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# Phenolic compounds from 'Hass' avocado peel are retained in the indigestible fraction after an in vitro gastrointestinal digestion

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## Abstract
Avocado peel is a source of bioactive compounds with antioxidant, anti-inflammatory, antiproliferative and antimicrobial capacities, among others. Physical and chemical interactions of phenolic compounds with indigestible polysaccharides could affect their bioaccessibility in the upper gastrointestinal tract, allowing them to reach the colon, where they exert antioxidant effects and related health effects. The objective of the present work was to evaluate the effect of chemical-enzymatic processes of an in vitro gastrointestinal digestion, on phenolic compounds, antioxidant capacity and chemical constituents of avocado peel, and to determine their content in the indigestible fraction. Results showed that most phenolic compounds resist intestinal digestion (66%), of which $954.72 \pm 19.45$ mg GAE/100 g of dry weight (dw) and $250.72 \pm 7.12$ mg CE/100 g dw are hydrolysable and condensed tannins, respectively. The highest antioxidant capacity remained in the indigestible fraction (79.4%, 59.8% and 79.6%), with greater predominance in the insoluble indigestible fraction (49.1%, 46.6% and 66.7%) for DPPH, ABTS and FRAP, respectively. Most of the indigestible fraction is insoluble (89.39%), while only 7.46% is soluble. The possible beneficial effects of phenolic compounds and antioxidant capacity of the indigestible fraction of avocado peel must be considered in further studies.

## Introduction

Avocado (*Persea Americana* Mill.) is highly-consumed in the Americas and in other parts of the world, with an estimated annual production of 6.5 million tons [1]. Industrial processing discards close to 34.6% of avocado as byproducts, namely, peel (16%) and seed (18.6%) [2].

Avocado peel contains numerous bioactive compounds like hydroxycinnamic acids, hydroxybenzoic acids, flavonoids, proanthocyanidins, procyanidins, phenolic alcohol derivatives, acerogenins, carotenoids, and organic acids, among others [3–5]. Phenolic compounds (PCs) have been widely studied and are known to induce various health-promoting effects related to their antioxidant, anti-inflammatory, antiproliferative and antimicrobial capacities, among others [6]. However, a significant proportion of bioactive compounds are often bound to complex macromolecules like polysaccharides, proteins and pectin, which could alter their release during the different digestion steps and their subsequent bioactivities [7].

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Blancas-Benitez et al. [8] reported that 40.53% of PCs from mango peel were bioaccessible, while Castrica et al. [9] reported that the in vitro digestibility of orange peel, grape pomace and *Camelina sativa* cake were 88.7%, 44.2% and 66.8%, respectively. This suggests that a significant proportion of PCs found in these byproducts is not released from the food matrix during digestion in the upper gastrointestinal tract (mouth-stomach-small intestine); they remain in the indigestible fraction (IF), which makes them available only after reaching the colon, and fermented by microbiota, subsequently favoring their release and have different biological effects.

IF is defined as the fraction of plant-derived food that is not digested or absorbed in the small intestine, thereby reaching the colon, providing a fermentable substrate for resident microbiota [10]. Main components of IF are non-digestible or resistant carbohydrates, lignin, resistant protein, ash and others. Because avocado peel is mainly made up of cellulose (27.6% dry weight, dw), hemicellulose (25.3% dw) and lignin (4.4% dw) [11], it is likely that its PCs remain in the IF when digested, thus, they may resist this process and exert health benefits in the colon by acting as prebiotics. Therefore, the objective of the present work was to evaluate the effect of an in vitro digestion on avocado peel, its PCs and their antioxidant capacity, with emphasis on the IF.

## Materials and methods

### Reagents and materials

Folin-Ciocalteu reagent, DPPH (2, 2-diphenyl-1-picryl-hydrazyl hydrate), Trolox (6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid), ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), TPTZ (2,4,2-tri(2-pyridyl)-S-triazine), pepsin, pancreatin, bile salts, calcium carbonate, rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose and myo-inositol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvents were purchased from J.T. Baker (Mexico City, Mexico).

