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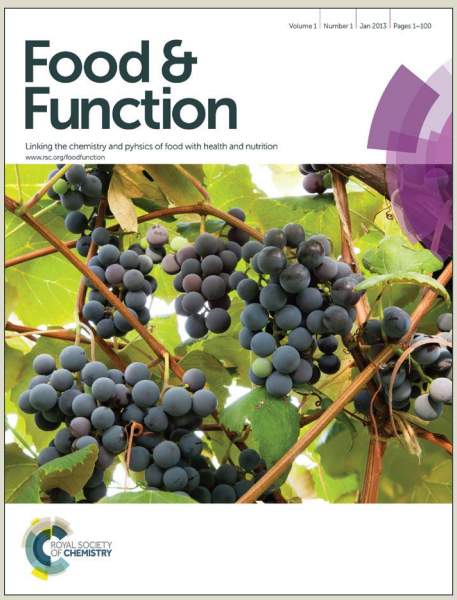

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Bioaccesibility of polyphenols associated with dietary fiber and *in vitro* kinetics release of polyphenols in Mexican 'Ataulfo' mango (*Mangifera indica* L) by-products

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ABSTRACT

The biological properties of polyphenol (PP) depend on its bioaccesibility and bioavailability. This means the process of releasing PP from the food matrix in the gastrointestinal tract through enzymatic hydrolysis which may be at least partially absorbed. The aim of this study is to determine the bioaccesibility of PP associated with dietary fiber (DF) and the kinetics release of PP in mango (*Mangifera indica* L.) 'Ataulfo' by-products by an *in vitro* model. Soluble and insoluble DF values were 7.99 and 18.56% in the mango paste and 6.98 and 22.78% in the mango peel, respectively. PP associated with soluble and insoluble DF was 6.0 and 3.73 g GAE/100 g in paste and 4.72 and 4.50 g GAE/100 g in peel. Bioaccesibility of PP was 38.67% in pulp and 40.53% in peel. Kinetics study shows a release rate of 2.66 and 3.27 g PP/min in paste and peel, respectively. Antioxidant capacity of paste increased as digestion reached a value of 2.87 mmol TE/min at 180 min. The antioxidant capacity of peel had its maximum (28.94 mmol TE/min) between 90 and 120 min of digestion; it started with a value of 2.58 mmol TE/min, and thereafter increased to 4.20 mmol TE/min at 180 min. The major PPs released during the digestion of paste were gallic and hydroxybenzoic acids, while in the peel, they were hydroxycinnamic and vanillic acids. It was concluded that these phenolics compounds are readily available for absorption in the small intestine and exert different potential health benefits.

Key words: Bioaccesibility, polyphenols, dietary fiber, *Mangífera indica*.

INTRODUCTION

Fresh fruits are widely consumed because of their taste, high nutrients and bioactive compounds that exert beneficial effects on human health.[1] These products are natural antioxidants and possess other biological properties of interest.[2] Fruits are important sources of some essential dietary micronutrients and dietary fiber (DF) and wide range of phytochemicals, which individually or in conjunction may have important biological activities that promote health benefits.[3] DF is not defined as a chemical group; rather it is a combination of chemically heterogeneous substances, with some physiological functions related to postprandial blood glucose/ insulin levels, gastrointestinal and cardiovascular health.[4] In fact nowadays, is considered that DF may serve as a carrier of a significant amount of polyphenols (PP) associated with food matrix through human intestine.[5]

Most fruits like mango (*Mangifera indica* L) are consumed fresh; however, there are many industrial processes that lead in products of high consumption such as juices and concentrates. Processing of tropical fruit produces high amounts of by-products that are not fully approached. They include ingredients used for preparing other products and untreated waste in the environment that causes contamination. It has been observed that by-products are a good source of DF and PP and that they could provide added value to different food products.[6] Therefore, the possibility of using these compounds as additives or active ingredients in the food industry is of great interest.[7] Mango pulp generates about 50 to 55% of waste represented by seeds, peel and paste.[8] The biological properties of antioxidants found in fruits depend on their bioaccesibility and bioavailability.[4] Some reports have shown poor bioavailability of several groups of PP,

which are reflected, for example, in a low concentration of PP in plasma.[9] PP bioavailability depends not only on the type but in other factors, such as its release from food matrix during gastrointestinal digestion (bioaccessibility), cellular uptake, metabolism and further transport in the circulatory system. Substances that reach the small intestine are removable [10] and constitute the soluble fraction in the gastrointestinal tract, whereas compounds not released (non- bioaccessible fraction) are passed out in feces.[11-13] The nutritional values of plant foods are usually estimated based on their native concentrations of nutrients, phytochemicals, and total antioxidant capacity. These data are usually obtained by direct extraction with aqueous-organic solvents.[14, 15] However, these conditions are different from the physiological conditions that ocurr in the digestive tract. Therefore, foods PP determined conventionally can have different values from that which is normally absorbed and assimilated.[10] The possible absorption metabolism, and excretion of PP was recently discussed. The impact of food matrix, nutrients, enzymes and pH in the upper gastrointestinal tract, colonic microbiota and physicochemical properties of phenolic compounds on their bioaccesibility and bioavialibility was also discussed. However, there is a need to fully characterize gastrointestinal factors that affect bioaccessibility of PCs bound tightly to the food matrix.[16] For this reason, the aim of this study is to evaluate the bioaccesibility of PP associated with DF and determine the kinetics release of PP in mango by-products (paste and peels) by an *in vitro* model.

MATERIALS AND METHODS

Sample preparation

'Ataulfo' mango by-products (paste and peels) from concentrate processing were provided by Mexifrutas, S.A. de C.V., from Nayarit, México. Samples were freeze-dried and subsequently ground, sifted with a mesh size of 0.5 microns, and stored in sealed bags at -20℃, until analysis.

Total soluble polyphenols (TSP) and hydrolysable polyphenols (HP) content

For the quantification of TSP, organic aqueous extraction was performed on samples with acidified methanol solution (0.8 N HCl 2N 50:50 v/v) and acetone-water solution (80:20 v/v). TSP contents were determined in the extracts previously obtained according to Montreau[17] with some modifications, using a microplate reader (BioTek ® Synergy HT, USA). The absorbance was read at 750 nm against a blank, and TSP was calculated using a calibration curve of gallic acid. Results were expressed as gallic acid equivalents (GAE)/100 g of sample dry weight (DW).

