Research Article

Received: 19 April 2023 Revised: 28 September 2023 Accepted article published: 27 October 2023 Published online in Wiley Online Library: 13 November 2023

(wileyonlinelibrary.com) DOI 10.1002/jsfa.13078

Unveiling the bioactivity and bioaccessibility of phenolic compounds from organic coffee husks using an *in vitro* digestion model

Thaianaly Leite Abreu,[a] Mario Estévez,[b*] Leila Moreira de Carvalho,[a] Lorena Lucena de Medeiros,[a] Valquíria Cardoso da Silva Ferreira,[a] Bruno Ramos Salu,[c] Maria Luiza Vilela Oliva,[c] Marta Suely Madruga[a] and Taliana Kênia Alencar Bezerra[a]

Abstract

BACKGROUND: The large quantities of by-products generated in the coffee industry are a problem. Studies related to the biological potential of organic coffee husks are still limited. The aim of this work was to investigate the occurrence of phenolic compounds in organic coffee husks and to evaluate their potential as a source of bioactive dietary components.

RESULTS: To achieve this objective, three extracts were prepared, namely extractable polyphenols (EPs), hydrolyzable non-extractable polyphenols (H-NEPs), and non-extractable polyphenols (NEPs). These extracts were characterized and evaluated for their bioactive properties after simulated gastrointestinal digestion. The results show that the extraction process affected the occurrence of phenols from coffee peels, especially for caffeic acid, gallic acid, and chlorogenic acid. The free and bound polyphenols found in the extracts and digests not only showed antioxidant properties against 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals but were also strongly bioavailable and had good anticoagulant potential.

CONCLUSION: These results highlight the potential health benefits of phytochemicals from coffee husks and open new perspectives for the use of such compounds in dietary supplements.

Keywords: polyphenols; bioaccessibility; antioxidant; anticoagulant; simulated digestion; circular economy

INTRODUCTION

Coffee holds the distinction of being the most widely consumed energy beverage globally, with Brazil emerging as the foremost producer and exporter. Coffee exerts a significant influence on the nation's economy, and the processing of coffee beans yield numerous by-products, since approximately 50% of the total industrial input turns into waste materials.[1]

The production of organic coffee aims to be environmentally sustainable by reducing the use of pesticides and fertilizers and optimizing production processes. One way to reduce the environmental impact of organic coffee production further is to reuse the husks as raw material in a circular economy system.[2] There is growing interest in using waste materials from the agricultural processing industry in the food industry due to their potential 'natural antioxidant' content.[3]

On the other hand, bioactive compounds must be bioaccessible so that they can display the desired health effects in internal target organs.[4] Knowledge concerning phenolic intake, together with the bioaccessibility/bioavailability of bioactive compounds throughout the gastrointestinal tract, is fundamental for the assessment of their biological importance for human health.[5]

These by-products have antioxidant properties; they also have anticoagulant and antihypertensive effects and other benefits due to the presence of bioactive compounds. They are widely found in plants and can be extracted using organic solvents.[6]

* *Correspondence to: M Estévez, IPROCAR Research Institute, TECAL Research Group, Universidad de Extremadura, 10003, Cáceres, Spain. E-mail: mariovet@unex.es*

a *Post-Graduate Program in Food Science and Technology, Department of Food Engineering, Technology Centre, Federal University of Paraiba, João Pessoa, Brazil*

b *IPROCAR Research Institute, TECAL Research Group, Universidad de Extremadura, Cáceres, Spain*

c *Department of Biochemistry, Federal University of São Paulo, São Paulo, Brazil*

Non-extractable polyphenols (NEPs) are a type of polyphenol found in plant by-products, which cannot be extracted using traditional methods. They are often associated with higher molecular weight compounds such as fibers, proteins, and melanoidins. Non-extractable polyphenols can be divided into three categories: hydrolyzable polyphenols, low molecular weight phenols, and non-extractable proanthocyanidins.[7,8] Proanthocyanidins are complex polymeric compounds belonging to the class of polyphenols commonly referred to as condensed tannins. They consist of repetitive units of flavanols, linked by C—C and C—O—C bonds, forming linear or branched structures. Their structural characteristics vary according to the type of flavanol and the degree of polymerization.[4] These polyphenols have been shown to have antioxidant, anticoagulant, cardio-protective, antithrombotic, and anti-inflammatory effects, and may be useful as dietary supplements. Further research is needed to explore the potential health benefits of these compounds and to find ways to extract and utilize them.[5,6]

The objective of this work was to investigate the phytochemical composition, *in vitro* bioaccessibility, and antioxidant and anticoagulant effects of polyphenolic extracts found in organic coffee processing by-products.

MATERIAL AND METHODS

Material

Organic coffee husks, comprising 100% Arabica and Typica varieties, were obtained from Taquaritinga do Norte, Pernambuco, Brazil (7° 88′ 81″ S, 36° 5′ 33″ W). These organic coffee beans were cultivated using shading techniques and were hand-harvested. Following the wet processing of coffee, the husks were meticulously separated from the beans and subsequently frozen at −18 °C until the commencement of the study.

Sample preparation

The coffee husks were dried in a forced circulation oven at 40 °C for 48 h to obtain the phenolic compounds. The conditions used in this process are similar to those described in a previous study by Silva *et al.*[9] The dried husks were then ground to approximately 2 mm using a Wiley-type grinder (Solab, SL-31, São Paulo, Brazil). They were subsequently placed within hermetically sealed containers, shielded from light, and refrigerated at −6 °C until the analysis was conducted.

Physical and chemical characterization of organic cultivated coffee flour

Physical and chemical characterization of organic coffee husk meal (in bulk) was performed. Lightness (L*), red/green intensity (a*), and yellow/blue intensity (b*) were measured using a Konica Minolta colorimeter (model CR −400, Osaka, Japan), under specified conditions: 8 mm diameter aperture, illuminant D65 and 0° standard observer. The chemical analysis of the organic coffee husk meal (moisture, ash, proteins, lipids and minerals) was performed according to the methodology described by AOAC.[10]

Sugar profile

The sugar profile was determined using the methodology described by Zeppa *et al.*[11] For this purpose, 2 g of the sample was used, diluted in 10 mL of ultrapure water, centrifuged for 10 min (6000 × *g*, 4 °C), and filtered through a 0.45 μm cellulose filter. The sugar profile was quantified using a Varian high-performance liquid chromatography system, model 1260 Infinity LC (Varian, Palo Alto, CA, USA), coupled to an Agilent (Santa Clara, CA, USA) Hi Plex Ca column (7.7 × 300 mm, 8 μ), at a temperature of 85 °C, with a refractive index detector (Varian) and a manual 20 μL loop sampler. The results were expressed in g sugar/100 g.

Organic acid profile

The profile of organic acids was determined following the methodology described by Zeppa *et al.*[11] using high-performance liquid chromatography (HPLC) and ultraviolet–visible spectroscopy (UV–visible). Two grams of the sample was used, diluted in 10 mL of ultrapure water, centrifuged for 10 min (6000 × *g*, at 4 °C), and filtered through a 0.45 μm cellulose filter. Quantification of the organic acid profile was performed using methods similar to those described for the determination of the sugar profile. The results are expressed in g of acid/100 g.

