



Bioaccessibility of apigenin from *Mangifera indica* (Water Lily Var.) during *in vitro* gastrointestinal digestion

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Abstract

The bioaccessibility property of apigenin from *Mangifera indica* (Water Lily var.) was investigated using *in vitro* gastrointestinal digestion model. Two digestion stages were simulated namely the gastric and small intestinal digestion by using different enzymes and physiological conditions that mimicked the digestion process. Following digestion, the bioaccessible apigenin was analysed using HPLD-DAD-MS. Results showed that the apigenin fraction in intestinal phase was higher than in gastric phase with amounts 1.03 ± 0.35 mg/100 g DW and 0.50 ± 0.08 mg/100 g DW, respectively. After 1 h exposure to gastric juice, the bioaccessibility of apigenin was $20.26 \pm 3.06\%$ with $79.74 \pm 3.06\%$ losses during the digestion, whereas in intestinal phase, the percentage of bioaccessibility significantly increased to $41.53 \pm 13.99\%$ and $58.47 \pm 13.99\%$ losses after 2 h treatment with pancreatin. It was concluded that the apigenin in Water Lily mango became bioaccessible, suggesting the absorption possibility of the compound in the upper part of intestine, which can lead to the health-related outcomes.

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Keywords

Bioaccessibility

Gastrointestinal digestion

Gastric phase

Intestinal phase

Water Lily mango

Apigenin

Introduction

Over the past decades, numerous studies on phytochemicals have considered their bioaccessibility using the latest physiological knowledge and technological breakthroughs (Briones-Labarca *et al.*, 2011). Bioaccessibility is defined as the fraction of a compound released from the food matrix and solubilised during digestion that is potentially available for absorption (Alminger *et al.*, 2014; Carbonell-Capella *et al.*, 2014). It is a digestive transformation of food into small components used for assimilation, absorption into intestinal epithelial cells, and subsequently the presystemic metabolism (Heaney, 2001). The amount of bioaccessible fraction may be equal or less than the amount of the compound released from the food (Stahl *et al.*, 2002).

Studies on bioaccessibility usually involve the *in vitro* digestion method, generally simulating gastric and small intestinal digestions, and sometimes followed by Caco-2 cells uptake (Courraud *et al.*, 2013). The scientific community has developed different digestion models which mimic the physiological and physicochemical conditions of the human gastrointestinal tract to measure the release of polyphenols from food matrices during digestion (Hur *et al.*, 2011). Several studies have reported different trends in the polyphenol profile after undergoing gastrointestinal digestion (Ortega *et al.*, 2009). During gastric digestion, the stability of certain polyphenol compounds may not change but increase as a result of the low pH after the digestion process. According to Bouayed *et al.* (2012), some polyphenols are unstable and poorly absorbed under the gastrointestinal environment.

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Several studies have reported different trends in the polyphenol profile after the gastrointestinal digestion process (Ortega *et al.*, 2009). Also, some studies have determined the stability and transformation of polyphenols during digestion, either by using synthesised compounds or by extraction from the food sources (Spencer *et al.*, 2000; Rios *et al.*, 2002). According to Maqueda (2012), the properties of polyphenolic compounds in a food matrix are not the same as they are tested as single components. During the digestion process, the effect exerted by the food matrix may change certain essential parameters such as the stability of compounds during digestion or bioavailability. There are some recent studies reporting the effect of food matrix on bioaccessibility of phytochemicals during digestion (Phan *et al.*, 2019).

Water Lily is one of the mango varieties found in Malaysia, Thailand and Indonesia. The fruit has a sweet taste, juicy, and firm texture but sometimes a little mushy. It is a delicious seasonal fruit that is generally available between June to August and November to February. Apigenin, a phenolic compound belonging to the flavone class that present in Water Lily mango, has been studied for to determine its bioaccessibility. It has gained much attention in recent years due to its benefits and health promotions, which are relatively non-toxic and non-mutagenic. Apigenin is commonly used in traditional medicines and herbal supplements for many pharmacological activities, including antioxidant properties and its role in free scavenging activity (Madunić *et al.*, 2018), anti-inflammatory effects (Funakoshi-Tago *et al.*, 2011), and growth inhibitory properties in several cancer lines including colon, skin (Tong *et al.*, 2007) and pancreas (Ujiki *et al.*, 2006). In order to associate apigenin consumption to any potential health benefits, it is necessary to first understand how mango and apigenin are digested as this will affect the bioavailability and bioactivity of the compound in the human body.