Avocados (*Persea americana* Mill.) cv. 'Hass' were purchased in a local market in the city of Hermosillo, Mexico. Avocados of commercial maturity and free of apparent defects were randomly selected. Ripeness stage of avocados was established according to the average L*(25.97 ± 0.90), hue (83.76 ± 5.03), chroma (8.11 ± 0.90) and oil percentage (18.44 ± 1.53%) values, which were similar to ripeness stage 3 (L* = 26.29 ± 0.52, hue = 90.62 ± 3.11, chroma = 8.79 ± 0.55 and % oil = 19.89), as previously reported by Villa-Rodríguez et al. [12]. They were disinfected with a sodium hypochlorite solution (200 µL/L, pH 7) for 2 min and dried at room temperature for 1 h. The peel

of 30 randomly selected fruits was manually separated from the avocados and lyophilized and stored in a desiccator until analysis.

### Methanolic extracts

Methanolic peel extracts were made as described by Palafox-Carlos et al. [13]. One g of sample was homogenized with 20 mL of 80% methanol, using an Ultra Turrax® T25 apparatus (IKA Works, Wilmington, DE, USA). The homogenate was sonicated for 30 min (Bransonic 2510, Danbury, CT, USA) and centrifuged (Hermle Z323, Labortechnik Technologies, Wehingen, Germany) at 15,000 rpm for 15 min at 4 °C.

Supernatant was collected, and extraction residues were washed twice more with 10 mL of 80% methanol under identical conditions. Supernatants were filtered through Whatman No. 1 filter paper, and their volume was adjusted to 30 mL with 80% methanol. Extract was used to determine total phenolic compounds and antioxidant capacity. Extraction residues were used to analyze hydrolysable and condensed tannins.

### Extraction of hydrolysable tannins

Extraction of hydrolysable tannins was carried out as described by Hartzfeld et al. [14]. A portion of methanol extraction residue was hydrolyzed with a methanol: sulfuric acid solution (10:1 v/v) for 20 h at 85 °C under constant stirring and then cooled to 25 °C and centrifuged at 15,000 rpm for 15 min at 4 °C. Supernatant was filtered (Whatman paper No. 1) and collected in a 50 mL volumetric flask. Residue was washed twice more with 10 mL distilled water per wash; sample was centrifuged and filtered as described. Recovered volume from hydrolysis and from the washes was made up to 50 mL with distilled water and was used to quantify hydrolysable tannins and antioxidant capacity.

### Extraction of condensed tannins

Residues from methanolic extraction were hydrolyzed to release anthocyanins from condensed tannins, as described by Reed et al. [15]. One gram of methanol extraction residue was mixed with 10 mL of a butanol: HCl solution (97.5:2.5 v/v) and placed in a water bath at 100 °C for 3 h. After hydrolysis, supernatant was separated by centrifugation (15,000 rpm, 15 min, 4 °C), and washed twice with 10 mL of the previous solution. Supernatants were made up to 50 mL, and condensed tannins were quantified from this solution as anthocyanin monomers, according to their absorbance at 555 nm in a FLUOstar Omega spectrophotometer (BMG Labtech, Durham, NC, USA). Results were reported as mg

cyanidin equivalents (CE)/100 g dw. A fraction of the extract was used to determine antioxidant capacity.

## Quantification of total phenolic compounds

Total phenolic compounds were quantified according to Singleton and Rossi [16]. Briefly, 15 μL of extract was mixed with 75 μL of Folin-Ciocalteu reagent (1 N) and 60 μL of 75% (v/v) sodium carbonate; mixture was incubated in the dark for 30 min at 25 °C. Absorbance was then read at 765 nm. Results were expressed as mg of gallic acid equivalents (GAE)/100 g dw.

## Antioxidant capacity

Antioxidant capacity of methanolic extracts, hydrolysable and condensed tannins was determined with the DPPH, ABTS and FRAP assays. DPPH was performed as described by Brand-Williams et al. [17], with slight modifications. Briefly, 10 μL of extract was mixed with 140 μL of DPPH solution (absorbance $0.70 \pm 0.02$ at 515 nm) in a microplate well and incubated for 30 min in the dark. Afterwards, absorbance was then read at 515 nm, and used to quantify antioxidant capacity, which was expressed as μmol Trolox equivalents (TE)/g dw.

ABTS was performed as described by Re et al. [18]. ABTS radical was generated by reacting a 7 mM ABTS solution with 2.45 mM sodium persulfate in the dark at 25 °C for 16 h. Resulting solution was diluted with ethanol until its absorbance at 734 nm was $0.70 \pm 0.02$. 245 μL of this solution was added to 5 μL of sample in a microplate well and incubated in the dark for 6 min. Absorbance was then read at 734 nm, and used to quantify antioxidant capacity, which was expressed as μmol TE/g dw.

