Supplementing corn chips with mango cv. 'Ataulfo' peel improves their sensory acceptability and phenolic profile, and decreases *in vitro* dialysed glucose

Running title: Mango peel-supplemented corn chips

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Abstract

Mango processing discards phenolic-rich by-products. The present work evaluated the effects of supplementing corn chips with mango cv. 'Ataulfo' peel (0, 10, 15 and 20 %), regarding sensory acceptability, phenolic content, profile and *in vitro* bioaccessibility, antioxidant activity and *in vitro* dialysed glucose. Addition of up to 15 % mango peel maintained or increased consumer acceptability. Phenolic content increased approximately nine-fold (from 0.9 mg GAE/g for control chips, to 8.9 mg GAE/g for chips with 15 % peel). Chips were enriched with mangiferin, quercetin and rutin, neither of which was found in control samples; antioxidant activity also increased significantly. Enriched chips had an increased bioaccessible (three-fold) and dialysable (two-fold) phenolic content, and decreased *in vitro* dialysed glucose. We conclude that 'Ataulfo' mango peel improves sensory acceptability and phenolic content of supplemented corn chips, as well as increasing antioxidant capacity and reducing *in vitro* dialysed glucose concentration, thereby exerting functional properties.

Keywords: antioxidant, bioaccessibility, by-product, functional food

Practical applications

Mango by-products are generated during industrial processing that are commonly discarded. Because of its content of bioactive phenolic compounds and the need to reduce waste,

mango peel has been explored as a potential ingredient in functional foods. Corn chips were selected to be enriched with mango peel in the present study, because corn-based products are highly consumed by various populations around the world. Their versatility makes them ideal to become everyday sources of bioactive phenolic compounds, thereby increasing the consumers' intake of these health-promoting molecules. Integrating by-products into the food-production chain also promotes a culture of zero food waste, which exerts positive impacts on society as a whole.

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1. **Introduction**

Mango (*Mangifera indica* L.) is a highly consumed tropical fruit, whose main industrial processing is destined for the production of juice, jam, and related items. Its processing yields peel as one of its main by-products, accounting for close to 20 % of the fruit's weight (Serna-Cock, García-Gonzales, & Torres-León, 2016). Mexico is the fifth worldwide mango producer, with 1.8 million tons/year, yielding 360 thousand tons of discarded peels. These are an environmental burden, since their high-water content makes them susceptible to bacterial proliferation, while also favouring emission of contaminating gases. To mitigate these problems, authors have supplemented some edible products, such as cookies and biscuits, to take advantage of this by-product, which also contains significant concentrations of bioactive compounds and fibre (Ajila, Leelavathi, & Rao, 2008; Goswami et al., 2017). Using by-products in the food industry as ingredients in functional foods and nutraceuticals, instead of discarding them has been proposed and evaluated with promising results (Ayala-Zavala et al., 2011; Velderrain-Rodríguez, Acevedo-Fani, González-Aguilar, & Martín-Belloso, 2019).

Mango cv. ‘Ataulfo’ is one of the most important and produced crops in Mexico. Its peel contains phytochemicals like phenolic compounds, carotenoids, vitamins and dietary fibre , most notably mangiferin and phenolic acids, whose health benefits have been recently studied and have been shown to exert antioxidant, anti-inflammatory and anti-

proliferative activities in different models (Lauricella, Emanuele, Calvaruso, Giuliano, & D'Anneo, 2017; Pacheco-Ordaz, Antunes-Ricardo, Gutiérrez-Uribe, & González-Aguilar, 2018). In addition to the bioactivities of phenolics, those of flavonoids, carotenoids, vitamins and dietary fibre have also been reported (Serna-Cock, Torres-León, & Ayala-Aponte, 2015). Because of its bioactive profile, others have explored mango peel as an ingredient in functional foods, such as cookies, snacks, and bread (Blancas-Benitez, de Jesús Avena-Bustillos, Montalvo-González, Sáyago-Ayerdi, & McHugh, 2015a; Palafox-Carlos, Yahia, & Gonzalez-Aguilar, 2012b; Pathak, Majumdar, Raychaudhuri, & Chakraborty, 2016). The health-promoting effects of mango have been well documented, for example, mango cv. 'Ataulfo' consumption improved the lipid profile and increased plasma antioxidant capacity in healthy volunteers, after 8 weeks of consumption (Robles-Sanchez et al., 2011). Mango by-product supplementation could therefore be a good alternative to add to various foods, in order to increase their bioactive compound content and antioxidant potential.

Corn is a staple food in Mexican households, since corn-based products are a significant portion of the daily caloric intake of a large segment of the population, consuming approximately 267 g/person/day (Ranum, Pena-Rosas, & Garcia-Casal, 2014). Thus, developing functional corn-based products by enriching them with mango peel, could increase the intake of bioactive compounds in large segments of the population that regularly consume them.