To date, limited data is available indicating on how apigenin is digested and metabolised through the gastrointestinal tract. Therefore, the purpose of the present work was to evaluate the bioaccessibility of apigenin in Water Lily mango through an *in vitro* gastrointestinal digestion model. The amount of bioaccessible fraction obtained from the digestion model was analysed using HPLC-DAD tandem mass spectrometry.

Materials and methods

Chemicals

Apigenin standard, pepsin (P-7000, from porcine stomach mucosa), pancreatin (P-1750, from porcine pancreas), and bile extract (B-8631, from porcine) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Phosphoric acid, hydrochloric acid, sodium hydroxide and HPLC-grade solvents were obtained from Fisher Scientific (Loughborough, UK).

Preparation of extract

Water Lily mango pulp extract (WLPE) was prepared following the method described by Ferreres *et al.* (2008) with slight modifications. The sample was cut into small pieces then freeze-dried at -40°C for 28 h, tempered for 1 h in liquid nitrogen, and freeze-dried again for 24 h under high vacuum conditions (0.04 mbar). Then, 3 g of freeze-dried sample was homogenised in 20 mL of 80% methanol using homogeniser. The mixture was sonicated for 30 min at 30°C in ultrasonic bath, followed by centrifugation at 12,000 g for 15 min at 5°C , and filtered through a Whatman filter paper No. 1. The extract was concentrated to dryness in a rotary evaporator. Then 100 mg of extract was mixed with 10 mL of 0.9% saline solution. All solutions were freshly prepared and immediately subjected to *in vitro* digestion following preparation.

In vitro gastric digestion

The WLPE was subjected to *in vitro* gastric digestion procedure, following the previously published method (Andlauer *et al.*, 2000) with minor modifications. The digestion by gastric phase was initiated by adjusting the pH of solution to 1.9 with 1 mol/L HCL. The sample was pre-heated in a water bath at 37°C for 5 min, then 2 mL of pepsin solution (17.5 g/L in 0.1 mol/L HCl) was added. The mixture was purged with nitrogen and container was tightly sealed. Incubation was carried out at 37°C for 1 h with continuous stirring. At the end of the gastric digestion, the aliquot was centrifuged (3,000 rpm at 4°C) for 30 min and the supernatant was analysed using HPLC-DAD tandem mass spectrometry.

In vitro intestinal digestion

The sample from gastric digestion underwent intestinal digestion by adjusting the pH to 6.5 with 1 mol/L NaOH with the addition of 2 mL of pancreatin (7 g/L) and bile salts (7.4 g/L) in 0.9% saline solution as described by Andlauer *et al.* (2000). Subsequently, oxygen was removed by blowing the mixture with

nitrogen then incubated in darkness for 2 h at 37°C with continuous stirring. Digestion process was stopped by cooling the mixture on ice bath and sample volume was made up to 20 mL with 0.9% NaCl. The sample was centrifuged at 3,000 rpm for 30 min at 4°C, and the supernatant was analysed using HPLC-DAD tandem mass spectrometry.

Determination of bioaccessible apigenin by HPLC-DAD analysis

To determine the bioaccessible apigenin, 1 mL of digested sample was taken after gastric and intestinal digestions, and mixed with 100 µL of 1% ascorbic acid and 0.28% phosphoric acid. The identification of apigenin fraction was carried out using HPLC (1100 series, Agilent Technologies, Germany) equipped with a diode array detector (DAD). The absorption spectrum was recorded at 280 nm. The system was equipped with Lichrosper C₁₈ column (250 × 4 mm, i.d., and particle size 5 µm). The mobile phase consisted of 0.5% (v/v) acetic acid in water (eluent A), and 100% methanol (eluent B), and gradient program was as follows: 0% to 90% B (0 - 20 min), 90% B isocratic (20 - 25 min), 90% to 0% B (25 - 30 min) at a flow rate of 0.6 mL/min. The injection volume was 20 µL. Quantification of apigenin was performed by recording the absorbance in a chromatogram analysis relative to external standard in the range 20 - 100 µg/mL at 280 nm. Apigenin compound was identified by chromatographic comparison with authentic synthetic standard.

