Journal of Food Measurement and Characterization
https://doi.org/10.1007/s11694-021-01117-z

ORIGINAL PAPER

Avocado paste from industrial byproducts as an unconventional source of bioactive compounds: characterization, in vitro digestion and in silico interactions of its main phenolics with cholesterol

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Received: 28 May 2021 / Accepted: 10 August 2021

Abstract

Avocado cv. 'Hass' paste is the main byproduct derived from industrial avocado processing, and contains various bioactive compounds (BCs). Consumption of these compounds from different sources has been associated with positive health effects, although studies of avocado byproducts remain minimal. Avocado paste was analyzed, regarding its physicochemical properties, proximate composition, main BCs, antioxidant capacity, in vitro digestion and in silico interactions of its three main phenolics with cholesterol. Avocado paste contained 14.2% of lipids and 13.8% of protein. Total phenolic compounds were 2.1 mg GAE/g dw, with a phenolic profile that contained phenolic acids (ferulic, protocatechuic and *p*-coumaric acids) as its main compounds. Carotenoids (mainly β-carotene), tocopherols (mainly α-tocopherol), fatty acids (mainly oleic acid) and other BCs were also identified and quantified. Release of its phenolic compounds and antioxidant capacity were highest in the gastric phase of an in vitro digestion. In silico analyses showed that ferulic acid had the strongest interaction with cholesterol, suggesting a possible mechanism to inhibit its intestinal absorption. According to its composition and characteristics, it is possible to suggest avocado paste may be used as an unconventional source of BCs, with possible effects on cholesterol digestion.

Keywords Phenolic acids · Waste valorization · Proximate analysis · Simulated digestion · Interactions

Introduction

Avocado (*Persea americana* Mill.) is endemic to Mesoamerica, while cv. 'Hass' is the most produced and consumed variety throughout the world; although Mexico is the main producer, its cultivation has spread to other countries. Avocado is considered a functional food due to the various bioactive compounds (BCs) that it has been shown to contain, such as phenolic compounds, tocopherols, carotenoids, fatty acids, fiber, among others [1–4], however, its BC content may vary depending on its ripening stage, as well as other postharvest factors [5, 6]. The industrial production of avocado oil and other products (for example, 'guacamole' or avocado sauce), generates byproducts such as defatted pulp, seed and peel, which results in a large amount of solid waste, since these represent 21–30% of the weight of fruit [3]. Avocado byproducts are not commonly used, and are therefore discarded directly, which represents a serious environmental problem [7]. Thus, it is important to take advantage of these

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Published online: 22 August 2021

byproducts, both to reduce their negative impact on the environment and to add value to them, since they can be important sources of bioactive phytochemical compounds [8, 9].

Recent works have studied avocado and its BCs for various purposes, for example, a comparative study by Ramos-Aguilar [4] on the phytochemical and nutrient composition of ripe 'Hass' and 'Hass type' (HT) avocado cultivars, showed significant variation between their phytochemical and nutrient composition. Overall, both samples showed a good balance between polar and non-polar compounds, and particularly noteworthy ratios of n-6-to-n-3 and polyunsaturated-to-saturated fatty acids, which are commonly associated with the prevention of cardiovascular diseases. A metabolomic analysis of ripe Mexican Creole (*Persea americana* var. Drymifolia) and'Hass' avocados also showed similar concentrations of lutein, chlorophyll α, β-sitosterol and α-tocopherol [10]. Knowledge about the chemical compositions of various avocado cultivars could be useful to understand their relevance in human health and nutrition, as well as the potential valorization of the byproducts generated by their processing industry.

Regarding avocado byproducts, Araujo [11] recovered BCs from avocado peel with an optimized microwave-assisted extraction (MAE, optimized temperature, time and ethanol/acetone), yielding a significant phenolic content with high antioxidant activity, which may be used on subsequent applications instead of discarding them. Alkaltham [5] studied the effects of ripening stages (ripe and unripe) and drying methods on the fatty acid content, physicochemical and bioactive properties of 'Hass' avocado and its byproducts. Total phenolics were shown to vary significantly in all tissues depending on ripening and drying method. Most notably, the byproducts were shown to have similar or higher concentrations and antioxidant activity in both ripe and unripe samples, but drying method had a significant effect. Thus, avocado byproducts are beginning to be recognized as sources of BCs, with phenolic compounds, procyanidins and catechins being of particular interest [4, 12]. It is also noteworthy that their concentration and antioxidant capacity may be even higher in byproducts than that found in the pulp [3, 13, 14]. Some have used avocado byproducts or extracts derived from them as larvicides, antifungals and antibacterials, while others highlight their antioxidant, antiprotozoal, antidiabetic, antihypertensive and hypocholesterolemic effects [15, 16]. These activities suggest that avocado byproducts contain important bioactive compounds that can be recovered and harnessed for different applications, yet they remain underutilized.

Various experiments have been carried out on peel and seed obtained under controlled laboratory conditions, but what is actually generated by the industry has not been studied, while their bioactivities have also not been considered. An interesting potential application of BCs is their ability to interact with cholesterol (dietary or biliary) during digestion, which prevents its incorporation into mixed micelles and/or inhibits its enteric absorption [17, 18]. This has been studied in some specific compounds [19] and some byproducts with promising success [20], however, there are currently no studies on avocado paste or the possible interactions of its phenolic compounds with cholesterol. Therefore, the objective of this study was to thoroughly characterize industrially generated avocado paste, in terms of its composition, release of its phenolic compounds during in vitro digestion and in silico interactions of its main phenolic acids with cholesterol. This will allow you to propose possible applications for it, instead of discarding it unused.

Materials and methods

Vegetable materials

Avocado cv.'Hass' paste used was kindly donated by a processing plant located in the state of Jalisco Mexico. It consisted of defatted pulp, peel and seed (in unspecified proportions), which were left over after oil extraction. Paste was shipped to the laboratory, where it was immediately stored at − 30 °C and freeze dried for 72 h.

Standards and reagents

HPLC-grade solvents [ethanol, methanol, butanol, methyl *tert*-butyl ether (MTBE), chloroform, acetone, triethylamine and hexane], sulfuric acid and hydrochloric acid (HCl) were obtained from JT-Baker (Mexico City, Mexico). Phenolphthalein, sodium hydroxide (NaOH), butylated hydroxytoluene (BHT), potassium hydroxide (KOH), ammonium acetate, potassium chloride (KCl), boron trifluoride (BF_3), sodium sulfate (Na_2SO_4), α-amylase, pepsin, pancreatin, pure standards of gallic acid, Trolox, catechin, quercetin, rutin, *p*-coumaric acid, ferulic acid, cyanidin, zeaxanthin, α-carotene, β-carotene, α- and γ-tocopherol, palmitic acid (16:0), palmitoleic acid (16:1, ω-7), stearic acid (18:0), oleic acid (18:1, ω-9), linoleic acid (18:2, ω-6) and α-linolenic acid (18:3, ω-3), as well as all other reagents used, were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Physicochemical and proximate analyses

The CIE L*, a* and b* color variables were measured at three points of the dry paste using a CR-400HS colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan). Total soluble solids (TSS) were analyzed as previously described [21]. Briefly, 10 g of avocado paste were dissolved in 50 mL of distilled water, mixture was filtered and TSS were measured directly from the filtered residue, using an Abbe digital

refractometer (E-Inginst Electron Corp., Fujian, China); results are expressed as Brix. Acidity was measured in 5 mL of avocado paste dissolved in 50 mL of distilled water containing 0.1% phenolphthalein. Mixture was titrated with 0.1 M NaOH to a final pH of 8.1. Acidity was expressed as g of citric acid/100 g fw [22]. pH was measured with an electronic pH meter (Fisher Scientific AB150, Ottawa, Canada). All measurements were performed in triplicate.

Proximate analysis (moisture, proteins, lipids, carbohydrates and ash) was performed using methods described by the Association of Official Analytical Chemists [21]. Analyses were performed in triplicate and expressed as percentage.

Phenolic compounds and flavonoids

Ethanolic extracts of avocado paste were obtained as previously described [23], and used to quantify total phenolic compounds and flavonoids. Briefly, 1 g of freeze-dried avocado paste was homogenized in 20 mL of ethanol:water (80:20, v/v), sonicated using a Bransonic 2510 sonicator (Bransonic Ultrasonic Co., Danbury, CT, USA) for 30 min and centrifuged (Allegra 64R, Beckman Coulter, Brea, CA, USA) at 9400 g for 15 min at 4 °C. Supernatant was collected and the extraction residues were washed twice more with 10 mL of 80% ethanol. Supernatants were pooled and filtered through Whatman No. 1 paper, and used to quantify phenolic compounds, flavonoids and antioxidant capacity.