Gallic acid, protocatechuic acid, gentisic acid, synapic acid, caffeic acid, mangiferin, quercetin and isoquercetin have been identified as main phytochemicals present in mango peel (Pacheco-Ordaz et al., 2018). Because of its bioactivity, mangiferin has been used to develop different products, for example, when this compound was added to milk nanoparticles, it significantly delayed carbohydrate digestion and reduced glycaemic index by 34.5 % (Samadarsi, Mishra, & Dutta, 2020). Others report that gallic acid and mangiferin competitively inhibit α-amylase and α-glucosidase, respectively, through non-covalent interactions with amino acids present in their active site (Bezerra et al., 2019; Wu, Liu, Qin, Wang, & Wu, 2019). This suggests that the bioactive profile of mango peel, which includes mangiferin and gallic acid, can potentially regulate

carbohydrate digestion. Phenolic compounds present in mango peel can also modulate systemic carbohydrate metabolism, as reported by Nomura et al. (2003), who describe the insulinotropic effect of some phenolic acids, suggesting that their effects extend beyond the digestive system. Authors have also described similar effects for other phenolics, for example, Khan, Amin, Tewari, Nabavi, and Atanasov (2019) report the inhibitory effects of various plant-derived glycosides on carbohydrate-digesting enzymes. Thus, the evidence suggests that enriching edible products with these compounds, may exert bioactivities that decrease their glycaemic index and modulate overall carbohydrate metabolism.

Although the phenolic profile of mango peel has been identified and reported, studies about its functionality when the whole peel is added to a food matrix are limited (Velderrain-Rodríguez et al., 2015). Indeed, when using it as a functional ingredient, most experiments have only considered the release of bioactives from the food matrix (bioaccessibility), their subsequent absorption (bioavailability) and antioxidant activity. Although these are crucial to validate a food's potential functionality, other effects must also be considered, such as how the presence of bioactive compounds modifies the digestion and absorption of macronutrients like starch and glucose, since this has been well documented to occur in *in vitro* and *in vivo* models (Dominguez-Avila et al., 2017). Sensory acceptability is also key to develop a healthy product that is offered to potential consumers. Therefore, the objective of the present work is to evaluate the effect of adding mango cv. 'Ataulfo' peel to corn chips, on their sensory acceptability, phenolic content, profile, bioaccessibility and bioavailability, antioxidant activity and *in vitro* dialysed glucose concentration.

2. Materials and methods

2.1. Production of mango peel flour

Ripe 'Ataulfo' mangos were selected according to previously-published criteria (18.4° Brix, pH 3.2, firmness 12.4 N, colour: 89° Hue and 72 L*) (Palafox-Carlos et al., 2012a). They were used as sources of peel, which was separated from the pulp using a sanitized knife; the seed and pulp were discarded. Recovered peel was frozen (-30 °C

for 24 h) before being freeze dried (FreeZone 6 Liter Benchtop, Labconco Corporation, Kansas City, MO, USA) at -50 °C for 72 h. Dry peel was blended in a commercial blender until a coarse powder was obtained, and sieved to obtain a flour with homogenous particle size of approximately 250 µm. Dry sieved flour was stored in amber bags at -20 °C, until used.

2.2. Corn chip preparation

Corn chips were produced as described by Luzardo-Ocampo et al. (2017) with slight modifications. Mango peel flour was mixed with commercially-available corn flour (Minsa, Hermosillo, Mexico) at 10, 15 and 20 % w/w, to produce three different samples, as well as a control sample with no mango peel. 5 kg per sample were prepared. Mixtures were kneaded in an industrial equipment (Kneader SP80R, Navatec, Torreón, Mexico) with 7 L of common-use water (35 °C), for 7 min at 37 °C at 20 rpm constant speed, until a dough was obtained. Dough was fed through the roller of a three-stage (pressing, cutting and cooking) tortilla-making apparatus (Tortilladora-8000, Navatec), which produced triangular pieces (7 cm per side, 2 mm thick) of homogenous weight (4 g). The pieces passed through a grid at 200 °C for 5 s, and were then baked in an industrial oven at 180 ± 2 °C for 5 min. Cooked samples were cooled at room temperature and stored in transparent polypropylene bags at 25 °C.

2.3. Proximate analysis

A proximate analysis of cooked corn chips was performed, in order to quantify their humidity, protein, fat and ash contents, according to standardized methods (AOAC, 2005); carbohydrates were obtained by difference. Nitrogen content was estimated by micro-Kjeldahl method and transformed to protein by using 6.25 as conversion factor.

2.4. Sensory acceptability

Organoleptic characteristics of mango peel-supplemented corn chips were analysed to determine their acceptability. Samples were coded with three random digits and

evaluated by an untrained panel of students, scientific and administrative staff at CIAD, in north-western Mexico (n=145). They were asked to evaluate odour, colour, flavour, texture and overall acceptability of each sample, and instructed to rank each attribute on a 9-point hedonic scale, as described by Meilgaard, Civille, and Carr (2007).

In order to provide an objective measure of their colour and texture, in addition to the subjective evaluation of the panel, these parameters were determined for all corn chips samples. Colour was measured on a colorimeter (Konica Minolta, Ramsey, USA); after calibration on a white tile, L*, a* and b* coordinates were directly read on 30 samples of each formulation. L* value ranges from 0 (black) to 100 (white), and describes luminosity, a* values indicate red (positive values) or green (negative values), while b* values describe yellow (positive values) or blue (negative values). Measured a* and b* were used to calculate C* and Hue, according to Equation 1 and Equation 2.

$$C^* = (a^* + b^*)^{1/2} \quad (1)$$

$$Hue\ (^\circ) = \mathrm{Arctan}\left(\frac{b^*}{a^*}\right) \quad (2)$$

Texture was measured on a texture analyser (TAXT2, Texture Technologies Corp., NY, USA). Samples were tested using a 25 kg load cell with a spherical probe (P/0.25S) of 10 mm diameter. Samples were placed on a fracture support platform and analysed at a constant speed of 1 mm/s, shooting force 100 x g and probe distance of 5 mm. Results were expressed in N.