Analysis of bioaccessible apigenin by LC-ESI-QTOF-MS

Analysis of apigenin fraction in the extract was carried out using Quadrupole Time-of-Flight (Q-TOF) mass spectrometer (Agilent Technologies Inc., Palo Alto, CA) equipped with electrospray ionisation (ESI) source. The mass spectra recorded from m/z 50 to 1100, in negative ionisation mode. High purity nitrogen applied was nebulising (30 psi) and drying gas (10 L/min). The gas and vaporiser temperature were set at 325°C, and the scan source parameters were capillary voltage and fragmenter with 4 kV and 175, respectively. Phenolic compounds were identified by comparing their retention time and absorption spectra with those obtained with reference standards as well as based on their mass spectra.

Bioaccessibility calculations

The estimation of the bioaccessibility of apigenin was calculated by analysing its concentration in the supernatant recovered after the *in vitro* simulated gastrointestinal digestion (Rodríguez-Roque *et*

al., 2015). The percentage of bioaccessibility was calculated as below:

$$\text{Bioaccessibility (\%)} = \left(\frac{A_{\text{digested}}}{A_{\text{non digested}}} \right) \times 100$$

Where, A_{digested} = concentration of apigenin in the supernatant, and A_{non digested} = amount of apigenin in the extract.

Statistical analysis

Data were expressed as means ± standard deviation. All experiments were carried out three times. The statistical analysis was performed using the SPSS statistical package for windows (Version 21.0; SPSS Inc., Chicago, IL, USA). t-test analysis was used to determine the significant differences with limit of probability of significance fixed at $p < 0.05$.

Results and discussion

Identification of bioaccessible apigenin by HPLC-DAD

In order to determine the fraction of apigenin following simulated digestion, HPLC-DAD analysis of digested sample was conducted. The digestion

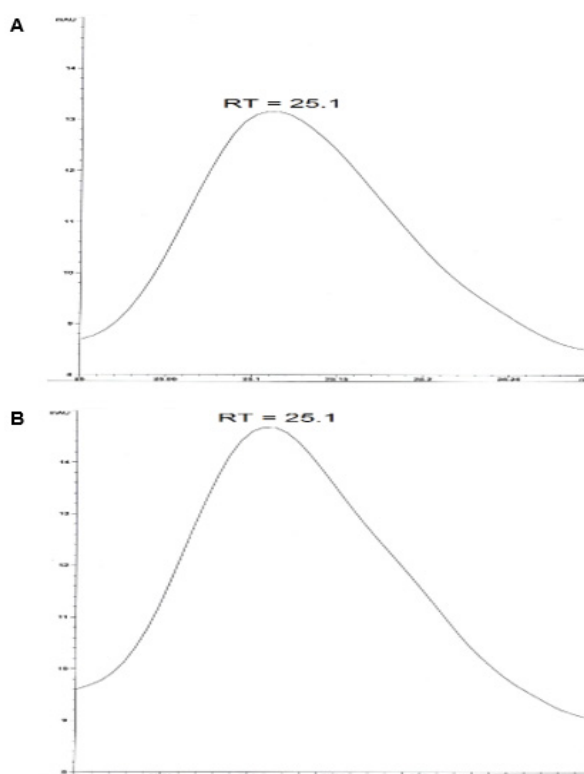


Figure 1. HPLC chromatograms of apigenin detected at 25.1 digestion time at 280 nm from: (A) Water Lily mango pulp extract (WLPE) subjected to 1 h of simulated gastric digestion with HCl-pepsin at pH 1.9; (B) Water Lily mango pulp extract (WLPE) after further 2 h of digestion with pancreatin-bile salts at pH 6.5.

Table 1. Mass spectrometry analysis of apigenin in Water Lily mango pulp extract (WLPE) and commercial apigenin standard after *in vitro* simulation gastrointestinal digestion.

Compound	Retention time (min)	Theoretical [M-H] ⁻ (m/z)	Experimental [M-H] ⁻ (m/z)	Abundance
Apigenin in WLPE	25.1	269.0467	269.0537	6379.98
Apigenin standard	25.1	269.0467	269.0465	123143.67

of the extract was performed for 1 h under stomach acidic condition and 2 h under intestinal condition. Figure 1 shows the HPLC chromatogram of apigenin compound after gastric and intestinal *in vitro* digestions. At 280 nm, the apigenin compound was observed in extract both at gastric and intestinal phase at 25.1 digestion time. The results obtained for digested sample were confirmed by comparing the retention time and mass spectra with pure commercial compound.