Total phenolic content was determined using the Folin-Ciocalteu method [24]. Absorbance was read at 765 nm using a FLUOstar Omega spectrophotometer (BMG Labtech, Durham, NC, USA). Results were expressed as mg gallic acid equivalents (GAE)/g dw. Total flavonoid content was determined using the spectrophotometric method described by Floegel [25]. Absorbance was measured at 496 nm on a FLUOstar Omega spectrophotometer (BMG Labtech). Flavonoid content was expressed as mg catechin equivalents (CaE)/g dw.

Phenolic profile was characterized by liquid chromatography. For this, extracts were obtained as described by Shivashankara [26] with some modifications. Freeze-dried avocado paste (0.5 g) was homogenized in 20 mL of 80% ethanol, using an Ultra Turrax T25 basic homogenizer (IKA Works, Wilmington, NC, USA) at room temperature, from which a 10 mL aliquot was taken. Aliquot was subjected to an alkaline hydrolysis using 10 mL of 4 M NaOH and incubated for 4 h at room temperature under dim lighting. After incubation, extracts were acidified to pH 2.0 with 4 N HCl. Hydrolyzed samples were injected into a UHPLC (ultra-high pressure liquid chromatography) apparatus (Acquity, Waters Co., Milford, MA, USA.), equipped with a diode detector (DAD), and analyzed as described by Velderrain-Rodríguez et al. (2018) with slight modifications (each phenolic acid was identified and quantified at its maximum absorbance wavelength).

Separation was performed on an Acquity BEH C18 column (Waters, Milford, MA, USA) (1.7 µm, 3.0 × 100 mm) at 60 °C. Solvent system was used, A (water with 0.5% formic acid) and B (100% methanol). The solvent gradient was as follows: 0–0.25 min 20% of B (flow 0.4 mL/min); 5 min 20% A (0.2 mL/min); 12 min 45% B (0.180 mL/min); 25 min 100% B (0.1 mL/min); 16 min (40% B, 0.2 mL/min); 30 min 20% B (0.4 mL/min). Detection of gallic acid, quercetin and rutin was performed at 240 nm, 280 nm and 360 nm, respectively. Detection of *p*-coumaric acid and ferulic acid was performed at 320 nm. Identification was performed by comparing retention time and UV–Vis absorption spectrum of analytes against those of authentic standards. Quantification was performed using standard curves. Results were expressed as µg/100 g dw.

Hydrolyzable and condensed tannins

Hydrolyzable and condensed tannins were quantified from the residues of the aforementioned ethanolic extraction, using the methodology of Hartzfeld [27]. To determine hydrolyzable tannins, samples were hydrolyzed with 20 mL of methanol and 2 mL of concentrated sulfuric acid for 20 h at 85 °C in a water bath (Precision 281, Thermo Scientific, Waltham, MA, USA), under continuous mixing. Subsequently, hydrolyzed tannins were incubated at room temperature and centrifuged at 15,000 rpm for 15 min at 4 °C. Supernatant was filtered through Whatman No. 1 paper and made up to 50 mL with distilled water. Two additional washes were performed with 10 mL of distilled water; sample was centrifuged and filtered after each wash. Recovered volume was adjusted to 50 mL using distilled water. Absorbance of the obtained supernatant was measured at 530 nm in a FLUOstar Omega spectrophotometer (BMG Labtech). Results were expressed as mg GAE/g dw.

To quantify condensed tannins, samples were dissolved in 10 mL of a solution containing 975 mL of butanol and 25 mL of HCl (37%). Mixture was incubated in a water bath at 100 °C for 3 h. The solution was centrifuged at 15,000 rpm, for 15 min at 4 °C, subsequently, 2 more washes were carried out with 10 mL of butanol/HCl. Supernatants were made up to 50 mL and their absorbance was read at 555 nm in a FLUOstar Omega spectrophotometer (BMG Labtech). Results were reported as mg of cyanidin equivalents (CyE)/g dw.

Carotenoids, tocopherols and chlorophylls

Carotenoid and tocopherol profile was characterized using the methodology of Hess [28] with some modifications. Freeze-dried avocado paste (0.2 g) was mixed with 1 mL

of ethanol with BHT and 1 mL of 50% KOH; mixture was vortexed for 15 s and incubated in a previously heated 70 °C water bath (Thermo Scientific). 5 mL of hexane were added and the mixture was centrifuged at 1500 rpm at 10 °C for 7 min (Allegra 64R, Beckman Coulter). 600 µL of the upper hexane layer were transferred to a microtube, where hexane was evaporated using nitrogen, and extracted solids were reconstituted in 200 µL of ethanol. Carotenoids were analyzed in an HP1100-series HPLC-DAD (Hewlett Packard, Palo Alto, CA, USA). Separation was performed using a 150×4.6 mm, 3 µm C30 reverse phase column (YMC Inc., Wilmington, NC, USA). Mobile phases were methanol/water (96:4, v/v) containing 0.05 M ammonium acetate and a volume fraction of 0.05% triethylamine (solvent A) and MTBE (solvent B). Elution gradient was (1) 10 min of solvent A from 100 to 90%, (2) from 9 min to 69% of solvent A and 31% of solvent B, (3) from 16 min to 35% of solvent A and 65% solvent B, and (4) a retention of 0.1 min to 65% solvent A and 35% solvent B. Absorbances were read at 430, 450, and 471 nm. Quantification of zeaxanthin, α-carotene and β-carotene was performed using calibration curves of pure standards (Sigma-Aldrich, St. Louis, MO, USA).

Tocopherols were quantified using an HP1100-series HPLC (Hewlett Packard) equipped with a fluorescence detector (FLD), using excitation and emission wavelengths of 294 and 326 nm, respectively. HPLC system was equipped with a Prodigy C18 250×4.6 mm ID, 3 µm column (YMC Inc.) maintained at 30 °C. Mobile phase was methanol/water (95:5 v/v), at a flow rate of 0.75 mL/min. α- and γ-tocopherol were quantified using calibration curves of pure standards (Sigma-Aldrich).

Quantification of α- and β-chlorophyll was carried out as described by León-Chan [29] with some modifications. Briefly, 0.3 g of sample were weighed and mixed with 30 mL of acetone. Their absorbance was read on a 1 cm spectrophotometer cell Varian Cary 50 UV–Vis (Varian Inc., Palo Alto, CA, USA); α-chlorophyll was read at 661.6 nm and β-chlorophyll at 644.8 nm. Results were expressed as µg/g dw.

Antioxidant capacity

Antioxidant capacity was determined using Trolox equivalent antioxidant capacity (TEAC) as described by Re [30], ferric reducing antioxidant power (FRAP) according to Benzie and Strain [31], and 2,2-diphenyl-1-picril-hydracyl (DPPH) methodology was performed as described by Brand-Williams [32]. Assays were performed in microplate wells and absorbances were read in a FLUOstar Omega spectrophotometer (BMG Labtech). Results of antioxidant capacity are the result of triplicate measurements, and were expressed as mg TE/g dw.

Fatty acid profile

Lipids were extracted from avocado paste in order to characterize its fatty acid profile, according to the methodology of Metcalfe and Schmitz [33] with slight modifications. Two g of freeze-dried paste were homogenized for 1 min with 10 mL of methanol using an Ultra Turrax homogenizer, and 20 mL of chloroform were then added. Mixture was transferred to a separating funnel, 25 mL of 0.88% KCl were added and the mixture was incubated until phase separation was observed. A sample from the lower phase was collected and evaporated to dryness using a rotary evaporator (R-215, Büchi Labortechnik, Switzerland).

Concentrated sample was placed in a distillation flask and attached to a condenser, after adding 10 mL of 0.5 N NaOH in methanol. Sample was then heated to boiling and refluxed for 10 min. Then, 12 mL of BF_3 were added and refluxed for 3 min. 4 mL of heptane were added and refluxed for 2 min. Sample was transferred to a test tube and NaCl was added until a white color was observed. 1 g of Na_2SO_4 was added and stirred until phase separation.

Fatty acids were identified after injecting 1 µL of final extract into a gas chromatograph (7820, Agilent, Santa Clara, CA, USA), equipped with a flame ionization detector (FID) and a capillary column of 30 m×0.32 mm×0.25 µm (Omegawax 320, Supelco, Bellefonte, PA, USA). Fatty acids were identified using authentic standards (Sigma-Aldrich), quantification was performed using calibration curves. Results are expressed as percentage.

In vitro digestion of avocado paste

Avocado paste was subjected to an in vitro digestion, following the methodology standardized by Minekus [34]. For oral phase, 1 g of freeze-dried avocado paste was mixed with 3.5 mL of simulated oral solution (containing 75 U/mL of α-amylase). Mixture was incubated at 37 °C in a shaking water bath for 2 min. For gastric phase, 6 mL of gastric solution (containing 2000 U/mL of pepsin) were added to the orally-digested sample, and pH was adjusted to 3.0 using 1 M HCl. Subsequently, total volume was adjusted to 8 mL with distilled water and the mixture was incubated in a shaking water bath at 37 °C for 2 h. For intestinal phase, gastric-digested samples were mixed with 7.7 mL of intestinal solution (containing 800 U/mL of pancreatin), pH was adjusted to 7.0 using 1 M NaOH and total volume was fixed to 14 mL with distilled water. Mixture was incubated in a shaking water bath at 37 °C for another 2 h. 2 mL aliquots were periodically collected from the intestinal phase at 30, 60, 90 and 120 min to analyze release kinetics of phenolic compounds and antioxidant activity during digestion. A blank digestion (without sample) was also performed under the same conditions, which was used to correct for enzyme

interferences. Total phenolic compound content and antioxidant activity were analyzed using methodologies described in previous sections.