The HP content was obtained based on the proposed method of Hartzfeld et al.[18] Residue from aqueous extraction was dispersed and 20 ml of methanol and 2 ml of H_2SO_4 were added. Extracts were incubated in a shaking water bath at 85℃ for 20 h. They were cooled at room temperature and centrifuged at 3000 rpm for 10 min. Then the supernatants were recovered. Subsequently, the residue was washed twice with 10 ml of distilled water and supernatants were mixed in a 50 ml volumetric flask. Quantification was performed as previously, calculating the concentration of HP with a calibration curve of gallic acid.

Antioxidant activity

2,2'-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The supernatants from aqueous extract were used to evaluate antioxidant activity with the reduction of DPPH radical assay. Determination was carried out with the method proposed by Prior et al.[19] with some modifications. DPPH (5 mM) was dissolved in pure methanol to a concentration of 190 µM, and was kept in the dark. Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic) was used as a standard and methanol as a blank. Samples of 20 µl of extract were added together with 200 µl of DPPH radical and incubated at room temperature in the dark for 30 min. Afterwards, absorbance was read at 517 nm in a microplate reader of 300 µl of capacity (Biotek, Synergy HT, Winooski, VT, USA). The results are reported in mmoL TE/100 g of sample DW.

2,20-Azinobis-3-ethylbenzotiazoline-6-sulfonic acid (ABTS) analysis

The supernatants from aqueous extract were used to evaluate antioxidant activity with the reduction of ABTS radical assay based on Re et al[20], with some modifications. For this determination, ABTS (7 mM) was dissolved in potassium persulphate (2.42 mM) and kept in the dark at room temperature for 14 h. The solution was adjusted with phosphate buffer at an absorbance of 0.70 (±0.02). Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic) was used as a standard and methanol as a blank. Samples of 10 µl of extract were added in a microplate reader (Biotek, Synergy HT, Winooski, VT, USA) of 300 µl of capacity and 280 µl of ABTS radical was added. Then, it was incubated at 37℃ in the dark and read for 6 min, at 734 nm; calibration curve was prepared using an aqueous solution of Trolox as standard. The results are reported in mmoL TE/100 g sample DW.

HPLC analysis of polyphenols (PP)

Partial identification of PP was performed using a Dionex ICS-5000 HPLC-PDA (proto-diode array) system. The separation was achieved using a reverse phase column Acclaim C_{18} (300 × 3.0 mm i.d., 5 µm particle size, Thermo Scientific, USA). The mobile phases were: acidified water with 0.1% trifluoroacetic acid (TFA) (A) and 85% acetonitrile with 0.085% TFA acidified (B). Separation was carried out in 50 min under the following conditions: 0 min, 92% A; 30 min, 60% A; 45 min, 35% A; 48 min, 92% A; 50 min, 92% A. The column was equilibrated for 5 min prior to each analysis. The mobile phase flow rate was 1 mL/min and the injection volume was 50 µL. UV detection was carried out from 214 to 520 nm.

Analysis of total soluble polyphenols (TSP) released from food matrix and associated with dietary fiber (DF)

In order to identify and quantify TSP released by enzymatic hydrolysis and PP associated with DF, aliquots of DF were taken and analyzed using the method (991.43 AOAC, 2000) modified by Mañas and Saura-Calixto [21] (Figure 1). The following steps were used to calculate these parameters.

(1) Paste and peel (500 mg) were incubated with a triple enzymatic hydrolysis with heat-stable α-amylase (25 µl, pH 6, 35 min, 100℃ A-3306, Sigma-Aldrich, St Louis, MO, USA), protease (50 µl of 50 mg/ml solution in phosphate buffer 0.08 M, pH 6, 60℃, 35 min, P-5380, Sigma) and amyloglucosidase (150 µl, pH 4.5, 60℃, 35 min, A-9913, Sigma).

(2) After *in vitro* digestion, an aliquot was taken to determine PP released from food matrix by enzymatic hydrolysis. Subsequently, the samples were centrifuged for 15 min, 4℃ at 8,000×*g* and the supernatant was removed and residue washed twice with

distilled water (10 ml). Supernatants were volumetric and transferred to dialysis tubes of cellulose membrane (D9652-3 0.48 m, 12,000-14,000 Da, Sigma Aldrich) for 24 h.

(3) After the dialysis, an aliquot was taken in this fraction to determine PP associated with soluble DF. (4) To quantify the soluble DF, dialysates were subjected to an hydrolysis with concentrated sulfuric acid to determine non-starch polysaccharides following the Englyst and Cummings,[22] method, using glucose as standard.

(5) After centrifugation, residues were hydrolyzed with H_2SO_4 12 M, 33 ml of water at 100℃ for 90 min to determine non-starch polysaccharides.[21]

(6) To identify and quantify the PP associated with insoluble fiber, the residue underwent a double organic extraction to evaluate the content of PP after the sulfuric acid hydrolysis. In order to identify PP released from food matrix and PP associated with soluble DF, aliquots obtained in previous steps were analyzed by HPLC-PDA, and compared to known standards. At the same time, TSP, ABTS and DPPH antioxidant activity was performed. All tests were carried out at least by triplicate.

Bioaccesibility of the polyphenols associated with dietary fiber

Bioaccesibility assessment was performed by difference based on the content of PP released by enzymatic hydrolysis, and the content of PP associated with soluble and insoluble DF. The following equation (1) describes the bioaccesibility considering that the difference of PP released after enzymatic hydrolysis and PP associated to soluble DF are the potential bioaccesible PP.

$$\text{Bioaccesibility}\ (\%) = \frac{(\text{PREH} - \text{PASF})}{(\text{PREH} + \text{PAIF})} \times 100 \qquad (1)$$

where PREH = PP released by enzymatic hydrolysis, PASF = PP associated with soluble DF, PAIF = PP associated with insoluble DF.