Preparation of the organic coffee husk extracts

Extractable polyphenols

Following the method proposed by Silva *et al.*,[9] the organic coffee husk flour was weighed directly into a Falcon tube and homogenized manually for 5 min with an extraction solution of water and ethanol (1:1). The ratio of flour to extraction solution was 1:10 (w:v). The resulting mixture was incubated in a water bath at 60 °C for 60 min and then centrifuged at 3500 × *g* for 20 min at 10 °C. The supernatant was then collected and filtered through filter paper. The extract obtained was rota-evaporated at a temperature of 60 °C for 5 consecutive hours (Fisatom 802, São Paulo, Brazil). Then, the extract was made up with ultrapure water to complete the original extract volume.

Hydrolyzable non-extractable polyphenols

First, 0.5 g of the flour was weighed in 20 mL of acidified methanol with HCl to pH 2.0 and water (50:50, v/v), and placed in an ultrasonic extraction bath (Unique, model USC-1800, São Paulo, Brazil) at 40 kHz for 30 min at 25 °C. The sample was centrifuged at 1372 × *g* for 15 min, centrifuge model SL −701 (Solab, São Paulo, Brazil). The supernatant was retrieved, and the remaining residue from the extract was once again subjected to extraction using 20 mL of acetone/water (70:30, v/v), following the same sonication and centrifugation procedures once more. The methanolic and acetone extracts were combined in a 25 mL volumetric flask and the volume was made up with deionized water.[7] The extract was concentrated to a final volume of 5 mL using a rotary evaporator (Fisatom 802, São Paulo, Brazil). An aliquot was filtered to obtain NEPs.

Non-extractable polyphenols

The supernatant of extractable polyphenols (EPs) was hydrolyzed with methanol and sulfuric acid (80:20 v/v) for 20 h at 85 °C. The extract was then centrifuged under the same conditions as EP, the pH was adjusted to 5.5 with NaOH, and the extract was filtered with a quality filter. After filtration, the extract was reconstituted twice, first with methanol (5 mL) and 50% methanol (50:50 v/v) (5 mL), and after the first wash, 1 mL of the sample was diluted again with methanol (1 mL) and 80% methanol (1 mL). Finally, the contents were combined and roto-evaporated under the same conditions as for the EP.[12]

In vitro gastrointestinal digestion assay

The study of released bioactive compounds in the particles with simulated gastric and intestinal fluids was performed following the method used by Minekus *et al.*[13] Aliquots of each extract were prepared: EPs, hydrolyzable NEPs (H-NEPs), and NEPs, to determine the effects of the simulated gastrointestinal conditions. Stock solutions of electrolytes and enzymes comprising the oral, gastric, and

intestinal phases were prepared in accordance with international consensus as reported by INFOGEST (INRAE, Rennes, France). Bioaccessibility was expressed as a percentage and determined according to Eqn (1).[14]

$$bioaccessibility\ (\%) = (CF\ digested / CF\ undigested) \times 100 \quad (1)$$

where: *CF digested* is the concentration of the analyzed compound from digested coffee husks (intestinal phase), and *CF undigested* is the concentration of analyzed compound from undigested coffee husks (initial extract).

Digestive enzymes like pepsin, pancreatin, and others are used to simulate the natural digestive process. The concentration of enzymes can vary based on the intended simulation (e.g., gastric or intestinal digestion). Pepsin, for example, is commonly used for gastric digestion and its concentration might be around 0.5-1 mg mL^{-1} in simulated gastric fluid. The pH conditions are adjusted to replicate the acidic environment of the stomach (gastric phase) and the more neutral environment of the small intestine (intestinal phase). The gastric phase typically has a lower pH (3.0) whereas the intestinal phase is usually around pH 7.0.

In vitro analysis of bioactive compounds from organic coffee husk extract in gastrointestinal digestion fluids

Determination of total phenolic compounds

The total phenolic content of the extracts was determined using the Folin–Ciocâlteu method. Aliquots of the standard were mixed with distilled water, Folin–Ciocâlteu reagent, and sodium carbonate, and the mixture were allowed to rest in the dark for 2 h before being read in a UV-visible spectrophotometer at 765 nm. The results were calculated using a standard curve for chlorogenic acid (10 to 50 μg mL^{-1}), and the total phenolic compound content was expressed in milligrams of chlorogenic acid equivalents (CAE) per 100 g of sample (mg CAE/100 g).[9]

Determination of total flavonoids

To measure the total flavonoid content of a sample, an aliquot of the extract was mixed with sodium nitrite and aluminum chloride to produce a colored complex. The absorbance of this complex was then measured at 510 nm using a UV-visible spectrophotometer (Quimis, Q798U, São Paulo, Brazil), and the total flavonoid content was expressed in milligrams of epicatechin (50–800 μg mL^{-1}) equivalent per gram of sample (mg epicatechin (EC)/100 g). A standard curve with catechin was used to determine the concentration of flavonoids in the sample.[9]

Phenolic compounds profile

The profile of phenolic compounds in organic coffee husk extracts was determined using reverse-phase HPLC and UV-visible with a C18 Shim-pack column (with an inner diameter of 4.6 mm and a length of 250 mm). A 20 μL aliquot of each sample was injected into the thermostat column at 40 °C, using a mobile phase composed of acetonitrile/0.1% formic acid (15:85, v/v) flowing at a rate of 0.8 mL min^{-1}. For all samples, the final concentration of compounds was determined by the average of the results of two consecutive injections.[15]

Assessment of organic coffee-husk extract digests bioactivity

2,2-Diphenyl-1-picrylhydrazyl radical sequestering activity

The ability of coffee husk extracts to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined using a method described by Brand-Williams *et al.*[16] The radical scavenging activity and antioxidant capacity of the coffee husk extracts were measured in triplicate using a UV–visible spectrophotometer (Quimis) 515 nm. The results were expressed in terms of equivalent Trolox antioxidant activity per gram of sample.

2,2-Azino-bis (3-ethylbeothiazoline)-6-sulfonic acid radical sequestering activity

The ability to sequester the 2,2-azino-bis (3-ethylbeothiazoline)-6-sulfonic acid (ABTS• +) radical was determined according to the method proposed by Re *et al.*[17] The radical elimination activity as the antioxidant capacity of the coffee husk extracts was verified at 734 nm in a UV-visible spectrophotometer (Quimis, Quito, Ecuador). Results were expressed as equivalent Trolox antioxidant activity per gram of sample.

Ferric reducing antioxidant power

The ferric reducing capacity was evaluated using the ferric reducing antioxidant power (FRAP) method described by Benzie *et al.*[18] The antioxidant potential, or ability of the coffee husk extracts to reduce iron (Fe^{3+}) to the ferrous form (Fe^{2+}) was verified at 593 nm in a UV-visible spectrophotometer (Quimis). Based on the calibration curve prepared with different concentrations of Trolox (50–1000 μM), the results were expressed as μmol of Trolox/ equivalent per gram of sample.