Quantum chemical methods

The three most abundant phenolic compounds quantified in avocado paste (see results) were chosen to analyze their in silico interactions with cholesterol, according to quantum chemical methods implemented in Gaussian 09 [35]. Initially, the molecular units were optimized using the B3LYP functional [36] and the standard basis set 6-31G(d,p) [37]. We then carried out single-point energy calculations, at the same level of theory, to calculate the atomic charges and generate the electrostatic potential (ESP) surfaces. The atomic charges were calculated by the Breneman and Wiberg scheme, where the atomic charges are fitted to reproduce the molecular ESP at a number of points around the molecule [38].

From these calculations, we identified the most electropositive and electronegative zones in the molecules to stablish a strategy to build dimers between cholesterol and the phenolic acids. The ESP surfaces are useful to predict molecular recognition and to analyze the intermolecular interactions [39, 40]. The above methodology generated three dimers: (1) cholesterol–protocatechuic acid; (2) cholesterol–ferulic acid; (3) cholesterol–*p*-coumaric acid. Geometry optimization and single point energy calculations were performed on these dimers at the same level of theory to obtain the binding energies. The output files were analyzed using GaussView 5.0.8 [41] and Mercury 4.0 [42].

Statistical analyses

Experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). A comparison of means was made using Tukey–Kramer method with a significance level of $p < 0.05$. Data was analyzed in NCSS 2012 (NCSS Software, Kaysville, UT, USA).

Results and discussion

Physicochemical characteristics and proximate analysis of avocado paste

Physicochemical characteristics and proximate composition of avocado paste are shown in Table 1. Avocado paste had low L*, hue and C* values, which are due to the presence of peel and seed, both of which have darker tonalities, as compared to avocado pulp. Thus, the coloration of our samples does not match that reported for pulp, for example, Villa-Rodríguez [6] reported higher L* values in pulp of avocado cv. 'Hass' (same used in the present study). Ramos-Aguilar [4] reported values of L* 38.5, a* 2.46, b* 1.70 in avocado peels which, in general, are indicative of the characteristic black coloration of 'Hass' avocados. Because color of avocado paste is dependent on its content of defatted pulp, peel and seed, it is expected to vary according to specific practices of the processing plant that generates it. Furthermore, decreases in L*, hue and C* are associated with chlorophyll degradation, which may occur in the fruit as a mechanism against solar radiation [43, 44], making it another source of color variation in avocado paste. After avocado is processed, byproducts are exposed to oxygen, light and other ambient conditions that enhance browning and degradation, thus, a rapid temperature reduction or addition of an antibrowning agent is recommended.

The TSS, titratable acidity and pH of avocado paste were all lower than those reported in pulp. For example, Astudillo-Ordóñez and Rodríguez [45] analyzed 'Hass' avocado

Table 1 Physicochemical characteristics and proximate composition of avocado paste

Variable	Value	Reported in the literature	References
L*	11.3 ± 0.7	38.52 ± 0.15 (P)	
a*	2.7 ± 0.3	2.46 ± 0.16 (P)	[90]
b*	11.2 ± 0.8	1.70 ± 0.20 (P)	
C*	11.5 ± 0.8	NQ	
Hue (°)	14.2 ± 13.3	NQ	
TSS (°Brix)	0.7 ± 0.1	8.20 ± 0.02 (P) 5.07–7.26 (PU)	[45, 90]
Titratable acidity (g of citric acid/100 g fw)	0.5 ± 0.0	0.02 ± 0.0 (P) 9.2–19.5 ± NR (PU)	
pH	4.3 ± 0.0	6.33 ± 0.03 (P) 6.6–7.1 ± NR (PU)	
Humidity (%)	27.8 ± 0.6	7.70 ± 0.40 (AF)	[48]
Protein (% dw)	13.8 ± 0.2	15.05 ± 0.35 (AF) 8.1 ± 0.4 (P) 4.9 ± 0.2 (S) 12.8 ± 1.3 (PU)	[48, 49]
Lipids (% dw)	14.2 ± 0.2	3.48 ± 0.15 (AF) 6.9 ± 2.0 (P) 3.7 ± 0.7 (S) 9.3 ± 6.4 (PU)	[48, 49]
Ash (% dw)	2.8 ± 0.1	7.25 ± 0.34 (AF) 3.6 ± 0.4 (P) 3.7 ± 0.2 (S) 7.0 ± 0.6 (PU)	[48, 49]
Carbohydrates (% dw)	69.1	1.2 ± 2.0 (P) 69.1 ± 1.1 (S) 0.6 ± 1.2 (PU)	[49]

Values are presented as mean ± standard deviation

NR not reported, *(P)* data reported in peel, *(S)* data reported in seed, *(AF)* data reported in defatted avocado flour, *(PU)* data reported in pulp

pulp harvested in different localities, and report TSS of 5.07–7.26, titratable acidity 9.2%-19.5% and pH 6.6–7.1. Likewise, Ramos-Aguilar [4] reported TSS values of 8.20, titratable acidity 0.02% and pH 6.3 in avocado peels. As with color, these differences suggest that oil extraction and combined characteristics of defatted pulp, peel and seed, all contributed significantly to changes in TSS, titratable acidity and pH. In addition, changes may be occurring during storage, since this can decrease TSS as sugars are being metabolized, while titratable acidity and pH also change in parallel to these and other metabolic reactions [46].

Proximate analysis of avocado paste showed high percentages of lipids and protein, which are comparable to those found in pulp. For example, Araújo et al. (2018) reported 12–24% lipids in avocado pulp, similar to the values reported herein. However, it should be noted that lipids were extracted to produce avocado oil, thus, a significant percentage of those found in our paste samples likely originated from its seed, since it is also a source of lipids [47]. Regarding protein, its concentration was higher than in pulp, due to becoming concentrated after oil extraction and to the presence of seed and peel. Wang [48] reported the proximate analysis of a defatted avocado flour, with a content of 7.70% humidity, 7.25% ash, 15% protein, 3.48% lipids; the latter is lower than that found in avocado paste analyzed in the present study. Permal [49] reported the proximate analysis of avocado byproducts in order to use them as a food preservative; they determined the content of protein, lipids, ash and carbohydrates of avocado peel (8.1, 6.9, 3.6 and 1.2%, respectively), seed (4.9, 3.7, 3.7 and 69.1%, respectively) and defatted pulp (12.8, 9.3, 7.0 and 0.6%, respectively). Thus, it is apparent that the avocado paste of the present study still retains a higher content of proteins and lipids, as compared to the data of Permal [49], which may be attributed to the content of all avocado byproducts. It is therefore remarkable that despite being considered a byproduct, avocado paste contains considerable amounts of both lipids and proteins, making it a potential source of these macronutrients [7].

Quantification of bioactive compounds (BCs) of avocado paste

Concentration of phenolic compounds identified and quantified in avocado paste are shown in Table 2. Although phenolic compounds are highly bioactive, they are prone to oxidation and other reactions that compromise their chemical structure and bioactivities. For example, changes in pH, metal ion-catalyzed oxidation, increases in temperature, exposure to light and oxygen all accelerate these reactions [50]. Susceptibility is further increased when cells that contain phenolic compounds are damaged, as in the case of avocado paste, where tissues have been mechanically processed and exposed to ambient conditions [51]. Furthermore, defatted pulp, peels and seeds generated during industrial processing are regularly discarded, thus, minimal or no precautions are taken to avoid harsh conditions. Should avocado paste be used as source of BCs, it should be handled and stored with care to avoid prolonged exposure to ambient conditions, otherwise, the concentrations of BC's may decrease, and their bioactivities may become compromised.