2.5. *Phenolic compounds and antioxidant activity*

In order to quantify phenolic compounds present in control and mango peel-supplemented corn chips, methanolic extracts were obtained according to the procedure of Alothman, Bhat, and Karim (2009). Total phenolic content was analysed with Folin-Ciocalteu's reagent as previously reported (Singleton, Orthofer, & Lamuela-Raventós, 1999), using a microplate reader (FLUOstar Omega, BMG Labtech, Durham,

NC, USA). A standard curve of gallic acid was used to determine the concentration of total phenolic compounds; data was expressed as mg gallic acid equivalents (GAE)/g of corn chips.

The phenolic profile was analysed in a UHPLC apparatus (Acquity, Waters, Milford, MA, USA) equipped with a diode array detector, as previously reported (Velderrain-Rodríguez et al., 2018). Separation was performed on a BEH C18 column (1.7 µm, 3.0 x 100 mm) under temperature-controlled conditions (60 °C). Solvents used were water with 0.5 % formic acid (A) and 100 % methanol (B), and used under the following gradient: 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 was performed at 240 nm (mangiferin), 280 nm (gallic acid), 320 nm (*p*-coumaric acid and ferulic acid) and 360 nm (quercetin and rutin). Retention time and UV-Vis spectrum of compounds detected were compared to those of authentic standards, in order to verify their identity. Compounds were also quantified with standard curves of the same standards. Results were expressed as µg/g of sample.

Antioxidant activity was measured by three methods, the ferric-ion reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and 2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. All results were expressed as mg of Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic) equivalents (TE)/g.

FRAP assay was performed as described by Benzie and Strain (1996); absorbance was measured at 595 nm after 30 min in a microplate reader. DPPH radical scavenging assay was performed as described by Brand-Williams, Cuvelier, and Berset (1995), changes in absorbance at 515 nm were determined spectrophotometrically. ABTS radical scavenging assay was evaluated according to the decrease in absorbance at 734 nm (Re et al., 1999).

2.6. In vitro gastrointestinal digestion

An *in vitro* simulation of the digestive process was performed according to Brodkorb et al. (2019), with some modifications, dividing the methodology into three stages, oral, gastric and intestinal. For the oral phase, chewing was simulated by grinding 1 g of mango-supplemented corn chips in a commercial food processor, and adding to it 5 mL of saliva from healthy volunteers. Samples and saliva were mixed in a 50 mL tube and incubated in a shaking water bath for 2 min at 37 °C.

Orally-digested samples were subjected to the gastric phase, by adding 8 mL of gastric simulation fluids and 5 µL of $CaCl_2$ (0.15 mM); pH was adjusted to 3.0 and 1 mL of pepsin was added. The gastric fraction was transferred to dialysis bags, which were sealed at both ends with thread. Bags were then suspended in glass containers with 50 mL of gastric simulation fluids and incubated for 2 h at 37 °C with constant 100 rpm mixing (Precision Scientific, Winchester, VA, USA) to obtain the gastric-digested phase. Aliquots (1 mL) were taken from inside of the dialysis bag (bioaccessible compounds) at the end of the incubation period, while the dialysate medium (outside the dialysis bag) was sampled at 0, 30, 60, 90 and 120 min.

Gastric-digested samples were transferred to a plastic tube, where 11 mL of intestinal simulation fluids, 40 µL of $CaCl_2$ (0.6 mM) and 2.5 mL of bile salts were also added. pH was then adjusted to 7.0 and 5 mL of pancreatin were added. The intestinal fractions were transferred to dialysis bags that were suspended in glass containers with 50 mL of intestinal simulation fluids, and incubated for 2 h at 37 °C with constant 100 rpm mixing to obtain the intestinal-digested phase. Aliquots (1 mL) were taken from inside of the dialysis bag (bioaccessible compounds) at the end of the incubation period, while the dialysate medium (outside the dialysis bag) was sampled at 0, 30, 60, 90 and 120 min. Aliquots taken during each digestion phase were used to analyse total phenolic compounds and antioxidant activity as described in previous sections, while *in vitro* dialysed glucose was quantified using the 3,5-dinitrosalicylate (DNS) method, as reported by Miller (1959).

2.7. Experimental design and statistical analysis

All measurements were carried out as independent experiments and performed in triplicate. Data was expressed as mean ± standard error (SE). Statistical analyses were

performed using the NCSS 2012 software (NCSS, LLC, Kaysville, UT, USA). A one-way analysis of variance (ANOVA) was performed with Tukey's test to determine significant differences at $p<0.05$.

3. Results and discussion

3.1. Sensory acceptability

Figure 1 shows the results of mango peel-supplemented corn chips sensory evaluation. Samples were rated favourably on all parameters evaluated, with an average score of at least 5. Odour acceptability had averages ranging from 6-7 with no significant differences among samples, suggesting that consumers could not perceive the possible volatiles or other compounds present in mango peel, which exerted negligible modifications. Pathak et al. (2016) reported that by supplementing products with a high percentage of mango peel, aromatic compounds present therein apparently interfered with the original aroma of the product. According to our results, the addition of up to 20 % mango peel preserves the acceptable odour of corn chips. It appears that processing or cooking methods used favoured the release of volatiles commonly present in mango peel, and its perception was not detectable by consumers.

