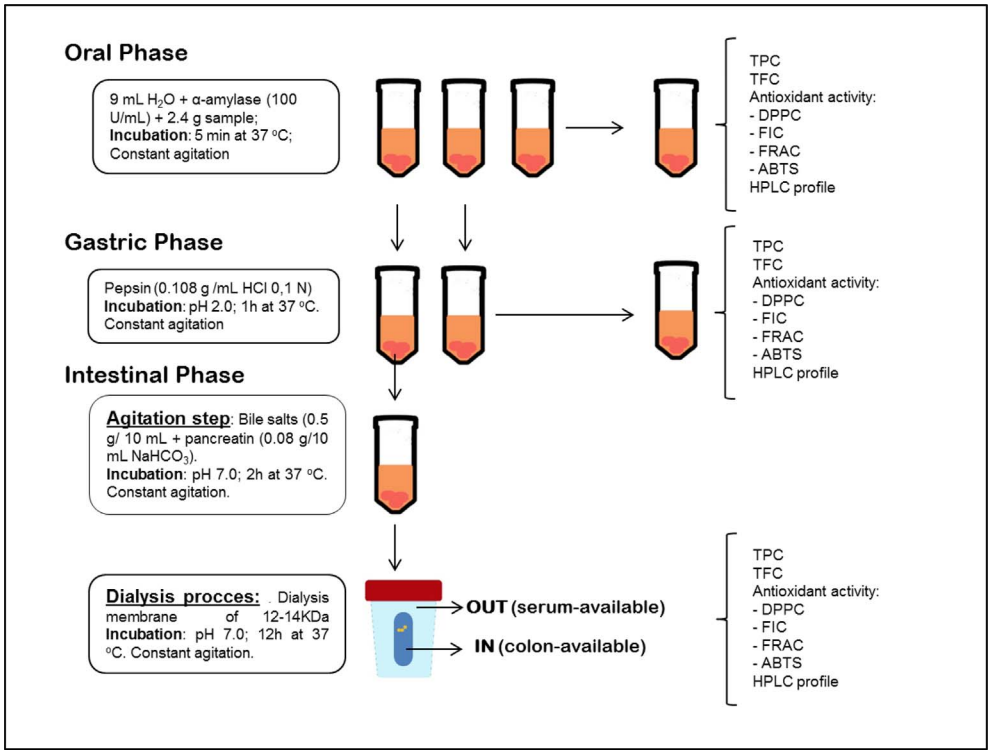

Fig. 1. Schematic representation of *in vitro* gastrointestinal digestion method carried out on persimmon flours.

sample (IN + OUT) after the dialysis phase.

2.5. Determination of polyphenolic compounds

Polyphenolic profiles of all samples obtained in each phase of *in vitro* GID were determined by High Performance Liquid Chromatography (HPLC) following the methodology described by Genskowsky et al. (2016). A Hewlett-Packard HPLC series 1200 instrument, equipped with C_{18} column (Mediterranea sea_{18}, 25 × 0.4 cm, 5 μm particle size) from Teknokroma, (Barcelona, Spain) was used. Phenolic compounds were analyzed, in standard and sample solutions, with a gradient elution of 1 mL/min. The mobile phases used were formic acid in water (1:99, v/v) as solvent A, and acetonitrile as solvent B. The chromatograms were recorded at 280, 320 and 360 nm. The identification of polyphenolic compounds was carried out by comparing UV absorption spectra and retention times of each compound with those of pure standards injected in the same conditions. When standards were unavailable, the compounds were tentatively identified by comparing their UV/Vis spectra with previously published data (Medina-Medrano, et al., 2015; Martínez-Las Heras, Quifer-Rada, Andrés, & Lamuela-Raventós, 2016). Quantification of phenolic acids and flavonoids were executed based on linear curves of authentic standards.

2.6. Antioxidant activity

To assess the antioxidant activity, four methods were used:

DPPH radical scavenging assay: The free radical scavenging activity was measured according to the methodology described by Brand-Williams, Cuvelier, and Berset (1995) using the stable radical DPPH. Absorbance values were measured on a spectrophotometer at 517 nm. Results were expressed as mg Trolox equivalent (TE)/g sample.

ABTS radical cation (ABTS·+) scavenging activity assay: This method was determined as described by Leite et al. (2011). Absorbance values were measured on a spectrophotometer at 734 nm. The results were expressed as mg Trolox equivalent (TE)/g of sample.

Ferric reducing antioxidant power (FRAP): This method was determined using the methodology described by Oyaizu (1986). The FRAP values were measured on a spectrophotometer at 700 nm and the results estimated in mg Trolox equivalents (TE)/g of sample.

Ferrous ion-chelating ability assay (FIC): Ferrous ions chelating activity was measured by inhibiting the formation of Fe2+-ferrozine complex, following the method of Carter (1971). Absorbance values were measured on a spectrophotometer at 532 nm. Results were expressed as mg EDTA equivalent/g sample.

2.7. Statistical analysis

The results were expressed as the mean ± SD of 2 parallel trials (n = 4). Data obtained for each digestion phase and persimmon flour were analyzed by means of a two-way ANOVA test with two factors: cultivar and digestion phase. Tukey's post hoc test was applied for comparisons of means; differences were considered significant at $p < 0.05$. Statistical analyses were carried out using the statistical package SPSS 19.0 (SPSS Inc., Chicago, IL.).

