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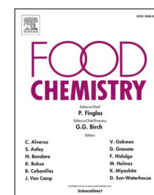

Simulated gastrointestinal digestion/Caco-2 cell model to predict bioaccessibility and intestinal permeability of *p*-coumaric acid and *p*-coumaroyl derivatives in peanut

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ABSTRACT

Data concerning physiological recovery of whole peanut major phenolics throughout the gastrointestinal tract are scarce. In our study, the bioaccessibility and intestinal permeability of peanuts major phenolics were predicted by simulated digestion followed by Caco-2 cells monolayer model. Phenolics identification and quantification were performed by HPLC-ESI-QTOF-MS and HPLC-PDA, respectively. As results, *p*-coumaroyl conjugates with tartaric, sinapic and ferulic acids, and *p*-coumaric acid were the major phenolics found in the non-digested extract and in the digested and transported fractions. The *in vitro* bioaccessibility and Caco-2 cell transport of *p*-coumaric acid was 370% and 127%, respectively, while it was much lower for *p*-coumaroyl derivatives (7–100% and 14–31%, respectively). Nonetheless, the peroxyl scavenging activity remained unaltered, likely, at least partly, due to synergies between some phenolics, which concentration proportions changed throughout the experiment. Hence, there is indication that whole peanut is a source of bioavailable antioxidant phenolics.

1. Introduction

Peanut or groundnut (*Arachis hypogaea* L.) is a low-cost seed widely produced in tropical and subtropical zones worldwide, including semi-arid areas, with high contents of unsaturated fatty acids, protein and minerals (Lozano, de Oliveira Sartori, Markowicz Bastos, & Bismara Regitano-d'Arce, 2019). Additionally, it has widely been recognized by the high content of bioactive phenolics with antioxidant activity, which are concentrated in its skin. Dietary antioxidants, such as those found in peanuts, can enhance the endogenous antioxidant system by playing a key role in keeping redox homeostasis and in alleviating oxidative stress, which is defined as the imbalance of pro-oxidants and antioxidants in favor of the former (Apak, 2019). Oxidative stress has been associated with the onset and development of cardiovascular diseases, diabetes, cancer, arthritis, premature aging, autoimmune disorders, and neurodegenerative diseases (Martinelli et al., 2021). Indeed, the complex composition of nutrients and bioactive compounds makes the regular and moderated peanut consumption related to a reduced risk of cardiovascular diseases, according to the Food and Drug Administration US agency (U.S. Food and Drug Administration, 2003). This claim is continuously supported by epidemiological and clinical studies demonstrating that the regular consumption of small amounts of peanuts decreases total cholesterol and low-density lipoprotein-cholesterol (Bansode et al., 2014; Tan, Tan, & Tan, 2020).

Among the bioactive compounds with antioxidant activity found in shelled whole peanuts (skin + kernel), *p*-coumaric acid and *p*-coumaroyl derivatives outstand in terms of concentration (Juliano et al., 2020; Phan-Thien, Wright, & Lee, 2014). *p*-Coumaric acid, which is a derivative of cinnamic acid with a phenyl hydroxyl group, plays a central role in the secondary metabolism, since it is a precursor of other relevant phenolic acids, such as caffeic acid, ferulic acid and chlorogenic acid (El-Seedi et al., 2012). The administration of free *p*-coumaric acid is related to a potent antioxidant activity in cells and to the attenuation of lipid peroxidation *in vivo* (Shen et al., 2019). However, *p*-coumaric conjugates are more abundant in nature than free *p*-coumaric acid and also exhibit biological activites, including antioxidant, anti-inflammatory,

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antimutagenic, anti-ulcer, antiplatelet and anti-cancer, as well as mitigating atherosclerosis, oxidative cardiac damage, UV-induced damage to ocular tissues, neuronal injury and diabetes (Pei, Ou, Huang, & Ou, 2016).

The diversity in the chemical structure of phenolics determines their biological activities, such as antioxidant activity, as well as their bioavailability, since it can affect the way they bond with other molecules such as enzymes, or cell receptors such as membrane transporters (Martinelli et al., 2021). Phenolics can be covalently bound to indigestible components such as dietary fiber within the food matrix, thereby not being absorbed in the small intestine, where nutrients and bioactive compounds are mostly absorbed (Quirós-Sauceda et al., 2014). It is important to mention that bioaccessibility, which can be defined as the quantity of a compound that is released from a food matrix in the gastrointestinal tract, influences the compound's quantity to be effectively absorbed, which is its bioavailability (Galanakis, 2017). Therefore, although *in vitro* antioxidant activity assays applied in non-digested foods are important for screening purposes, their results often overestimate the biological role of complex food matrices, when compared with results of *in vivo* studies, which are more expensive and time-consuming (Martinelli et al., 2021). In order to mimic physiological conditions and predict health-related food compounds bioavailability, the costly effective combination of simulated *in vitro* digestion followed by the transport assay using the enterocyte-like differentiated human colon adenocarcinoma (Caco-2) cell monolayer model have been used (Chukwumah, Walker, Vogler, & Verghese, 2011; Ding et al., 2021; Wu et al., 2017).

Additionally, food fractions can have their antioxidant activities evaluated through the gastrointestinal system using *in vitro* antioxidant activity assays (Martinelli et al., 2021). The total phenolic content (TPC) and the ORAC assay values for Georgia peach extracts were significantly associated with the cellular antioxidant activity determined using a Caco-2 model (Liao, Brock, Jackson, Greenspan, & Pegg, 2020). The ORAC assay is one of the most important *in vitro* assays to estimate antioxidant activity, since it generates peroxyl radicals (ROO•), which are commonly formed in biological systems (Martinelli et al., 2021).

In this study, the bioaccessibility and intestinal permeability of the major phenolics of whole peanuts were predicted by simulated gastrointestinal digestion followed by an enterocyte-like differentiated Caco-2 cells monolayer transport assay. Additionally, the peroxyl scavenging activity of peanut before and after simulated gastrointestinal digestion and after Caco-2 cell transport was assessed.

2. Material and methods

2.1. Sample

Peanut from the BR1 cultivar (Valencia market type) belonging to the Peanut Breeding Program from Embrapa Algodão, which is drought tolerant, was grown in Campina Grande-PB, Brazil (7° 13′ 51″ S, 35° 52′ 54″ W, 512 m) during raining season, between May and September 2015. After harvesting, seeds were dried until reaching moisture ranging from 8 % to 10 %, shelled and kept at −20 °C until analysis.

2.2. Preparation of the non-digested extract

For comparison purposes and representing the non-digested peanut, an extract was prepared using the shelled seeds (kernels covered with the skins) according to Regueiro et al. (2014), with modifications. Ground sample (0.5 g) was homogenized with 4 mL acetone:water (60:40; v/v), extracted in an ultrasound water bath for 5 min, and centrifuged at 4000*g* (Eppendorf 5810R, Eppendorf AG, Hamburg, Germany) for 10 min at 4 °C. Supernatant was collected and proceeding was repeated twice. Then, supernatants were combined and kept at −20 °C until analysis. Extraction was conducted in triplicate. Fig. 1 illustrates the performed experiments.