Supplementing corn chips with mango peel improved colour acceptability, which increased from 5.4 ± 0.2 for the control, to 7.3 ± 0.1 and 7.2 ± 0.1 for samples supplemented with 10 % and 15 % mango peel, respectively. Because samples were baked, and the control had no ingredient other than corn flour, it maintained its natural colour, mostly white. In contrast, the presence of mango peel was evident, since it imparted a golden tonality, which was to the consumers' liking. In order to provide an objective measurement of colour and texture, which were subjectively evaluated by the panellists, an instrumental analysis of these variables was performed; results are shown in Table 1. It is apparent that they preferred intermediate values of L*, a* and b*, according to higher scores given to samples with these characteristics (10% and 15% mango peel). However, it should be noted that statistical differences in the instrumental analysis were only found between the control and peel-supplemented samples in all

variables, suggesting that their perception of colour may have been influenced by another variable, such as taste, likely due to the fact that they were an untrained panel.

Others have reported similar changes when adding mango peel to different foods, showing that peel adds a yellow colour to them, in fact, it has been suggested that it could be used as a natural food colorant (Ajila, Aalami, Leelavathi, & Rao, 2010; Serna-Cock et al., 2015). Colours of commercially-available corn chips are often yellow and brown, which are sometimes due to artificial colouring (FCF yellow, caramel I, II and II). These are added to make them more visually appealing, since this variable significantly influences the consumer's decision to accept or reject a product. According to our results, adding 10 or 15 % mango peel to corn chips provides a golden tonality, thereby making it possible to avoid adding artificial colouring.

Adding 10 % mango peel significantly increased the flavour acceptability of corn chips, 15 % maintained it, while 20 % decreased it, as compared to the control. Some consumers mentioned that the sample with 20 % mango peel had a slightly bitter aftertaste, which may explain their perception. This bitterness or astringency is characteristic of phenolic compounds, specifically of soluble tannins that are highly concentrated in unripe mango peel and whose concentration decreases during ripening (Li, Du, & Ma, 2011). Peel from ripe mango contains lower tannin and phenolic compound concentration than unripe mangos, however, it is possible that their concentration in corn chips with 20 % peel may have been high enough to be detectable, and were responsible for the lowest flavour acceptability. Ajila et al. (2008) reported a similar aftertaste when adding 20 % mango peel to muffins; acceptability of these samples also decreased, as reported in the present work. In contrast, others report that supplementing cookies with 20 % mango peel increased their sensory acceptability, which suggests that the particular type of food to which mango peel is added, also plays an important role in masking the aftertaste imparted by phenolic compounds (Bandyopadhyay, Chakraborty, & Bhattacharyya, 2014). There are different possible molecular and physical interactions between phenolic compounds within the food matrix, which could affect their release bioaccessibility during digestion (Blancas-Benitez et al., 2015b; Palafox-Carlos et al., 2012b; Velderrain-Rodriguez et al., 2016).

Texture is another important attribute that determines consumer preference for corn chips. Results showed a higher preference for the texture of samples supplemented with 10 % (13.3 N) and 15 % (13.1 N) mango peel, as compared to the control (13.3 N), while those with 20 % mango peel were evaluated similarly to all other samples. However, instrumental analysis only found a significant difference between 20% peel-supplemented samples and all others. Similar to colour, their perception of texture may have been influenced by another variable. This suggests that adding mango peel to corn chips increases or maintains acceptability of their texture. Changes reported to texture may be due to fibre contained in mango peel, which, when added to breads and cookies, has been shown to increase their hardness (Pathak et al., 2016). Because the amount of fibre significantly influences a food's texture, the amount of mango peel-derived fibre appears to be optimal when supplementing corn chips with 10-15 % mango peel. It should also be mentioned that the effects on texture of adding mango peel, depend on the amount added, on the type of food being supplemented and on processing technology used.

Finally, results of overall acceptability show that consumers preferred samples supplemented with 10 and 15 % mango peel, as compared to the control and sample with 20 % mango peel, both of which had similar values. This suggests that supplementing corn chips with up to 15 % mango peel results in a product with better overall quality, as compared to flour-only controls. This is consistent with colour, flavour and texture results, all of which were significantly improved by the addition of mango peel.

3.2. Proximate analysis, phenolic compounds and antioxidant activity

Proximate analysis, phenolic profile and antioxidant activity results are shown in **Table 2**. Carbohydrates were the main component of all samples, accounting for approximately 81 % of all macronutrients. Although there were some significant differences ($p<0.05$) between samples, numerical values were minor (close to 1 %). Finally, lipid content was low in all samples, with values < 1 % and differences of approximately 0.3 %. According to these results, supplementing corn chips with mango

peel maintained a consistent macronutrient profile in samples analysed, where variations found were of approximately ±1 %.

Total phenolic compounds and antioxidant activity are shown in **Figure 2**, while phenolic compounds identified and quantified by UHPLC are presented in **Table 3**. Total phenolic compounds ranged from 0.90 to 10.26 mg GAE/g for control and samples supplemented with 20 % mango peel, respectively. Significant differences ($p<0.05$) were observed between all samples, as well as an evident trend which shows that concentration of phenolics increases by approximately 3 mg GAE/g, for each additional 5 % of mango peel that is added. A similar pattern has been reported by other authors, for example, Ajila et al. (2008) reported increases of 2 mg GAE/g for every 5 % mango peel added to bread. Bandyopadhyay et al. (2014) also reported that adding 10 % mango peel to cookies increases their concentration of phenolics by 3 mg GAE/g. Differences between studies are likely due to the variety of mango used, as well as processing conditions and method used to quantify phenolics, although the trend is still evident.