The determination of bioaccessibility requires quantitative analysis of polyphenols: (i) the total amount obtained in extract, and (ii) the concentration in each gastric and intestinal digestion. The apigenin standard was also subjected to the same *in vitro* conditions and results were similar as in the extract. One particular finding was that the signal intensity of digested sample at intestinal phase was significantly higher than gastric phase, indicating that the concentration of bioaccessible apigenin was higher during digestion in the intestinal environment. This could probably be explained by the longer incubation time during intestinal environment along with the

influence of intestinal enzyme on the complex food matrix resulting in the release of apigenin bound to the matrix (Carbonell-Capella *et al.*, 2014). Meanwhile, Blancas-Benitez *et al.* (2018) claimed that the gastrointestinal tract acted as an extractor that liberates the polyphenolic compounds from food matrices.

Analysis of bioaccessible apigenin by ESI-QTOF-MS

The confirmation of apigenin compound in extract following *in vitro* simulated gastrointestinal digestion achieved by ESI-QTOF-MS is presented in Figure 2. Results showed that the deprotonated parent ion peak at m/z 269.0537 was the characteristic of apigenin compound in digested sample. Further confirmation with apigenin standard (m/z 269.0465) was also conducted and results indicated the same ion mass for both standard and bioaccessible fractions.

ESI-QTOF-MS facilitates the identification and characterisation of known and unknown constituents based on their molecular formula, exact mass measurements, and MS ion. Table 1 depicts the bioaccessible fraction of apigenin in extract identified by LC-ESI-MS following *in vitro* gastrointestinal digestion. As seen in Table 1, the parent ion peak of bioaccessible fraction of apigenin was similar to the parent ion peak of pure apigenin and theoretical mass ion of apigenin at 269.0537, 269.0465, and 270.0528, respectively. Thus, these data strengthen the fact that bioaccessible apigenin was observed at retention time of 25.1 during the simulated gastrointestinal digestion. Bergantin *et al.* (2017) identified the presence of apigenin glucoside in red chicory (leafy vegetable) using HPLC-MS/MS with m/z 431 after undergoing gastrointestinal digestion.

In vitro gastrointestinal digestion of apigenin

The concentration of bioaccessible apigenin following *in vitro* simulated gastrointestinal digestion was expressed as mg/100 g of dried weight, and is depicted in Figure 3. The result showed that the concentration of bioaccessible apigenin significantly decreased from 2.48 ± 0.09 mg/100 g DW to 0.5 ± 0.08 mg/100 g DW following digestion in acidic gastric condition. There was an increase (1.03 ± 0.35 mg/100 g DW) of about 51.46% ($p < 0.05$) following 2 h exposure in the intestinal environment. Despite

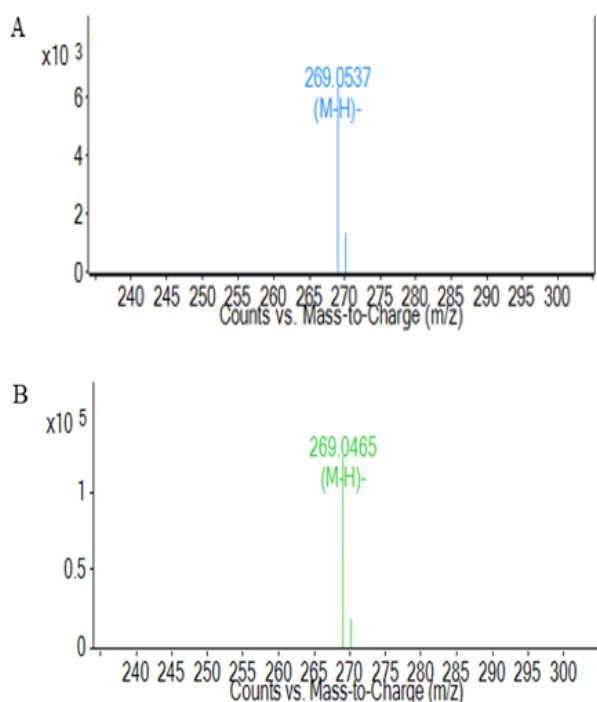


Figure 2. LC-ESI-MS spectra acquired in negative full scan mode corresponding to the [M-H]⁻ ions of: (A) apigenin compound in Water Lily mango pulp extract (WLPE); (B) commercial apigenin standard compound after *in vitro* simulation gastrointestinal digestion.