Kinetics release of polyphenols (PP) from food matrix, in 'Ataulfo' mango by-products (paste and peel)

Kinetics release of PP in food matrix was determined according to the *in vitro* digestion method of Granfeldt,[23] with some modifications. Dried sample (300 mg) was weighed into 50 ml centrifuge tubes, 10 ml of phosphate buffer (0.05 M pH 1.5) was added, then 0.2 ml of pepsin solution (from porcine pancreas, powder, ≥ 250 units / mg, 300 mg / mL, P-7000, Sigma) was added and incubated at 37℃ for 1 h. Afterwards, phosphate buffer (4.5 ml, 0.05 M, pH 6.9) was added and transferred to cellulose dialysis bags (D9652-30.48 m, 12,000 - 14,000 Da, Sigma Aldrich). One milliliter of pancreatic α-amylase (110 U Sigma/ml, 40 µl/7 ml, A6255, Sigma) was added to each dialysis bag and sample was adjusted to a volume of 30 ml and dialysis tube was sealed. The tubes were placed in a glass vessel with 200 ml of phosphate buffer (0.05 M, pH 6.9), previously stabilized at 37℃. Samples were incubated for 3 h with continuous stirring. At 30 min intervals, 1 ml of external liquid containing the dialyzed compounds was taken and TPS compounds and antioxidant capacity were analyzed in triplicate. Samples were injected in an HPLC to determine the kinetic release of PP during incubation. Data were used to calculate the release rates. A linear regression model was used to obtain values of the slope, which correspond to the rates of PP released, and the changes in antioxidant capacity.

Data analysis

A completely randomized design was used. All analyses were performed in triplicate; means and standard deviations from each determination were calculated.

RESULTS AND DISCUSSION

Total soluble phenols (TSP), hydrolysable polyphenols (HP), HPLC polyphenols profile and antioxidant activity in 'Ataulfo' mango by-products

In general, PP is an important parameter in the study of fruits and their by-products due to its high content and contribution to antioxidant activity. The results obtained for TSP values were 9.51 ± 1.72 g GAE/100 g sample in paste and 7.22 ± 1.80 g GAE/100 g DW in peel (Table 1). Kim et al.[24] reported similar values in mango peels in the same cultivar. These values are higher than those reported for whole mango pulps, for which values have been reported as 0.2 g GAE/100 g.[25] The major phenolic compounds found in mango paste were gallic, hydroxycinnamic and hydroxybenzoic acids. It was previously reported that the major phenolics of mango pulp were gallic, chlorogenic, vanillic and protocateic[26] similar to those found in the pulp and the peel by-products studied, however other glycosylated compounds or some other flavonoids identified in other works[27] were not identified in the present study, this may be due to the purification that takes place in other studies or the sensitivity of identification and quantification methods, which are more accurate than those made in the present work. Meanwhile, in the case of peels, the major phenolic compounds were chlorogenic and vanillic acids. It has been reported that chlorogenic acid participates in lignin biosynthesis that is related as possible responses to different stresses, such as, mechanical damage and fungal attack.[28] HP contents are usually ignored in PP analyses, even their biological relevance

as anti-atherogenic on cellular cholesterol metabolism and uptake, protection of lipoproteins against oxidation [29], anti-thrombotic on the development of atherosclerotic plaque [30] and anti-inflammatory decreasing PGE_2 levels in macrophages effect, [31] HP in mango peels had 5.54 ± 0.40 g GAE/100 g DW, while in paste it was 2.6 ± 0.22 g GAE/100 g DW. It has been reported that mango peel contains gallotannins from 5 to 13 units which are normally presented in HP fraction.[32] The major PP identified in HP fraction in paste was hydroxycinnamic, caffeic, coumaric, gallic, chlorogenic and vanillic acids, as well as naringerine. However, the main PPs found in the HP of the peel were hydroxycinnamic acid, ellagic acid, hydroxybenzoic acid, caffeic acid, coumaric acid and naringerin. HP is the result of a strong acidic treatment that may degrade some PPs; however, it allows high recovery of PP and can be considered as a good alternative method to evaluate HP.[5] Whole fruits are generally a good source of TSP, such as apple that contains close to 1.81 g GAE/100 g DW;[33] pink guava, 0.61 g GAE/100 g DW;[34] mango, 0.34 g GAE/100 g DW.[35] However, these values are lower than those obtained in this study. Most fruits have higher contents of PP in their edible portion as well as in their by-products. Phenolic acids or their derivatives are usually bound covalently to polysaccharides in the plant cell wall, forming ester bonds with arabinose in hemicellulose or with lignin.[36]

Antioxidant capacity has been evaluated in food products using various methodologies with different mechanisms.[37] ABTS assay is generally recommended for measuring the antioxidant activity of hydrophilic compounds, while DPPH method is commonly used for aqueous/organic extracts with hydrophilic and lipophilic compounds.[38] The antioxidant activities of TSP and HP in paste and peels are shown in Table 1, the

reported values for both by-products are much higher than those reported for whole pulps, which are in a range of between 3.87±0.01 to 4.01±0.09 mmol Trolox/100 g determined by ABTS.[2] It is important to address that DPPH assay showed higher values in antioxidant capacity (approximately 50%) compared to ABTS assay. This result agrees with that of Arnao[34] and Almeida et al.[39], where they argue that colored compounds such as anthocyanins and carotenoids present in the sample might have a spectrum, which overlaps DPPH at 515 nm, and thus interferes with the measurements. This phenomenon is influenced by the chemical structure, distribution and number of OH groups in the molecules. Therefore, the small molecules can interact greatly with the radical and apparently possess greater antioxidant capacity with this method.[19] Reversible reactions of DPPH with certain phenols, such as eugenol and its derivatives, result in low values of antioxidant activity.[40] The major polyphenols in mango are phenolic acids, which are small molecules, and therefore can react with the radical and be more reactive, resulting in higher DPPH values. Paste and peel of 'Ataulfo' mango by-products are an excellent source of PP (between 790 and 118 mg GAE/100 g DW) with a considerable antioxidant capacity between 303.04 and 790.79 mmol TE/100 g DW.