The phenolic profile of control corn chips (with 0 % mango peel) shows compounds normally present in corn, such as gallic acid (11.0 µg/g), *p*-coumaric acid (18.9 µg/g) and ferulic acid (332.7 µg/g) (Lee, Garcia, & Parkin, 2010). Concentration of gallic acid increased proportionately in supplemented samples, showing that mango peel is a significant contributor of gallic acid, which is consistent with previous findings which show gallic acid is the main phenolic acid found in mango cv. 'Ataulfo', and the one with the highest antioxidant activity (Palafox-Carlos et al., 2012b). In contrast, samples with mango peel showed a 50 % and 25 % decreased concentration of *p*-coumaric acid and ferulic acid, respectively. This is likely due to the formation of complexes with dietary fibre present in mango peel, a phenomenon that has been known to occur under multiple conditions (Domínguez-Avila, Villegas-Ochoa, Alvarez-Parrilla, Montalvo-Gonzalez, & González-Aguilar, 2018). When such interactions occur, fibre protects phenolics from gastric and intestinal conditions, delivering them to the large intestine where they are released and could exert several health-promoting actions (Janicke et al., 2011; Quiros-Sauceda et al., 2014).

Mangiferin (894-2800 µg/g), quercetin glucoside (32.6-134.6 µg/g) and quercetin rutinoside (rutin, 23.7-104.9 µg/g) were only detected in supplemented samples, in accordance with mango peel percentage. Mangiferin is a glycosylated xanthone characteristic of mango, particularly its peel, and was the most abundant compound detected. Quercetin is a flavonoid ubiquitous in various fruits, vegetables and cereals; it was found glycosylated with glucose and rutinose, commonly referred to as quercetin and rutin, respectively. All three compounds are highly bioactive and are responsible for numerous bioactivities of mango consumption. For example, mangiferin exerts antioxidant activity in the liver of diabetic rats and antidiabetic actions in general, due to its intestinal glucosidase and amylase inhibiting effects (Fernández-Ochoa et al., 2020; Sekar, Chakraborty, Mani, Sali, & Vasanthi, 2019). Quercetin and rutin are potent antioxidants and hepatoprotective agents (Bonechi et al., 2018; Lee, Lee, Lee, & Sung, 2019). This suggests that supplementing corn chips with mango peel increases their concentration of phenolic compounds, as well as enriching their phenolic profile with bioactives not found in control samples. Therefore, consumption of mango-supplemented corn chips could be a good alternative for consumers, for example, because of their high quality and increased concentration of bioactive compounds and antioxidant capacity. Different studies have reported that foods supplemented with by-products, could prevent different oxidation processes, and is therefore a good alternative that must be considered (Ayala-Zavala et al., 2011; Ayala-Zavala, Rosas-Dominguez, Vega-Vega, & Gonzalez-Aguilar, 2010).

Antioxidant activity increased significantly in samples supplemented with mango peel. When measured with the FRAP method, values increased approximately 15-fold when adding 10 % mango peel, with only minor increases with 15 and 20 %, as compared to the control. DPPH and ABTS values increased approximately 4- and 10-fold, respectively, when supplementing samples with 10 % mango peel. Each subsequent 5 % mango peel that was added to the samples (up to 15 and 20 %) resulted in a linear increase in values obtained by both methods. This is likely due to mangiferin and gallic acid, which, as previously mentioned, were the most abundant compounds present in mango peel, and both exert significant antioxidant activity.

Mangiferin and gallic acid are the most important phenolics present in mango peel, and their biological activities are well-documented (Pacheco-Ordaz et al., 2018; Palafox-Carlos et al., 2012b; Quiros-Sauceda et al., 2014). In the case of mangiferin, the four hydroxyl groups present in its structure demonstrate remarkable antioxidant properties both *in vitro* and *in vivo* (Stohs et al., 2018). Its antioxidant-related bioactivities have also been shown to increase with prolonged consumption, by stimulating the endogenous antioxidant system, in addition to direct effects exerted by the molecule itself (Fernández-Ochoa et al., 2020). Furthermore, it can also modulate carbohydrate metabolism by inhibiting carbohydrate-digesting enzymes along the digestive tract (Bezerra et al., 2019; Sekar et al., 2019), which, together with its antioxidant effects, can potentially mitigate postprandial oxidative stress in the consumer. It should also be mentioned that other compounds may contribute to this effect, since mango peel is a rich source of various bioactives (Serna-Cock et al., 2016). Antioxidant activity results suggest that increasing the concentration of mango peel, and therefore phenolic compounds, will also increase the antioxidant activity of the resulting product. This makes mango peel, and likely other byproducts, a readily available source of bioactive compounds that can be used to increase the antioxidant potential of edible products.

3.3. *In vitro bioaccessibility of phenolic compounds*

Bioaccessibility of phenolic compounds and antioxidant activity during *in vitro* digestion (oral, gastric and intestinal stages) of corn chips supplemented with mango peel are shown in **Table 4**.

Concentration of phenolic compounds coincides with the pattern described in Section 3.2, where this value depends on mango peel percentage. However, in contrast with the solvent extraction described in Section 3.2, *in vitro* digestion was less efficient at extracting these compounds, since their concentration was lower than the ones shown in **Figure 2**. This is also congruent with antioxidant activity, as measured with the FRAP, DPPH and ABTS methods.