3. Results and discussions

3.1. Recovery and bioaccessibility indexes

Recovery index for the TPC and TFC after each digestion phase (oral, gastric and intestinal) can be observed in Fig. 2.

As regard the recovery index for the TPC, no statistical differences ($p > 0.05$) were found between 'Rojo Brillante' (RB) and 'Triumph' (TH) flours after each digestion phase, except for the oral phase. In this phase, TPC recovery index for the RB flour didn't change ($p < 0.05$) with respect to undigested sample, whereas in the case of TH flour the recovery index value was lower than undigested sample ($p < 0.05$). The highest recovery index values were showed after the gastric phase ($p < 0.05$), whereas the lowest recovery index values were found in the last phase of gastrointestinal digestion ($p < 0.05$). A similar situation was detected for the TFC, although in this case, after the oral phase, the recovery index was significantly higher for RB than in undigested sample ($p < 0.05$).

These results showed that oral digestion phase affected the persimmon flours and bioactive compounds in different ways, since the release of polyphenol compounds from persimmon flour matrix was dependent on the cultivar and type of bioactive compounds ($p < 0.05$). These facts could be due to: (*i*) the different composition of persimmon flours, mainly related to dietary fiber content (total dietary fiber (TDF) is higher in TH than RB flour, and also their content in insoluble dietary fiber (Lucas-González et al., 2017); (*ii*) the interaction of polyphenolic compounds with α-amylase, thus, some authors have reported antidiabetic effects in persimmon leaves and fruits, due to their interactions (Lee et al., 2006; Kawakami, Aketa, Nakanami, Iizuka, & Hirayama, 2010). In the literature, Martínez-Las Heras, Pinazo, Heredia, & Andrés (2017) reported a similar recovery index for phenolic and flavonoids compounds in peel-fiber from RB persimmon after the oral phase, whereas the decreases of phenolic compounds after the oral phase have been previously reported by Pellegrini et al. (2017) in different quinoa seeds.

The differences showed in TH flour between digestion phases could be explained by their higher TDF content and also due to the different pH of the digestion phases; the acid medium in the gastric phase promotes the break of bonds between bioactive compounds and nutrients, like fiber, protein and carbohydrates, helping to the remain of polyphenolic compounds in the food matrix (Alminger et al., 2014). Other authors have reported increases in polyphenol content in different vegetable products after gastric digestion (Chandrasekara & Shahidi, 2011; Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2013).

The decrease in the amount of polyphenols after intestinal digestion has been widely reported by the scientific community (Ortega et al., 2011; Rodríguez-Roque et al., 2013; Lucas-González, et al., 2016). The drastic losses in bioactive compounds after intestinal digestion, probably were due to different factors: (*i*) interactions with other dietary compounds, like fiber, protein, carbohydrate and minerals, (*ii*) chemical reactions, mainly oxidation and polymerization, affording the formation of other phenolic derivatives, such as chalcones (Gil-Izquierdo, Gil, Ferreres, & Tomás-Barberán, 2001) or, (*iii*) changes in molecular structures due to enzymatic action and consequently in its solubility (Kroll et al., 2003).

The intestinal digestion is the largest phase in the GID, where the majority of nutrients are absorbed in the intestinal epithelium. The bioaccesibility can be defined as the amount of an ingested chemical compound that is available for absorption (Etcheverry et al., 2012). The bioaccesibility index for the TPC (Fig. 3) was similar ($p > 0.05$) in both flours. Additionally, the bioaccesibility for flavonoids was dependent on persimmon cultivar (Fig. 3), since significant differences were found in their bioaccesibility index value ($p < 0.05$), showing TH flour higher values (45.31%) than RB flour (21.54%) ($p < 0.05$). This fact is probably due to the different flavonoid profile of both persimmon flours, as can be observed in Table 1. According with these results, a substantial amount of phenolic and flavonoid compounds remained into the IN fraction (colon-available) and could be metabolized by colonic bacteria, transforming dietary polyphenols in simple phenolic compounds, which could result in metabolites more biologically active than the original compounds (Selma, Espín, & Tomás-Barberá, 2009). This fact could be due to the complex matrix of persimmon flours, which are rich in sugars and insoluble fiber, with presence of protein