Coagulation test: determination of activated partial thromboplastin time (APPT) and prothrombin time (PT)

Both determinations were performed in a dual-channel semiautomated coagulometer designed for low-volume laboratories (BF II coagulometer, Dade Behring, Glasgow, Delaware, USA), according to the procedure described by Salu *et al.*[19] Plasma for the assays was obtained from whole blood of three healthy volunteers, collected in 3.8% (w/v) trisodium citrate (1/10), and centrifuged at 1726 × *g* for 15 min (25 °C). Assays were performed in duplicate, and results were expressed in minutes.

Statistical analysis

The entire experimental trial was performed in triplicate, where the differences between the phenolic compound content and antioxidant activity of organic coffee husk extracts were evaluated using analysis of variance (ANOVA) and mean (Tukey test, $P \leq 0.05$). All statistical analyses were performed with Statistica Software 5.1.

RESULTS AND DISCUSSION

Physical and chemical characterization of organic coffee husk flour

In summary, coffee husks are a by-product of coffee processing, which can be dried and ground into flour.[9] According to the mass yield result, after drying it was 20% of the initial volume of the untreated husks (prior to drying), which is valuable for both economic and environmental reasons as it can reduce waste and result in more efficient utilization of the raw material in its entirety.[8]

Coffee husks are naturally acidic and the pH can vary depending on factors such as soil, climate, and processing conditions. The Codex Alimentarius[20] recommends a moisture content of 15.5% for flours. There are no specific guidelines for the moisture content of vegetable by-product flours but the moisture content (which is related to the low pH of 3.97) found in coffee husk flour is considered favorable for microbiological stability and safety.

The lightness (L*) measure determined in this study (45.37) showed a tendency towards a darker color, but approximately in the middle range of the scale. The variable a* places the color on the green-red axis and the variable b* on the blue-yellow axis. For the red intensity, the determined value of 5.13 shows a predominance of the red/brown color, characteristic of coffee samples. In total, b* (9.03) was characterized as a pronounced yellowish tendency.

The chemical composition of coffee (beans and by-products) depends on the origin, variety, development, and harvest. Different chemical compounds may also be present due to organic farming.[21] Proteins and lipids from coffee husks play significant roles in their composition and potential applications. Proteins can serve as sources of essential amino acids and contribute to the nutritional value of the product, especially when coffee husks are considered for human consumption or animal feed. Proteins can also influence the texture and quality of coffee flour in specific culinary or baking applications.[8]

Lipids in coffee husks also hold nutritional and functional relevance. They can contain essential fatty acids and other beneficial compounds. Lipids can enhance the palatability and flavor of the end product and contribute to texture and ingredient binding in food products.[23] As can be seen in Table 1, the results of the present study are consistent with data from the literature, as dry coffee husks are composed of approximately 80% carbohydrates, 8–11% proteins, and 0.5–2% lipids.[8,22]

Although the chemical composition of coffee processing by-products varies, the values found in the present study for proteins (9.41%) and lipids (0.50%) are similar to those reported by the above authors. Coffee husks contain chemical compounds with high potential for use in various biotechnological processes but they also have environmental problems due to the toxicity of caffeine and tannins. These issues can be reduced by reusing coffee husks.[23]

The Brazilian agribusiness industry is required to certify its management systems through organizations like NBR-ISO14001 to demonstrate to society and the market that it is reducing negative impacts on biodiversity.[24] Organic farming in the coffee industry offers various benefits, including environmental and nutritional advantages and improved food safety. It also contributes to the control and regulation of coffee production and can be internationally certified.[21]

Coffee husks are a lignocellulosic by-product that contains a large quantity of dietary fiber and polyphenols, as well as polysaccharides, which are attached to the cell wall. They have the potential to be converted into food ingredients and can be used as substrates for fermentation by microorganisms and bioconversion reactions due to their high organic content, low cost, and availability.[7,25]

The major sugars found in coffee husks were glucose, xylose, fructose, and arabinose. These sugars can be used by microorganisms in bioprocesses to produce bioethanol, xylitol, and arabitol, and they also have prebiotic potential that can be exploited from coffee processing by-products.[26] The concentrations of xylose and arabinose in the present coffee husks were 3.40 mg mL^{-1} and 6.44 mg mL^{-1}, respectively.

Shankar *et al.*[27] reports that bioconversion of assorted husks by-products is a complex process that requires the use of pretreatments to break down polysaccharides and produce fermentable sugars. In the study, the authors carried out the saccharification of pretreated corn, peanut, and coffee hulls with lignocellulolytic enzymes to eventually produce bioethanol. However, the pretreatments of the husks for saccharification reported by these authors were optimized by the use of enzymes. Considering the data found in the present study and in the literature, it is evident that there is a strong biotechnological potential for coffee residues.

In addition to carbohydrates, we detected nine organic acids (citric acid, tartaric acid, ascorbic acid, malic acid, pyruvic acid, succinic acid, lactic acid, formic acid, and propionic acid) in coffee husks. These results are shown in Table 2. Malic acid, succinic acid, and ascorbic acid were all found at high concentrations (5.05 > 3.46 > 2.09 mg/100 g). Bressani *et al.*[28] reported values for fermented coffee beans with concentrations of citric acid (2.78 to 4.65 mg g^{-1}), succinic acid (0.58 and 2.40 mg g^{-1}), and tartaric acid (0.02–0.93 mg g^{-1}). Succinic acid had a higher concentration in the coffee husks. Phenolic compounds are generally found in conjugation with organic acids and sugars[4] and the findings of the present study help to explain many aspects of coffee husk metabolism, although they remain to be clarified because few data are available.

Table 1. Determination of physical and chemical parameters of organic coffee husk flour

Parameters	Organic coffee husk meal	Coffee husk*
Yield	20.09%	-
pH	3.97 ± 0.06	4.70–6.63
Color L*	45.37 ± 0.73	-
Color a*	5.13 ± 0.52	-
Color b*	9.03 ± 0.29	-
Humidity (g/100 g)	13.81 ± 0.49	12.00
Lipids (g/100 g)	0.50 ± 0.03	0.50–2.00
Proteins (g/100 g)	9.41 ± 0.17	9.20
Maltose (g/100 g)	3.72 ± 0.95	-
Glucose (g/100 g)	3.40 ± 0.35	-
Xylose (g/100 g)	3.32 ± 0.11	-
Fructose (g/100 g)	1.62 ± 0.10	-
Arabinose (g/100 g)	6.44 ± 0.04	-

*Values presented by the literature in research related to coffee husk.[8,22]

Bioaccessibility of phenolic compounds from polyphenol extracts in organic coffee husk

In this study, three organic coffee husk extracts were obtained using different extraction methods. To evaluate the stability and metabolism of the phenolic compounds in the extracts (EPs, H-NEPs, and NEPs), each treatment was subjected to an *in vitro* gastrointestinal simulation that mimics human organism conditions.