Dialysable phenolic compounds and their antioxidant activity during gastric and intestinal phases of an *in vitro* digestion is shown in **Figure 3**. It appears that phenolics were steadily released and dialysed during both digestion phases, however, maximum values were greater during the gastric phase by approximately two-fold. This also occurs for antioxidant activity, which regularly correlates well with the concentration of phenolic compounds.

This lower concentration of phenolics and antioxidant activity observed in intestinal phases, as compared to gastric ones, has been previously reported. For example, Bouayed, Hoffmann, and Bohn (2011) showed that apple phenolics are released mainly during gastric phase, with antioxidant activity closely mimicking this pattern, as measured with the FRAP and ABTS methodologies. They also proposed that chemical extraction, like that performed for the present study, may overestimate the concentration of bioaccessible and bioavailable compounds. Likewise, a comprehensive review by Shahidi and Peng (2018) argues that pH and enzyme activities may be responsible for the observed decrease in concentration/activity during the intestinal stage, due to molecular changes suffered by phenolic compounds on key hydroxyl groups. These changes interfere with the compounds' ability to react with the Folin-Ciocalteu reagent and radicals used for each antioxidant activity methodology, thereby yielding decreased values. According to our results, *in vitro* digestion patterns documented to occur in fruits and vegetables where phenolic compounds are naturally found, also take place on corn chips supplemented with mango peel.

Previous work has been done using mango cv. 'Ataulfo' by-products to enrich edible products. For example, Bertha, Alberto, Tovar, Sayago-Ayerdi, and Zamora-Gasga (2019) produced mango-enriched snack that contained mango paste, peel and seed in varying proportions. They determined that paste and peel were better suited to be incorporated into the product, according to an increased concentration of phenolics and an enriched profile, similar to data presented herein. Complementary studies showed that phenolics present in mango pulp- and peel-enriched fruit bars had a 53.78 % bioaccessibility, while also being fermented in the colon to hydroxyphenolic acids and short chain fatty acids (Hernandez-Maldonado et al., 2019). Accordingly, the phenolic

profile of mango-enriched corn chips has the potential to exert bioactivities related to their antioxidant capacity and as fermentation metabolites.

3.4. In vitro dialysed glucose

Results of dialysed glucose after *in vitro* digestion of mango peel-supplemented corn chips are presented in **Figure 4**. All samples had a similar and minor dialysable glucose concentration during the gastric phase. In contrast, dialysed glucose during the intestinal phase was significantly ($p<0.05$) greater in control samples, as compared to mango peel-supplemented corn chips, independently of its percentage. Decreased *in vitro* dialysed glucose suggests that, in the absence of mango peel, amylase efficiently hydrolyses corn starch. Furthermore, this process occurs rapidly, since the maximum concentration was already reached after only 30 min. The remarkable difference shown by supplemented samples suggests that bioactives present in mango peel, most notably phenolic compounds and fibre, interfere with the enzyme's ability to hydrolyse starch for at least 2 h of an *in vitro* intestinal digestion. Our results therefore suggest that supplementing corn chips with mango peel may reduce the product's glycaemic index, although this hypothesis remains to be tested in *in vivo* models to conclusively demonstrate.

Foods rich in dietary fibre are recognised for their ability to decrease glycaemic index, which often results in significant benefits for the consumer (Evans et al., 2017; Rizkalla, Bellisle, & Slama, 2002). Because mango peel contains approximately 51 % fibre (Ajila et al., 2010; Pathak et al., 2016), it is likely that it was at least partially responsible for the observed effect. A similar result was reported by Vergara-Valencia et al. (2007), who added mango fibre to breads, and found lower glycaemic indices during an *in vitro* digestion.

Phenolic compounds are also capable of altering carbohydrate digestion, due to inhibiting pancreatic amylase and glycosidase. For example, Taslimi and Gulçin (2017) report that phenolic compounds are effective amylase and glycosidase inhibitors, with IC_{50} values in the nanomolar range against both enzymes. Mangiferin has been shown to exert a postprandial pro-hypoglycaemic activity by modulating glucose digestion and its subsequent metabolism (Telang, Dhulap, Mandhare, & Hirwani, 2013), which is a

highly desired advantage due to the fact that carbohydrate-rich foods are abundant in modern diets, leading to marked glycaemic and insulinemic spikes. Because of its effects on carbohydrate metabolism, mangiferin has been evaluated as a functional ingredient in multiple forms, for example, it has been administered from a mango extract (Sekar et al., 2019), in micelles (Bezerra et al., 2019) and loaded into nanoparticles (Samadarsi et al., 2020). In addition to its effects on carbohydrate digestion, its potent antioxidant activity also makes it an ideal molecule to add to corn chips.

According to our results, dietary fibre and phenolic compounds present in mango peel exert complementary effects, which decreased dialysable glucose concentration of supplemented corn chips by approximately 70 %. This effect was observed with at least 10 % mango peel supplementation; increasing this value up to 20 % did not further decrease dialysed glucose.