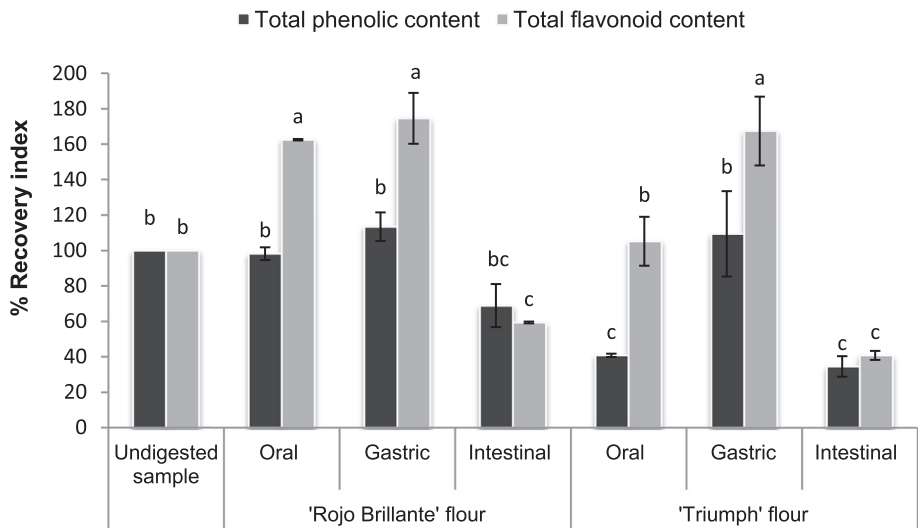

Fig. 2. Recovery index percentage calculated for total phenol content and total flavonoid content after the simulated gastrointestinal digestion of persimmon flours. Bars followed by the same lower-case letter (a–c) are not significantly different ($p > 0.05$) according to Tukey's Multiple Range Test.

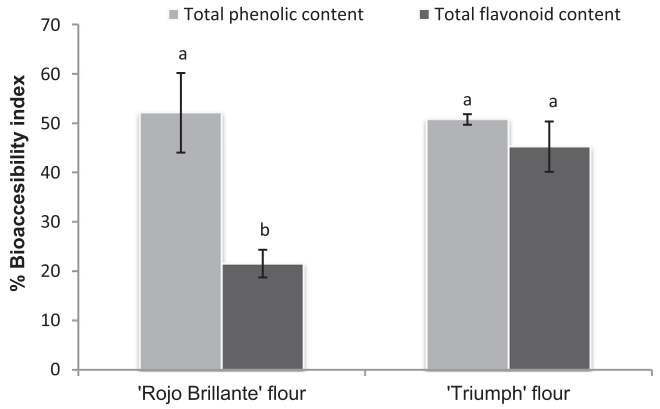

Fig. 3. Total phenolic and total flavonoid content bioaccessibility index after intestinal phase of *in vitro* gastrointestinal digestion from persimmon flours (cultivars 'Rojo Brillante' and 'Triumph'). Values with same lower case letter (a–b) are not significantly different among digestion ($p > 0.05$) according to Tukey's Multiple Range Test.

and minerals (Lucas-González et al., 2017). Because of this composition, multiple interactions can occur between dietary components and bioactive compounds, which could produce a reduction in their bioaccessibility. In the scientific literature Gullón et al. (2015a) reported a value of 64.02% for the bioaccessibility index of flavonoids in pomegranate peel flour, while the bioaccessibility index of polyphenolic compounds present in date pits flour and apple bagasse flour, at the end of intestinal digestion, were 78.54 and 91.58%, respectively (Gullón, et al., 2015b).

3.2. Stability of polyphenolic compounds during simulated in vitro gastrointestinal digestion

Polyphenols detected after *in vitro* GID are showed in Table 1. In both persimmon flours, RB and TH, the following compounds have been identified: gallic acid, 4-hydroxibenzoic acid, epicatechin, quercetin-*o*-pentoside I, quercetin, kaempferol-*o*-rhamnoside and ellagic acid. Furthermore, in TH flour, the following flavonoid compounds have been detected: quercetin-*o*-hexoside, quercetin-*o*-pentoside II, kaempferol-*o*-hexoside I and kaempferol); and in RB flour: coumaric acid-*o*-hexoside and kaempferol-3-*o*-glucoside. The majority compounds in both studied persimmon flours were gallic acid and 4-hydroxybenzoic acid, furthermore the results showed that RB had more phenolic acids than TH flour ($p < 0.05$) and TH flour had more presence of flavonoids compounds than RB flours ($p < 0.05$). The polyphenols identified have been previously detected in persimmon fruit and leaves by different authors (Martínez-Las Heras et al., 2016; Lucas-González, Fernández-López, Pérez-Álvarez, & Viuda-Martos, 2018).

The GID had different repercussions in the stability of the polyphenols detected in RB and TH flours. After the oral and gastric digestion all polyphenols identified in both persimmon flours were detected. However, both factors, digestion phases and flour type, affected, in different way, their stability and release from food matrix. Thus, after the oral digestion the phenolic acids concentration increased 160.6% and 176.7% in RB and TH flours, respectively; begin gallic acid, which presented the highest release from food matrix. The stability of flavonoid compounds was compromised in this phase and a significant decrease was observed in epicatechin, quercetin (only in TH flour) and in all glycoside flavonoids ($p < 0.05$), with the exception of kaempferol-3-*o*-glucoside, detected in RB flour, whose concentration didn't change compared to undigested sample ($p > 0.05$). As regard to ellagic acid, their concentration didn't change in RB flour, whereas in TH flour significant losses were observed ($p < 0.05$). Similar behavior was observed in polyphenols stability after the gastric digestion; phenolic acids increased their concentration, showing significant differences with oral phase ($p < 0.05$); with exception of coumaric acid-*o*-hexoside, which didn't show variation in their concentration in oral and gastric phases with respect to undigested sample. The most remarkable fact was that the concentration of glycoside flavonoids present in TH flour increased their concentration with respect to oral phase ($p < 0.05$), whereas in RB flour not significant differences ($p > 0.05$) were observed. The increase in polyphenolic compounds after gastric phase could be due to the breakage of the bonds with the dietary components of the persimmon flours, proteins and fiber, induced by the acid medium. These facts, highlight the different behavior of flavonoids after GID, and confirm the TFC recovery index showed in both persimmon flours. From the 17 polyphenols detected in persimmon flours only six appeared after intestinal phase: 4-hydroxybenzoic acid, ellagic acid and the glycosylated flavonoids (with exception of quercetin-*o*-pentoside II and kaempferol-3-*o*-glucoside). All these compounds showed a decrease in their concentration with respect to other phases and to undigested sample ($p < 0.05$); with the exception of 4-hydroxybenzoic acid, which reported the biggest concentration in this phase ($p < 0.05$). Rodríguez-Roque et al. 2013 also reported an increase of 4-hydroxybenzoic acid from soymilk after the intestinal phase respect to gastric and undigested sample. On the other hand, the distribution of these compounds in the intestinal fractions, IN and OUT, were