FRAP assay was performed as described by Benzie and Strain [19]. FRAP reagent (280 μL) was mixed with 20 μL of methanol extract, hydrolysable or condensed tannin extract and incubated in the dark for 30 min. Absorbance was read at 630 nm, and results were expressed as μmol TE/g dw.

## Extraction of indigestible soluble (IF) and insoluble fraction

Composition of IF was determined as described by Saura-Calixto et al. [10], which makes it possible to differentiate between PCs associated with macromolecules in the soluble and insoluble forms. This method combines enzymatic treatments under physiological temperature and pH, while separating digestible and indigestible compounds by dialysis. Total IF was calculated as the sum of insoluble IF [nonstarch polysaccharides (NSPs), Klason lignin, resistant proteins, ash, PCs, condensed tannins and hydrolysable tannins) and soluble IF (NSPs and PCs)].

To extract soluble IF, 300 g of lyophilized peel were sequentially treated with 0.2 mL pepsin solution (300 mg/mL in 0.08 M HCl-KCl buffer, pH 1.5, 40 °C, 1 h), 1 mL pancreatin (5 mg/mL in 0.1 M phosphate buffer, pH 7.5, 37 °C, 6 h) and 1 mL α-amylase (120 mg/mL in 0.1 M Tris-maleate buffer, pH 6.9, 37 °C, 16 h). Samples were then centrifuged (15 min, 15,000 rpm) and supernatants recovered. Residues were washed twice with 5 mL of distilled water, and all supernatants combined and used to extract the soluble IF. Residues correspond to the insoluble IF [10].

Supernatant was incubated with 100 μL of amyloglucosidase (45 min, 60 °C). To eliminate digestible compounds, supernatants were transferred to cellulose membranes (12,000 and 14,000 Da MW cutoff) and dialyzed under constant waterflow (7 L/h) for 48 h and 25 °C. After dialysis, content of the membranes was placed in glass containers and stored at −20 °C, until subsequent analyses.

## Quantification of neutral sugars, uronic acids and Klason lignin

NSPs from the soluble IF were hydrolyzed with 1 M sulfuric acid at 100 °C for 90 min; NSPs were evaluated as the sum of neutral sugars and uronic acids and expressed as % dw [10].

Neutral sugars were derivatized to alditol acetates as described by Blakeney et al. [20]. For this, 500 μL of hydrolyzed soluble IF were first dried under nitrogen flow; 100 μL of sodium borohydride were added, and the mixture was incubated for 1 h at 25 °C. Two hundred μL of acetic anhydride, 20 μL of 1-methylimidazole, 2 mL of water and 3 mL of chloroform were then added to the mixture. The chloroform phase was recovered and dried with nitrogen. Derivatized product was suspended in 150 μL of acetone and injected into a gas chromatograph (Varian CP-3800, Varian, Palo Alto, CA, USA) equipped with an FID detector (250 °C) and DB-23 capillary column (30 m × 0.25 mm, 210 °C), using helium (3 mL/min) as a carrier gas. Myo-inositol was used as internal standard. Neutral sugars were quantified from calibration curves of rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose.

Uronic acids were quantified using the method of Ahmed and Labavitch [21]. Briefly, a 200 μL aliquot of the soluble IF hydrolyzate was mixed with 1.2 mL of 12.5 mM sodium borate in concentrated sulfuric acid. Mixture was incubated for 5 min in a 100 °C water bath, and 20 μL of *m*-phenyl phenol (0.15%, v/v) diluted in 0.5% sodium hydroxide (w/v) were then added. Absorbance was read at 520 nm, and galacturonic acid was used as reference standard.

Klason lignin was determined as described by Southgate [22]. Briefly, insoluble IF was hydrolyzed with 3 mL of 12 M sulfuric acid for 1 h in a 37 °C water bath. 33 mL of distilled water were added and incubated in a water bath at 100 °C

for 90 min. Samples were then centrifuged at 14,000 rpm for 15 min at 4 °C; supernatant was recovered and filtered through Whatman paper No. 1. Residues were washed three additional times with 10 mL distilled water, centrifuged and supernatants recovered. Five hundred μL and 200 μL were sampled from this hydrolysate, and used to quantify neutral sugars and uronic acids, respectively, according to the previously described methodologies. Residues that remained after the washes were oven-dried at 100 °C for 12 h, cooled and weighed. Recorded weight was reported as content of Klason lignin of the insoluble IF.