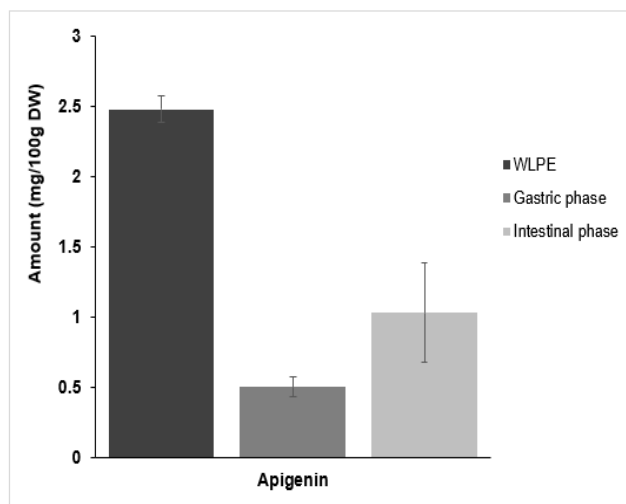


Figure 3. Amount of apigenin in Water Lily mango pulp extract (WLPE) and bioaccessible fraction after gastric and intestinal *in vitro* digestion. Data are means of three determinations ($n = 3$) with bars indicating standard deviation.

noticeable degradation during the digestion process, apigenin compound was still present in both gastric and intestinal juices (Xie *et al.*, 2013).

The gastric phase revealed the reduction of apigenin concentration in WLPE, in comparison with the initial amount, believed to occur due to the less stability of apigenin in digestive fluid that can affect the concentration available to the intestinal epithelia (Neilson *et al.*, 2007). Digestion process can alter and degrade the phenolic structure, thereby affecting the bioaccessibility and possible beneficial effects (Antunes-Ricardo *et al.*, 2017). The increase in amount of bioaccessible apigenin after the 2-h intestinal digestion suggested that despite mild alkaline conditions, the protein-polyphenol interactions were typically weak thus reversed reactions may occur (Santos-Buelga and Scalbert, 2000). Hence, part of the bound apigenin was released from the protein-polyphenol complex, which then made the fraction become more bioaccessible.

Meanwhile, the measurement of biological parameter on the *in vitro* digestion model is the percentage of bioactive compounds absorbed during digestion process (Gil-Izquierdo *et al.*, 2002; Ortega *et al.*, 2009). In the present work, the bioaccessibility of phenols was approximated *in vitro* using the centrifugation technique instead of semipermeable cellulose membrane or filtration methods. This technique involves the separation of soluble fraction from the digested sample. The determination of bioaccessibility of apigenin in WLPE was based on the amount of solubilised component during the digestion process.

In vitro gastrointestinal digestion is a model

applying physiologically based conditions such as the chemical composition of digestive fluids, pH, and residence time typical of each compartment which then simulates the digestion process in the gastrointestinal tract in humans (D'Antuono *et al.*, 2015). When studying the influence of *in vitro* digestion on polyphenols, two important factors need to be considered which are oxygen and light as they can change the properties and structure of phenolic components, caused by oxidative reactions (Talcott and Howard, 1999). According to Bermúdez-Soto and Tomás-Barberán (2004), *in vitro* systems with less levels of oxygen may be a more accurate representation of physiological conditions in the stomach and small intestine rather than digestion conducted in open-air flasks.

Table 2. Percentage bioaccessibility of apigenin in Water Lily mango pulp extract (WLPE) after *in vitro* gastrointestinal digestion.

Digestion phase	% bioaccessibility (recovery)	% loss
Gastric juice	20.26 ± 3.06 ^a	79.74 ± 3.06 ^a
Intestinal juice	41.53 ± 13.99 ^b	58.47 ± 13.99 ^b

Data are means of three determinations ($n = 3$) ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).

Bioaccessibility of apigenin

Table 2 shows the detailed bioaccessible percentages of apigenin compound following *in vitro* gastrointestinal digestion. The results demonstrate that after 1 h of exposure to gastric environment, the bioaccessibility of apigenin was 20.26 ± 3.06%, with 79.74 ± 3.06% losses during the digestion. The transition from the acidic gastric to the mild alkaline intestinal condition caused a significant increase in the percent recovery of apigenin, with 41.53 ± 13.99% of bioaccessibility and 58.47 ± 13.99% losses.