Table 1
Polyphenolic profile of undigested and digested samples (oral, gastric and intestinal phase) of persimmon flours: 'Rojo Brillante' (RB) and 'Triumph' (TH).

		Undigested sample	Oral phase	Gastric phase	Intestinal phase		
					IN	OUT	Total
Phenolic acids							
Gallic acid (mg/g)	RB	3 ± 1^{c}	6 ± 0^{b}	7 ± 1^{a}	ND	ND	–
	TH	2 ± 0^{b}	4 ± 0^{c}	4 ± 0^{c}	ND	ND	–
4-Hydroxybenzoic acid (μg/g)	RB	200 ± 20^{c}	220 ± 10^{c}	300 ± 10^{b}	270 ± 20^{x}	240 ± 10^{x}	510 ± 40^{a}
	TH	110 ± 10^{d}	89 ± 15^{d}	200 ± 7^{c}	110 ± 6^{y}	230 ± 40^{x}	340 ± 30^{b}
Coumaric acid-*o*-hexoside (μg/g)	RB	24 ± 5^{a}	21 ± 1^{a}	20 ± 3^{a}	ND	ND	–
	TH	ND	ND	ND	ND	ND	–
Flavonoids							
Epicatechin (μg/g)	RB	36 ± 4^{a}	22 ± 2^{b}	19 ± 2^{b}	ND	ND	–
	TH	33 ± 3^{a}	18 ± 4^{b}	23 ± 3^{b}	ND	ND	–
Quercetin-*o*-hexoside (μg/g)	RB	ND	ND	ND	ND	ND	–
	TH	110 ± 20^{a}	45 ± 2^{c}	67 ± 1^{b}	11 ± 0^{x}	14 ± 2^{x}	25 ± 2^{c}
Quercetin-*o*-pentoside I (μg/g)	RB	54 ± 1^{c}	44 ± 1^{d}	36 ± 2^{f}	15 ± 4^{x}	16 ± 3^{x}	31 ± 1^{g}
	TH	110 ± 20^{a}	45 ± 4^{d}	71 ± 2^{b}	14 ± 0^{y}	19 ± 3^{x}	33 ± 2^{g}
Kaempferol-3-*o*-glucoside (μg/g)	RB	5 ± 1^{a}	5 ± 0^{a}	4 ± 1^{a}	ND	ND	–
	TH	ND	ND	ND	ND	ND	–
Quercetin-*o*-pentoside II (μg/g)	RB	ND	ND	ND	ND	ND	–
	TH	23 ± 3^{a}	16 ± 1^{b}	17 ± 1^{b}	ND	ND	–
Kaempferol-*o*-hexoside I (μg/g)	RB	ND	ND	ND	ND	ND	–
	TH	19 ± 3^{a}	8 ± 1^{bc}	12 ± 0^{b}	3 ± 1^{x}	3 ± 0^{x}	7 ± 1^{c}
Kaempferol-*o*-rhamnoside (μg/g)	RB	12 ± 1^{a}	5 ± 0^{c}	4 ± 1^{c}	3 ± 0^{x}	1 ± 0^{y}	5 ± 0^{c}
	TH	14 ± 2^{a}	8 ± 1^{b}	9 ± 0^{b}	3 ± 0^{x}	2 ± 0^{y}	5 ± 0^{c}
Quercetin (μg/g)	RB	1 ± 0^{c}	1 ± 0^{c}	2 ± 0^{c}	ND	ND	–
	TH	17 ± 3^{a}	9 ± 0^{b}	12 ± 0^{b}	ND	ND	–
Kaempherol (μg/g)	RB	ND	ND	ND	ND	ND	–
	TH	2 ± 0^{a}	2 ± 0^{a}	2 ± 0^{a}	ND	ND	–
Ellagictannins							
Ellagic acid (μg/g)	RB	8 ± 0^{b}	6 ± 1^{b}	16 ± 2^{a}	4 ± 1^{x}	2 ± 0^{y}	6 ± 1^{b}
	TH	8 ± 2^{b}	4 ± 1^{c}	4 ± 0^{c}	1 ± 0^{x}	1 ± 0^{x}	3 ± 0^{c}

For the same polyphenol, values with same lower case letter (a–g) are not significantly different among digestion phases and cultivar ($p > 0.05$) according to Tukey's Multiple Range Test.
For the same polyphenol and different intestinal fraction (IN and OUT) values with same lower case letter (x–y) are not significantly different ($p > 0.05$) according to Tukey's Multiple Range Test.

dependent on the individual compound and persimmon flour type ($p < 0.05$).