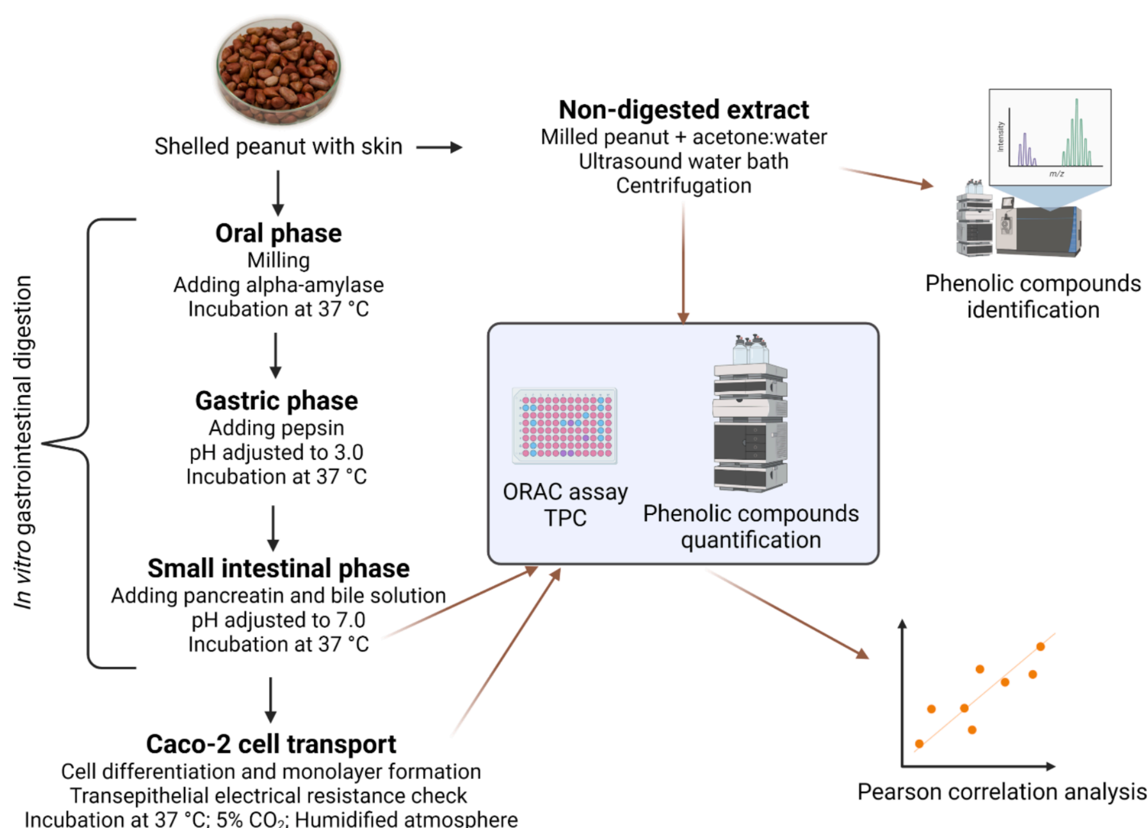

Fig. 1. Diagram of the performed experiments and key assessed parameters. ORAC: Oxygen Radical Absorbance Capacity. TPC: total phenolic content.

2.3. In vitro bioaccessibility of phenolic compounds

In vitro bioaccessibility of peanut phenolics was evaluated through a simulated gastrointestinal digestion according to Rodrigues, Mariutti, and Mercadante (2016), with modifications. Simulated salivary fluid (SSF), containing 0.325 g/L of α-amylase; gastric fluid (SGF), containing 26.4 g/L of pepsin; and small intestinal fluid (SIF), containing 20 g/L of pancreatin, and 11.25 g/L bile solution, were prepared according to Minekus et al. (2014).

Oral phase: seeds were ground in a mill (IKA A11 Basic) with liquid N_2 to simulate food mastication. Ground seeds (5 g) were mixed with SSF (5 mL), incubated at 37 °C in a thermostatted water bath for 2 min under agitation (100 rpm), and transferred to an ice bath to stop the reaction. The solution was centrifuged at 10,000 *g* for 10 min at 4 °C, and the supernatant was collected and kept at −20 °C until analysis.

Gastric phase: 10 mL of SGF were added with the resulting solution from oral phase, pH was adjusted to 3.0 with 1 M HCl, the mixture was incubated at 37 °C for 2 h under agitation (100 rpm), and transferred to an ice bath in order to stop the reaction. Mixture was centrifuged at 10,000 *g* for 10 min at 4 °C, and the supernatant was collected and kept at −20 °C until analysis.

Small intestinal phase: 20 mL of SIF were added with the resulting solution from gastric phase, pH was adjusted to 7.0 with 1 M NaOH, the mixture was incubated at 37 °C for 2 h under agitation (100 rpm), and transferred to an ice bath in order to stop the reaction. Mixture was centrifuged at 10,000 *g* for 10 min at 4 °C, and the supernatant (accessible fraction) was collected and kept at −20 °C until analysis.

For every phase (oral, gastric and small intestinal), two controls were submitted to the same conditions: 1) containing samples with digestive fluids without the enzymes; and 2) containing digestive fluids with enzymes but without samples. The simulated gastrointestinal digestion was performed in triplicate. *In vitro* bioaccessibility was calculated as percentage according to the equation: *In vitro* bioaccessibility (%) = [concentration of phenolics in the solution after digestion/concentration of phenolics in the non-digested extract] × 100.

2.4. Cell culture

Caco-2 HTB-37™ cell line (ATCC, Manassas, USA) was cultivated according to a protocol (Hubatsch, Ragnarsson, & Artursson, 2007), in medium containing Advanced DMEM (Sigma–Aldrich, Buchs, Switzerland) supplemented with 10 % FBS (Sigma–Aldrich, Buchs, Switzerland), 1 % non-essential amino acids and 1 % penicillin/streptomycin, at 37 °C in a humidified atmosphere containing 5 % CO_2 in air. Medium was replaced every 48 h and cells were trypsinized with 0.25 % trypsin-EDTA solution (Sigma–Aldrich, Buchs, Switzerland) until 70–80 % confluence was reached. Cultures passages 20 at a density of 2.6×10^5 cells/cm^2 were seeded in 6-well Transwell® plates (0.4 µm pore size, 24 mm i.d., Corning Life Sciences, Acton, USA). Cell medium was added in both apical (1.5 mL) and basolateral (2.5 mL) chambers and incubated (37 °C, humidified 5 % CO_2 in humidified atmospheric air) for 21 days until cell differentiation and monolayer formation for the Caco-2 cell transport assay.