4. Conclusions

Supplementing corn chips with mango peel maintained or improved their odour, colour, flavour, texture and overall acceptability; those supplemented with 10 or 15 % were evaluated more favourably than those not supplemented, or supplemented with 20 % mango peel. Mango peel increased the content of total phenolic compounds, it enriched its phenolic profile with mangiferin, quercetin and rutin, and increased their antioxidant activity. Phenolics had a bioaccessibility of approximately 47 %, of which about 15 % were dialysable. Mango peel supplementation also decreased *in vitro* dialysed glucose. According to our results, mango peel can be used to produce supplemented corn chips with good sensory acceptability, high phenolic concentration and antioxidant, as well as low glucose absorption (which suggests that the products may have a low glycaemic index). Additional research is required in *in vivo* models to support results reported in the present work.

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Table 1. Instrumental evaluation of mango-peel supplemented corn chips.

Variables	Mango peel percentage			
	0 %	**10 %**	**15 %**	**20 %**
L*	38.6 ± 8.3^{a}	31.3 ± 6.0^{b}	29.0 ± 5.8^{bc}	27.2 ± 3.4^{c}
a*	0.2 ± 0.1^{a}	1.5 ± 0.1^{b}	2.4 ± 0.1^{c}	2.4 ± 0.1^{c}
b*	13.1 ± 0.3^{a}	18.8 ± 0.2^{b}	21.5 ± 0.2^{c}	21.9 ± 0.2^{c}
C*	13.1 ± 2.0^{c}	18.8 ± 1.5^{b}	21.6 ± 1.7^{a}	22.0 ± 1.5^{a}
Hue (°)	91.4 ± 4.3^{a}	85.4 ± 1.3^{b}	83.8 ± 1.5^{c}	83.7 ± 1.2^{c}
Texture (N)	13.3 ± 0.4^{b}	13.3 ± 0.3^{b}	13.1 ± 0.2^{b}	14.7 ± 0.3^{a}

Different letters in the same row indicate significant differences ($p<0.05$).

Table 2. Proximate analysis of mango peel-supplemented corn chips.

Variables	Mango peel percentage			
	0 %	**10 %**	**15 %**	**20 %**
Humidity	6.7 ± 0.0^{b}	8.5 ± 0.0^{a}	5.9 ± 0.0^{c}	6.7 ± 0.1^{b}
Carbohydrate	81.7 ± 0.2^{b}	80.8 ± 0.0^{c}	82.0 ± 0.1^{ab}	82.3 ± 0.0^{a}
Protein	6.9 ± 0.2^{b}	6.6 ± 0.1^{bc}	7.4 ± 0.0^{a}	6.3 ± 0.0^{c}
Lipid	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}	0.6 ± 0.0^{b}	0.8 ± 0.0^{a}
Ash	4.0 ± 0.0^{a}	3.4 ± 0.0^{c}	3.9 ± 0.0^{a}	3.7 ± 0.0^{b}

Macronutrient and ash values are expressed as % dry weight. Data is expressed as mean ± S.E. of 3 independents experiments. Different letters in the same row indicate significant differences ($p<0.05$).

Table 3. Phenolic profile of mango peel-supplemented corn chips.

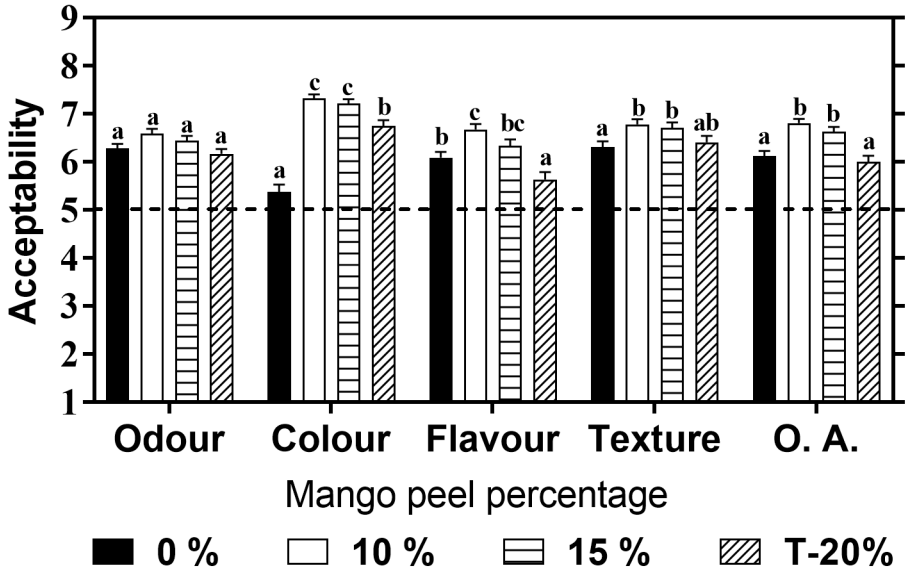
Variables	Mango peel percentage			
	0 %	**10 %**	**15 %**	**20 %**
Gallic acid	11.0 ± 0.1^{d}	310.4 ± 0.7^{c}	503.8 ± 0.5^{b}	754.8 ± 0.2^{a}
p-coumaric acid	18.9 ± 0.5^{a}	9.9 ± 0.1^{c}	10.4 ± 0.0^{b}	10.5 ± 0.3^{b}
Ferulic acid	332.7 ± 29.1^{a}	275.2 ± 2.6^{b}	252.2 ± 9.4^{c}	226.6 ± 4.1^{d}
Mangiferin	ND	894.0 ± 12.9^{c}	1950.0 ± 27.2^{b}	2800.5 ± 169.6^{a}
Quercetin-3-β-D-glucoside	ND	32.6 ± 3.3^{c}	63.0 ± 1.9^{b}	134.6 ± 5.0^{a}
Quercetin-3-β-D-rutinoside	ND	23.7 ± 2.9^{c}	49.8 ± 0.3^{b}	104.9 ± 2.1^{a}

Values are expressed µg GAE/g. Data is expressed as mean ± S.E. of 3 independents experiments. Different letters in the same row indicate significant differences ($p<0.05$). ND: not detected.