In the present work, a decreasing trend in bioaccessibility in apigenin after digestion with pepsin could be due to the low stability of this compound in gastric environment. Mosele *et al.* (2015) stated that the chemical structure of the phenolic compounds as well as their stability during simulated digestion can affect these kinds of results. The bioaccessibility of apigenin after 2 h in intestinal condition demonstrated a higher recovery percentage since its solubility increased in mild alkaline environment and observed to be 2-fold higher than the gastric phase. Losses during gastrointestinal digestion could be due to the changes in pH between these two phases and interaction with other components, which differ in their chemical structures (Krook and Hagerman, 2012; Velderrain-Rodríguez *et al.*, 2016).

In agreement with these findings, Weathers *et al.* (2014) stated that the bioaccessibility of flavonoids extracted from *Artemisia annua* leaves was higher in the intestinal phase than its amount at the end of the gastric digestion phase. Chandrasekara and Shahidi (2012) also reported a similar trend after a gastrointestinal digestion of different varieties of grains. A study by Velderrain-Rodríguez *et al.* (2016) revealed that the bioaccessibility of phenolic compounds in mango was stable with about 50% of its total polyphenols during the gastric and intestinal phases. However, a study by Lingua *et al.* (2018) observed a decrease in bioaccessible polyphenols during intestinal digestion. This could be associated with the pH changes along the gastrointestinal digestion, as the phenolic compounds are unstable in a neutral or alkaline condition but resistant to gastric environment (Arenas and Trinidad, 2017; Burgos-Edwards *et al.*, 2017).

Conclusion

From the results obtained in the present work, it is possible to conclude that the apigenin compound from Water Lily mango pulp extract was more accessible in the intestinal digestion by using the *in vitro* digestion model, thus indicating the possibility of absorption in the small intestine, which can lead to the beneficial relation between mango consumption and health-related outcomes. Losses mostly occurred during gastric digestion, suggesting that the bioaccessible apigenin was more stable in an intestinal environment as compared to an acidic gastric condition, and this could be due to the difference in pH and enzymes involved in both digestion phases. For further investigations, the bioavailability of apigenin fraction should be carried out to determine whether it is available or susceptible to be absorbed in the upper gastrointestinal tract, subsequently passing through biological membranes, then entering the systemic circulation to exert a specific biological activity.

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References

- Alminger, M., Aura, A.-M., Bohn, T., Dufour, C., El, S. N., Gomes, A., ... and Santos, C. N. 2014. *In vitro* models for studying secondary plant metabolite digestion and bioaccessibility. *Comprehensive Reviews in Food Science and Food Safety* 13(4): 413–436
- Andlauer, W., Kolb, J. and Fürst, P. 2000. Isoflavones from tofu are absorbed and metabolized in the isolated rat small intestine. *Journal of Nutrition* 130(12): 3021–3027.
- Antunes-Ricardo, M., Rodríguez-Rodríguez, C., Gutiérrez-Urbe, J. A., Cepeda-Cañedo, E. and Serna-Saldívar, S. O. 2017. Bioaccessibility, intestinal permeability and plasma stability of isohamnetin glycosides from *Opuntia ficus-indica* (L.). *International Journal of Molecular Sciences* 18(8): article ID E1816.
- Arenas, E. H. and Trinidad, T. P. 2017. Fate of polyphenols in pili (*Canarium ovatum* Engl.) pomace after *in vitro* simulated digestion. *Asian Pacific Journal of Tropical Biomedicine* 7(1): 53–58.
- Bergantin, C., Maietti, A., Cavazzini, A., Pasti, L., Tedeschi, P., Brandolini, V. and Marchetti, N. 2017. Bioaccessibility and HPLC-MS/MS chemical characterization of phenolic antioxidants in red Chicory (*Cichorium intybus*). *Journal of Functional Foods* 33: 94–102.
- Bermúdez-Soto, M. J. and Tomás-Barberán, F. A. 2004. Evaluation of commercial red fruit juice concentrates as ingredients for antioxidant functional juices. *European Food Research and Technology* 219(2): 133–141.
- Blancas-Benitez, F. J., Pérez-Jiménez, J., Montalvo-González, E., González-Aguilar, G. A. and Sáyago-Ayerdi, S. G. 2018. *In vitro* evaluation of the kinetics of the release of phenolic compounds from guava (*Psidium guajava* L.) fruit. *Journal of Functional Foods* 43: 139–145.
- Bouayed, J., Deußer, H., Hoffmann, L. and Bohn, T. 2012. Bioaccessible and dialyzable polyphenols in selected apple varieties following *in vitro* digestion vs. their native pattern. *Food Chemistry* 131(4): 1466–1472.
- Briones-Labarca, V., Venegas-Cubillos, G., Ortiz-Portilla, S., Chacana-Ojeda, M. and Maureira, H. 2011. Effects of high hydrostatic pressure (HHP) on bioaccessibility, as well as antioxidant activity, mineral and starch contents in Granny Smith apple. *Food Chemistry* 128(2): 520–529.
- Burgos-Edwards, A., Jiménez-Aspee, F., Thomas-Valdés, S., Schmeda-Hirschmann, G. and 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.