2.5. Caco-2 cell transport assay

Medium was removed and cell monolayers were washed with Hank's balanced salt solution (HBSS) supplemented with 25 mmol/L of HEPES (Sigma–Aldrich, Buchs, Switzerland). Integrity of the cell monolayer was then assessed by the transepithelial electrical resistance (TEER) using a Millicell® ERS-2 (Millipore Corporation, Bedford, MA, USA). TEER value was calculated using the following equation: TEER value ($\Omega \times cm^2$) = [Ω cell monolayer (Ω) – Ω filter (cell-free) (Ω)] × filter area (cm^2). The obtained TEER values $\geq 252\ \Omega \times cm^2$ before the transport and $\geq 229\ \Omega \times cm^2$ after the transport were considered qualified, since they were $> 165\ \Omega \times cm^2$, which indicates Caco-2 cell monolayer integrity (Hubatsch et al., 2007).

The digested fraction was diluted 1:40 with HBSS (v/v) and added at the apical chamber. HBSS (2.5 mL) was also added at the basolateral chamber. Transwell® plates were incubated at 37 °C under smooth agitation for 4 h, the TEER value was reevaluated, and then, both apical and basolateral fractions were collected and stored at −80 °C until analysis. The experiment was conducted in triplicate. Caco-2 cell transport was calculated as percentage according to the equation: Caco-2 cell transport (%) = [concentration of phenolics in the basolateral solution after Caco-2 cell transport, named as transported fraction/concentration of phenolics in the non-digested extract] × 100.

2.6. Identification of phenolic compounds by HPLC–ESI-QTOF-MS

A Shimadzu chromatograph (Shimadzu Co., Tokyo) equipped with a LC-30AD quaternary pump, photodiode array detector, and a SIL-30AC self-injector was used. Separation was performed on a Phenomenex Luna C18 column (4.6 × 250 mm and i.d. 5 µm) at 30 °C. The mobile phase consisted of water:formic acid (99.75:0.25, v/v) (A) and acetonitrile:formic acid:water (80.00:0.25:19.75, v/v/v) (B). The mobile phase flow rate was 1.0 mL/min, and elution gradient was 10 % of solvent B, increasing to 20 % in 10 min, 30 % in 20 min, 50 % in 30 min, then decreasing to 10 % in 38 min. A MAXIS 3G Bruker Daltonics high-resolution mass spectrometer (Bruker Daltonics, Bremen, Germany) was equipped with an electrospray ionization (ESI) source operating in negative mode under the following operating conditions: *m/z* interval of 100–2,000, nebulizer gas at 29 psi; dry gas at 8 L/min; and HV of 4.5 kV. Data analysis was performed using the DataAnalysis software (Bruker Daltonics, version 4.3). Compounds were tentatively identified by comparing the exact masses, MS/MS mass spectra and molecular formulas with the literature. The exception was for *p*-coumaric acid, which had its identity confirmed by comparison with an authentic standard (Sigma–Aldrich, Buchs, Switzerland).

2.7. Quantification of phenolic compounds by HPLC-PDA

A Shimadzu chromatographic system (Shimadzu Co., Tokyo) equipped with a SPD-M 10AVp photodiode array detector was used. Separation was performed on a C18 column with the same dimensions of that used for the mass spectra analysis (4.6 × 250 mm and i.d. 5 µm, Agilent, Santa Clara, California, USA) at 30 °C. The mobile phase, its flow rate, as well as the elution gradient were the same used for the HPLC–ESI-QTOF-MS analysis. The major compounds were analyzed at 310 nm wavelength, which is the maximum absorption of *p*-coumaric acid, and had their retention times compared with those identified by HPLC–ESI-QTOF-MS.

Results for the non-digested extract, the digested fraction and the transported fraction were expressed as µg *p*-coumaric acid equivalents per gram of shelled whole peanut on a fresh weight basis (µg *p*-CAE/g). Limit of detection (LOD) and limit of quantification (LOQ) were calculated using the parameters of the *p*-coumaric acid analytical curve equation (estimated standard deviation of the linear coefficient and slope) as 0.8739 µg/mL and 2.6483 µg/mL, respectively. Linearity (R^2) obtained from the linear regression was 0.9997 for a concentration interval from 0.002 µg/mL to 40 µg/mL.

2.8. Total phenolic content (TPC) and peroxyl scavenging activity

A microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA) was used for both methods. TPC was determined according to the spectrophotometric method using the Folin–Ciocalteau reagent, as described by Melo et al. (2015), and results were expressed as µg of gallic acid equivalents per gram of peanut on a fresh weight basis (µg GAE/g). The peroxyl scavenging activity was conducted according to the Oxygen Radical Absorbance Capacity (ORAC) assay (Melo et al., 2015), the reaction temperature was carefully kept constant at 37 °C, and results

were expressed as μmol of trolox equivalents per gram of peanut on a fresh weight basis (μmol TE/g).

2.9. Statistical analysis

Analyzes were carried out in triplicate and results were expressed as means ± standard deviation (SD). Normality of distributions (Shapiro-Wilk's test) and homoscedasticity (Bartlett's test) were verified. Means were compared by using the *post hoc* Tukey's test, after performing One-way ANOVA (analysis of variance), at a level of confidence of 0.05. Pearson's linear correlation was determined to detect associations between TPC, peroxyl scavenging activity, total identified compounds and identified compounds, as taken individually. R software, version 4.1.1 (R Core Team. (2020), 2020), was used for almost all statistical analyzes, except the Pearson's linear correlation, which was performed using Minitab software, version 20 (Minitab, 2021).

3. Results and discussion

3.1. Identification of major phenolic compounds in peanut by HPLC-ESI-QTOF-MS

Seven major compounds were found in the non-digested extract and in the digested and transported (basolateral) fractions, and tentatively identified as *p*-coumaroyl derivatives and free *p*-coumaric acid (Table 1).

RT: retention time. Concentration results expressed as mean ± standard deviation. Different letters within the same row indicate statistically significant differences (ANOVA and *post hoc* Tukey' test, $p < 0.05$). FW: fresh weight. 1: *p*-Coumaroyltartaric (isomer 1); 2: *p*-Coumaroyltartaric (isomer 2); 3: *p*-Coumaric acid; 4: Unidentified *p*-coumaroyl derivative; 5: Di-*p*-coumaroyltartaric acid; 6: *p*-Coumaroylsinapoyltartaric acid; 7: *p*-Coumaroylferuloyltartaric acid.

Compounds 1 and 2 were tentatively identified as isomers of *p*-coumaroyltartaric acid, since both compounds gave [*p*-coumaric-H]$^-$, [*p*-coumaric-H-CO_2]$^-$ and [tartaric-H]$^-$ as their characteristic ions in MS^2, at *m/z* 163.0416, 119.0510 and 149.0109, respectively. Although it was not possible to specify the isomers, a study using similar chromatographic conditions in peanut leaf extracts reported that the *trans*- isomer presented a shorter retention time (7.5 min) and a higher peak area than the *cis*-isomer (8.0 min) (Sullivan, 2014). Therefore, compounds 1 is likely to be the *trans-p*-coumaroyltartaric acid, named in our study as *p*-coumaroyltartaric acid (isomer 1) and compound 2 is likely to be the *cis-p*-coumaroyltartaric acid, named in our study as *p*-coumaroyltartaric acid (isomer 2).