It is apparent that most phenolics were not freely available for extraction, that is, they were covalently bound and required an initial hydrolysis to be released. Acid hydrolysis released the majority of all compounds present, followed by alkaline hydrolysis. Ferulic acid was the most abundant compound released after both hydrolyses, while gallic acid was mainly extracted directly, since its yield did not improve

Table 2 Phenolic compounds identified and quantified in avocado paste

Compound	Free	Ac H	Alk H	Reported in the literature	Reference
Total phenolic compounds	2.1 ± 0.1	NQ	NQ	NQ	
Gallic acid	0.9 ± 0.0	ND	Tr		
Protocatechuic acid	ND	9.9 ± 0.5	13.1 ± 1.8	0.0220 ± 0.0023 (P) 0.0028 ± 0.0012 (S)	[54]
p-coumaric acid	ND	6.7 ± 0.9	15.8 ± 0.3	0.0024 ± 0.0011 (P) 0.0020 ± 0.0002 (S)	
Ferulic acid	ND	11.0 ± 1.7	29.0 ± 1.2	0.0025 ± 0.0010 (P) 0.0005 ± 0.0001 (S)	
Quercetin	ND	4.6 ± 0.0	ND	0.0069 ± 0.0002 (P) 0.0039 ± 0.0000 (S)	
Quercetin 3-β-D-glucoside	0.2 ± 0.0	0.7 ± 0.0	0.1 ± 0.0	NQ	
Kaempferol	ND	2.4 ± 0.1	ND	NQ	

Values are presented as mean ± standard deviation. Total phenolic compounds are expressed as mg GAE/g dw, all others are µg/g dw. Compounds were directly extractable with solvents (Free) or released after an acid hydrolysis (Ac H) or an alkaline hydrolysis (Alk H)

NQ not quantified, *Tr* trace amounts, *ND* Not Detected, *(P)* data reported in peel, *(S)* data reported in seed

after hydrolysis. Gallic acid is apparently present as free compound and bound to other macromolecules to a lesser extent. Other compounds released include *p*-coumaric acid, quercetin aglycone and a glycoside and kaempferol. This phenolic profile is similar to the one reported by Villa-Rodriguez [52] in ripe avocado pulp, however, there are differences in concentrations. For example, they report catechin as the most abundant compound (7.2 µg/g), followed by vanillic acid (2.4 µg/g) and chlorogenic acid (0.9 µg/g). Likewise, Pahua-Ramos [53] identified protocatechuic acid (128.2 µg/g) and kaempferol (2.2 µg/g) in avocado seed meal. The profile reported in the present study is similar to the one found by Velderrain-Rodríguez [54], but with higher concentrations; they analyzed the phenolic composition of avocado peel and seed, where protocatechuic acid (0.0220 and 0.0028 µg/g, respectively), *p*-coumaric acid (0.0024 and 0.0020 µg/g, respectively), ferulic acid (0.0025 and 0.0005 µg/g, respectively) and quercetin (0.0069 and 0.0039 µg/g, respectively) were found. Likewise, López-Cobo [3] identified phenolic compounds present in avocado pulp, showing that their concentration varies in ripe and overripe fruits, for example, in the case of *p*-coumaric acid glucoside (84.4 and 93.5 µg/g, respectively), ferulic acid glucoside (19.5 and 24.4 µg/g, respectively), protocatechuic acid-4-glucoside (2.40 and 2.70 µg/g, respectively). This is expected, since various metabolic processes are known to take place during ripening which alter phenolic concentration, such as rates of synthesis, degradation and biotransformation [52]. Furthermore, the effects of exposure to ambient conditions, such as those discussed for total phenolic compounds, are more noticeable when analyzing specific compounds.

Concentrations of other BCs found in avocado paste are shown in Table 3. Total flavonoid concentration found in the present study is higher than that reported in peel (0.84 mg CE/g dw) and pulp (0.31 mg CE/g dw) by Amado [55]. In contrast, it is lower than values reported in seed by Athaydes [56], who found 28.09 mg CE/g dw. According to these results, it is likely that seed is a significant contributor to flavonoid concentration in avocado paste, since these compounds are less abundant in pulp and peel. It is also plausible that flavonoids that were originally found in pulp, may have been co-extracted during oil extraction, making defatted pulp a minor contributor to flavonoid concentration.

Content of hydrolyzable tannins was approximately 18-fold higher than condensed tannins. Others have analyzed tannins in avocado seed, for example, Ejiofor [57] report that it contains 0.06 mg GAE/g dw of total tannins, a lower value than the one found in our samples. Tannins are complex, high-molecular-weight polymeric compounds, whose presence can be easily perceived when orally consumed, due to the astringent sensation that they produce [58]. Tannin concentration found in avocado paste makes it likely to result in noticeable astringency if orally ingested, thus, its applications within the food industry should take this fact into consideration. Tannins have also been reported to exert significant bioactivities, for example, they have high antioxidant properties due to their chemical composition (gallate moieties for hydrolyzable and anthocyanins for condensed). This in turn is responsible for their anticancer and antimutagenic potentials, by protecting against oxidative damage [59, 60]. Tannins contained in avocado pulp and its byproducts are also capable of exerting numerous bioactivities on health, in addition to those of other BCs present in avocado [7].

Chlorophyll concentrations found are higher than those quantified by Wang [16], who report 28.7 µg/g dw, 41.2 µg/g dw and 28.8 µg/g dw in avocado pulp, seed and peel, respectively. Ramos-Aguilar [4] reported the content of α (1217 µg/g dw) and β (8319 µg/g dw) chlorophyll in avocado peel. Chlorophylls have been studied for their bioactivities in various models, for example, reports suggest that they may exert chemopreventive effects, which are partially related to their antioxidant and antimutagenic activities, in addition to some antimicrobial and anti-inflammatory effects [61]. However, bioeffects of avocado chlorophylls have been poorly studied.

Carotenoid and tocopherol profile of avocado paste is shown in Table 4. The most abundant carotenoid was

Table 3 Concentrations of bioactive compounds (BCs) in avocado paste

Compound	Concentration	Reported in the literature	References
Total flavonoids (mg CaE/g dw)	3.87 ± 0.34	0.84 ± 0.04 (P) 28.09 ± 0.02 (S) 0.31 ± 0.02 (PU)	[55, 56]
Hydrolyzable tannins (mg GAE/g dw)	11.07 ± 1.79	NQ	
Condensed tannins (mg CyE/g dw)	0.63 ± 0.04	NQ	
Chlorophylls (µg/g dw)	145.47 ± 0.29	28.8 ± 6.2 (P) 41.2 ± 5.7 (S) 28.7 ± 3.3 (PU)	[16]

Values are presented as mean ± standard deviation

GAE gallic acid equivalents, *CaE* catechin equivalents, *CyE* cyanidin equivalents, *NQ* not quantified, *(P)* data reported in peel, *(S)* data reported in seed, *(PU)* data reported in pulp

Table 4 Carotenoid and tocopherol profile of avocado paste

Compound	Concentration	Reported in the literature	References
α-carotene	17.9 ± 2.5	270 ± 6 (P) 19–30 ± NR (PU)	[62, 90]
β-carotene	98.8 ± 8.6	135 ± 3 (P) 250 ± NR (AWW) 48–81 ± NR (PU)	[49, 62, 90]
Zeaxanthin	63.0 ± 5.3	6.0 ± 0.0 (P) 8.0–18.0 ± NR (PU)	[62, 90]
α-tocopherol	10,412 ± 436	3057 ± 25 (P) 9970 ± 1681 (AWW)	[62, 90]
γ-tocopherol	3120 ± 361	NR	NR

Units are μg/100 g dw. Values are presented as mean ± standard deviation

(P) data reported in peel, *(AWW)* data reported in avocado wastewater, *NR* not reported, *(PU)* data reported in pulp

Table 5 Antioxidant capacity of avocado paste

Methodology	Antioxidant capacity	Reported in the literature	References
FRAP	3.52 ± 0.33	47.88 ± 2.34 mg Fe (II)/g dw (P) 131.76 ± 0.72 mg Fe (II)/g dw (P) 7.65 ± 0.18 mg Fe (II)/g dw (S)	[65, 66]
DPPH	1.57 ± 0.14	8.36 ± 4.04 (P) 1.83 ± 0.07 (S)	[54, 68]
TEAC	5.89 ± 0.34	24.12 ± 0.18 (P) 133.74 ± 0.72 (P) 6.73 ± 0.00 (S)	[65, 66]

Units are mg TE/g dw. Values are presented as mean ± standard deviation

(P) data reported in peel, *(S)* data reported in seed

β-carotene, followed by zeaxanthin and α-carotene. Their concentration is comparable to those found by Lu [62] in avocado pulp, who report 48–81 μg/100 g of β-carotene, 8–18 μg/100 g of zeaxanthin and 19–30 μg/100 g of α-carotene, with variations due to sampling date. Likewise, Ramos-Aguilar [4] reported the carotenoid content in avocado peel, where they obtained a content of α-carotene of 270 μg/100 g, 135 μg/100 g of β carotene and 6 μg/100 g of zeaxanthin. Carotenoids are precursors of vitamin A, most notably β-carotene, but their bioactivities as antioxidants are also well-known. Consumption of carotenoids is associated with a low incidence of certain types of cancer and cardiovascular diseases [63].