Dietary fiber content, polyphenols (PP) released by enzymatic hydrolysis, and polyphenols associated with soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) in 'Ataulfo' mango paste and peel

Fruits and many by-products are sources of DF that can embed different compounds such as PP which are able to interact chemically and physically with food matrix.[4] PP

has hydrophobic aromatic rings and hydrophilic hydroxyl groups that can be linked to polysaccharides and proteins at several sites on the cell wall (cellulose, hemicellulose and lignin)[41]. Some of them can exert antioxidant activity once they are hydrolyzed and released by enzymatic reaction. Table 2 shows the total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) in 'Ataulfo' mango by-products (paste and peel). Our results coincided with those reported by García-Magaña et al.[42] for mango by-products (6.22% SDF and 17.67% IDF in paste and 11.11% SDF and 16.53% IDF in peel). Previous investigation reported values of 1.0% in TDF in mango pulp and 28.0% in TDF in mango peel.[43] These differences may be due to the cultivars and maturity stage. Other conditions include exposed food matrix such as heat treatment, enzymatic hydrolysis and chemical processing during the industrialization of mango.

PP released by the enzymatic hydrolysis is shown in Table 2. In paste, TSP released content was 12.77 g GAE/100g and major PPs identified were gallic, hydroxycinnamic, hydroxybenzoic, caffeic and ferulic acids (Figure 2). While in peel, TSP released content was 9.34 g GAE/100 g, where ellagic, hydroxybenzoic, caffeic, ferulic and gallic acids (Figure 2) were identified as the major compounds. It appears that these compounds are potentially bioaccesible. After the gastric phase, pepsin digestion and low pH favor the inseparable PP, which may release the diffusion from the food matrix to the aqueous phase due to reduced ionic interactions.[44] It is well known that PP biological properties depends on their release-absorption process and the release rate from food matrix in the upper gastrointestinal tract.[45] TSP values obtained by enzymatic hydrolysis were higher than those quantified by aqueous organic extraction (Tables 1 and 2).

Perez-Jimenez et al.[37] and Saura-Calixto[46] showed that boiled rice extracted under simulated gastric digestion conditions had 15% more TSP than that extracted in an aqueous organic solvent. This could be due to the partial release of PP bounded to the cell wall material of the endosperm fraction of the grain.[47] After dialysis process the PP linked to SDF in paste was 6.01 g GAE/100g, being ellagic, hydroxycinnamic, and gallic acids (the major phenolic compounds identified) (Figure 2). It is important to highlight that in this stage only few compounds were detected. In the case of ellagic acid, which was not identified in the previous step, the paste has ellagitannins, consisting of mainly ellagic acid, which could be released during the dialysis process by enzymatic hydrolysis.[48]

These compounds linked to SDF in the paste are not accessible in the small intestine and thus they cannot be absorbed; although they can reach the colon and be fermented by microbiota releasing a significant amount of phenolics that can create an antioxidant environmental and prevent oxidative stress of membranes.[10] Almost all SDFs are fermentable and it has been proven that increase in the microbiota fermentation of some polyphenols enhances the bioavailability of aglycons increasing rates of deglycosylation, but reduces the bioavailability of native polyphenols.[49]

On the other hand, PP linked to SDF in peel has values of 3.73 g GAE/100 g, being caffeic acid, naringerin and chlorogenic acid (the major phenolics identified). Other phenolic compounds in lower concentrations were identified in the peel (Figure 2). Two phenolic acids and naringenin which is a flavanone are not identified in the previous stage of dialysis. This may be due to a possible glycosylation process linked to disaccharide[50] that reduces its release during the early stages of *in vitro* digestion.

However, they probably be released by the action of amyloglucosidase used in DF determination process and this allows that PP be more available and be detected in more extent during the HPLC assay. A clear decrease in certain compounds such as gallic acid, hydroxybenzoic acid, caffeic and ferulic was observed before and after dialysis (Figure 2). This could be a good indicator that these compounds have the possibility of being absorbed by passive transport in the small intestine.[51] However, further analyses involving other methodologies such as line cells as Caco-2 commonly used in different studies are necessary to validate this asseveration.[11]

The value of PP linked to IDF in paste was 4.73 g GAE/100 g DW. Compounds identified were ferulic and coumaric acids with major percentage; also chlorogenic and vanillic acids were identified (Table 2). While in the peel, PP in IDF was on average, 4.51 g GAE/100 g. The major compounds identified were hydroxycinnamic, hydroxybenzoic and ferulic acids (Table 2). It has been reported that PP, generally hydroxycinnamic acid derivatives such as gallic, sinapic, p-coumaric and ferulic acids, can be found in the fiber fractions forming cross links with the polysaccharides of the cell wall, as those reported in lemon, orange and grapes.[7] The compounds related to the IDF, but not released from food matrix, apparently are not absorbed in the small intestine. It has been observed that these compounds have various biological effects, including inhibition of *in vitro* and *in vivo* oxidation of LDL and protection against oxidative DNA damage, showing antithrombotic, anti-inflammatory, antimicrobial, anticarcinogenic and antimutagenic properties.[52]

Antioxidant capacity of the studied samples decreased during the digestion process. This is consistent with that reported by Bermudez-Soto et al.[53] that observed a reduction

of various PP following similar digestion simulation of various fruit juices, which displayed high increase of PP content after the gastric phase of digestion (similar to the current study), but lower levels after the duodenal phase. Bioaccesibility was calculated according to Equation 1, where paste showed about 38.67% of PP potentially bioaccesible, whereas peel showed about 40.53% of PP potentially bioaccesible. This indicates that both by-products have around 40% of PP contents that are apparently available for absorption in the small intestine, and possibly may have beneficial effect on the organism.