Studies with phenols generally underestimate the content of these compounds, because they include only low or medium molecular weight soluble polyphenols or EPs extracted using aqueous-organic solvents.[29]

A significant fraction of phenols remain in the residues of phenolic extractions, the so-called NEPs or macro-antioxidants, which can be divided into several fractions: i) H-NEPs, which are low molecular weight phenols strongly associated with polysaccharides and proteins, and ii) NEPs and non-extractable proanthocyanidins (PANE), which are high molecular-weight compounds and hence, difficult to extract.[7]

After being subjected to simulated *in vitro* digestion, the extracts (EP, H-NEP, and NEP) showed total phenolic concentrations ranging between 60.62 and 2.430 mg/100 g DW; with total

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Table 2. Organic acid profile of organic coffee husk flour

Organic acids (mg/100 g)	Organic coffee husk meal
Citric	1.59 ± 0.22
Tartaric	1.10 ± 0.17
Ascorbic	2.09 ± 0.51
Malic	5.05 ± 1.00
Pyruvic	0.14 ± 0.04
Succinic	3.46 ± 0.26
Lactic	0.71 ± 0.15
Formic	0.26 ± 0.05
Propionic	0.68 ± 0.85

flavonoids ranging from between 0.59 and 9.00 mg/100 g DW (Table 3). However, a study by Silva *et al.*,[9] which investigated different methods for the extraction of phenolic compounds and the antioxidant activity of coffee husks, found flavonoid levels ranging between 0.63 and 9.93 mg g^{-1} DW. The results are similar to those of this study and are relevant to EP. However, they indicated that the extraction method for polyphenols/macroantioxidants does not affect the retention of total flavonoids present in coffee husk.[4] Although flavonoids are the most abundant phenolics in teas and some fruits, coffee is considered the better source of phenolic acids.[5]

The retention of phenols was satisfactory and statistically different only in the NEP extracts, which had the lowest content (60.62 mg/100 g DW). However, Hartzfeld *et al.*,[12] reported an efficient H-NEP polyphenol extraction, using and fast chemical hydrolysis procedure that reduced the interaction between the polyphenols and the matrix, leading to a greater release due to bond cleavage.[4] In a study by Pérez-Jiménez *et al.*,[7] significantly different results were obtained when two hydrolysis methods were used to extract macro-antioxidants in peels of different fruits. The EP content of apple and pear peels were 1278 and 721 mg/100 g DW, respectively. In the same study, based on EP extraction, the polyphenol content in banana peels was 1961.3 mg/100 g DW, while for NEP it was 7667.2 mg/100 g DW. In the present study, similar behavior was observed, with the extraction being crucial for the retention of phenols in the H-NEP extracts. This result confirms that the selection of the extraction and processing procedure is crucial for the recovery of compounds with bioactive potential.[4,7]

There is a lot of information available in the literature about the presence of phenolic compounds in coffee husk by-products, but there are fewer studies on the bioavailability and bioaccessibility of these compounds. To have an effect on the body, bioactive compounds must be capable of being absorbed by the intestine and remain in circulation until they reach their target organ.[23] In terms of digestion/bioaccessibility, it was generally found that all extracts (EP, H-NEP, and NEP) had a significant decrease in concentration during the gastric phase of the *in vitro* digestion. The NEP for total phenols was the only group of compounds that were found to increase in concentration at this stage. Non-extractable polyphenols often display a chemical structure that renders them more susceptible to the acidic environment of the stomach. This acidity contributes to protein denaturation and the cleavage of

Table 3. Bioaccessibility of phenolic compounds and flavonoids from organic coffee husk polyphenol extracts

		Digestion *in vitro*			
Determination	Extracts types	Extract	Gastric phase	Intestinal phase bioaccessible	Bioaccessibility (%)
Total Phenolics mg CAE/100 g	**EP**	501.00 ± 1.84^{aB}	160.46 ± 1.00^{bB}	141.04 ± 4.00^{bB}	28.51^{Z}
	H-NEP	2.430 ± 3.05^{aA}	1039.60 ± 1612^{cA}	1355.5 ± 9.70^{bcA}	55.78^{Y}
	NEP	60.62 ± 12.45^{dB}	101.63 ± 8.11^{cB}	180.05 ± 13.42^{bB}	297^{X}
Total flavonoids mg EC/100 g	**EP**	9.00 ± 1.05^{aA}	1.38 ± 0.30^{cA}	1.15 ± 0.19^{cA}	12.7^{Z}
	H-NEP	0.99 ± 0.02^{bB}	0.42 ± 0.66^{bA}	0.68 ± 0.53^{bA}	68.9^{Y}
	NEP	0.59 ± 0.41^{bB}	0.41 ± 0.68^{bA}	1.09 ± 0.06^{bA}	184.7^{X}
Gallic acid μg g^{-1}	**EP**	30.37 ± 0.00^{aA}	15.05 ± 0.02^{bA}	3.33 ± 0.03^{cA}	10.96^{X}
	H-NEP	4.18 ± 0.00^{aB}	0.74 ± 0.01^{bB}	0.27 ± 0.01^{cB}	6.45^{Y}
	NEP	3.47 ± 0.00^{aC}	2.00 ± 0.01^{bB}	0.2 ± 0.00^{cC}	5.76^{Z}
Caffeic acid μg g^{-1}	**EP**	33.7 ± 0.36^{aA}	9.79 ± 0.01^{bA}	3.74 ± 0.00^{cA}	11.09^{X}
	H-NEP	5.24 ± 0.00^{aB}	1.06 ± 0.01^{bB}	0.56 ± 0.01^{cB}	10.68^{Y}
	NEP	-	-	-	-
Chlorogenic acid μg g^{-1}	**EP**	7.52 ± 0.02^{aA}	2.28 ± 0.01^{bA}	0.51 ± 0.01^{cA}	6.78^{Z}
	H-NEP	1.05 ± 0.00^{aB}	0.30 ± 0.00^{bB}	0.47 ± 0.00^{cB}	44.76^{X}
	NEP	0.55 ± 0.00^{aC}	0.10 ± 0.00^{bC}	0.17 ± 0.01^{cC}	30.9^{Y}
Syringic acid μg g^{-1}	**EP**	0.27 ± 0.01^{aA}	0.03 ± 0.00^{b}	-	-
	H-NEP	0.07 ± 0.01^{aB}	-	0.06 ± 0.00^{bA}	-
	NEP	-	-	-	-
Quercetin μg g^{-1}	**EP**	N.m	N.m	N. m	-
	H-NEP	1.17 ± 0.00^{aA}	0.28 ± 0.01^{bA}	-	-
	NEP	1.20 ± 0.00^{aB}	0.21 ± 0.00^{bB}	0.17 ± 0.00^{cC}	14.16^{Z}

CAE: chlorogenic acid equivalents; EC: epicatechin. EP: extractable polyphenols; H-NEP: hydrolyzable non-extractable polyphenols; NEP: non-extractable polyphenols; Different lower case letters indicate significant differences ($P < 0.05$) between means located within the same row (gastrointestinal simulation phases); different upper case letters indicate significant differences ($P < 0.05$) between means located within the same column (extracts). X, Y, and Z indicate significant differences ($P < 0.05$) between means from the column: "Bioaccessibility (%)" of extracts.

chemical bonds within complex molecules, such as non-extractable polyphenols. These characteristics differ and impact the retention of these compounds in the extracts.[4]

In the intestinal phase, NEP showed the same behavior in both determinations, with a significant retention of the compounds, a threefold increase for phenols, and an approximately twofold increase for total flavonoids.[5,30] It is important to emphasize that alkaline conditions and enzymatic hydrolysis of complex food components facilitates the release of phenolic acids from the food matrix, and increases their concentration in the intestine through the formation of monomeric forms.[4]

The phenolic compounds in coffee husk extracts were stable during the digestive process and have potential as prebiotics that support the colonization by, and balance of, the microbial flora in the colon. The NEP, specifically, has the potential to promote health if the bioactive compounds remain stable during metabolism in the colon and can circulate in the blood.[7,31] For total phenolics, the observed bioaccessibility was NEP 297% > H-NEP 55.78% > EP 28.51%. Extraction differences were critical for the release and retention of phenols between phases. The flavonoids had bioaccessibilities of 184.7% for H-NEP, 68.6% for NEP, and 12.7% for EP.