Other authors have detected glycoside flavonoids, like rutin, quercetin-3-galactoside, cyanidin-3-glucoside, quercetin-*o*-(rhamnosyl)rutinoside or kaempferol-*o*-rutinoside and ellagic acid and 4-hydroxybenzoic acid after *in vitro* digestion in different vegetable products (Lucas-Gonzalez et al., 2016; Pellegrini et al., 2017; Pinto, Spínola, Llorent-Martínez, Fernández-de Córdova, Molina-García, & Castilho, 2017). These results were in agreement with the results showed in the current work and would indicate that glycoside compounds are more stable to gastrointestinal digestion than aglycone compounds. Furthermore, the instability of gallic acid and kaempferol after intestinal digestion has been previously reported in different vegetable extracts (Schulz et al., 2017).

The losses of polyphenol compounds after intestinal digestion could be due to alkaline pH values (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010), interaction with other dietary compounds and/or interaction with bile salts, since Kida, Suzuki, Matsumoto, Nanjo, and Hara (2000) have identified biliary metabolites of (−)-epigallocatechin gallate in rats after oral administration.

3.3. Antioxidant properties

Bioactive compounds have different mechanism for developing their antioxidant activity, such as hydrogen atom, single electron transfer or metal chelation (Leopoldini, Russo, & Toscano, 2011). There is not a unique method to evaluate all antioxidant mechanism, so in this work, different methods have been used to evaluate the antioxidant activity of the undigested and digested samples of persimmon flours (Table 2).

From the four methods used (DPPH, ABTS·+, FRAP and FIC) only the ABTS·+ values showed in the undigested samples of both flours were significantly different ($p < 0.05$). However, the antioxidant activity of both persimmon flours was affected by the GID in different way, highlighting the dependence on cultivar and gastrointestinal phase in their antioxidant activity. After *in vitro* GID, the DPPH radical scavenging activity of RB flour decreased gradually from the oral phase to intestinal phase ($p < 0.05$). A similar behavior was observed in TH flour, however the highest loss in their DPPH radical scavenging activity was observed after gastric digestion (25.8%). Martínez-Las Heras et al. (2017) reported similar results in peel and pulp fiber obtained from persimmon cv. RB undergoing an *in vitro* GID. Other authors have informed an increase in DPPH radical scavenging activity after gastric and intestinal digestion in different vegetable samples (Burgos-Edwards, Jiménez-Aspee, Thomas-Valdés, Schmeda-Hirschmann, & Theoduloz, 2017). As regards to ABTS·+ after oral digestion, the ABTS·+ radical scavenging activity of both persimmon flours didn't change with respect to undigested flours ($p > 0.05$). After gastric digestion, RB and TH flours showed an increase of 25.37 and 32.73%, respectively ($p < 0.05$). However, the intestinal digestion compromises their ABTS·+ radical scavenging activity in both persimmon flours ($p < 0.05$). The losses in the antioxidant activity measured by ABTS·+ assay after intestinal digestion and dialysis phase have been previously reported in onion, lettuce and tomato (Gawlik-Dzik, 2012).

The ability of RB flours to reduce Fe^{+3} to Fe^{+2} didn't change after oral and gastric digestion, however, after intestinal digestion, a significant decrease ($p < 0.05$) in their metal reduction activity (60.1%) was observed. As regards to TH flour, the oral digestion didn't affect their metal reduction ability ($p < 0.05$), however, after gastric digestion, a significant increase in their antioxidant activity ($p > 0.05$) was observed, followed by a drastic decrease (66.6%) in their metal

Table 2
Antioxidant properties of the two fractions (pellet fraction and soluble chime fraction) obtained after each phase (oral, gastric and intestinal) of *in vitro* gastrointestinal digestion of persimmon flours cultivars 'Rojo Brillante' (RB) and 'Triumph' (TH) measured with DPPH, FRAP, FIC and ABTS·+ assays.

		DPPH (mg TE/g)		FRAP (mg TE/g)		FIC (mg EDTA eq./g)		ABTS (mg TE/g flour)	
		RB	TH	RB	TH	RB	TH	RB	TH
Test matrix		3.2 ± 0.1^{a}	3.4 ± 0.0^{a}	5.3 ± 0.4^{b}	5.6 ± 0.1^{b}	0.1 ± 0.0^{a}	0.1 ± 0.00^{a}	2.0 ± 0.1^{c}	3.0 ± 0.0^{b}
Oral phase	Total	3.1 ± 0.1^{ab}	2.7 ± 0.1^{b}	5.4 ± 0.6^{b}	3.1 ± 0.2^{c}	0.1 ± 0.0^{a}	0.1 ± 0.01^{a}	2.0 ± 0.2^{c}	3.0 ± 0.5^{b}
	% Var.	−2.4	−21.2	2.5	−44.9	−0.6	−14.6	2.9	−2.1
Gastric phase	Total	2.7 ± 0.2^{b}	2.5 ± 0.1^{bc}	5.1 ± 0.7^{b}	7.3 ± 0.3^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	2.4 ± 0.1^{bc}	4.0 ± 0.2^{a}
	% Var.	−13.1	−25.8	−2.8	30.8	−82.2	−85.1	25.4	32.7
Intestinal phase	IN	1.2 ± 0.1^{B}	2.0 ± 0.4^{A}	1.1 ± 0.2^{A}	0.9 ± 0.2^{A}	0.2 ± 0.0^{A}	0.1 ± 0.0^{B}	0.8 ± 0.0^{A}	0.3 ± 0.0^{B}
	OUT	1.0 ± 0.0^{B}	0.7 ± 0.1^{B}	1.0 ± 0.1^{A}	0.9 ± 0.2^{A}	0.1 ± 0.0^{C}	0.0 ± 0.0^{C}	0.5 ± 0.0^{B}	0.7 ± 0.1^{A}
	Total	2.2 ± 0.1^{c}	2.7 ± 0.2^{b}	2.1 ± 0.5^{d}	1.9 ± 0.4^{d}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	1.2 ± 0.1^{d}	1.1 ± 0.1^{d}
	% Var.	−30.8	−7.2	−60.1	−66.6	17.3	8.7	−35.9	−61.8