Compound 3 was identified as *p*-coumaric acid by comparison with an authentic standard and since it gave [*p*-coumaric acid-H]$^-$ at *m/z* 163.0403 as the characteristic ion in MS^2. Compound 4 was unidentified, although, based on the given deprotonated ions at *m/z* 119.0513 and 163.0414 in MS^2, it is clearly a *p*-coumaroyl derivative (named in our study as unidentified *p*-coumaroyl derivative).

Compound 5 was tentatively identified as di-*p*-coumaroyltartaric acid, since it gave [*p*-coumaroyltartaric-H]$^-$, [M−H−*p*−coumaroyl−H_2O]$^-$, [*p*-coumaric-H]$^-$ and [*p*-coumaric-H-CO_2]$^-$ as their characteristic ions in MS^2, at *m/z* 295.0482, 277.0373, 163.0413 and 119.00512, respectively.

Compound 6 was tentatively identified as *p*-coumaroylsinapoyltartaric acid, since it gave [M−H−sinapoil−H_2O−H]$^-$ at *m/z* 277.0375, [M−H−*p*−coumaroyl−H_2O]$^-$ at *m/z* 337.0588, [M−H−sinapoil−H_2O−74-($C_2H_2O_3$)]$^-$ at *m/z* 203.0360, [tartaric-H]$^-$ at *m/z* 149.0254, and [sinapic-H]$^-$ at *m/z* 223.0633 as characteristic ions in MS^2.

Compound 7 was tentatively identified as *p*-coumaroylferuloyltartaric acid, since it gave [M−H−feruloyl−H_2O−H]$^-$ at *m/z* 277.0373, [M−H−feruloyl−H_2O−74-($C_2H_2O_3$)]$^-$ at *m/z* 203.0366, [M−H−*p*−coumaroyl−H_2O]$^-$ at *m/z* 307.0489, [*p*-coumaric-H-CO_2]$^-$ at *m/z* 119.0510, and [ferulic-H]$^-$ at *m/z* 193.0539 as characteristic ions in MS^2. *p*-Coumaroyl derivatives were also among the major phenolics found in hydroacetonic and methanolic extracts of shelled whole peanuts (Juliano et al., 2020; Ma et al., 2014).

3.2. In vitro bioaccessibility of p-coumaric acid and p-coumaroyl derivatives from peanut

p-Coumaroyltartaric acid (isomer 1) was the most abundant identified phenolic (681.42 μg *p*-CAE/g) in the non-digested extract (Table 1). The order of the other compounds in terms of concentrations were: di-*p*-coumaroyltartaric acid (362.96 μg *p*-CAE/g), *p*-coumaroyltartaric acid (isomer 2) (150.21 μg *p*-CAE/g), *p*-coumaric acid, the unidentified *p*-coumaroyl derivative (compound 4) and *p*-coumaroylsinapoyltartaric acid (from 66.99 to 68.55 μg *p*-CAE/g), and *p*-coumaroylferuloyltartaric acid (35.30 μg *p*-CAE/g).

Compared with the concentrations found in the non-digested extract, the concentrations of *p*-coumaroyltartaric (isomer 1) and of the unidentified *p*-coumaroyl derivative (compound 4) did not statistically increase ($p < 0.05$) in the digested fraction (Table 1). On the other hand, the concentrations of di-*p*-coumaroyltartaric acid, *p*-coumaroylsinapoyltartaric acid and *p*-coumaroylferuloyltartaric acid decreased ($p < 0.05$); while *p*-coumaric acid was the only compound showing a significant concentration increase in this fraction ($p < 0.05$). Consequently, the *in vitro* bioaccessibility of each phenolic compound varied greatly, as shown at Fig. 2.

Free *p*-coumaric acid outstood with the highest *in vitro* bioaccessibility of ca. 370 %, followed by *p*-coumaroyltartaric acid (isomer 1) and the unidentified *p*-coumaroyl derivative (compound 4) (ca. 100 %), *p*-coumaroyltartaric acid (isomer 2) (ca. 80 %), di-*p*-coumaroyltartaric acid (30 %), *p*-coumaroylsinapoyltartaric acid (25.9 %) and

Table 1
Identification and quantification of *p*-coumaric acid and *p*-coumaroyl derivatives in the non-digested extract and in the digested and transported (basolateral) fractions of peanut by HPLC–ESI-QTOF-MS and HPLC-PDA, respectively.

Compound	RT (min)	[M−H]$^-$	MS fragments (*m/z*)	Concentration (μg *p*-coumaric acid equiv./g FW peanut)		
				Non-digested extract	Digested fraction	Transported fraction (Basolateral fraction)
1	13.2	295.0494	163.0416; 149.0109; 119.0510	681.42 ± 47.06a	757.51 ± 41.41a	203.69 ± 6.69b
2	16.5	295.0482	163.0415; 149.0109; 119.0514	150.21 ± 7.27a	115.50 ± 4.72b	33.34 ± 1.53c
3	19.4	163.0403	1,190,511	68.55 ± 5.84b	252.86 ± 12.48a	86.71 ± 4.45b
4	18.8	603.1395	163.0414; 457.1015; 119.0513	67.97 ± 5.57a	71.21 ± 2.03a	21.18 ± 0.74b
5	29.3	441.0861	295.0482; 163.0413; 277.0373; 119.0512	362.96 ± 24.96a	108.83 ± 2.55b	75.29 ± 2.11b
6	29.7	501.1071	277.0375; 203.0360; 337.0588; 149.0254; 223.0633	66.99 ± 5.54a	17.34 ± 0.24b	11.80 ± 0.97b
7	30.1	471.0970	277.0373; 203.0366; 307.0489; 193.0539; 119.0510	35.30 ± 6.92a	2.33 ± 0.94b	4.68 ± 0.51b
Total identified compounds:				1,468.70	1,352.58	436.69

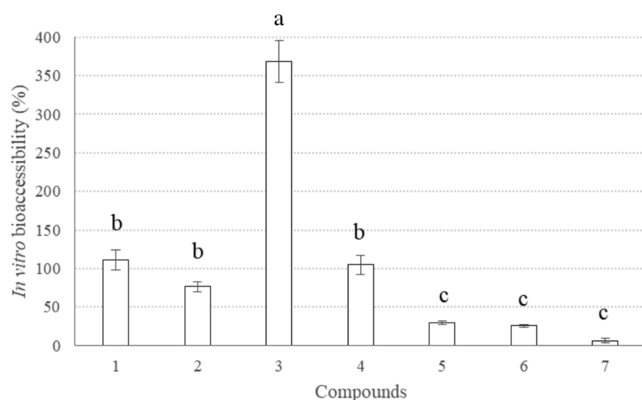

Fig. 2. *In vitro* bioaccessibility of *p*-coumaric acid and *p*-coumaroyl derivatives from peanut. 1: *p*-Coumaroyltartaric (isomer 1); 2: *p*-Coumaroyltartaric (isomer 2); 3: *p*-Coumaric acid; 4: Unidentified *p*-coumaroyl derivative; 5: Di-*p*-coumaroyltartaric acid; 6: *p*-Coumaroylsinapoyltartaric acid; 7: *p*-Coumaroylferuloyltartaric acid. Different letters indicate statistically significant differences (ANOVA and *post hoc* Tukey' test, $p < 0.05$).