The physiological effects attributed to phenoliccompounds highly depend on their concentration in blood and target organs. Although NEP and H-NEP did not contain remarkable amounts of flavonoids compared with the undigested extract (EP), it was generally observed that these compounds had good bioavailability (concentration reaching the intestinal phase) because, as previously reported, pH and hydrolysis conditions favored retention. Nieto-Figueroa *et al.*[32] showed that the highest concentration of total and individual phenols from cocoa shells occured in the intestinal phase of digestion.

This may be due to the release of other phenols at the higher pH in the intestine, which can lead to the cleavage of other components and the production of phenolic acids as degradation products. It is therefore necessary to study the properties of the matrix and the metabolism of the dietary polyphenols themselves to better understand their retention and absorption. Mcdade *et al.*[4] noted that polyphenols must be available at a certain concentration in the target tissue to exert a health-related effect. The properties of certain compounds, such as phenolic acids, are related to their interactions with other compounds and stages of metabolism. These interactions can include release from the food matrix through enzymatic hydrolysis or the bacterial microbiota, absorption, and biotransformations. To understand the impact of phenolic intake on human health, it is important to know the levels of these compounds and their bioaccessibility and bioavailability throughout the gastrointestinal tract.[28]

Table 3 shows the profile of the main phenolic compounds and their bioavailability. The data show significant differences ($P < 0.05$) between both, types of extracts and digestion phases. Another common observation for all samples was the concentration of gallic acid and chlorogenic acid, as well as a decrease in phenolic content depending on the stage of digestion. New perspectives are emerging in the literature to identify variations in phenolic composition between organic and conventional coffee samples, and some compounds are particularly associated with organic varieties.[21] The differences between contents for EP, H-NEP, and NEP samples could be attributed to the differences in cultivation as they were obtained from organic production systems. However, there is a need for further investigation because, to date, there is no research specifically addressing the dietary macroantioxidant/polyphenol content or bioaccessibility of a matrix derived from an organic coffee by-product. Syringic acid and quercetin made the lowest contribution to the profile, ranging from 0.06 μg g^{-1} to 0.26 μg g^{-1} and from 0.16 μg g^{-1} to 1.19 μg g^{-1}, respectively. The EP extract stood out with the highest phenolic retention. However, the other treatments, H- NEP, and NEP, showed significant decreases before and after digestion, probably due to degradation under the more stringent processing conditions (temperature, solvent polarity, and pH).

Digestion had a positive effect on the bioaccessibility of the individual phenolic compounds; with the exception of syringic and caffeic acids, the other compounds were detected in all simulated gastric treatments. Chlorogenic acid showed higher bioaccessibility in H-NEP and NEP extractions (44.7% and 30.9%, respectively). Variations in bioaccessibility of phytochemicals are influenced by key interactions between compounds and/or digestive enzymes, variable pH, and other factors.[5] Gallic acid exhibited bioaccessibility ranging from 10.96% to 5.76%. Gallic acid, a type of phenolic acid, was found to have high bioaccessibility (meaning that it was easily absorbed by the body) after being digested, according to a study by Zeng *et al.*[30] The study also found that the most common chlorogenic acid in coffee by-products is 5-*O*-caffeoylquinic acid (also known as 5-ACG or chlorogenic acid). During *in vitro* digestion, chlorogenic acid binds to pepsin in the gastric phase and forms a complex through Van der Waals interactions and hydrogen bonding. It also decreases the rate of pancreatin hydrolysis in the intestinal phase. Caffeic acid is less stable after digestion by the pancreas and is retained more in the gastric phase than the intestinal phase.[5]

The presence of high concentrations of phenolic compounds such as gallic acid, chlorogenic acid, and caffeic acid is a good indicator of bioavailability because these compounds remain stable during digestion. The concentration of these compounds can be reduced by digestion and metabolism.[32] To understand the bioactivity of phenolic compounds isolated from their matrix of origin it is necessary to observe the effects of digestion on their biological activity. The literature provides a significant contribution to studies on macro-antioxidants from coffee by-products. The *in vitro* model based on human physiology, which was applied in the present study, contributes to a better understanding of these aspects. However, to confirm these claims, it is necessary to explore new perspectives through *in vivo* and randomized clinical trials with the analyzed extracts (EP, H-NEP, and NEP) as dietary supplements.

Assessment of bioactivity of organic coffee husk extracts

Antioxidant activity

Antioxidant potential is the best known bioactivity of polyphenols in food systems. Studies suggest that non-extractable polyphenols are important contributors to the total antioxidant activity of fruit peels and are equivalent or even superior to soluble antioxidants.[7] Although there is no unified method to estimate the actual antioxidant activity of phenol-rich materials,[3] three methods have been used in a complementary way to assess the total antioxidant capacity, taking into account different mechanisms of action.[28] The ABTS and DPPH assays are used to determine the antiradical activity of phenolic compounds, while the reducing capacity of iron (III) (FRAP) is mainly used to determine their reducing capacity.[7]

The results of the application of the antioxidant capacity assays (DPPH, ABTS, and FRAP) are shown in Table 4. Coffee husk extracts have been found to have high antioxidant capacity, particularly in

the undigested form (known as the EP extract). This may be due to the presence of phytochemicals such as chlorogenic acid, which is known to have antioxidant properties.[9] The ABTS assay demonstrated more efficient antioxidant activity for the extracts when compared to the other methods. However, the radical scavenging effects observed in laboratory tests may not necessarily be seen in the human body.[3,7]

During digestion, complex polyphenols and other condensed compounds are naturally broken down and reach the intestine in reduced amounts.[4] When the extracts (PE, H- NEP and NEP) of coffee husks were digested *in vitro* and the concentrations of the compounds were diluted, a decrease in antiradical activity was observed. The presence of phenols such as gallic acid, caffeic acid, and chlorogenic acid likely contributes to the scavenging activity of these radicals. In addition to their ability to neutralize radicals, these phenolic compounds may also act as antioxidants by chelating metals involved in the formation of free radicals.