Concentration of α-tocopherol was approximately three-fold higher than γ-tocopherol. Both compounds have been reported in avocado, for example, Villa-Rodriguez [52] found a similar concentration of α-tocopherol (approximately 10,000 μg/100 g), but a lower one for γ-tocopherol (approximately 700 μg/100 g). Ramos-Aguilar [4] reported the α-tocopherol content in avocado peel, where they obtained a content of 3057 μg/100 g. Permal [49] analyzed avocado wastewater, the most abundant byproduct generated when producing avocado oil by cold pressing, and reported 9970 μg/100 g of α-tocopherol and 250 μg/100 g of β-carotene. This is a lower value than that reported in avocado paste, which may be due to the presence of all byproducts (defatted pulp, seed and peel). Due to their lipophilic nature, it is likely that most carotenoids and tocopherols present in avocado pulp were co-extracted during oil extraction, thus, compounds quantified in avocado paste may be derived from seed rather than pulp.

Antioxidant capacity of avocado paste

Table 5 shows the antioxidant capacity of avocado paste, as determined with three different methods. Antioxidant activity ranged from 1.57–5.89 mg TE/g dw for DPPH and TEAC, respectively. Since each methodology has a different mechanism of action, differences in antioxidant capacity usually arise when measuring it with more than one, thus, differences obtained here can be attributed to these inter-method variations. More specifically, affinity of phenolic compounds (and other antioxidant molecules present in the sample) towards the compound used by each assay varies according to structural differences like hydroxylation and methylation patterns, food matrix and pH [64]. Other authors have measured antioxidant capacity of avocado pulp and its byproducts, for example, Pahua-Ramos [53] reports 43.32 mg TE/g dw when using the TEAC method to measure it in avocado seed meal. This value is significantly higher than the one reported here, which can be attributed to the controlled conditions under which they processed their samples, as compared to samples from industrial sources reported here. Thus, increased precautions are required when handling avocado byproducts if BCs with antioxidant capacity are to be extracted from them, in order to avoid degradation and loss of their biological activities.

Figueroa [65] reported a high antioxidant capacity in ethanolic avocado peel extracts [47.88 mg Fe (II)/g dw and 24.12 mg TE/g dw for FRAP and TEAC, respectively], higher values than those found on this study. This may be due to the use of an optimized a microwave-assisted extraction method, which maximizes the extraction of bioactive phenolic compounds, while minimizing the degradation of other sensitive compounds, furthermore, their samples were obtained under laboratory conditions. Likewise, Ortega-Arellano [66] reported the antioxidant capacity

of methanolic avocado peel [131.76 mg Fe (II)/g dw and 133.74 mg TE/g dw] and seed extracts [7.65 mg Fe (II)/g dw and 6.73 mg TE/g dw] by the FRAP and TEAC methods. They found higher values in the peel, likely due to the fact that it has a higher content of extractable BCs like condensed tannins, phenolic acids and flavonoids [67]. Other authors reported the antioxidant capacity (DPPH method) of avocado peel and seed, with the peel having 8.36 mg TE/g dw [54] and the seed 1.83 mg TE/g dw [68], similar values to those found in the present study. The choice of extraction solvent is critical, since this can affect the amount and type of extracted compounds. Ethanol was used in the present work, since it is suitable to extract compounds with antioxidant activity from food matrices or byproducts, its high efficiency and low toxicity, since it is recognized as a safe solvent that does not produce adverse health effects, as compared to methanol or similar solvents [69].

Fatty acid profile of avocado paste

Fatty acid profile of avocado paste is shown in Table 6. Six fatty acids were identified, with oleic acid as the most abundant, followed by linoleic and palmitic acid with similar percentages. Profile contained mostly monounsaturated fatty acids (MUFAs), with similar percentages of polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs). These results differ from those reported by Amado [70] in avocado pulp, where palmitic acid (4398 mg/100 g) was found in highest concentration, and with similar concentration as that of oleic acid (4306 mg/100 g). They also report a general profile of 48.85% MUFAs, 17.61% PUFAs and 33.53% SFAs. Thus, fatty acid profile of avocado paste contains an increased concentration of MUFAs and PUFAs, with less SFAs, as compared to avocado pulp. As previously stated, most lipids present in pulp were extracted during oil extraction, making it likely that those found in paste originate from its seed, peel and some that remained after pressing. Biotic and abiotic factors like temperature, maturation, production area, handling and agronomic practices, processing, among others may influence the lipid content of avocado and its byproducts. Alkaltham [5] reported the percentage of oleic, palmitic, linoleic and stearic acids present in avocado pulp (51.96, 20.40, 18.99 and 0.69%, respectively), peel (54.57, 18.59, 17.00 and 0.63%, respectively) and seed (36.53, 29.34, 18.78 and 3.26%, respectively). Said compounds were also found in avocado paste of the present study. As previously stated, most lipids present in pulp were extracted during oil production, making it likely that those found in paste originate from its seed, peel and some that remained after pressing. Biotic and abiotic factors like temperature, ripening, production area, handling, agronomic practices, processing, among others may influence the lipid content of avocado and its byproducts. The fatty acid content of avocado paste could be attributed to the breakdown of the cell matrix and the opening of oil-containing vacuoles, thus enhancing the leaching of oil from cells [5].

Table 6 Fatty acid profile of avocado paste

Fatty acid	Percentage	Reported in the literature	References
Palmitic acid (16:0)	17.8 ± 0.9	18.59 ± 0.01 (P) 18.78 ± 0.24 (S) 20.40 ± 0.04 (PU)	[5]
Palmitoleic acid (16:1, ω-7)	8.0 ± 0.1	NR	
Stearic acid (18:0)	1.5 ± 0.2	0.63 ± 0.00 (P) 3.26 ± 0.03 (S) 0.69 ± 0.04 (PU)	
Oleic acid (18:1, ω-9)	49.8 ± 0.9	54.57 ± 0.17 (P) 36.53 ± 0.28 (S) 51.96 ± 0.05 (PU)	
Linoleic acid (18:2, ω-6)	20.5 ± 0.3	29.34 ± 0.16 (P) 17.00 ± 0.07 (S) 18.99 ± 0.05 (PU)	
α-linolenic Acid (18:3, ω-3)	2.4 ± 0.2	NR	
MUFAs	57.9 ± 1.0%	NR	
PUFAs	22.8 ± 0.5%	NR	
SFAs	19.3 ± 1.1%	NR	

Values are presented as mean ± standard deviation

MUFAs sum of monounsaturated fatty acids, *PUFAs* sum of polyunsaturated fatty acids, *SFAs* sum of saturated fatty acids, *NR* not reported, *(P)* data reported in peel, *(S)* data reported in seed, *(PU)* data reported in pulp

Release of phenolic compounds during an in vitro digestion of avocado paste

Figure 1 shows the concentration of phenolic compounds during different phase of an in vitro digestion of avocado paste. After oral phase, approximately 0.3 mg GAE/g dw of phenolic compounds were bioavailable (released from the food matrix), which increases approximately three-fold after gastric phase and decreases slightly after the intestinal. Pre-processing of avocado pulp during oil extraction may have also exerted positive effects on the bioavailability of its phenolic compounds. Most phenolics are not found free, but are instead covalently bound to components the food matrix, such as carbohydrates, proteins, fiber and cell walls [71–73]. Phenolic digestion happens mainly during the intestinal phase, but their interaction with the food matrix can influence their release and absorption.

Antioxidant capacity during each phase of an in vitro digestion of avocado paste was evaluated by three different methods, as shown in Fig. 2. Data demonstrates that, independent of method used, significant differences ($p < 0.05$) were found between digestion phases, with gastric showing the highest values. This is consistent with the concentration of phenolic compounds, where the highest concentration was reported during the gastric phase. Indeed, a similar pattern has been reported when digesting other fruits,

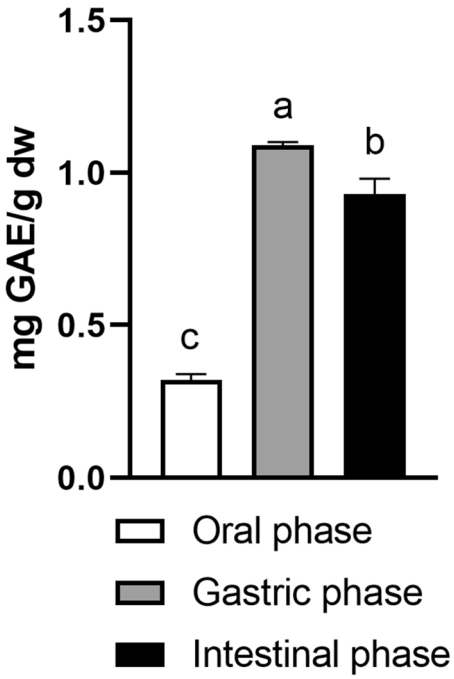

Fig. 1 Phenolic compounds released after each phase of an in vitro digestion of avocado paste. Values are presented as mean ± standard deviation (n = 3). Different literals indicate significant differences ($p < 0.05$)