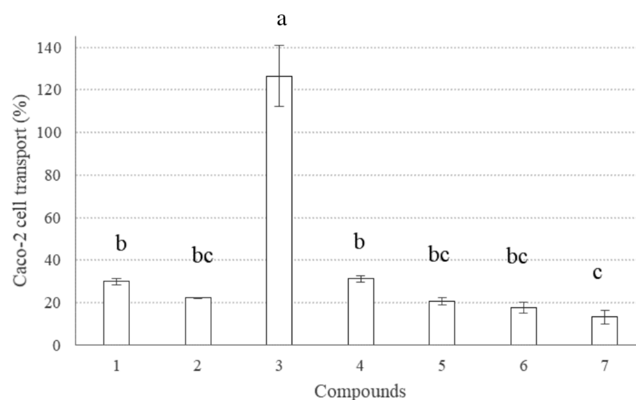

Fig. 3. Percentage of *p*-coumaric acid and *p*-coumaroyl derivatives from the digested fraction of peanut transported across the Caco-2 cell monolayer. 1: *p*-Coumaroyltartaric (isomer 1); 2: *p*-Coumaroyltartaric (isomer 2); 3: *p*-Coumaric acid; 4: Unidentified *p*-coumaroyl derivative; 5: Di-*p*-coumaroyltartaric acid; 6: *p*-Coumaroylsinapoyltartaric acid; 7: *p*-Coumaroylferuloyltartaric acid. Different letters indicate statistically significant differences (ANOVA and *post hoc* Tukey' test, $p < 0.05$).

p-coumaroylferuloyltartaric acid (6.6 %).

The outstanding result for free *p*-coumaric acid and the decrease in the concentrations of some intact *p*-coumaroyl derivatives is expected, since esterases present in the small intestine and colon of humans are able to cleave the ester bonds of esterified hydroxycinnamic acids, and release a proportion of their free forms into the lumen (Nićiforović & Abramovič, 2014).

Conversely, the lower *in vitro* bioaccessibility of *p*-coumaroylferuloyl-tartaric acid may be related to the lower bioaccessibility of ferulic acid, when compared with *p*-coumaric acid, observed in all phases of the rat gastrointestinal tract (Zhao & Moghadasian, 2010). Additionally, sinapic acid showed higher bioaccessibility than ferulic acid in wheat bran, likely because it is more water-soluble (Hemery et al., 2010), which may explain the higher *in vitro* bioaccessibility for *p*-coumaroylsinapoyl-tartaric acid than for *p*-coumaroylferuloyl-tartaric acid in our study.

3.3. Transport of p-coumaric acid and p-coumaroyl derivatives from the digested fraction of peanut through Caco-2 cells

As observed in the non-digested extract and in the digested fraction, *p*-coumaroyltartaric acid (isomer 1) was the compound found in the highest concentration (203.69 μg *p*-CAE/g) in the intestinal absorbed fraction (basolateral fraction) (Table 1). The order of the other compounds in terms of concentration in the intestinal absorbed fraction was: *p*-coumaric acid (86.21 μg *p*-CAE/g), di-*p*-coumaroyltartaric acid (75.29 μg *p*-CAE/g), *p*-coumaroyltartaric acid (isomer 2) (33.34 μg *p*-CAE/g), the unidentified *p*-coumaroyl derivative (peak 4) (21.18 μg *p*-CAE/g), *p*-coumaroylsinapoyltartaric acid (11.80 μg *p*-CAE/g), and *p*-coumaroylferuloyltartaric acid (4.68 μg *p*-CAE/g).

Compared with the digested fraction, almost all quantified compounds had a significant decrease ($p < 0.05$) after Caco-2 cell transport, except the *p*-coumaroylferuloyltartaric acid, which small quantity in the digested fraction doubled in the transported fraction. However, when compared with the non-digested extract, almost all compounds had decreased concentrations in the basolateral fraction, giving Caco-2 cell transport of 14 % to *p*-coumaroylferuloyltartaric acid, ca. 20 % to *p*-coumaroyltartaric acid (isomer 2), di-*p*-coumaroyltartaric acid and *p*-coumaroylsinapoyltartaric acid and of ca. 30 % to the unidentified *p*-coumaroyl derivative and *p*-coumaroyltartaric acid (isomer 1), as shown at Fig. 3.

Those differences in transport across the Caco-2 cell monolayer may be related with differences in chemical structures of the compounds, resulting in different affinities for enzymes or membrane transporters found in the enterocyte cells (Teng et al., 2012). Additionally, since whole peanut was used for simulated digestion and Caco-2 cell transport, some phenolics may have formed complexes with dietary fiber or other peanut components, hampering their epithelial transport (Quirós-Sauceda et al., 2014).

Our results for Caco-2 cell transport of the intact *p*-coumaroyl derivatives are comparable with the amounts of resveratrol from boiled and roasted peanuts transported across the Caco-2 cells after *in vitro* digestion, which varied from ca. 5 % to 28 % (Chukwumah et al., 2011). Bioactive compounds from food matrices generally show low bioavailability, which ranges between 2 % and 30 % (Carbonell-Capella, Buniowska, Barba, Esteve, & Frígola, 2014; Feng et al., 2021; Wu et al., 2017).

The exception was the free *p*-coumaric acid, with 127 % of Caco-2 cell transport, which was outstanding, although its concentration after transport was not statistically different from its concentration in the non-digested extract ($p < 0.05$) (Table 1). The transepithelial transport of free *p*-coumaric acid is likely via two mechanisms, namely: 1) passive diffusion; and 2) the monocarboxylic acid transporter, which is more effective than the paracellular pathway used for other phenolics, such as gallic acid (Konishi, Kobayashi, & Shimizu, 2003). According to *in vivo* studies, the free form of *p*-coumaric acid is easily absorbed in the gastrointestinal tract, while *p*-coumaric acid conjugates with tartaric acid are absorbed in a very low proportion (Pei et al., 2016).