All extracts (PE, H-NEP, and NEP) showed effective antioxidant activity in both ABTS and FRAP assays and bioavailability. Only EP showed activity against the DPPH radical. It is known that the main structural features of polyphenols are responsible for effective antioxidant activity.[3] The antioxidant capacity of the samples in the two fractions analyzed, with the exception of NEP, decreased significantly in the intestinal phase (1526.3 μM ET/g sample). However, the behavior toward the ABTS radical was unexpected and exceeded the potential of the undigested extract itself (1376.9 μM ET/g sample). Variations in the antioxidant activity of coffee husks may be caused by changes during digestion in the gastrointestinal tract. The high pH during digestion allows for reactions such as self-oxidation to occur in the intestinal lumen.[4] Inhibition of DPPH radicals was observed only with the extracts of EP and H-NEP. The other treatments did not show antioxidant activity. The antioxidant capacity of DPPH was affected by the H-NEP and NEP extraction methods ($P < 0.05$).

The antioxidant activity of caffeic acid was found to be lower than that of its derivatives, suggesting that its antioxidant potential decreases with increasing polarity of the environment. Factors such as the solvents used, the antioxidant determination method, and the samples after *in vitro* digestion may have contributed to the lower retention of caffeic acid in some samples (EP, H-NEP, and NEP). The chemical and enzymatic reactions during digestion can also affect the integrity of caffeic acid and its effectiveness as an antioxidant.[5]

Wang *et al.*[33] reported that lipophilic reactions aimed at improving its hydrophobicity could also increase its antioxidant potential. In the extracts analyzed, a significant difference ($P < 0.05$) was found between the extracts and the phases after digestion, with the ability to scavenge ABTS radicals decreasing in the gastric phase and increasing in the intestinal phase. The increase in antioxidant content and activity in the simulated intestinal phase could also be related to the interactions between the oxidizing factors of the pancreatic enzymes themselves (lipid oxidation and lipophilic enzymes),[4,5] making the samples more susceptible to reactions.

In a study on fermented coffee, Bressani *et al.*[28] observed the inactivation of DPPH radicals between 49.67–91.60 μM Trolox g^{-1}, ABTS 316.05–232.92 μM Trolox g^{-1}, and FRAP 106.00–232.54 μM FS g^{-1} before fermentation. The antioxidant compounds present in beans (they are also present in the husk) are rich in soluble and non-extractable polyphenols that act against oxidative stress. However, the antioxidant activity of macromolecular compounds present in coffee husks needs to be measured by *in vivo* studies.[4,7]

The extracts showed better bioaccessibility in the ABTS and FRAP determinations, with absorption greater than 50%. For DPPH, as previously reported, bioaccessibility was found only in the EP (10.28%), indicating that soluble antioxidants and their metabolites resist gastrointestinal tract conditions even at low concentrations.

The EP, H-NEP, and NEP extracts might play a crucial role in bioavailability in the digestive tract and body.[4] There is little information available on the fate of phenolic compounds from coffee husks during digestion, which makes it difficult to compare and equate absorption values between different matrices and methods. To determine the potential of food matrices as functional or nutraceutical ingredients, *in vitro* gastrointestinal digestion combined with bioacceptance is a reliable alternative.[32] The antioxidant capacity of extracts can be correlated with the phenolic compound content and the retention of these compounds until the final stage of digestion (bioaccessibility).

Table 4. Antioxidant evaluation of organic coffee husk polyphenol extracts and bioaccessibility

		Digestion *in vitro*			
Antioxidant	Extract types	Extract	Gastric phase	Intestinal phase bioaccessible	Bioaccessibility (%)
DPPH μmol EqT g^{-1}	**EP**	1995.5 ± 24.7^{aA}	467.6 ± 62.7^{b}	205.3 ± 27.2^{c}	10.28
	H-NEP	215.55 ± 8.32^{B}	N.d	N.d	-
	NEP	N.d	N.d	N.d	-
ABTS μmol EqT g^{-1}	**EP**	2953 ± 62.5^{aA}	2072.5 ± 95.7^{cA}	2672.5 ± 52.4^{bA}	90.50^{Z}
	H-NEP	1332.63 ± 69.4^{bB}	1039.37 ± 7.6^{cC}	1245.9 ± 47.2^{bC}	93.49^{Y}
	NEP	1376.9 ± 42.5^{bcB}	1210.5 ± 31.9^{cB}	1526.5 ± 7.5^{bB}	110.86^{X}
FRAP μmol EqT g^{-1}	**EP**	2043.1 ± 8.21^{aA}	1924.18 ± 2.83^{bA}	1812.88 ± 3.9^{dA}	201.08^{X}
	H-NEP	773.82 ± 13.32^{bB}	109.33 ± 5.53^{dB}	489.4 ± 40.8^{cB}	88.72^{Y}
	NEP	607.79 ± 6.41^{bC}	68.16 ± 6.19^{dC}	374.5 ± 23.6^{cC}	61.61^{Z}

EP: extractable polyphenols; H-NEP: hydrolyzable non-extractable polyphenols; NEP: non-extractable polyphenols; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: (2-2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)); FRAP: Ferric reducing ability of plasma. Different lower case letters indicate significant differences ($P < 0.05$) between means wthin the same row (gastrointestinal simulation phases; different upper case letters indicate significant differences ($P < 0.05$) between means within the same column (extracts. X, Y, and Z indicate significant differences ($P < 0.05$) between means within the column "Bioaccessibility (%)" of the extracts.

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Coagulation assessment: activated partial thromboplastin time and prothrombin time

The anticoagulant effect of gastrointestinal digestive products from coffee husks was evaluated using activated partial thromboplastin time (APTT) and prothrombin time (PT) assays, shown in Fig. 1. Activated partial thromboplastin time is an assessment of intrinsic clotting factors, whereas PT is used to characterize extrinsic clotting factors.[19] The results show the anticoagulant properties of the coffee husk extracts obtained in the study before and after simulated gastrointestinal digestion *in vitro*. In general, all extracts showed prolonged antithrombotic activity compared with NaCl (0.15 M), except for the EP gastric digestion samples. All intestinal phase samples showed significantly prolonged APTT.

With respect to PT, only the crude extract of H-NEP showed a significantly longer retention time, whereas the other extracts remained close to the retention time estimated in NaCl (20.3 s). In the gastric phase, a similar behavior to APTT is observed. On the other hand, in all samples (EP, H-NEP, and NEP), the intestinal phase was critical in increasing the ability of the digestive products to delay prothrombin action. In both determinations (APTT and PT), digestion phases had no effect on the anticoagulant properties of H-NEP.