Resistant protein of the insoluble IF was determined using the micro Kjeldahl 960.52 l-AOAC method [23], while ash was determined using method 942.05-AOAC [23].

### Quantification of phenolic compounds (PCs) and antioxidant capacity of the indigestible fraction (IF)

Total PCs of the soluble IF solution obtained after dialysis were quantified using the Folin-Ciocalteu Reagent, while antioxidant capacity was quantified using DPPH, ABTS and FRAP methods, as described in previous sections.

For insoluble IF, a methanolic extraction was first performed to quantify total PCs, hydrolysable tannins [14] and condensed tannins [15]. PCs and antioxidant capacity were quantified according to methods described in previous sections (Fig. 1).

### Statistical analyses

Data were analyzed using a Student's t-test for two independent samples (total PCs from IF and dialyzable PCs), using NCSS version 8.0 (Number Cruncher Statistical System, 2012, Kaysville, UT, USA). Results were considered significant when $p < 0.05$.

## Results and discussion

### Total phenolic compounds (PCs)

Concentration of PCs and tannins (hydrolysable and condensed) quantified in avocado peel is shown in Table 1. Samples contained 5367.3 mg GAE/100 g dw of total PCs, 579.9 mg GAE/100 g dw of hydrolysable tannins and 163.2 mg CE/100 g dw of condensed tannins.
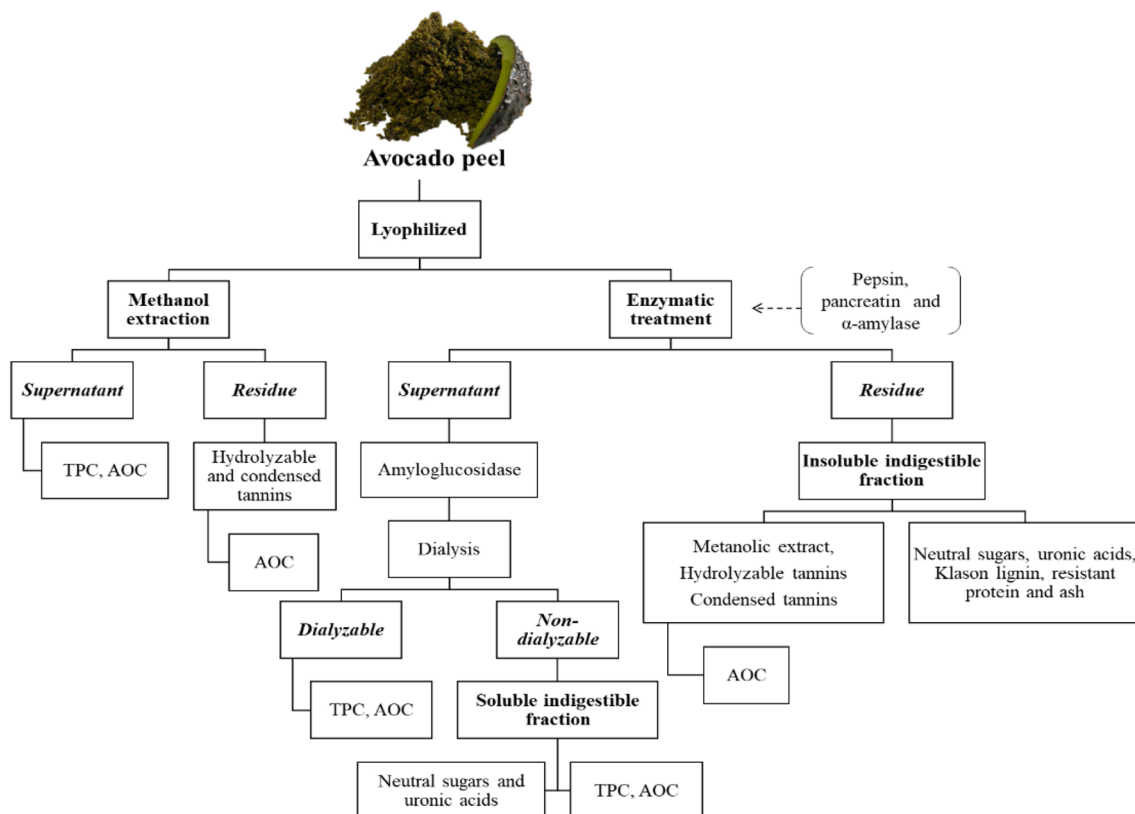