In vitro polyphenols kinetics release

It is widely recognized that not all components present in food matrix are completely bioaccessible.[12, 54] This bioaccesibility is a function of several parameters including the initial concentration of the components and composition of the food matrix, physiological factors, such as enzyme concentrations and pH of the gastrointestinal environment.[36] Bioaccessibility analysis performed in this study revealed that a large number of compounds, released from the food matrix, could be available and absorbed in the small intestine. PP can be present in the matrix as an individual molecule bound to cell organelles or entrapped in complex macromolecular matrices with other macronutrients such as carbohydrates or proteins. Food matrices affect many aspects of PP bioaccessibility, bioavailability, and bioactions.[55] However, the information in this regard is scarce. This fact is of great importance. The released kinetics of PP could give good information about the bioaccesibility of PP in this type of matrix. Figure 3 shows the PP kinetics released which showed average release rates of 2.66 g of PP/min in the paste and 3.27g of PP/min in the peel. This showed in both cases an apparent rapid release

of PP from the food matrix by enzymatic action. However, this could be attributed to the possible interference of the free sugars present in the samples analyzed. The major PPs released during the kinetics in the paste were gallic and hydroxybenzoic acids (Figure 4), whereas in the peel, they were vanillic and hydroxycinnamic acids (Figure 5). These results not only reveal the potential bioaccesibility of PP linked to DF, but the ratio with which these compounds could be released and absorbed in the small intestine by passive transport mechanism; as well as their different health benefits. These results indicate that these phenols apparently interact in less extent than other phenols present in the DF of peel and pulp. In the same way, aliquots were taken during the experiment to determine the antioxidant activity, which increases with values of 2.87 mmol TE/min in the paste. However, for the case of peel, the kinetic shows three stages, in which the speed was different over time: a) stage 1, an average increase rates of 2.58 mmol TE/min, from 0 to 90 min; b) stage 2 between 90 and 120 min, a pronounced increase occurred, reaching an antioxidant capacity rate of 28.94 mmol TE/min; c) stage 3, at 180 min, an average rate increase in antioxidant capacity of 4.20 mmol TE/min was found. PPs released were mainly phenolic acids, which due to their low molecular weight, can easily pass through the dialysis membrane by a passive transport mechanism. These phenolics acids apparently are weakly linked to DF, and this allows release during the digestion simulation. It is important to notice that gallic acid as well as hydroxybenzoic acids present in mango pulp and peel demonstrated the highest antioxidant capacity.[56] However, during the dialysis process some other compounds, such as carotenoids and lipids, which contribute to the antioxidant capacity measured in this experiment may be released.[57] These lipophilic compounds are being studied in a

separate experiment to confirm the possible additive effect they could have with PP to determine the individual contribution to the antioxidant activity of these groups of antioxidants. An increase in the antioxidant activity of the external compounds that passed through the membrane of dialysis was observed, which can be an indicator of the amount of PP released during the kinetics study.

Conclusion

Major phenolic compounds found in soluble and insoluble DF in the paste and the peel of mango were ellagic, gallic, caffeic, chlorogenic and hydroxycinnamic acids. According to our results, 38.67 and 40.53% of the PP found in the paste and peel are potentially bioaccessible. The current methods for determining PP could be underestimation of the actual content of PP compounds, which may be available for absorption in the gut. The study of *in vitro* release kinetics of PP could contribute to learning more about *in vivo* digestion and absorption of the PP in fruits. This would provide a scientific basis for further studies on bioaccessibility and bioavailability of PP and their possible mechanisms of action in the different metabolic pathways that are involved. The high contents of PP with good antioxidant activity of mango by-products are factors to consider for their integral approach. The knowledge of PP stability under certain physiological conditions facilitates development of new PP-rich functional foods and consequently the reduction of contamination to environment.

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511 **Table 1.** Total soluble polyphenols (TSP) hydrolysable polyphenols (HP), and antioxidant capacity, on mango 'Ataulfo' by-
512 products (paste and peel)[1].
513

Parameter	Paste	Peel
TSP (g GAE/100 g sample)	9.51 ± 1.72	7.22 ± 1.80
Polyphenols profile (%)		
Gallic acid	76	n.d[2]
Hydroxycinnamic acid	12.67	n.d
p-hydroxybenzoic acid	9.26	n.d
Chlorgenic acid	n.d	82
Vanillic acid	n.d	17
Antioxidant activity (mmol TE/100 g)		
DPPH	303.04 ± 5.66	790.79 ± 11.57
ABTS	35.32 ± 6.60	116.01 ± 7.15
HP (g GAE/100 g)	2.60 ± 0.22	5.54 ± 0.40
Polyphenols profile (%)		
Hydroxycinnamic acid	44.2	45.26
Caffeic acid	22.08	12.42
Coumaric acid	13.72	6.05
Gallic acid	10.08	n.d
Ellagic acid	n.d	19.07
p-hydroxybenzoic acid	n.d	14.25
Antioxidant activity (mmol TE/100 g sample)		
DPPH	118.04 ± 2.17	154.58 ± 4.39
ABTS	63.6 ± 4.8	109.8 ± 7.9

514
515 [1]Data are means of three repetitions ± standard deviation.[2] n.d: Not detected.
516