3.4. Effect of the simulated gastrointestinal digestion and Caco-2 cell transport on total phenolic content (TPC) and peroxyl scavenging activity

As *p*-coumaric acid and *p*-coumaroyl derivatives act as antioxidants *in vivo*, TPC and peroxyl scavenging activity were evaluated throughout our experiment (Fig. 4). TPC increased continuously from 2,074.06 μg GAE/g in the non-digested extract to 4,930.18 μg GAE/g in the digested fraction (138 % higher), reaching 5992.81 μg GAE/g in the transported fraction (189 % higher), as shown at Fig. 4A. However, peroxyl scavenging activity was not statistically different among the non-digested extract and the other fractions ($p < 0.05$), as shown at Fig. 4B, and no linear correlation between TPC and peroxyl scavenging activity was found, as shown at Table 2 ($r = -0.068$, $p = 0.862$).

Furthermore, negative correlation coefficients were obtained between TPC and total identified compounds, as well as between TPC and the majority of identified compounds, as taken individually (Table 2). Since peanut is a complex matrix, one hypothesis to explain the increase on TPC throughout simulated digestion would be the release of non-

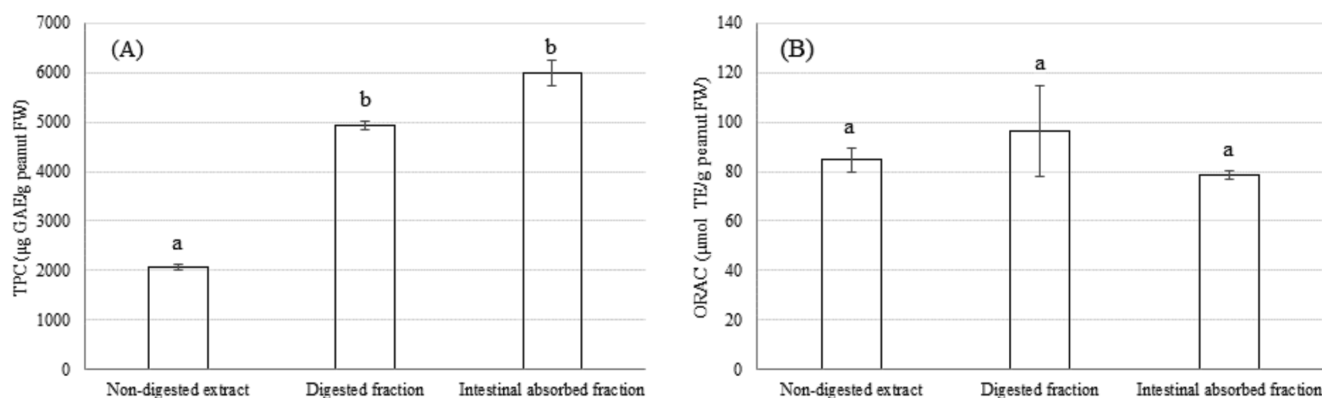

Fig. 4. Total phenolic content (TPC) (A) and peroxyl scavenging activity (B) of the non-digested extract, the digested fraction and the transported fraction (basolateral fraction) of peanut. GAE: gallic acid equivalent. TE: Trolox equivalent. FW: fresh weight. Different letters indicate statistically significant differences (ANOVA and *post hoc* Tukey' test, $p < 0.05$).

Table 2
Pearson correlation coefficients (p value) between total phenolic content, peroxyl scavenging activity, total identified compounds and the identified compounds in peanut.

	Total phenolic content	Peroxyl scavenging activity	Total identified compounds
Peroxyl scavenging activity	−0.068 (p = 0.862)		
Total identified compounds	−0.770 (p = 0.015)	0.452 (p = 0.222)	
1	−0.609 (p = 0.082)	0.521 (p = 0.150)	0.974 (p = 0.000)
2	−0.879 (p = 0.020)	0.345 (p = 0.363)	0.980 (p = 0.000)
3	0.339 (p = 0.372)	0.629 (p = 0.069)	0.328 (p = 0.389)
4	−0.664 (p = 0.051)	0.537 (p = 0.136)	0.987 (p = 0.000)
5	−0.984 (p = 0.000)	−0.034 (p = 0.931)	0.668 (p = 0.049)
6	−0.979 (p = 0.000)	−0.056 (p = 0.886)	0.656 (p = 0.055)
7	−0.926 (p = 0.000)	−0.092 (p = 0.815)	0.531 (p = 0.142)
1 + 2	–	0.498 (p = 0.172)	–
1 + 3	–	0.605 (p = 0.084)	–
1 + 4	–	0.523 (p = 0.149)	–
1 + 2 + 3	–	0.583 (p = 0.099)	–
1 + 3 + 4	–	0.602 (p = 0.086)	–
1 + 2 + 4	–	0.501 (p = 0.169)	–
2 + 3 + 4	–	0.668 (p = 0.049)	–
2 + 3	–	0.677 (p = 0.045)	–
2 + 4	–	0.412 (p = 0.270)	–
3 + 4	–	0.673 (p = 0.047)	–
1 + 2 + 3 + 4	–	0.581 (p = 0.101)	–

1: *p*-Coumaroyltartaric (isomer 1); 2: *p*-Coumaroyltartaric (isomer 2); 3: *p*-Coumaric acid; 4: Unidentified *p*-coumaroyl derivative; 5: Di-*p*-coumaroyltartaric acid; 6: *p*-Coumaroylsinapoyltartaric acid; 7: *p*-Coumaroylferuloyltartaric acid. –: not determined. Level of confidence of 0.05.

phenolics that do not act as peroxyl scavengers during digestion, such as amino acids and peptides, which could have been transported across the differentiated Caco-2 cell monolayer and reacted with the Folin-Ciocalteau reagent (Pavan, Sancho, & Pastore, 2014).

Conversely, positive and strong linear correlations were found between total identified compounds and all identified compounds as taken individually, although it was not statistically significant ($p > 0.05$) for *p*-coumaric acid, *p*-coumaroylsinapoyltartaric acid and *p*-coumaroylferuloyltartaric acid (Table 2). Therefore, those compounds followed similar patterns of concentration changes throughout the experiment.

No statistically significant correlation coefficients ($p > 0.05$) were obtained between peroxyl scavenging activity and total identified compounds or any identified compound as taken individually (Table 2). However, moderate (r between 0.6 and 0.7) and statistically significant ($p < 0.05$) linear correlations were found between peroxyl scavenging activity and different combinations of *p*-coumaroyltartaric acid (isomer 2), *p*-coumaric acid, and the unidentified *p*-coumaroyl derivative, as shown at Table 2. Hence, one hypothesis is that synergism between the major phenolics to scavenge the peroxyl radical is influenced more by their concentration proportions than by their concentrations as taken individually. Synergistic antioxidant effects of phenolics under physiological conditions have been reported (Martinelli et al., 2021).

Other hypothesis is the formation of other compounds able to scavenge peroxyl radicals even when found in very small concentrations, which were not investigated in our study. It is noteworthy that the differentiated Caco-2 cell monolayer have intracellular glucuronosyltransferases and sulfotransferases able to form glucuronide and sulfate conjugates of hydroxycinnamates as metabolites, as well as *O*-methylated derivatives of those compounds (Farrell, Poquet, Dionisi, Barron, & Williamson, 2011).