Research has shown that many phenolic compounds, including flavonoids and polyphenols, have significant biological effects, including anticoagulant, antithrombotic, and antiplatelet activity. Chlorogenic acid present in coffee peel extracts has been found to have these effects in some studies, and it has also been tested in clinical trials with no cytotoxicity at safe doses between 0.5–1 g, where it has shown neuroprotective, cardiovascular, and antiplatelet effects. There are fewer studies on the homeostatic functions of coffee peels, but some research has been conducted in this area.[6,9] In a study investigating the enzymatic extract of decaffeinated coffee beans treated with chlorogenase, caffeic acid was reported to be the major blood clotting factor.[34]

The presence of phenolic acids and the differences between the profiles of the extracts that were studied indicated the influence of *in vitro* digestion on the anticoagulant activity of all extracts (EP, H-NEP, and NEP). However, it is possible to observe a milder effect in gastric samples. Bijak *et al.*[35] explain that the binding of polyphenols to plasma proteins may delay the anticoagulant

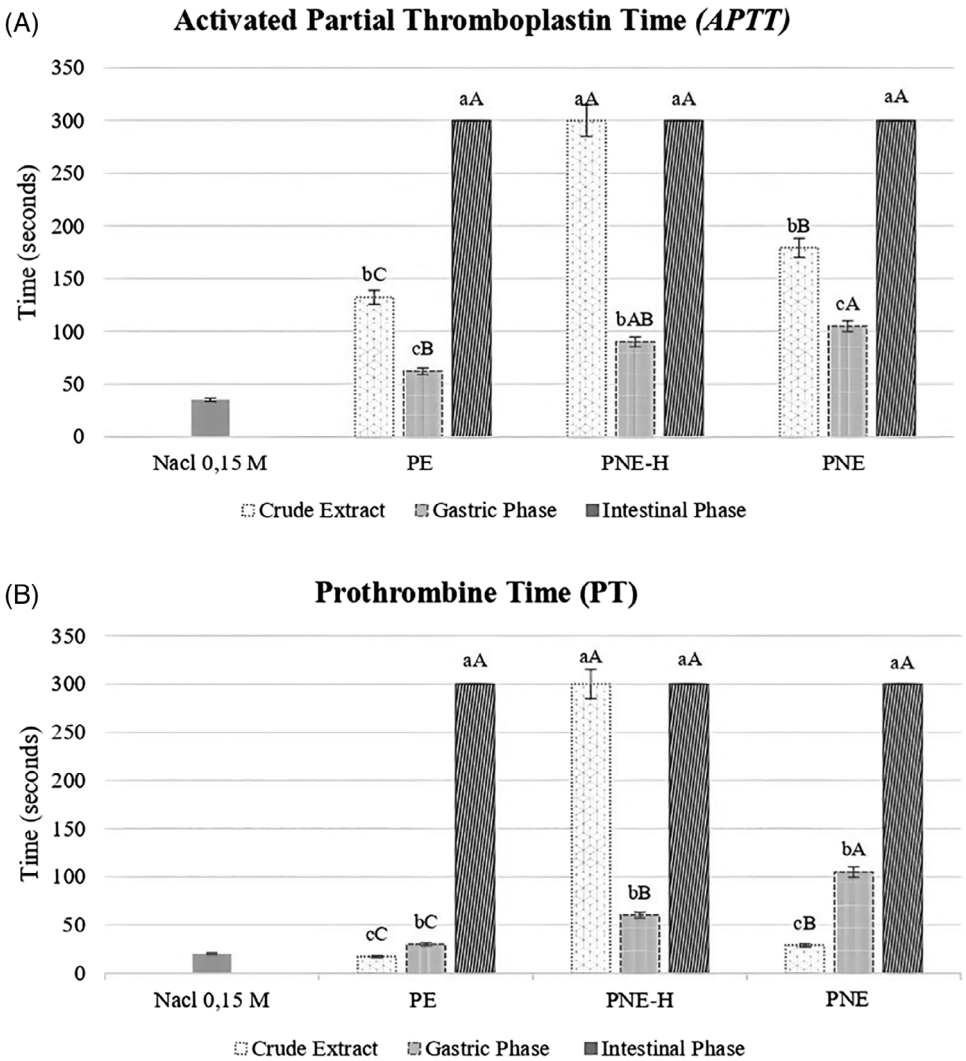

Figure 1. Assay of anticoagulant activity. (A) Activated partial thromboplastin time (APTT). (B) Prothrombine time (PT). EP: Extractable polyphenols; H-NEP: Hydrolyzable non-extractable polyphenols; NEP: Non-extractable polyphenols. Different lower case letters indicate significant differences ($P < 0.05$) between extracts; different upper case letters indicate significant differences ($P < 0.05$) between digestion stages in the same extract. Source: Prepared by the author, 2022.

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

effect. This can be explained by the presence of pepsin as an interaction between anticoagulants and proteins and/or digestive enzymes has been demonstrated.[36]

The results of this research demonstrate the potential to obtain anticoagulant compounds from coffee by-products. The bioactive compounds caffeic acid and chlorogenic acid in coffee peel extracts may be used as alternatives for oral anticoagulation therapy as antiplatelet and antithrombotic agents and may have fewer side effects than other drug treatments. Bioavailability is an important factor in the effectiveness of these phenols in the body.[36,37]

CONCLUSION

From our results, the three organic coffee husk extracts tested were good sources of macro-antioxidants, exhibiting antioxidant effects, bioaccessibility, and anticoagulant activity. When comparing extracts, EP proved to be more efficient but H-NEP and NEP also showed favorable results (in some cases even better than EP). Coffee husk extracts have the potential to be used as sources of nutraceutical compounds due to their antioxidant and anticoagulant properties. The extractive process, including drying and grinding, and the extracts' abilities to sequester the DPPH radical and prolong thromboplastin time, were studied. The non-extractable polyphenolic fractions of the coffee husks also contributed to the retention and bioaccessibility of the phenolic compounds. These findings suggest that coffee husk extracts can be a valuable resource for the development of new nutraceutical compounds.