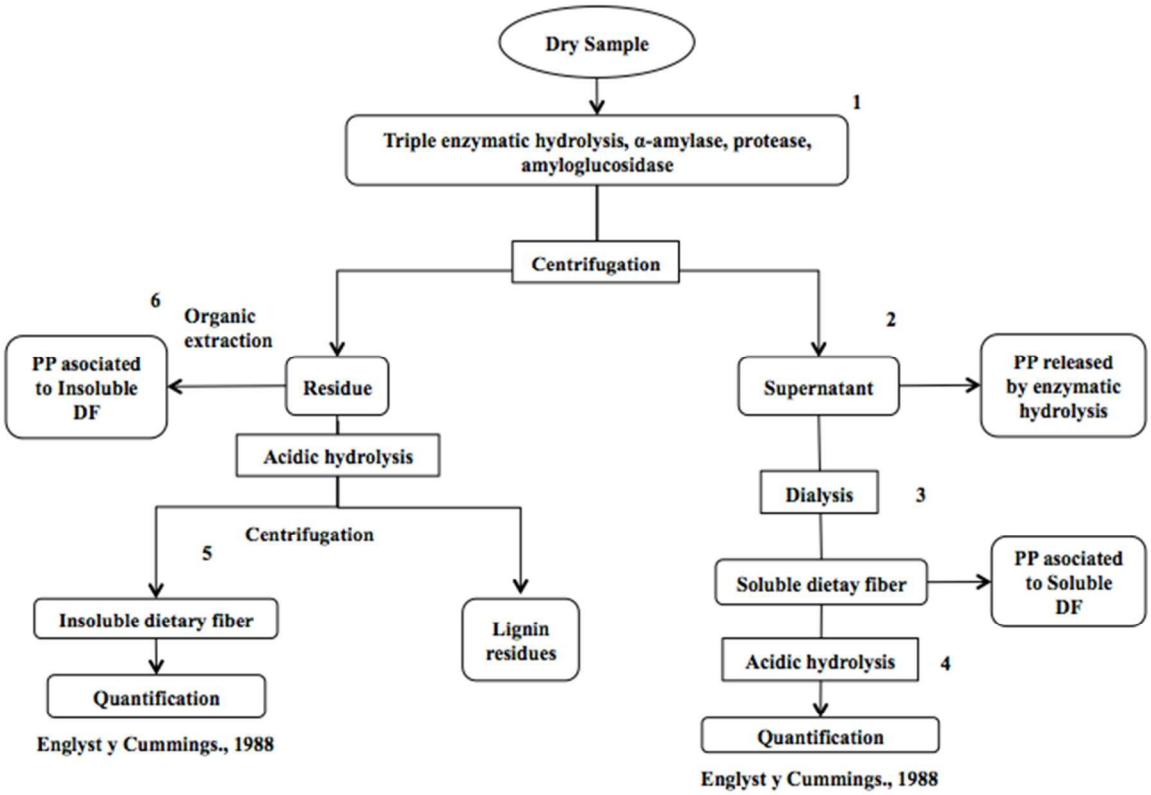
517 **Table 2.** Dietary fiber (DF), polyphenols (PP) released by enzymatic hydrolysis, PP associated to soluble and insoluble DF
518 and antioxidant capacity in 'Ataulfo' mango by-products (paste and peel).
519

Parameter	Paste	Peel
Dietary fiber		
Total dietary fiber[1]	14.97	41.34
Soluble dietary fiber	7.99 ± 0.50	18.56 ± 1.33
Insoluble dietary fiber	6.98 ± 1.29	22.78 ± 2.30
Polyphenols released in enzymatic hydrolysis		
TSP (g GAE/100 g DW)	12.77 ± 1.38	9.35 ± 2.15
Polyphenols profile (%)		
Gallic acid	40.0	8.1
Hydroxycinnamic acid	21.0	n.d.[2]
p-hydroxybenzoic acid	15.0	24.7
Caffeic acid	10.0	16.7
Ferulic acid	8.0	13.8
Ellagic acid	n.d.	36.0
Antioxidant capacity (mmoL TE/100 g DW)		
DPPH	83.5 ± 0.4	91.0 ± 1.1
ABTS	29.4 ± 1.4	54.0 ± 0.8
Polyphenols associated to soluble DF		
TSP (g GAE/100 g DW)	6.01 ± 0.58	3.37 ± 0.08
Polyphenols profile (%)		
Ellagic acid	47.0	n.d
Hydroxycinnamic acid	43.0	n.d
Gallic acid	10.0	n.d
Caffeic acid	n.d	71.0

Naringerin	n.d	22.3
Chlrorogenic acid	n.d	7.0
Antioxidant capacity (mmol ET/100 g DW)		
DPPH	34.2 ± 0.4	39.1 ± 1.2
ABTS	9.5 ± 0.02	11.4 ± 0.8
Polyphenols associated to insoluble DF		
TSP (g GAE/100 g DW)	4.73 ± 0.67	4.51 ± 0.34
Polyphenols profile (%)		
Ferulic acid	45.0	18.26
Coumaric acid	45.0	n.d
Chlorogenic acid	7.38	n.d
Vanillic acid	1.54	n.d
Hydroxycinnamic acid	n.d	46.9
p-hydroxybenzoic acid	n.d	32.4
Antioxidant capacity (mmoL TE/100 g DW)		
DPPH	61.5 ± 0.04	57.8 ± 0.23
ABTS	9.1 ± 0.48	5.40 ± 0.34
Polyphenols bioaccesibility percentage (%)	38.67	40.53

[1]Total dietary fiber as a sum of soluble DF+ insoluble DF; data are means of three replicates ± standard deviation. [2]n.d: not detected.

[3]Bioaccesibility (%) = $\frac{(\mathrm{PREH}-\mathrm{PASF})}{(\mathrm{PREH}+\mathrm{PAIF})} \times 100$; PREH = PP released by enzymatic hydrolysis, PASF = PP associated to soluble DF, PAIF = PP associated to insoluble DF.



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526

527 **Figure 1.** Total soluble polyphenols (PP) released from food matrix and associated to dietary fiber: 1) Triple enzymatic
528 hydrolysis, 2) Identification of PP released by the enzymatic hydrolysis, 3) PP associated to soluble dietary fiber, 4) Soluble
529 dietary fiber quantification, 5) Insoluble dietary fiber quantification, 6) PP associated to insoluble dietary fiber.