- Carbonell-Capella, J. M., Buniowska, M., Barba, F. J., Esteve, M. J. and Frígola, 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(2): 155–171.
- Carbonell-Capella, J. M., Buniowska, M., Barba, F. J., Esteve, M. J. and Frígola, A. 2014. Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables. *Comprehensive Reviews in Food Science and Food Safety* 13(2): 155–171.
- Chandrasekara, A. and 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(1): 226–237.
- Courraud, J., Berger, J., Cristol, J. P. and Avallone, S. 2013. Stability and bioaccessibility of different forms of carotenoids and vitamin A during *in vitro* digestion. *Food Chemistry* 136(2): 871–877.
- D'Antuono, I., Garbetta, A., Linsalata, V., Minervini, F. and Cardinali, A. 2015. Polyphenols from artichoke heads (*Cynara cardunculus* (L.) subsp. *scolymus* Hayek): *in vitro* bioaccessibility, intestinal uptake and bioavailability. *Food and Function* 6(4): 1268–1277.
- Ferreres, F., Valentão, P., Pereira, J. A., Bento, A., Noites, A., Seabra, R. M. and Andrade, P. B. 2008. HPLC–DAD–MS/MS–ESI screening of phenolic compounds in *Pieris brassicae* L. reared on *Brassica rapa* var. *rapa* L. *Journal of Agricultural and Food Chemistry* 56(3): 844–853.
- Funakoshi-Tago, M., Nakamura, K., Tago, K., Mashino, T. and Kasahara, T. 2011. Anti-inflammatory activity of structurally related flavonoids, Apigenin, Luteolin and Fisetin. *International Immunopharmacology* 11(9): 1150–1159.
- Gil-Izquierdo, A., Zafrilla, P. and Tomás-Barberán, F. A. 2002. An *in vitro* method to simulate phenolic compound release from the food matrix in the gastrointestinal tract. *European Food Research and Technology* 214(2): 155–159.
- Heaney, R. P. 2001. Factors influencing the measurement of bioavailability, taking calcium as a model. *Journal of Nutrition* 131(4 Suppl): 1344S–1348S.
- Hur, S. J., Lim, B. O., Decker, E. A. and McClements, D. J. 2011. *In vitro* human digestion models for food applications. *Food Chemistry* 125(1): 1–12.
- Krook, M. A. and Hagerman, A. E. 2012. Stability of polyphenols epigallocatechin gallate and pentagalloyl glucose in a simulated digestive system. *Food Research International* 49(1): 112–116.
- Lingua, M. S., Wunderlin, D. A. and Baroni, M. V. 2018. Effect of simulated digestion on the phenolic components of red grapes and their corresponding wines. *Journal of Functional Foods* 44: 86–94.
- Madunić, J., Madunić, I. V., Gajski, G., Popić, J. and Garaj-Vrhovac, V. 2018. Apigenin: a dietary flavonoid with diverse anticancer properties. *Cancer Letters* 413: 11–22.
- Maqueda, A. S. 2012. Polyphenol metabolism: from *in vitro* to *in vivo* approaches. Spain: Universiti de Lleida, PhD Thesis.
- Mosele, J. I., Macià, A., Romero, M.-P., Motilva, M.-J. and 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.
- Neilson, A. P., Hopf, A. S., Cooper, B. R., Pereira, M. A., Bomser, J. A. and Ferruzzi, M. G. 2007. Catechin degradation with concurrent formation of homo- and heterocatechin dimers during *in vitro* digestion. *Journal of Agricultural and Food Chemistry* 55(22): 8941–8949.
- Ortega, N., Reguant, J., Romero, M. P., Macià, A. and Motilva, M. J. 2009. Effect of fat content on the digestibility and bioaccessibility of cocoa polyphenol by an *in vitro* digestion model. *Journal of Agricultural and Food Chemistry* 57(13): 5743–5749.
- Ortega, N., Reguant, J., Romero, M. P., Macià, A. and Motilva, M. J. 2009. Effect of fat content on the digestibility and bioaccessibility of cocoa polyphenol by an *in vitro* digestion model. *Journal of Agricultural and Food Chemistry* 57(13): 5743–5749.
- Phan, M. A. T., Bucknall, M. P. and Arcot, J. 2019. Co-ingestion of red cabbage with cherry tomato enhances digestive bioaccessibility of anthocyanins but decreases carotenoid bioaccessibility after simulated *in vitro* gastro-intestinal digestion. *Food Chemistry* 298: article ID 125040.
- Rios, L. Y., Bennett, R. N., Lazarus, S. A., Rémésy, C., Scalbert, A. and Williamson, G. 2002. Cocoa procyanidins are stable during gastric transit in humans. *American Journal of Clinical Nutrition* 76(5): 1106–1110.
- Rodríguez-Roque, M. J., De Ancos, B., Sánchez-Moreno, C., Cano, M. P., Elez-Martínez, P. and Martín-Belloso, O. 2015. Impact of food matrix and processing on the *in vitro* bioaccessibility of vitamin C, phenolic compounds and hydrophilic antioxidant activity from fruit juice-based beverages. *Journal of Functional Foods* 14: 33–43.
- Santos-Buelga, C. and Scalbert, A. 2000. Proanthocyanidins and tannin-like compounds - nature, occurrence, dietary intake and effects on nutrition and health. *Journal of the Science of Food and Agriculture* 80(7): 1094–1117.
- Spencer, J. P., Chaudry, F., Pannala, A. S., Srini, S. K., Debnam, E. and Rice-Evans, C. 2000. Decomposition of cocoa procyanidins in the gastric milieu. *Biochemical and Biophysical Research Communications* 272(1): 236–241.
- Stahl, W., van den Berg, H., Arthur, J., Bast, A., Dainty, J., Faulks, R. M., ... and Astley, S. B. 2002. Bioavailability and metabolism. *Molecular Aspects of Medicine* 23(1-3): 39–100.
- Talcott, S. T. and Howard, L. R. 1999. Phenolic autoxidation is responsible for color degradation in