**Fig. 1** General workflow diagram used to quantify total PCs, antioxidant capacity and other chemical components of the IF of avocado peel. *TPC* total phenolic compounds, *AOC* antioxidant capacity

**Table 1** Total phenolic compounds (PCs) and tannins in methanolic extracts of avocado peel, and in the soluble (dialyzable) and insoluble (non-dialyzable) indigestible fraction (IF) obtained after an in vitro gastrointestinal digestion

| Treatment | Fraction | Total phenolic compounds mg GAE/100 g dw | Hydrolysable tannins | Condensed tannins mg CE/100 g dw |
| --- | --- | --- | --- | --- |
| Methanolic extraction | | 5367.27 ± 115.06 | 579.88 ± 4.64 | 163.25 ± 1.32 |
| In vitro digestion | Soluble (dialyzable) | 2006.49 ± 34.59 | n.d | n.d |
| | Soluble IF | 2165.52 ± 40.11 | n.d | n.d |
| | Insoluble IF | 492.85 ± 4.94 | 954.72 ± 19.45 | 250.72 ± 7.12 |

Values are expressed as the mean ± standard deviation (n = 5)

*GAE* gallic acid equivalents, *CE* cyanidin equivalents, *dw* dry weight, *n. d.* not determined

This coincides with a previous report for 'Hass' avocados (6300 mg GAE/100 g), but is lower than 'Fuerte' (12,030 mg GAE/100 g) [24]. Concentration of total PCs in avocado peel is higher than in mango (4553 mg GAE/100 g dw) [25], lemon (4980 mg GAE/100 mg dw) and orange (3560 mg GAE/100 g dw) peel, but lower than grapefruit peel (7730 mg GAE/100 g dw) [26]. On the other hand, extracts of phenolic compounds from avocado peel have been associated with different health benefits [27], such as anti-diabetic effects, via the inhibition of key enzymes of carbohydrate digestion and metabolism, such as α-amylase (IC$_{50}$ values of 36.02 ± 0.23 μg/ml) [28]. In this sense, the anti-diabetic effects of an avocado peel and red ginger-based functional drink were recently reported and were exerted via the inhibition of α-amylase [29].

An in vitro digestion was performed on avocado peel, in order to determine what percentage of PCs are dialyzable or remain in its IF. Approximately 33% (2006.49 ± 34.59 mg GAE/100 g dw) were dialyzable (bioaccessible), that is, they were released from the food matrix during digestion. Release of PCs during digestion in the upper gastrointestinal tract is crucial to enhance absorption and distribution to other organs via systemic circulation [30, 31].

Dialyzable compounds are associated with monomers that may possibly present intestinal permeability [32, 33]. Previously, monomers of phenolic compounds such as catechins, epicatechins, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, vanillic acid, protocatechuic acid, quercetin and derivatives, apigenin, kaempferol and others, have been reported in peel of different avocado varieties [4, 24, 34]. In contrast, approximately 66% of PCs remain bound to the IF (37% soluble and 29% insoluble). From these compounds, 954.72 ± 19.45 mg GAE/100 g dw and 250.72 ± 7.12 mg CE/100 g dw were hydrolysable and condensed tannins, respectively. This shows that most PCs from avocado peel are resistant to in vitro digestion and may therefore reach the colon. This can be explained because PCs can be linked to macromolecules like carbohydrates and proteins in different sites of the cell walls, through glycosidic bonds, ester bonds or hydrophobic interactions [35].

Andreasen et al. [36] reported that diferulic acids linked to cell wall polysaccharides resist gastrointestinal digestion, while esterases produced by colonic microbiome make them bioavailable once they reach the colon. Rosero et al. [34] reported that condensed tannins from avocado peel are mainly procyanidins (B1, B2 and A oligomers). This is in accordance with Wang et al. [37], who reported that procyanidins are present in the peel of eight avocado varieties (8.6–8.9 mg/g of fresh weight, fw), in the form of monomers to decamers and even longer polymers. Tremocoldi et al. [24] reported procyanidin B2 concentrations of 48.38 ± 0.04 μg/g and 28.34 ± 0.23 μg/g in peel of 'Hass' and 'Fuerte' avocados, respectively.