TPC and antioxidant activity throughout simulated digestion seems to vary greatly, depending on the food matrix. TPC and ORAC values increased>10-fold after digestion in five millet varieties (Chandrasekara & Shahidi, 2012). Conversely, despite a 51 % increase in the TPC, the peroxyl scavenging activity decreased 70 % in cooked lentils after digestion (Zhang et al., 2017).

In summary, the simulated gastrointestinal digestion method followed by an enterocyte-like differentiated Caco-2 cell monolayer transport assay was useful to predict the bioaccessibility and intestinal permeability of *p*-coumaric acid and *p*-coumaroyl conjugates with tartaric, sinapic and ferulic acids in whole peanut. The *p*-coumaroyl derivatives were cleaved at a certain extension, producing more *p*-coumaric acid in its free form. There is indication that the *in vitro* bioaccessibility of the *p*-coumaroyl derivatives found in whole peanut is related with their solubility, since the *p*-coumaroyl conjugates with sinapic acid were more accessible than the *p*-coumaroyl conjugates with tartaric acid. Caco-2 cell transport (%) was the highest for the free form of *p*-coumaric acid, which was previously shown to be highly absorbed through the gastrointestinal tract in *in vivo* studies. Despite the lowered total concentrations of the identified phenolics in the digested and transported fractions, the peroxyl scavenging activity of peanut remained unaltered ($p < 0.05$), which is likely related, at least partly, to a synergistic antioxidant effect of the concentration proportions of some phenolics, which changed throughout the experiment. Nevertheless, further studies to investigate other peanut compounds with peroxyl scavenging activity formed during digestion and intestinal epithelial transport should be encouraged. However, our results obtained by

simulated gastrointestinal digestion and a cellular assay for epithelial transport indicate that whole peanut is a food source of bioavailable antioxidant phenolics.

CRediT authorship contribution statement

Adna Prado Massarioli: Formal analysis, Investigation, Writing – review & editing, Visualization, Data curation. **Alan Giovanini de Oliveira Sartori:** Writing – original draft, Visualization, Visualization. **Fernanda Francetto Juliano:** Formal analysis, Validation. **José Eduardo Pedroso Gomes do Amaral:** Investigation, Validation. **Roseane Cavalcanti dos Santos:** Conceptualization, Resources, Supervision. **Liziane Maria de Lima:** Resources. **Severino Matias de Alencar:** Conceptualization, Resources, Supervision, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Apak, R. (2019). Current Issues in Antioxidant Measurement. *Journal of Agricultural and Food Chemistry, 67*(33), 9187–9202. https://doi.org/10.1021/acs.jafc.9b03657

Bansode, R. R., Randolph, P., Ahmedna, M., Hurley, S., Hanner, T., Baxter, S. A. S., … Williams, L. L. (2014). Bioavailability of polyphenols from peanut skin extract associated with plasma lipid lowering function. *Food Chemistry, 148*, 24–29. https://doi.org/10.1016/j.foodchem.2013.09.129

Carbonell-Capella, J. M., Buniowska, M., Barba, F. J., Esteve, M. J., & 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. https://doi.org/10.1111/1541-4337.12049

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*(1), 226–237. https://doi.org/10.1016/j.jff.2011.11.001

Chukwumah, Y., Walker, L., Vogler, B., & Verghese, M. (2011). In vitro absorption of dietary trans -resveratrol from boiled and roasted peanuts in Caco-2 cells. *Journal of Agricultural and Food Chemistry, 59*(23), 12323–12329. https://doi.org/10.1021/jf202535q

Ding, X., Hu, X., Chen, Y., Xie, J., Ying, M., Wang, Y., & Yu, Q. (2021). Differentiated Caco-2 cell models in food-intestine interaction study: Current applications and future trends. *Trends in Food Science and Technology, 107*(November 2020), 455–465. https://doi.org/10.1016/j.tifs.2020.11.015

El-Seedi, H. R., El-Said, A. M. A., Khalifa, S. A. M., Göransson, U., Bohlin, L., Borg-Karlson, A. K., & Verpoorte, R. (2012). Biosynthesis, natural sources, dietary intake, pharmacokinetic properties, and biological activities of hydroxycinnamic acids. *Journal of Agricultural and Food Chemistry, 60*(44), 10877–10895. https://doi.org/10.1021/jf301807g

Farrell, T., Poquet, L., Dionisi, F., Barron, D., & Williamson, G. (2011). Characterization of hydroxycinnamic acid glucuronide and sulfate conjugates by HPLC – DAD – MS 2: Enhancing chromatographic quantification and application in Caco-2 cell metabolism. *Journal of Pharmaceutical and Biomedical Analysis, 55*(5), 1245–1254. https://doi.org/10.1016/j.jpba.2011.03.023

Feng, S., Yi, J., Li, X., Wu, X., Zhao, Y., Ma, Y., & Bi, J. (2021). Systematic Review of Phenolic Compounds in Apple Fruits: Compositions, Distribution, Absorption, Metabolism, and Processing Stability. *Journal of Agricultural and Food Chemistry, 69*(1), 7–27. https://doi.org/10.1021/acs.jafc.0c05481

Galanakis, C. (2017). *Nutraceutical and Functional Food Components* (1st ed). Elsevier.

Hemery, Y. M., Anson, N. M., Havenaar, R., Haenen, G. R. M. M., Noort, M. W. J., & Rouau, X. (2010). Dry-fractionation of wheat bran increases the bioaccessibility of phenolic acids in breads made from processed bran fractions. *Food Research International, 43*(5), 1429–1438. https://doi.org/10.1016/j.foodres.2010.04.013

Hubatsch, I., Ragnarsson, E. G. E., & Artursson, P. (2007). Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. *Nature Protocols, 2*(9), 2111–2119. https://doi.org/10.1038/nprot.2007.303

Juliano, F. F., Alvarenga, J. F. R., Lamuela-Raventos, R. M., Massarioli, A. P., Lima, L. M., Santos, R. C., & Alencar, S. M. (2020). Polyphenol analysis using high-resolution mass spectrometry allows differentiation of drought tolerant peanut genotypes. *Journal of the Science of Food and Agriculture, 100*(2), 721–731. https://doi.org/10.1002/jsfa.10075

Konishi, Y., Kobayashi, S., & Shimizu, M. (2003). Transepithelial transport of p-coumaric acid and gallic acid in Caco-2 cell monolayers. *Bioscience, Biotechnology and Biochemistry, 67*(11), 2317–2324. https://doi.org/10.1271/bbb.67.2317

Liao, X., Brock, A. A., Jackson, B. T., Greenspan, P., & Pegg, R. B. (2020). The cellular antioxidant and anti-glycation capacities of phenolics from Georgia peaches. *Food Chemistry, 316*(July 2019), 126234. https://doi.org/10.1016/j.foodchem.2020.126234