ACKNOWLEDGEMENTS

The authors would like to thank the Coordination for Higher Education Personnel Improvement-CAPES, for the grant, and the Research Support Foundation of the State of Paraíba (Fapesq PB), where the present line of research is carried out through the project approved in the Support Program for Centers of Excellence – (Edital 006/2018-PRONEX 2018). The authors thank the National Council for Scientific and Technological Development (CNPq) for financial support. They would also like to thank Fazenda Yaguara Ecológico for the partnership and availability of the raw material.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1 ICO (International Coffee Organization), Trade Statistic Table – Exports of coffee by exporting countries [Internet]. 2021 Available from: www.ico.org/prices/m1-exports.pdf.
2 Castaldo L, Graziani G, Gaspari A, Izzo L, Luz C, Mañes J *et al*., Study of the chemical components, bioactivity and antifungal properties of the coffee husk. *J Food Res* **7**:43 (2018).
3 Estévez M, Critical overview of the use of plant antioxidants in the meat industry: opportunities, innovative applications and future perspectives. *Meat Sci* **181**:108610 (2021).
4 Mcdade D, Patrick RM, Rizvi SSH, Knorr D and Labuza TP, Dietary Polyphenols (2021).
5 Soares MJ, Sampaio GR, Guizellini GM, Figueira MS, da Costa Pinaffi AC, Soares Freitas RAM *et al*., Regular and decaffeinated espresso coffee capsules: unravelling the bioaccessibility of phenolic compounds and their antioxidant properties in milk model system upon in vitro digestion. *LWT* **135**:110255 (2021).
6 Choi JH and Kim S, Investigation of the anticoagulant and antithrombotic effects of chlorogenic acid. *J Biochem Mol Toxicol* **31**:1–6 (2017).
7 Pérez-Jiménez J and Saura-Calixto F, Fruit peels as sources of non-extractable polyphenols or macromolecular antioxidants: analysis and nutritional implications. *Food Res Int* **111**:148–152 (2018).
8 Gemechu FG, Embracing nutritional qualities, biological activities and technological properties of coffee byproducts in functional food formulation. *Trends Food Sci Technol* **104**:235–261 (2020).
9 de Oliveira SM, Honfoga JNB, de Medeiros LL, Madruga MS and Bezerra TKA, Obtaining bioactive compounds from the coffee husk (Coffea arabica L.) using different extraction methods. *Molecules* **26**:1-13 (2020).
10 AOAC Association, *Chemists of Official Analytical*. Official Methods of Analysis, Gaithersburg, Maryland, USA (2010).
11 Zeppa G, Conterno L and Gerbi V, Determination of organic acids, sugars, diacetyl, and acetoin in cheese by high-performance liquid chromatography. *J Agric Food Chem* **49**:2722–2726 (2001).
12 Hartzfeld PW, Forkner R, Hunter MD and Hagerman AE, Determination of hydrolyzable tannins (gallotannins and ellagitannins) after reaction with potassium iodate. *J Agric Food Chem* **50**:1785–1790 (2002).
13 Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C *et al*., A standardised static in vitro digestion method suitable for food-an international consensus. *Food Funct* **5**:1113–1124 (2014).
14 Brandon EFA, Bakker MI, Kramer E, Bouwmeester H, Zuidema T and Alewijn M, Bioaccessibility of vitamin A, vitamin C and folic acid from dietary supplements, fortified food and infant formula. *Int J Food Sci Nutr* **65**:426–435 (2014).
15 Andrade KS, Gonalvez RT, Maraschin M, Ribeiro-Do-Valle RM, Martínez J and Ferreira SRS, Supercritical fluid extraction from spent coffee grounds and coffee husks: antioxidant activity and effect of operational variables on extract composition. *Talanta* **88**:544–552 (2012).
16 Brand-Williams W, Cuvelier ME and Berset C, Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol* **28**: 25–30 (1995).
17 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* **26**:1231–1237 (1999).
18 Benzie IFF and Strain JJ, Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol* **1999**:15–27 (1995).
19 Salu BR, Pando SC, de Brito MV, Fernando A, Odei-addo F, Frost C *et al*., Improving the understanding of plasma kallikrein contribution to arterial thrombus formation using two plant protease inhibitors improving the understanding of plasma kallikrein contribution to arterial thrombus formation using two plant protease inhibito. *Platelets* **00**:1–9 (2018). https://doi.org/10.1080/09537104.2018.1428738.
20 FAO, Standard for Wheat Flour. Codex alimentarius [Internet]. 2019 Available from: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B152-1985%252FCXS_152e.pdf.
21 Consonni R, Polla D and Cagliani LR, Organic and conventional coffee differentiation by NMR spectroscopy. *Food Control* **94**:284–288 (2018).
22 Moreira MD, Melo MM, Coimbra JM, dos Reis KC, Schwan RF and Silva CF, Solid coffee waste as alternative to produce carotenoids with antioxidant and antimicrobial activities. *Waste Manage* **82**:93–99 (2018). https://doi.org/10.1016/j.wasman.2018.10.017.
23 Iriondo-DeHond A, Aparicio García N, Fernandez-Gomez B, Guisantes-Batan E, Velázquez Escobar F, Blanch GP *et al*., Validation of coffee by-products as novel food ingredients. *Innov Food Sci Emerg Technol* **51**:194–204 (2019).
24 ABNT, NBR ISO 14001-Sistemas de gestão ambiental-Requisitos com orientações para uso. Associação Brasileira de Normas Técnicas 2015;3° Ed.:35.
25 Belmiro RH, de Carvalho Oliveira L, Geraldi MV, Maróstica Junior MR and Cristianini M, Modification of coffee coproducts by-products by dynamic high pressure, acetylation and hydrolysis by cellulase:

a potential functional and sustainable food ingredient. *Innov Food Sci Emerg Technol* **68**:102608 (2021).
26 Ratnadewi AAI, Amaliyah Zain MH, Nara Kusuma AAN, Handayani W, Nugraha AS and Siswoyo TA, Lactobacillus casei fermentation towards xylooligosaccharide (XOS) obtained from coffee peel enzymatic hydrolysate. *Biocatal Agric Biotechnol* **23**:101446 (2020).
27 Shankar K, Kulkarni NS, Jayalakshmi SK and Sreeramulu K, Saccharification of the pretreated husks of corn, peanut and coffee cherry by the lignocellulolytic enzymes secreted by Sphingobacterium sp. ksn for the production of bioethanol. *Biomass Bioenergy* **127**:105298 (2019).
28 Bressani APP, Batista NN, Ferreira G, Martinez SJ, Simão JBP, Dias DR *et al*., Characterization of bioactive, chemical, and sensory compounds from fermented coffees with different yeasts species. *Food Res Int* **150**:110755 (2021).
29 Jakobek L, Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chem* **175**:556–567 (2015). https://doi.org/10.1016/j.foodchem.2014.12.013.
30 Zeng W, Jin L, Zhang F, Zhang C and Liang W, Naringenin as a potential immunomodulator in therapeutics. *Pharmacol Res* **135**:122–126 (2018). https://doi.org/10.1016/j.phrs.2018.08.002.
31 Pérez-Jiménez J, Arranz S and Saura-Calixto F, Proanthocyanidin content in foods is largely underestimated in the literature data: an approach to quantification of the missing proanthocyanidins. *Food Res Int* **42**:1381–1388 (2009).
32 Nieto-Figueroa KH, Mendoza-García NV, Gaytán-Martínez M, Wall-Medrano A, Guadalupe Flavia Loarca-Piña M and Campos-Vega R, Effect of drying methods on the gastrointestinal fate and bioactivity of phytochemicals from cocoa pod husk: in vitro and in silico approaches. *Food Res Int* **137**:109725 (2020).
33 Wang S, Li Y, Meng X, Chen S, Huang D, Xia Y *et al*., Antioxidant activities of chlorogenic acid derivatives with different acyl donor chain lengths and their stabilities during in vitro simulated gastrointestinal digestion. *Food Chem* **357**:129904 (2021). https://doi.org/10.1016/j.foodchem.2021.129904.
34 Cai W, Xie L, Chen Y and Zhang H, Purification, characterization and anticoagulant activity of the polysaccharides from green tea. *Carbohydr Polym* **92**:1086–1090 (2013). https://doi.org/10.1016/j.carbpol.2012.10.057.
35 Bijak M, Bobrowski M, Borowiecka M, Podsędek A, Golański J and Nowak P, Anticoagulant effect of polyphenols-rich extracts from black chokeberry and grape seeds. *Fitoterapia* **82**:811–817 (2011).
36 Moradi S, Farhadian N, Balaei F, Ansari M and Shahlaei M, Multi spectroscopy and molecular modeling aspects related to drug interaction of aspirin and warfarin with pepsin; structural change and protease activity. *Spectrochim Acta A Mol Biomol Spectrosc* **228**:117813 (2020). https://doi.org/10.1016/j.saa.2019.117813.
37 Nam GS, Park HJ and Nam KS, The antithrombotic effect of caffeic acid is associated with a cAMP-dependent pathway and clot retraction in human platelets. *Thromb Res* **195**:87–94 (2020). https://doi.org/10.1016/j.thromres.2020.07.024.

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.