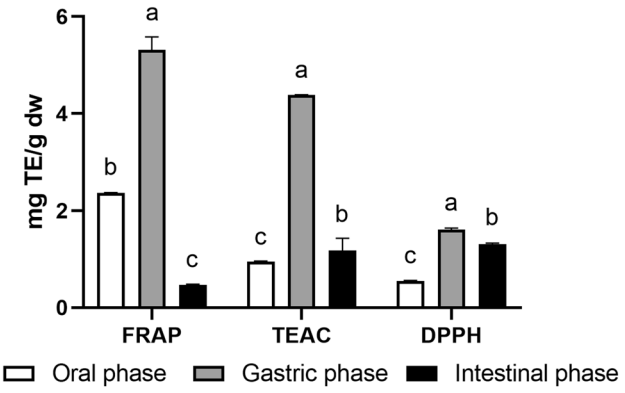

Fig. 2 Antioxidant capacity during different phases of an in vitro digestion of avocado paste, determined by three different methods (FRAP, TEAC and DPPH). Values are presented as mean ± standard deviation (n = 3). Different literals indicate significant differences ($p < 0.05$) for each method

such as mango, where an increased release of phenolics is associated with an increased antioxidant capacity [74]. This suggests that phenolic compounds are major contributors to the antioxidant capacity of avocado paste, when digested in an in vitro model, which is in accordance with other studies. For example, a previous study showed that the highest contribution to the antioxidant activity was provided by phenolic compounds, rather than vitamin C or carotenoids [75, 76]. Furthermore, even while avocado is rich in lipids and hydrophobic antioxidants, the contributions of its phenolic compounds appear to be significant, and may be tentatively attributed to its tannin, flavonoid and phenolic acid composition.

Figure 3 shows concentration of phenolic compounds that passively diffused through a dialysis membrane during the intestinal phase of an in vitro digestion of avocado paste. Dialysis was used to simulate passive transport of bioaccessible phenolic compounds, where those that are dialyzable represent the fraction that would be absorbed in the intestinal lumen. It is apparent that there is a marked increase in concentration of phenolic compounds during the first 30 min, thereafter, significant differences ($p < 0.05$) continue over time, with the highest concentration found at 120 min. After avocado paste has been digested, compounds released from the food matrix (fiber, protein, free sugars) can interact with phenolics and/or the dialysis membrane, which affects their passage across the membrane. Regardless, a significant proportion of phenolics present in our samples was still able to cross, likely due to their small size, which allows them to overcome these interactions. Some ex vivo studies indicated that phenolic acid absorption takes place in the intestinal portion [77]. Certain chemical characteristics, for example, molecular weight, lipophilicity, stereochemistry and the presence of groups capable of hydrogen bonding, affect

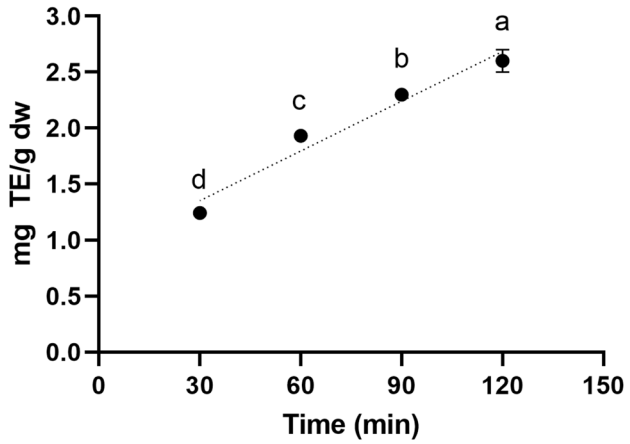

Fig. 3 Passive diffusion of phenolic compounds from avocado paste, during the intestinal phase of an in vitro digestion. Values are presented as mean ± standard deviation (n = 3). Different literals indicate significant differences ($p < 0.05$)

their transport and permeability into the enterocytes [78]. It is also believed that phenolic compounds are absorbed by a passive diffusion mechanism or by carriers present in the intestine, such as P-glycoprotein and cotransporters for SGLT1, for example, in the case of aglycones that cross the membrane of the epithelial cells via passive diffusion, whereas glycosides, esters and polymers require active transport [76].

Antioxidant capacity of the dialysis medium of an in vitro digestion of avocado paste is shown in Fig. 4. A linear increase in antioxidant capacity was apparent when measuring it with FRAP and TEAC, where significant increases ($p < 0.05$) were found between each sampling time, reaching its highest value at 120 min. When DPPH was used to measure it, maximum value was recorded at 60 min, remaining stable thereafter. The pattern recorded with FRAP and TEAC is similar to that of dialyzable phenolic compounds (Fig. 3), however, different sensitivity and behavior of each method used was observed, most notably with DPPH. Increases in antioxidant capacity are likely due to phenolic compounds, since they are characterized for this effect, although contribution of carotenoids, tocopherols and other minor compounds cannot be ruled out [79].

In silico interactions of main phenolic compounds found in avocado paste and cholesterol

It has been widely reported that phenolic compounds from various sources affect the digestive process overall, and that of cholesterol in particular, through different mechanisms [80, 81]. We therefore focused on the interactions of the three most abundant phenolic compounds identified and quantified in avocado paste (protocatechuic acid, ferulic acid and *p*-coumaric acid) with cholesterol, to determine if they are likely to interact with it and possibly disrupt its absorption. The quantum chemical method chosen to

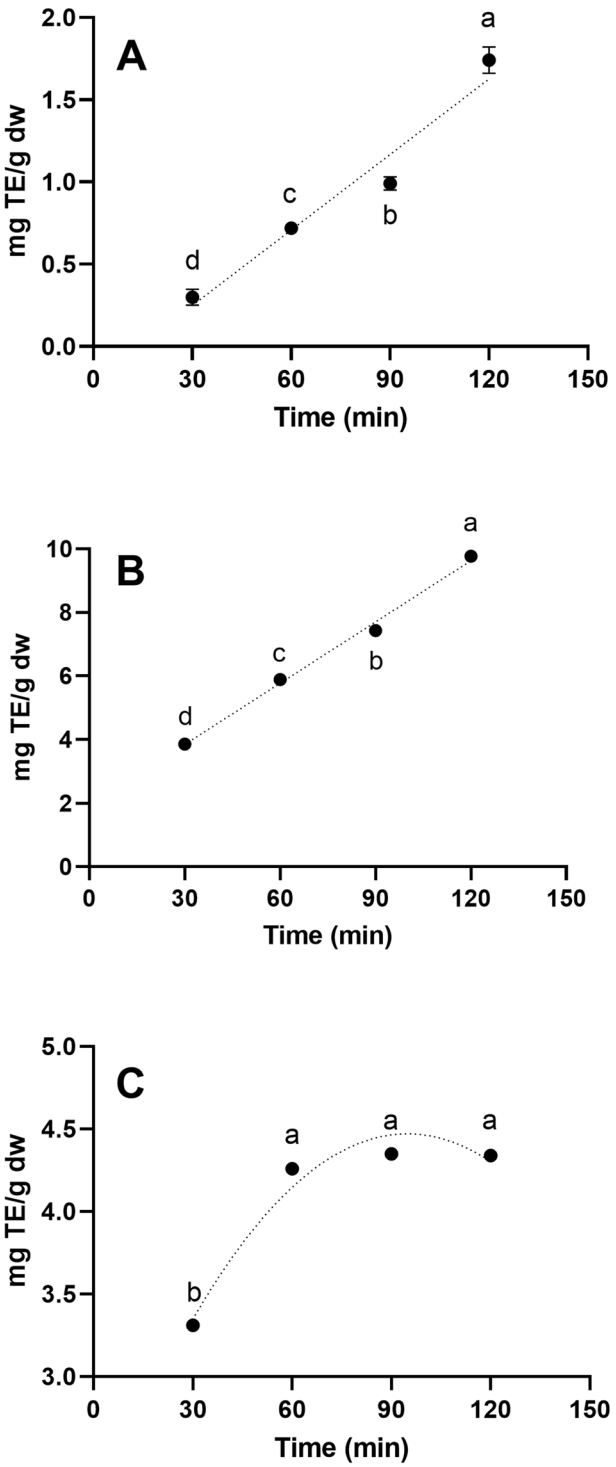

Fig. 4 Antioxidant capacity of the dialysis medium of an in vitro digestion of avocado paste. **A** FRAP, **B** DPPH and **C** TEAC. Values are presented as mean ± standard deviation (n = 3). Different literals indicate significant differences ($p < 0.05$) for each method

produce optimized molecules [B3LYP/6-31G(d,p)], aims to identify the molecular geometry that minimizes local or global energy of the molecules, where the atomic force on each atom is reasonably close to zero, and the superficial potential energy is in a stationary point [82].

An electrostatic map of all molecules of interest (cholesterol and the three phenolic compounds) was produced, as shown in Figs. 5, 6, 7 and 8. The most electronegative areas were localized over the hydroxyl and carboxyl groups, where it is likely that the phenolic-cholesterol interactions may take place. Particularly, the most electropositive area is located over the hydrogen atom of the hydroxyl group; this means that the molecule of cholesterol is prone to form hydrogen bonds through this hydrogen bond donor. At the same time, the most electronegative area is located over the oxygen atom. This atom is part of a hydroxyl group, and is a feature that gives some hydrophilic properties to cholesterol, since it allows it to form hydrogen bonds and interact with water and other molecules.