Lozano, M. G., de Oliveira Sartori, A. G., Markowicz Bastos, D. H., & Bismara Regitano-d'Arce, M. A. (2019). Selected nutrients and antinutrients in peanut cultivars harvested in Brazil. *Journal of the Science of Food and Agriculture, 99*(12), 5334–5340. https://doi.org/10.1002/jsfa.9772

Ma, Y., Kosińska-Cagnazzo, A., Kerr, W. L., Amarowicz, R., Swanson, R. B., & Pegg, R. B. (2014). Separation and characterization of phenolic compounds from dry-blanched peanut skins by liquid chromatography-electrospray ionization mass spectrometry. *Journal of Chromatography A, 1356*, 64–81. https://doi.org/10.1016/j.chroma.2014.06.027

Martinelli, E., Granato, D., Azevedo, L., Gonçalves, J. E., Lorenzo, J. M., Munekata, P. E. S., … Lucini, L. (2021). Current perspectives in cell-based approaches towards the definition of the antioxidant activity in food. *Trends in Food Science and Technology, 116*(April), 232–243. https://doi.org/10.1016/j.tifs.2021.07.024

Melo, P. S., Massarioli, A. P., Denny, C., Dos Santos, L. F., Franchin, M., Pereira, G. E., … De Alencar, S. M. (2015). Winery by-products: Extraction optimization, phenolic composition and cytotoxic evaluation to act as a new source of scavenging of reactive oxygen species. *Food Chemistry, 181*, 160–169. https://doi.org/10.1016/j.foodchem.2015.02.087

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., … Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food-an international consensus. *Food and Function, 5*(6), 1113–1124. https://doi.org/10.1039/c3fo60702j

Minitab, I. (2021). *Minitab 20 Statistical Software* (20.4). www.minitab.com.

Nićiforović, N., & Abramovič, H. (2014). Sinapic acid and its derivatives: Natural sources and bioactivity. *Comprehensive Reviews in Food Science and Food Safety, 13*(1), 34–51. https://doi.org/10.1111/1541-4337.12041

Pavan, V.Ô., Sancho, R. A. S., & Pastore, G. M. (2014). The effect of invitro digestion on the antioxidant activity of fruit extracts (Carica papaya, Artocarpus heterophillus and Annona marcgravii). *LWT - Food Science and Technology, 59*(2P2), 1247–1251. https://doi.org/10.1016/j.lwt.2014.05.040

Pei, K., Ou, J., Huang, J., & Ou, S. (2016). p-Coumaric acid and its conjugates: Dietary sources, pharmacokinetic properties and biological activities. *Journal of the Science of Food and Agriculture, 96*(9), 2952–2962. https://doi.org/10.1002/jsfa.7578

Phan-Thien, K. Y., Wright, G. C., & Lee, N. A. (2014). Peanut antioxidants: Part 2. Quantitation of free and matrix-bound phytochemicals in five selected genotypes with diverse antioxidant capacity by high performance liquid chromatography (HPLC). *LWT - Food Science and Technology, 57*(1), 312–319. https://doi.org/10.1016/j.lwt.2013.12.020

Quirós-Sauceda, A. E., Palafox-Carlos, H., Sáyago-Ayerdi, S. G., Ayala-Zavala, J. F., Bello-Perez, L. A., Álvarez-Parrilla, E., … González-Aguilar, G. A. (2014). Dietary fiber and phenolic compounds as functional ingredients: Interaction and possible effect after ingestion. *Food & Function, 5*(6), 1063–1072. https://doi.org/10.1039/C4FO00073K

R Core Team. (2020). *R: A Language and Environment for Statistical Computing.* https://www.r-project.org.

Regueiro, J., Sánchez-González, C., Vallverdú-Queralt, A., Simal-Gándara, J., Lamuela-Raventós, R., & Izquierdo-Pulido, M. (2014). Comprehensive identification of walnut polyphenols by liquid chromatography coupled to linear ion trap-Orbitrap mass spectrometry. *Food Chemistry, 152*, 340–348. https://doi.org/10.1016/j.foodchem.2013.11.158

Rodrigues, D. B., Mariutti, L. R. B., & Mercadante, A. Z. (2016). An in vitro digestion method adapted for carotenoids and carotenoid esters: Moving forward towards standardization. *Food & Function, 7*(12), 4992–5001. https://doi.org/10.1039/C6FO01293K

Shen, Y., Song, X., Li, L., Sun, J., Jaiswal, Y., Huang, J., … Guan, Y. (2019). Protective effects of p-coumaric acid against oxidant and hyperlipidemia-an in vitro and in vivo evaluation. *Biomedicine and Pharmacotherapy, 111*(December 2018), 579–587. https://doi.org/10.1016/j.biopha.2018.12.074

Sullivan, M. L. (2014). Perennial peanut (Arachis glabrata Benth.) leaves contain hydroxycinnamoyl-CoA:Tartaric acid hydroxycinnamoyl transferase activity and accumulate hydroxycinnamoyl-tartaric acid esters. *Planta, 239*(5), 1091–1100. https://doi.org/10.1007/s00425-014-2038-x

Tan, S. S., Tan, C. X., & Tan, S. T. (2020). Cold pressed peanut (Arachis hypogaea L.) oil. In *Cold Pressed Oils* (pp. 357–364). Elsevier Inc.. https://doi.org/10.1016/B978-0-12-818188-1.00032-3

Teng, Z., Yuan, C., Zhang, F., Huan, M., Cao, W., Li, K., Yang, J., Cao, D., Zhou, S., & Mei, Q. (2012). Intestinal absorption and first-pass metabolism of polyphenol compounds in rat and their transport dynamics in caco-2 cells. *PLoS ONE, 7*(1), 1–9. https://doi.org/10.1371/journal.pone.0029647

U.S. Food and Drug Administration. (2003). *Food Qualified Health Claims : Letter of Enforcement Discretion - Nuts and Coronary Heart Disease (Docket No 02P0505)*.

Wu, T., Grootaert, C., Voorspoels, S., Jacobs, G., Pitart, J., Kamiloglu, S., … Van Camp, J. (2017). Aronia (Aronia melanocarpa) phenolics bioavailability in a combined in vitro digestion/Caco-2 cell model is structure and colon region dependent. *Journal of Functional Foods, 38*, 128–139. https://doi.org/10.1016/j.jff.2017.09.008

Zhang, B., Deng, Z., Tang, Y., Chen, P. X., Liu, R., Dan Ramdath, D., … Tsao, R. (2017). Bioaccessibility, in vitro antioxidant and anti-inflammatory activities of phenolics in cooked green lentil (Lens culinaris). *Journal of Functional Foods, 32*, 248–255. https://doi.org/10.1016/j.jff.2017.03.004

Zhao, Z., & Moghadasian, M. H. (2010). Bioavailability of hydroxycinnamates: A brief review of in vivo and in vitro studies. *Phytochemistry Reviews, 9*(1), 133–145. https://doi.org/10.1007/s11101-009-9145-5