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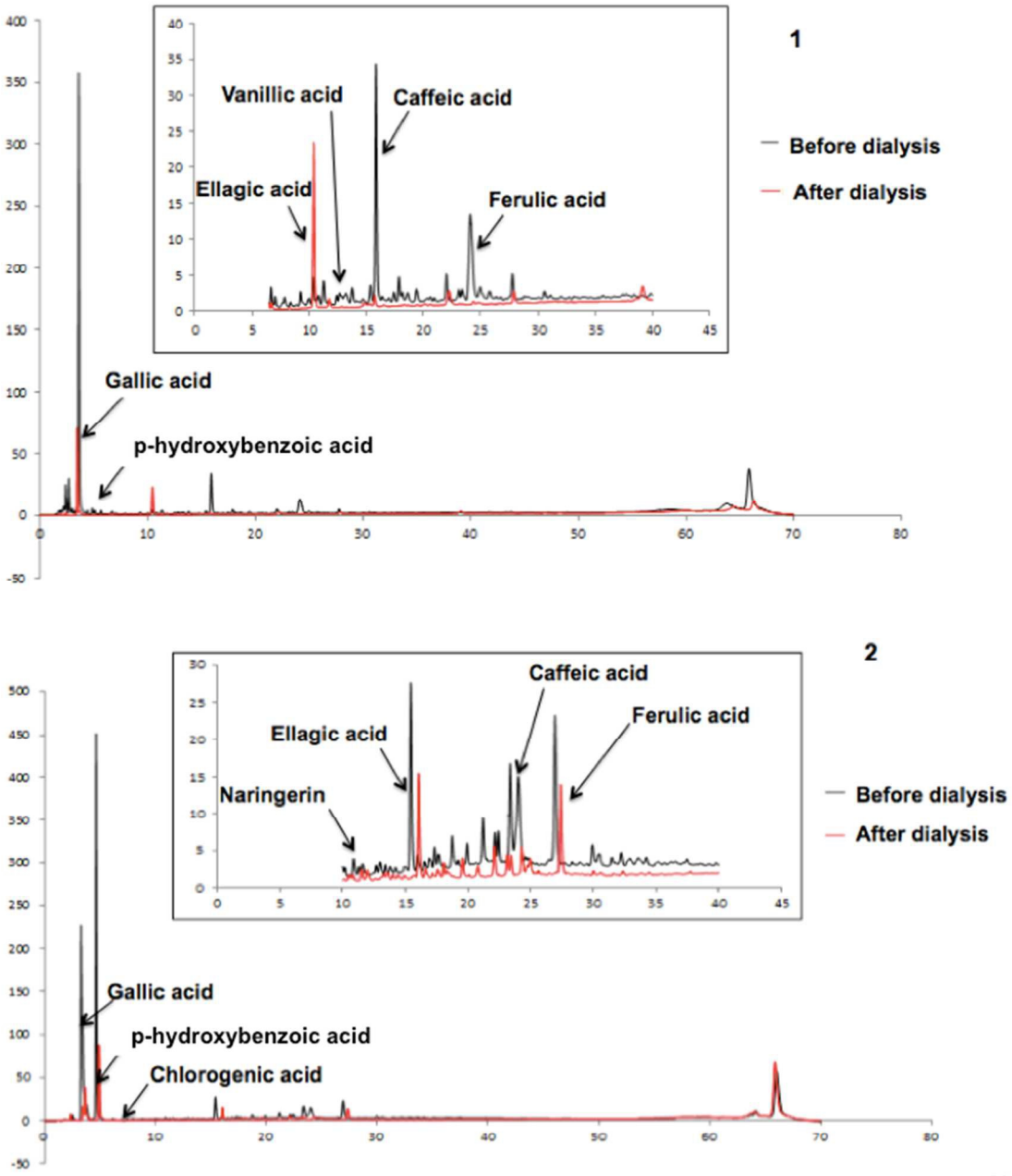
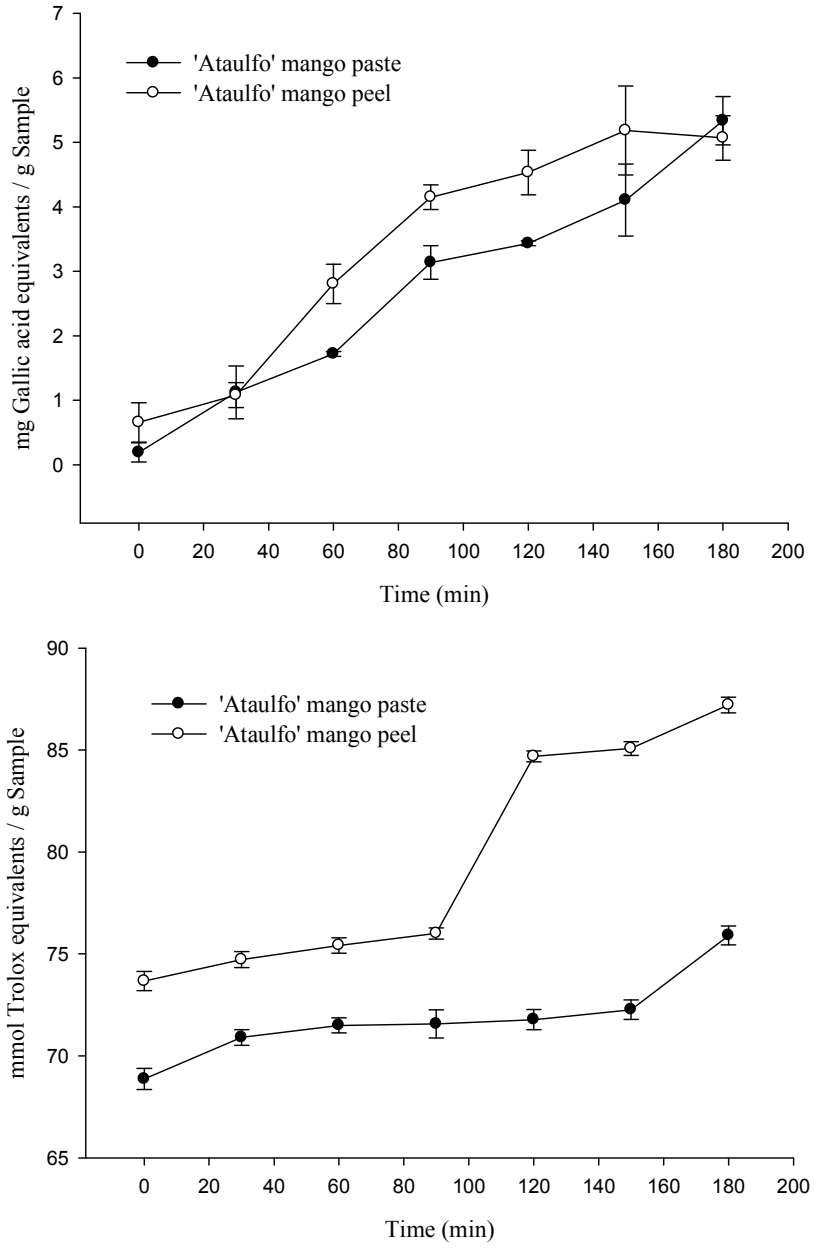


Figure 2. HPLC-PDA chromatogram of polyphenols (PP) before and after dialysis process in 'Ataulfo' mango paste (1) and peel (2) in dietary fiber analysis. Screening on 280-320 nm.