% Var.: Percentage of variation between the initial values and the values obtained after digestion.
For the same antioxidant assay, values with same lower case letter (a–f) are not significantly different among digestion phases and cultivar ($p > 0.05$) according to Tukey's Multiple Range Test.
For the same antioxidant assay, values followed with same upper case letter (A–B) are not significantly different among the same digestion phase and cultivar ($p > 0.05$) according to Tukey's Multiple Range Test.

reduction activity after the intestinal phase ($p > 0.05$). Lucas-González et al. (2016) also reported an increase (24.8%) in the ferric reduction activity power after gastric phase in lyophilized maqui. The negative influence of the intestinal digestion on the reducing power activity has been previously reported by Neto et al. (2017).

As regard to the ability to chelate metals, determined by FIC method, both persimmon flours, experimented the same behavior after GID: after oral phase, their chelate metals ability was not affected ($p > 0.05$), while after gastric digestion, significant losses were observed ($p < 0.05$). Whereas, after intestinal phase their antioxidant activity increased with respect to gastric phase ($p > 0.05$) and was similar to undigested samples ($p > 0.05$). This increase could be due to the fact that several compounds with chelating activity, like reducing carbohydrates, tocopherols and carotenoids, could have been solubilized and probably, the antioxidant activity showed were mediated by these bioactive compounds (Gullón et al., 2015a). Other authors have reported some increase in FIC values in different antioxidant products after intestinal phase (You, Zhao, Regenstein, & Ren, 2010; Pellegrini et al., 2017). These results showed that a high part of the antioxidant activity of these persimmon flours could arrive to the large intestine and to play a protective role against oxidation reactions.

The distribution of antioxidant activity between both intestinal fractions, IN and OUT, was dependent on cultivar and antioxidant method, as can be observed in Table 2. Thus, the antioxidant activity determined by DPPH assay in RB flour showed the same activity in both fractions ($p > 0.05$), whereas TH flour reported the highest antioxidant activity in IN fraction ($p < 0.05$). In the case of ABTS·+ radical scavenging activity, RB and TH flour showed higher activity in IN fraction and OUT fraction, respectively. The same behavior was observed in the distribution of their antioxidant activity determined by FRAP and FIC methods ($p > 0.05$).

In the scientific literature, there are contradictory works about the antioxidant activity obtained in the serum and colon available fractions. Thus, in the case of lyophilized maqui, the antioxidant activity determined with different methods (DPPH, FRAP, FIC and ABTS·+) after dialysis phase, was higher in the serum-available fraction than in colon-available fraction (Lucas-González et al., 2016). However, in pomegranate peel flour, the antioxidant activity measured by FRAP, DPPH, ABTS·+ and ORAC, was higher in the colon-available fraction than in the serum-available fraction (Gullón et al., 2015a). Probably, the content of total dietary fiber and their nature in the raw material, determines the release of polyphenol compounds from food matrix.

4. Conclusion

The best of our knowledge is the first time that polyphenols profile in persimmon flours from juice co-products of cultivars 'Rojo Brillante' and 'Triumph', has been studied after *in vitro* gastrointestinal digestion. Recovery index for total phenolic and flavonoids content was dependent on persimmon flour type and digestion phase, being intestinal phase which showed the highest effect on their recuperation. The bioaccesibility index for total phenol and flavonoid content, as well as, individual polyphenols detected, were strongly affected after gastrointestinal digestion; however, some polyphenol compounds remained as such at the end of the digestion, especially the glycosylated flavonoids and 4-hydroxibenzoic acid, which increased their concentration after gastrointestinal digestion. Their antioxidant activity also was affected, showing that their protective action against free radicals and their ability to reduce metals increased after the gastric phase, whereas after the intestinal phase, their metal chelating activity improved. For these reasons, persimmon flours of cvs. 'Rojo Brillante' and 'Triumph' could be included in the formulation of foods to improve either their scarcity of bioactive compounds or an unbalanced nutritional composition.

References

Alminger, M., Aura, A. M., Bohn, T., Dufour, C., El, S. N., Gomes, A., et al. (2014). *In vitro* models for studying secondary plant metabolite digestion and bioaccessibility. *Comprehensive Reviews in Food Science and Food Safety, 13*, 413–436.