In the case of phenolic acids, all of them present remarkable electronegative areas over the carboxyl groups. We propose that this feature is the key to molecular recognition, as the most electropositive area in cholesterol is located over the hydroxyl group, and can therefore expect strong attraction between the carboxyl groups of phenolic acids and the hydroxyl group of cholesterol.

Figure 9 shows the dimers formed between cholesterol and the phenolic acids, according to DFT. The image also shows the binding energies of the optimized systems, where it is apparent that these supramolecular complexes have similar binding energies. The ferulic acid-cholesterol complex (Fig. 9b) had the lowest binding energy (15.37 kcal/mol), followed by the cholesterol—protocatechuic acid complex (Fig. 9a) (15.62 kcal/mol) and finally the

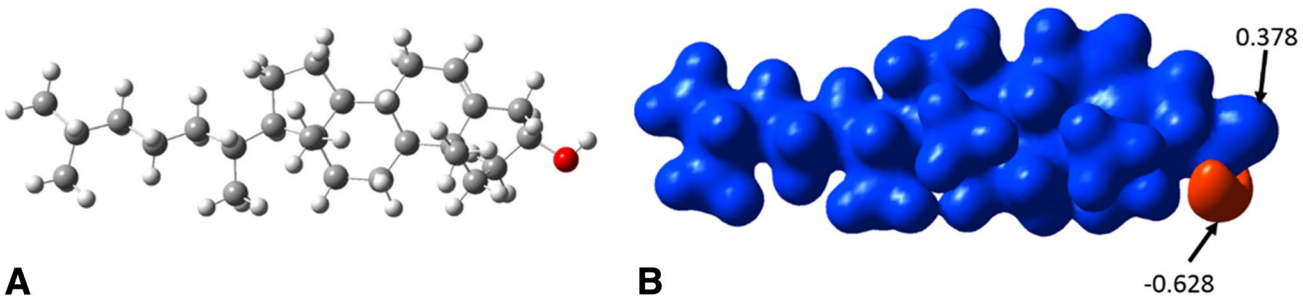

Fig. 5 Molecule of cholesterol, **a** optimized and **b** ESP surface, analyzed through the B3LYP/6-31G(d,p) level of theory. Carbon (grey), hydrogen (white) and oxygen (red). ESP charges are expressed in atomic units

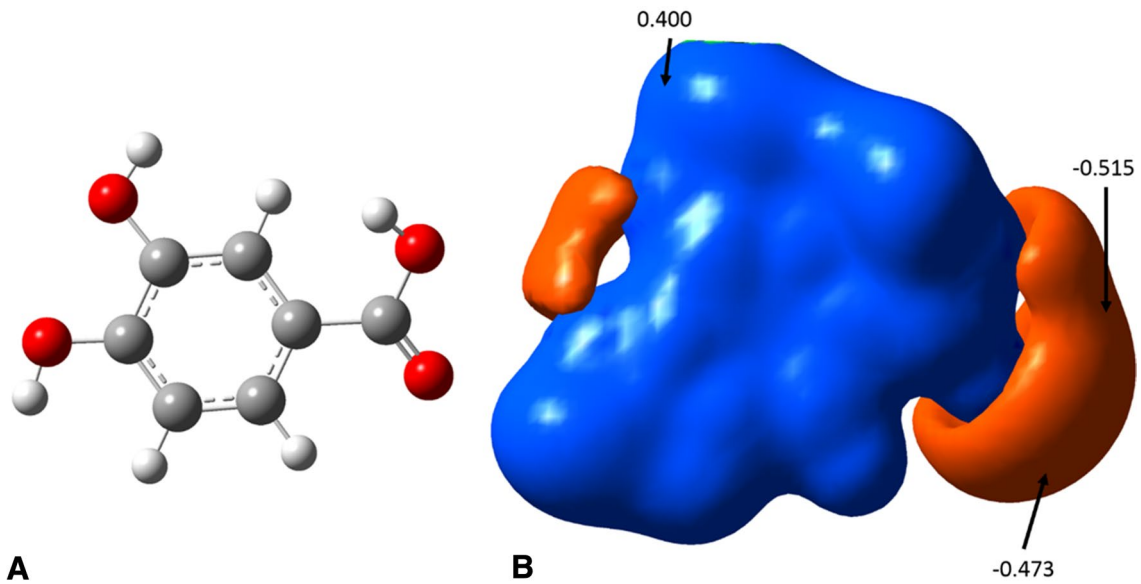

Fig. 6 Molecule of protocatechuic acid, **a** optimized and **b** ESP surface, analyzed through the B3LYP/6-31G(d,p) level of theory. Carbon (grey), hydrogen (white) and oxygen (red). ESP charges are expressed in atomic units

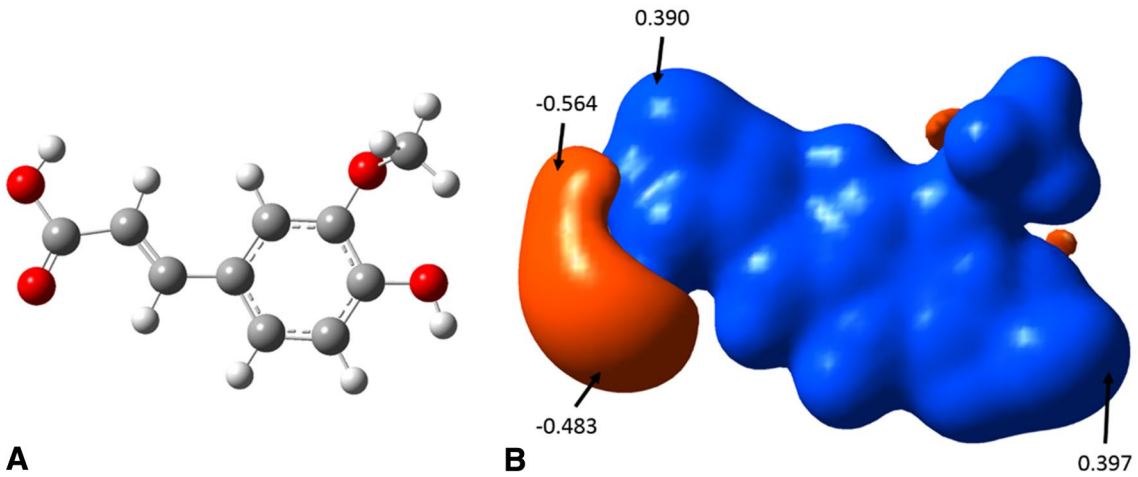

Fig. 7 Molecule of ferulic acid, **a** optimized and **b** ESP surface, analyzed through the B3LYP/6-31G(d,p) level of theory. Carbon (grey), hydrogen (white) and oxygen (red). ESP charges are expressed in atomic units

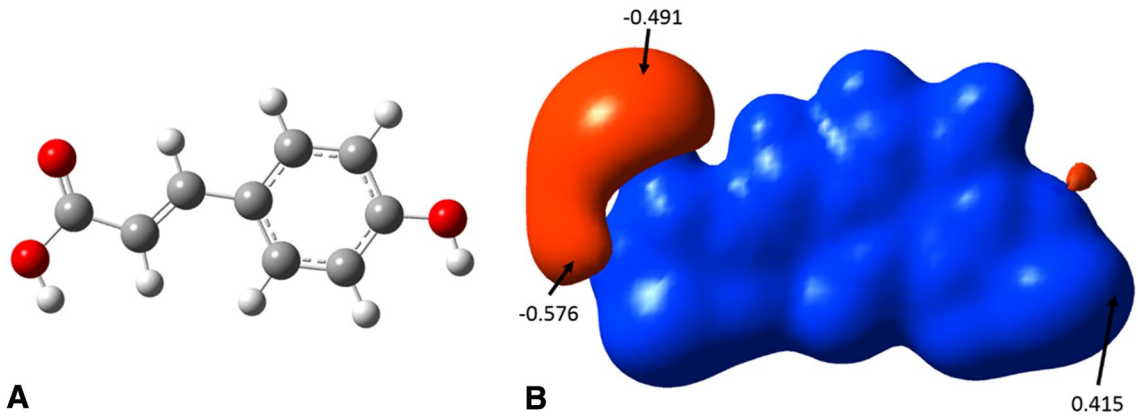

Fig. 8 Molecule of *p*-coumaric acid, **a** optimized and **b** ESP surface, analyzed through the B3LYP/6-31G(d,p) level of theory. Carbon (grey), hydrogen (white) and oxygen (red). ESP charges are expressed in atomic units

cholesterol–*p*-coumaric acid complex (Fig. 9c) (16.14 kcal/mol). These results also revealed that the supramolecular are stabilized by two hydrogen bonds between the hydroxyl–carbonyl interaction. The distances of all hydrogen bonds were similar, ranging from 2.674 to 2.759 Å, which are in agreement with typical values for O–H···O H-bonds [83]. The angles of the hydrogen bonds range from 140.16° to 159.59°. Again, these values are consistent with O–H···O H-bonds [83]. Hydrogen bonds are considered strong if they are close to 180°, while those that are < 120° are considered weak [84]. Thus, it appears that the strength of the hydrogen bonds of these systems is moderate, but strong enough to interact with cholesterol even in aqueous media, avoiding the interactions between cholesterol molecules and the formation of bigger aggregates of molecular complexes. Also, from the results, it is suggested that the interaction between *p*-coumaric acid and cholesterol would have a more significant effect on cholesterol digestion. However, additional experimentation is required to fully substantiate this claim.