- processed carrot puree. *Journal of Agricultural and Food Chemistry* 47(5): 2109–2115.
- Tong, X., Van Dross, R. T., Abu-Yousif, A., Morrison, A. R. and Pelling, J. C. 2007. Apigenin prevents UVB-induced cyclooxygenase 2 expression: coupled mRNA stabilization and translational inhibition. *Molecular and Cellular Biology* 27(1): 283–296.
- Ujiki, M. B., Ding, X. Z., Salabat, M. R., Bentrem, D. J., Golkar, L., Milam, B., ... and Adrian, T. E. 2006. Apigenin inhibits pancreatic cancer cell proliferation through G2/M cell cycle arrest. *Molecular Cancer* 5: article ID 76.
- Velderrain-Rodríguez, G., Quirós-Sauceda, A., Mercado-Mercado, G., Ayala-Zavala, J. F., Astiazarán-García, H., Robles-Sánchez, R. M., ... and González-Aguilar, G. A. 2016. Effect of dietary fiber on the bioaccessibility of phenolic compounds of mango, papaya and pineapple fruits by an *in vitro* digestion model. *Food Science and Technology* 36(2): 188–194.
- Weathers, P. J., Jordan, N. J., Lasin, P. and Towler, M. J. 2014. Simulated digestion of dried leaves of *Artemisia annua* consumed as a treatment (pACT) for malaria. *Journal of Ethnopharmacology* 151(2): 858–863.
- Xie, Y., Kosińska, A., Xu, H. and Andlauer, W. 2013. Milk enhances intestinal absorption of green tea catechins in *in vitro* digestion/Caco-2 cells model. *Food Research International* 53(2): 793–800.