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538 **Figure 3.** Kinetics of the release of polyphenols and antioxidant capacity in paste and peel in 'Ataulfo' mango.

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540

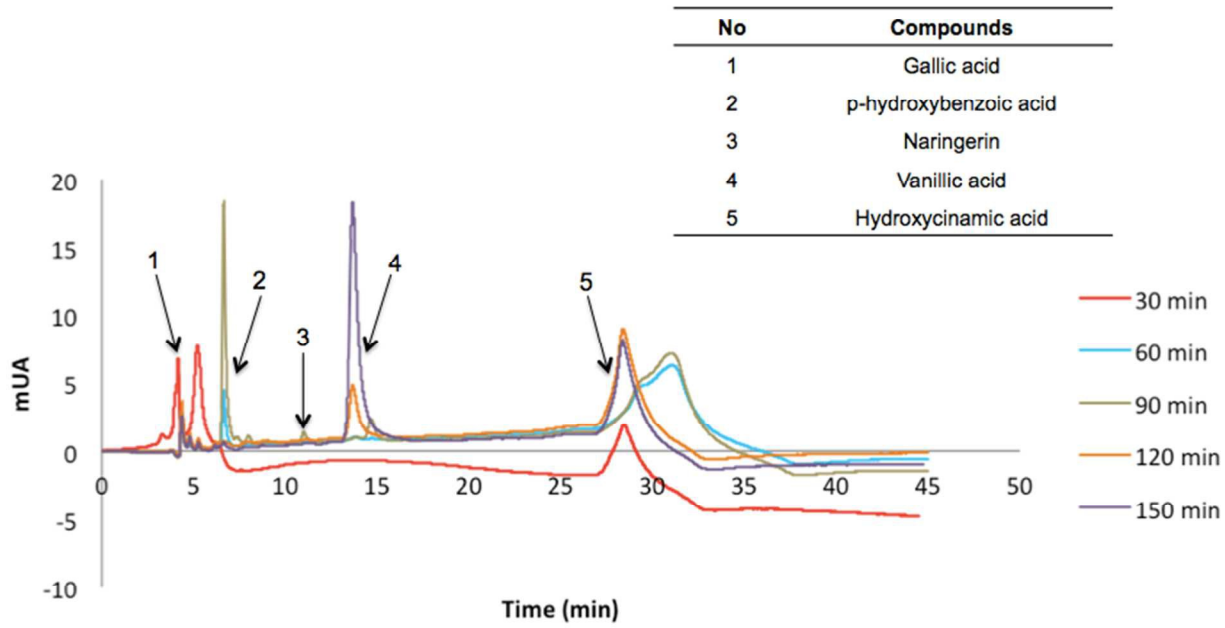
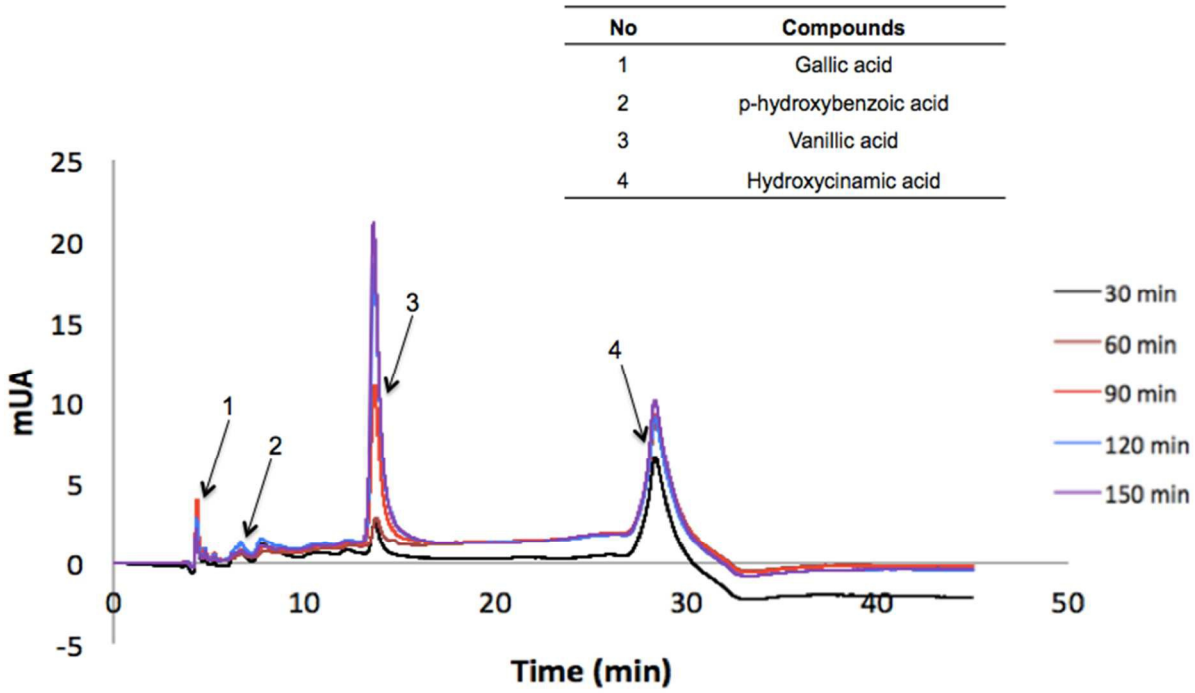


Figure 4. HPLC-PDA chromatogram of polyphenols in kinetic release in 'Atafulfo' mango paste. Screening on 280-320 nm.



548

549 **Figure 5.** HPLC-PDA chromatogram of polyphenols in kinetic release in 'Atafulfo' mango peel. Screening on 280-320 nm.

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