The presence of procyanidins in the IF could exert health benefits once they reach the colon, where they are transformed into bioaccessible metabolites like urolithins A and B, phenolic monomers, among others, with numerous documented bioactivities [35, 38–42]. In this regard, it has been reported that colonic fermentation of procyanidin B2 leads to the formation of epicatechin, phenylacetic acid, *p*-hydroxybenzoic acid and protocatechuic acid monomers [42], all of which have been documented to possess high bioactivity. For example, epicatechin has shown colon-protective effects in mouse and cell line assays, exerted through its antioxidant and anti-inflammatory effect by modulation of the NF-κB pathway [43]. Catechin induces apoptosis in human colon cancer cells (SW480) [44], while protocatechuic acid ameliorates dextran-induced ulcerative colitis in rats [45] and exerts a protective effect against 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis in mice [46]. Similarly, fermentation of hydrolysable tannins produces urolithins [40], which also exert important health-related bioactivities, such as an antiproliferative effect by reducing the glycolytic potential via p53/TIGAR axis in colon cancer cells [47]. Giménez-Bastida et al. [48] reported the anticancer effect of urolithin A in human colon cells (via increased senescence-associated β-galactosidase activity), by causing cellular senescence (via p53-dependent chemoprevention). Furthermore, urolithins decreased cellular clonogenic efficiency and proliferation of HT-29 cells, by cell

cycle arrest and induction of apoptosis [49], which could hinder tumorigenesis in the colon.

## Antioxidant capacity

Antioxidant capacity of methanolic extracts, obtained after chemical hydrolysis (hydrolysable and condensed tannins), as well as the soluble and insoluble fractions obtained after in vitro digestion of avocado peel are shown in Table 2.

Antioxidant capacity of methanolic extracts of avocado peel, according to DPPH (683.19 ± 9.57 μmol TE/g dw), ABTS (751.54 μmol TE/g dw) and FRAP (471.8 μmol TE/g dw) assays, were higher than those obtained for chemical tannin extraction (hydrolysable tannins and condensed tannins), which coincides with total PC content. These results obtained after chemical extraction are higher than those reported by Wang et al. [37], who evaluated antioxidant capacity of peel from eight avocado varieties (DPPH 38–189 μmol TE/g fw). This difference can be associated with the extraction method, since Wang et al. [37] used acetone/water/acetic acid (70:29.7:0.3, v/v/v) and 10 min sonication, while the methodology used in this study consisted of 1.5 h of sonication and chemical hydrolysis.

When avocado peel was digested, only 20.6%, 40.2% and 20.3% of antioxidant capacity (DPPH, ABTS and FRAP, respectively) was due to the dialyzable fraction (bioavailable). In contrast, 79.4%, 59.8% and 79.6% (DPPH, ABTS and FRAP, respectively) is exerted by the IF, with greater predominance in the insoluble IF (49.1%, 46.6% and 66.7%, respectively). Furthermore, highest antioxidant capacity according to all three methods comes from tannins present in the insoluble IF. This suggests that PCs in avocado peel could exerted a significant antioxidant effect, after being enzymatically released during gastrointestinal digestion. Therefore, it can be suggested that avocado peel releases its PCs in the colon, where they exert different effects, such as inducing an antioxidant environment or acting as prebiotics. Other vegetable byproducts have been reported to have a similar behavior, for example, berry byproducts obtained during industrial processing [50], olive oil wastewater [51] and cranberry pomace [52], among others.

## Chemical composition of the indigestible fraction (IF)

Chemical composition of avocado peel IF is presented in Table 3, where it is apparent that 96.85% of dry matter resisted the effect of enzymes used during gastrointestinal digestion. IF is mainly insoluble (89.39%), with only a small percentage (7.46%) being soluble. Main constituents of IF

**Table 3** Chemical composition of avocado peel indigestible fraction (IF)

| Variable | Soluble IF | Insoluble IF | Total IF |
|---|---|---|---|
| Total neutral sugars | 0.48 ± 0.15 | 34.40 ± 0.37 | 34.88 |
| Arabinose | 0.04 ± 0.001 | 2.55 ± 0.01 | 2.59 |
| Fucose | 0.02 ± 0.0002 | 4.75 ± 0.02 | 4.77 |
| Galactose | n.d | 1.01 ± 0.03 | 1.01 |
| Glucose | 0.26 ± 0.01 | 22.24 ± 0.74 | 22.50 |
| Mannose | 0.008 ± 0.0004 | 1.71 ± 0.02 | 1.72 |
| Rhamnose | n.d | n.d | n.d |
| Xylose | 0.15 ± 0.0001 | 2.14 ± 0.01 | 2.29 |
| Uronic acids | 6.98 ± 0.16 | 12.87 ± 0.20 | 19.85 |
| Klason lignin | n.d | 23.19 ± 0.71 | 23.19 |
| Resistant protein | n.d | 13.53 ± 1.19 | 13.53 |
| Ash | n.d | 5.40 ± 0.10 | 5.40 |
| Total IF | 7.46 ± 0.31 | 89.39 ± 0.78 | 96.85 |