Blasa, M., Candiracci, M., Accorsi, A., Piacentini, P. M., Albertini, M. C., & Piatti, E. (2005). Raw Millefiori honey is packed full of antioxidants. *Food Chemical, 97*, 217–222.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology, 28*, 25–30.

Burgos-Edwards, A., Jiménez-Aspee, F., Thomas-Valdés, S., Schmeda-Hirschmann, G., & Theoduloz, C. (2017). Qualitative and quantitative changes in polyphenol composition and bioactivity of Ribes magellanicum and R. punctatum after *in vitro* gastrointestinal digestion. *Food Chemistry, 237*, 1073–1082.

Carter, P. (1971). Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). *Analytical Biochemistry, 40*, 450–458.

Chandrasekara, A., & Shahidi, F. (2012). Bioaccessibility and antioxidant potential of millet grain phenolics as affected by simulated *in vitro* digestion and microbial fermentation. *Journal of Functional Foods, 4*, 226–237.

De Ancos, B., González, E. M., & Cano, M. P. (2000). Ellagic acid, vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. *Journal of Agricultural & Food Chemistry, 48*, 4565–4570.

Etcheverry, P., Grusak, M. A., & Fleige, L. E. (2012). Application of *in vitro* bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B6, B12, D, and E. *Frontiers in Physiology, 3*, 317.

Gawlik-Dzik, U. (2012). Changes in the antioxidant activities of vegetables as a consequence of interactions between active compounds. *Journal of Functional Foods, 4*, 872–882.

Genskowsky, E., Puente, L. A., Pérez-Álvarez, J. A., Fernández-López, J., Muñoz, L. A., & Viuda-Martos, M. (2016). Determination of polyphenolic profile, antioxidant activity and antibacterial properties of maqui [*Aristotelia chilensis* (Molina) Stuntz] a Chilean blackberry. *Journal of the Science of Food and Agriculture, 96*, 4235–4242.

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

Gullón, B., Pintado, M. E., Barber, X., Fernández-López, J., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2015a). *In vitro* gastrointestinal digestion of pomegranate peel (*Punica granatum*) flour obtained from co-products: Changes in the antioxidant potential and bioactive compounds stability. *Journal of Functional Foods, 19*, 617–628.

Gullón, B., Pintado, M. E., Barber, X., Fernández-López, J., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2015b). Bioaccessibility, changes in the antioxidant potential and colonic fermentation of date pits and apple bagasse flours obtained from co-products during simulated *in vitro* gastrointestinal digestion. *Food Research International, 78*, 169–176.

Kawakami, K., Aketa, S., Nakanami, M., Iizuka, S., & Hirayama, S. (2010). Major water-soluble polyphenols, proantocyanidins, in leaves of persimmon (*Diospyros kaki*) and their α-amylase inhibitory activity. *Bioscience, Biotechnology, and Biochemistry, 74*, 1380–1385.

Kida, K., Suzuki, M., Matsumoto, N., Nanjo, F., & Hara, Y. (2000). Identification of biliary metabolites of (-)-epigallocatechin gallate in rats. *Journal of Agricultural and Food Chemistry, 48*, 4151–4155.

Kim, G. N., Shin, M. R., Shin, S. H., Lee, A. R., Lee, J. Y., Seo, B. I., et al. (2016). Study of antiobesity effect through Inhibition of pancreatic lipase activity of *Diospyros kaki* fruit and Citrus unshiu peel. *BioMedical Research International, 1723042*. http://dx.doi.org/10.1155/2016/1723042.

Kroll, J., Rawel, H. M., & Rohn, S. (2003). Reactions of plant phenolics with food proteins and enzymes under special consideration of covalent bonds. *Food Science and Technology Research, 9*, 205–218.

Lee, S. O., Chung, S. K., & Lee, I. O. (2006). The antidiabetic effect of dietary persimmon (*Diospyros kaki* L. cv. Sangjudungsi) peel in Streptozotocin-induced diabetic rats. *Journal of Food Science, 71*, S293–S298.

Leite, A., Malta, L. G., Riccio, M. F., Eberlin, M. N., Pastore, G. M., & Marostica, J. M. R. (2011). Antioxidant potential of rat plasma by administration of freeze-dried jaboticaba peel (*Myrciaria jaboticaba* Vell Berg). *Journal of Agricultural and Food Chemistry, 59*, 2277–2283.

Leopoldini, M., Russo, N., & Toscano, M. (2011). The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chemistry, 125*, 288–306.

López-Marcos, M. C., Bailina, C., Viuda-Martos, M., Pérez-Alvarez, J. A., & Fernández-López, J. (2015). Properties of dietary fibers from agroindustrial coproducts as source for fiber-enriched foods. *Food and Bioprocess Technology, 8*, 2400–2408.

Lucas-González, R., Navarro-Coves, S., Pérez-Álvarez, J. A., Fernández-López, J., Muñoz, L. A., & Viuda-Martos, M. (2016). Assessment of polyphenolic profile stability and changes in the antioxidant potential of maqui berry (*Aristotelia chilensis* (Molina) Stuntz) during *in vitro* gastrointestinal digestion. *Industrial Crops and Products, 94*, 774–782.