The interactions of cholesterol with some phenolic compounds have been described before. For example, Zheng [85] analyzed the interactions of catechins with cholesterol. They report hydrogen bond distances of 2.01 and 2.15 Å with an energy of 2.6 kcal/mol for the epigallocatechin-cholesterol complex, while the epicatechin-cholesterol complex had distances of 1.78 and 2.12 Å with an energy of 1.8 kcal/mol. According to their results, authors propose that the interactions of catechins with cholesterol is mainly stabilized by hydrogen bonds, similar to the data reported herein.

There is also in vitro evidence that shows that catechins (as well as other phenolic compounds) decrease cholesterol solubility by preventing its incorporation into mixed micelles, which delays or inhibits its absorption. For example, apple catechins form insoluble precipitates with

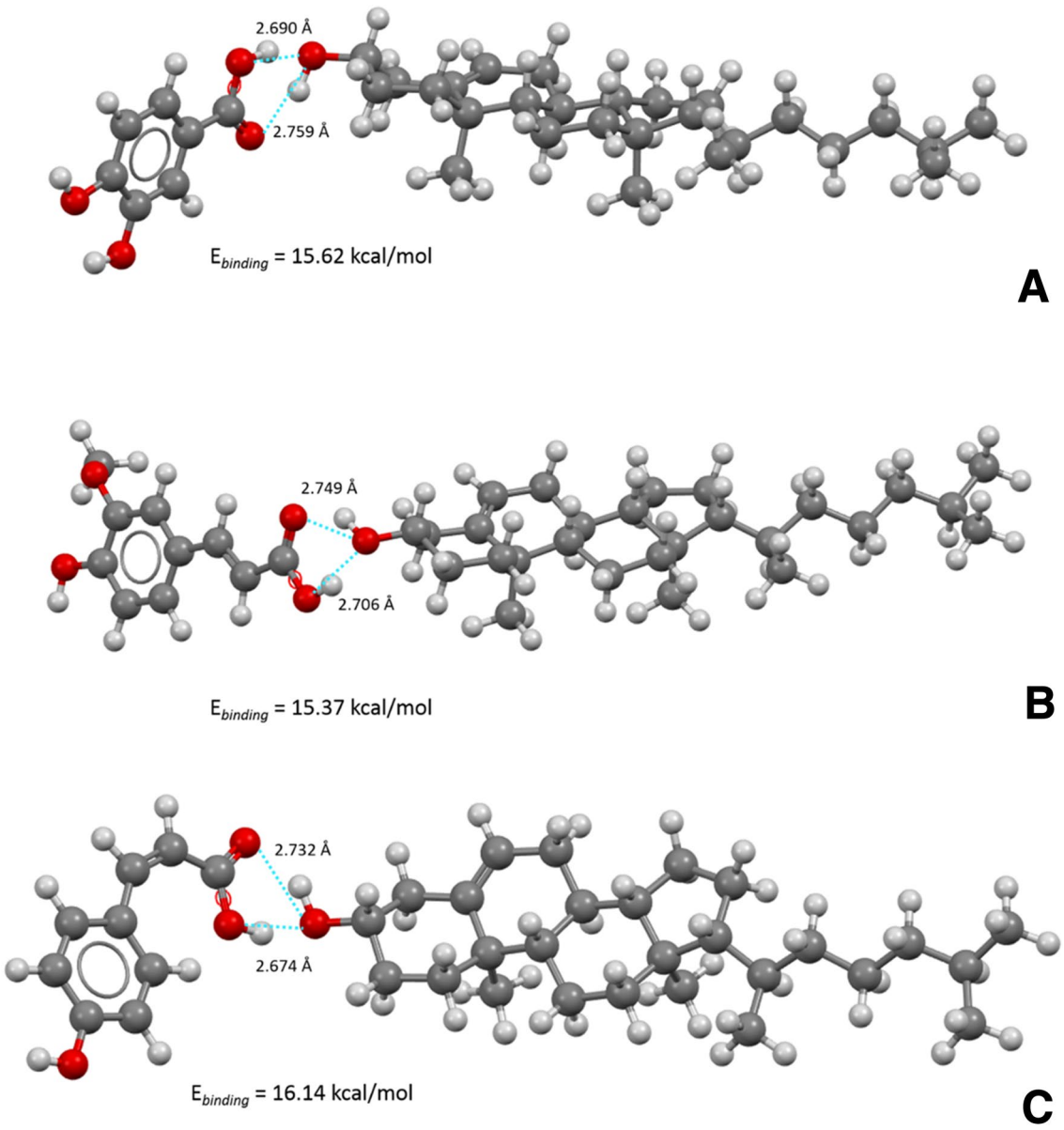

Fig. 9 Molecular dimers and binding energies obtained through the B3LYP/6-31G(d,p) level of theory: **a** dimer 1 (cholesterol–protocatechuic acid); **b** dimer 2 (cholesterol–ferulic acid) and **c** dimer 3 (cholesterol–*p*-coumaric acid). Carbon (grey), hydrogen (white) and oxygen (red)

oxidized cholesterol products, thus inhibiting their absorption [86]. In the case of phenolic acids, such as those studied here, Grzelak-Blaszczyk [87] showed that protocatechuic acid present in onion byproducts increased serum HDL concentration in rats fed high fat diets, thereby decreasing the risk of atherogenic lesions. A ferulic acid-rich extract of *Prosopis cineraria* (of the Fabaceae family) has also shown significant hypolipidemic effects in rats fed high fat diets [88], however, the exact phenolic composition of this extract was not evaluated, thereby making it likely that other compounds also contributed to this effect. Amalan [89] evaluated the effect of *p*-coumaric acid in diabetic rats, where significant decreases in serum cholesterol and triacylglycerols were found, in addition to increases in HDL. Tannins have shown remarkable in vitro and in silico lipase-inhibiting potential [19], thus, the effects of these compounds present in avocado paste may also exert other digestion-modulating actions whose impact may reach beyond cholesterol.

According to our results and to the evidence reported in the literature, we propose that the major phenolic compounds identified in avocado paste may interact with cholesterol, mainly through hydrogen bonds. If these interactions were to take place in the intestinal milieu, they may form insoluble complexes which inhibit incorporation of

cholesterol (dietary or biliary) into mixed micelles and/or its enteric absorption. This is consistent with the evidence reported by other authors in different in vitro and in vivo models, where the consumption of these compounds results in significant improvements of the serum lipid profile.

Conclusion

Avocado paste is a byproduct that contains significant concentrations of lipids and proteins. Its minor components include bioactive phenolic compounds, carotenoids, tocopherols and chlorophylls. Its fatty acid profile mainly contains MUFAs, with oleic acid being the most abundant compound. In vitro digestion showed that total phenolic compounds had a greater release after the gastric phase. Dialyzed phenolic compounds during intestinal phase increased with time, a pattern that was consistent with the antioxidant capacity of this medium. In silico analyses showed that the main phenolic compounds found in avocado paste interact with cholesterol through hydrogen bonds, which suggests that they may exert effects on its digestion. Composition of avocado paste suggests that it may be used as an unconventional source of molecules with health-related bioactivities, thus, additional in vivo studies are required to determine their actions once consumed.

Acknowledgements B. S. Zuñiga-Martínez thanks CONACYT for the scholarship that she received to obtain her Master's Degree. This work was supported by Instituto de Bebidas de la Industria Mexicana de Coca-Cola through project "Inducción de saciedad y modulación de la digestión intestinal de lípidos ejercidos por los compuestos fenólicos de aguacate Hass" (Premio Nacional en Ciencia y Tecnología de Alimentos 2019); and by Centro de Investigación en Alimentación y Desarrollo A.C. (CIAD). The authors thank ACARUS (Área de Computo de Alto Rendimiento, Universidad de Sonora) for allowing access to computational resources.

Funding This work was supported by Instituto de Bebidas de la Industria Mexicana de Coca-Cola through project "Inducción de saciedad y modulación de la digestión intestinal de lípidos ejercidos por los compuestos fenólicos de aguacate Hass" (Premio Nacional en Ciencia y Tecnología de Alimentos 2019); and by Centro de Investigación en Alimentación y Desarrollo A. C. (CIAD).

Data availability Available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare they have no conflict of interest.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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