All units are % dw. Values are expressed as the mean ± standard deviation (n = 5)

*n. d.* not determined

**Table 2** Antioxidant capacity of avocado peel methanolic extracts obtained after an in vitro gastrointestinal digestion

| Treatment | Fraction | Sample | DPPH | ABTS | FRAP |
|---|---|---|---|---|---|
| Methanolic extraction | | Total PCs | 683.19 ± 9.57 | 751.54 ± 59.06 | 472.85 ± 13.59 |
| | | Hydrolysable tannins | 31.19 ± 0.39 | 34.62 ± 1.62 | 70.24 ± 0.38 |
| | | Condensed tannins | n.d | 115.82 ± 3.29 | 204.78 ± 4.62 |
| In vitro digestion | Soluble (dialyzable) | Total PCs | 33.92 ± 7.51 | 159.19 ± 11.71 | 108.72 ± 8.11 |
| | Soluble IF | Total PCs | 49.85 ± 0.95 | 52.37 ± 0.23 | 68.79 ± 4.29 |
| | Soluble (dialyzable) | Hydrolysable tannins | n.d | n.d | n.d |
| | | Condensed tannins | n.d | n.d | n.d |
| | Insoluble IF | Total PCs | 33.24 ± 1.76 | 33.48 ± 1.80 | 39.37 ± 1.76 |
| | | Hydrolysable tannins | 47.54 ± 0.69 | 44.92 ± 1.14 | 119.91 ± 1.55 |
| | | Condensed tannins | n.d | 106.39 ± 0.60 | 196.66 ± 7.24 |

All units are μmol TE/g dw. Values are expressed as the mean ± standard deviation (n = 5)

*n. d.* not determined, *IF* indigestible fraction, *PCs* phenolic compounds

were NSPs (54.73%, made up of neutral sugars and uronic acids) and lignin (23.19%). Glucose and xylose were most abundant neutral sugars in soluble IF, while fucose and glucose were the most abundant in insoluble IF. Fucose is an aldose constituent of pectins, hemicellulose and some glycoproteins [53], while a high glucose content (22.50%) suggests the presence of cellulose, one of the main polysaccharides in avocado, in addition to hemicellulose and pectin [11]. These NSPs are fermented by the microbiota once they reach the colon [54]. Some of their metabolites, especially butyrate, have been described as beneficial for intestinal and overall health [55]. It has been reported that short-chain fatty acids (SCFAs) are produced by microbiota in the colon and the distal small intestine from resistant starch, dietary fiber, and other low-digestible polysaccharides in a fermentation process [56].

Dávila et al. [11] reported lower lignin values in avocado peel (4.37%) than those obtained in the present study (23.19%). In plants, lignin plays an important role by covalently binding to hemicelluloses, providing strength and stiffness to tissues that make up the cell wall [57]. However, lignification is known to decrease the digestibility of some components of plant cell walls [58]. Some PCs like *p*-coumaric acid and ferulic acid are usually associated with these polymeric structures [7], thus making them less bioaccessible, which could reduce the possible health benefits of these linked compounds.

Adding byproducts with a high content of indigestible compounds (hemicellulose, cellulose, and lignin), such as avocado peel, to functional foods, has the advantage of reducing or maintain its caloric content, as compared to adding soluble dietary fiber (pectin, β-glucans, galactomannans, fructans, oligosaccharides, guar gums or mucilage) [59], and can be therefore considered when using plant byproducts as a source of functional ingredients. Although lignin in avocado peel affects the digestibility of other compounds, its presence can be considered advantageous, because it can enhance the release of some PCs in the colon. Casler and Jung [60] reported that the presence of Klason lignin and etherified ferulate are limiting factors for digestibility, which could retain avocado peel PCs in the IF, making them available to colonic microbiota and favoring subsequent effects in the colon. Therefore, avocado peel could be used as a base material for the targeted delivery of bioactive compounds to the colon.