Lucas-González, R., Viuda-Martos, M., Pérez-Álvarez, J. A., & Fernández-López, J. (2017). Evaluation of particle size influence on proximate composition, physicochemical, techno-functional and physio-functional properties of flours obtained from persimmon (*Diospyros kaki* Trumb.) coproducts. *Plant Foods for Human Nutrition, 72*, 67–73.

Lucas-González, R., Fernández-López, J., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2018). Effect of particle size on phytochemical composition and antioxidant properties of two persimmon flours from *Diospyros kaki* Thunb. vars. 'Rojo Brillante' and 'Triumph' coproducts. *Journal of the Science of Food & Agriculture, 98*, 504–510.

Martínez-Las Heras, R., Pinazo, A., Heredia, A., & Andrés, A. (2017). Evaluation studies of persimmon plant (*Diospyros kaki*) for physiological benefits and bioaccessibility of antioxidants by *in vitro* simulated gastrointestinal digestion. *Food Chemistry, 214*, 478–485.

Martínez-Las Heras, R., Quifer-Rada, P., Andrés, A., & Lamuela-Raventós, R. (2016). Polyphenolic profile of persimmon leaves by high resolution mass spectrometry (LC-ESI-LTQ-Orbitrap-MS). *Journal of Functional Foods, 23*, 370–377.

Medina-Medrano, J. R., Almaraz-Abarca, N., González-Elizondo, M. S., Uribe-Soto, J. N., González-Valdez, L. S., & Herrera-Arrieta, Y. (2015). Phenolic constituents and antioxidant properties of five wild species of *Physalis* (Solanaceae). *Botanical Studies, 56*, 24–37.

Minekus, M., Alminger, M., Alvito, P., Balance, S., Bohn, T., Bourlieu, C., et al. (2014). A standardised static *in vitro* digestion method suitable for food – An international consensus. *Food & Function, 5*, 1113–1124.

Neto, J. J., de Almeida, T. S., de Medeiros, J. L., Vieira, L. R., Moreira, T. B., Maia, A. I., et al. (2017). Impact of bioaccessibility and bioavailability of phenolic compounds in biological systems upon the antioxidant activity of the ethanolic extract of *Triplaris gardneriana* seeds. *Biomedicine & Pharmacotherapy, 88*, 999–1007.

ONU. (2016). General assembly resolution 70/259 of 1 April 2016. United Nations Decade of Action on Nutrition (2016–2025).

Ortega, N., Macia, A., Romero, M. P., Reguant, J., & Motilva, M. J. (2011). Matrix composition effect on the digestibility of carob flour phenols by a digestion model. *Food Chemistry, 124*, 65–71.

Oyaizu, M. (1986). Studies on products of browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition, 44*, 307–315.

Park, Y. S., Jung, S. T., Kang, S. G., Delgado-Licon, E., Martinez-Ayala, A. L., Tapia, Maria S., et al. (2006). Drying of persimmons (*Diospyros kaki* L.) and the following changes in the studied bioactive compounds and the total radical scavenging activities. *LWT – Food Science and Technology, 39*, 748–755.

Pellegrini, M., Lucas-Gonzalez, R., Fernández-López, J., Ricci, A., Pérez-Álvarez, J. A., Lo Sterzo, C., et al. (2017). Bioaccessibility of polyphenolic compounds of six quinoa seeds during in vitro gastrointestinal digestion. *Journal of Functional Foods, 38*, 77–88.

Pinto, J., Spínola, V., Llorent-Martínez, E. J., Fernández-de, Córdova M. L., Molina-García, L., & Castilho, P. C. (2017). Polyphenolic profile and antioxidant activities of Madeiran elderberry (*Sambucus lanceolata*) as affected by simulated in vitro digestion. *Food Research International, 100*, 404–410.

Rodríguez-Roque, M. J., Rojas-Graü, M. A., Elez-Martínez, P., & Martín-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.

Schulz, M., Biluca, F. C., Gonzaga, L. V., Borges, G.d. S. C., Vitali, L., Micke, G. A., & Fett, R. (2017). Bioaccessibility of bioactive compounds and antioxidant potential of juçara fruits (Euterpe edulis Martius) subjected to *in vitro* gastrointestinal digestion. *Food Chemistry, 228*, 447–454.

Selma, M. V., Espín, J. C., & Tomás-Barberán, F. A. (2009). Interaction between phenolics and gut microbiota: Role in human health. *Journal of Agricultural and Food Chemistry, 57*, 6485–6501.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Son, J. E., Hwang, M. K., Lee, E., Seo, S. G., Kim, J. E., Jung, S. K., et al. (2013). Persimmon peel extract attenuates PDGF-BB-induced human aortic smooth muscle cell migration and invasion through inhibition of c-Src activity. *Food Chemistry, 141*, 3309–3316.

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

Viuda-Martos, M., Ruiz-Navajas, Y., Martín-Sánchez, A., Sánchez-Zapata, E., Fernández-López, J., Sendra, E., et al. (2012). Chemical, physico-chemical and functional properties of pomegranate (*Punica granatum* L.) bagasses powder co-product. *Journal of Food Engineering, 110*, 220–224.

You, L., Zhao, M., Regenstein, J. M., & Ren, J. (2010). Changes in the antioxidant activity of loach (*Misgurnus anguillicaudatus*) protein hydrolysates during a simulated gastrointestinal digestion. *Food Chemistry, 120*, 810–816.