Resistant protein found in IF was 13.53%, and is the fraction that resists gastrointestinal digestion, reaches the colon and becomes the main source of nitrogen for resident microbiota. It is estimated that between 3 and 9 g/day of resistant protein reaches the colon, a value that varies according to food matrix [61]. Tannins easily form complexes with proteins, compromising the digestibility of this macronutrient [62]. However, this has been shown to exert some positive effects, for example, experimental models of gastric ulcers show that tannin-protein complexes promote tissue repair and have anti-*Helicobacter pylori* effects [63]. In previous work, we observed that tannins present in mango peel inhibited lipase activity, thereby having a potential anti-obesogenic effect [64]. A similar behavior can be found for PCs and tannins present in avocado peel. This highlights the potential of tannins as potential nutraceuticals with an inhibitory effect on the digestive process. The anti-obesogenic effect of avocado hydroalcoholic extracts, via inhibition of key enzymes of lipid metabolism (fatty acid synthase and HMG CoA reductase in liver), was reported in obesity-induced rats that were fed high-fat diets [65].

Inorganic compounds that remain in the residues after digestion were also quantified as part of avocado peel IF. Some minerals (ashes) have been shown to adhere to vegetable cell walls after enzymatic digestion [10], minimizing their absorption in the small intestine. Once in the colon, these micronutrients can be absorbed after polysaccharide fermentation by microbiota. In avocado peel IF, these compounds represent a small fraction of its composition (5.40%). Tannins have also been reported to interact with divalent minerals, making it likely to have tannin-mineral complexes in avocado peel, which are not bioaccessible in the small intestine [66]. The bonds and interactions of PCs with other constituents of avocado peel allow them to resist digestion in the upper gastrointestinal tract, making avocado peel a biocompatible material that can deliver dietary antioxidants to the colon, or as a material that exposes them to the action of colonic microorganisms [35, 67]. Content of phenolic compounds and antioxidant capacity of the indigestible fraction of avocado peel must be considered in further studies, in order to elucidate if its release in the colon, provide an antioxidant environmental that can be beneficial for this organ's health.

The use of natural polysaccharides, such as pectin and others, has previously been proposed for the controlled administration of oral therapies [68] or for colonic drug delivery [69]. For example, Dai et al. [70] created lignin nanostructures to use as green carriers to deliver resveratrol, while Cheng et al. [71] created ibuprofen-loaded nanomicelles. Lee and Chang [72] used oligochitosan and deesterified yuzu peel (*Citrus junos*) pectin to create a colon-targeted quercetin delivery system. Delivery systems made from by-product-derived materials from natural sources have certain advantages, for example, they are biocompatible, biodegradable and contribute to the reduction of waste. PCs are usually associated with different constituents of the food matrix. Avocado peel IF includes components that usually associate with different PCs, some of which are fermentable substrates, which could allow their gradual release after bacterial metabolism in the colon. Metabolites of this process are able to exert health-promoting bioactivities,

including maintaining an antioxidant environment in this organ, thereby mitigating pro-oxidant damage. However, further studies on PC-macromolecule interactions and their colonic fermentation are necessary to verify this statement. We previously reported possible physical and chemical interactions that can be take place between dietary fiber and PCs, however, the wide variety and amount of fiber and PCs present in fruits and vegetables requires individual studies for each specific case [73]. The beneficial effects of tannins and phenolic compounds contained in avocado byproducts have been recently studied by our group, for example, satiety was induced in a murine model, which was due to increased serum concentration of anorexigenic hormones (data not yet published). This suggests that avocado, its byproducts and the phytochemicals contained therein have potential bioactivities that require further study.

## Conclusion

'Hass' avocado peel is resistant to in vitro gastrointestinal digestion. The main constituents of its indigestible fraction are non-starch polysaccharides and resistant protein. Its phenolic compounds are capable of exerting antioxidant capacity, although most become available after reaching the colon. This suggests that avocado peel may serve to deliver phenolic compounds to the lower gastrointestinal tract, where they may exert bioactivities that promote intestinal and overall health and act as prebiotics. Additional in vivo studies are required to validate the possible effects of consuming avocado peel or when using it as a functional ingredient in new foods and colon-targeted delivery systems.

**Author contributions** NJSL wrote the manuscript, MLSR and MAVO carried out the work, JADA edited the manuscript, JFAZ corrected the manuscript, and GAGA was responsible for conceiving the idea and edited the manuscript.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest, financial or otherwise.

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