Table 4. Bioaccessibility of phenolic compounds and antioxidant activity during *in vitro* digestion of mango peel-supplemented corn chips.

Digestion stage	Mango peel percentage			
	0 %	10 %	15 %	20 %
Phenolic compounds				
Oral	0.89 ± 0.13^{a}	2.11 ± 0.07^{b}	3.37 ± 0.08^{c}	5.46 ± 0.12^{d}
Gastric	1.07 ± 0.02^{a}	0.78 ± 0.02^{a}	2.56 ± 0.21^{b}	4.88 ± 0.23^{c}
Intestinal	0.83 ± 0.08^{a}	2.32 ± 0.02^{b}	2.66 ± 0.12^{b}	5.71 ± 0.46^{c}
Antioxidant activity				
FRAP-Oral	1.93 ± 0.01^{a}	5.58 ± 0.10^{b}	10.89 ± 0.09^{c}	15.81 ± 0.43^{d}
FRAP-Gastric	4.59 ± 0.10^{a}	5.48 ± 0.09^{a}	14.11 ± 0.46^{b}	28.01 ± 0.73^{c}
FRAP-Intestinal	8.66 ± 0.07^{a}	9.63 ± 0.08^{a}	24.44 ± 0.81^{b}	22.89 ± 0.27^{b}
DPPH-Oral	1.52 ± 0.05^{a}	1.98 ± 0.07^{a}	6.10 ± 0.29^{b}	14.75 ± 0.09^{c}
DPPH-Gastric	2.52 ± 0.07^{a}	3.50 ± 0.08^{ab}	4.90 ± 0.37^{b}	13.25 ± 0.61^{c}
DPPH-Intestinal	4.98 ± 0.21^{a}	5.07 ± 0.05^{a}	9.07 ± 0.57^{b}	13.29 ± 1.01^{c}
ABTS-Oral	0.39 ± 0.03^{a}	1.17 ± 0.11^{b}	3.10 ± 0.11^{c}	4.66 ± 0.09^{d}
ABTS-Gastric	0.29 ± 0.04^{a}	1.31 ± 0.04^{a}	5.09 ± 0.43^{b}	6.31 ± 0.34^{c}
ABTS-Intestinal	1.71 ± 0.14^{a}	1.39 ± 0.05^{a}	10.73 ± 0.89^{b}	6.68 ± 0.31^{b}

Content of phenolic compounds is expressed as mg GAE/g; antioxidant activities are expressed as mg TE/g. Data is expressed as mean ± S.E. of 3 independents experiments. Different letters in the same row indicate significant differences ($p<0.05$).



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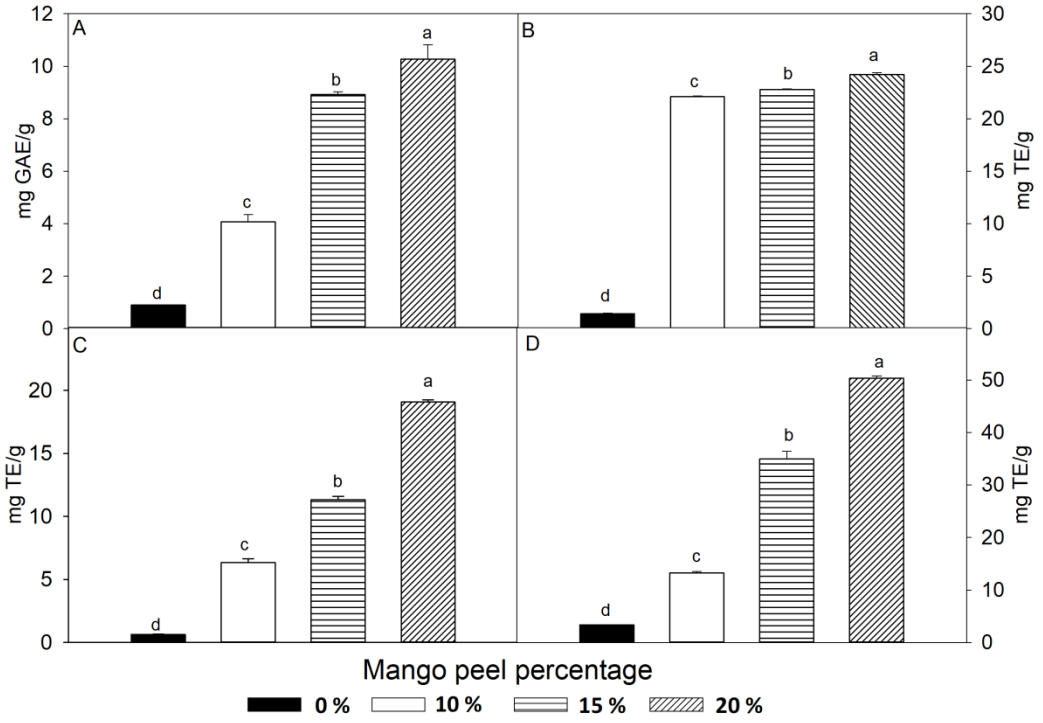

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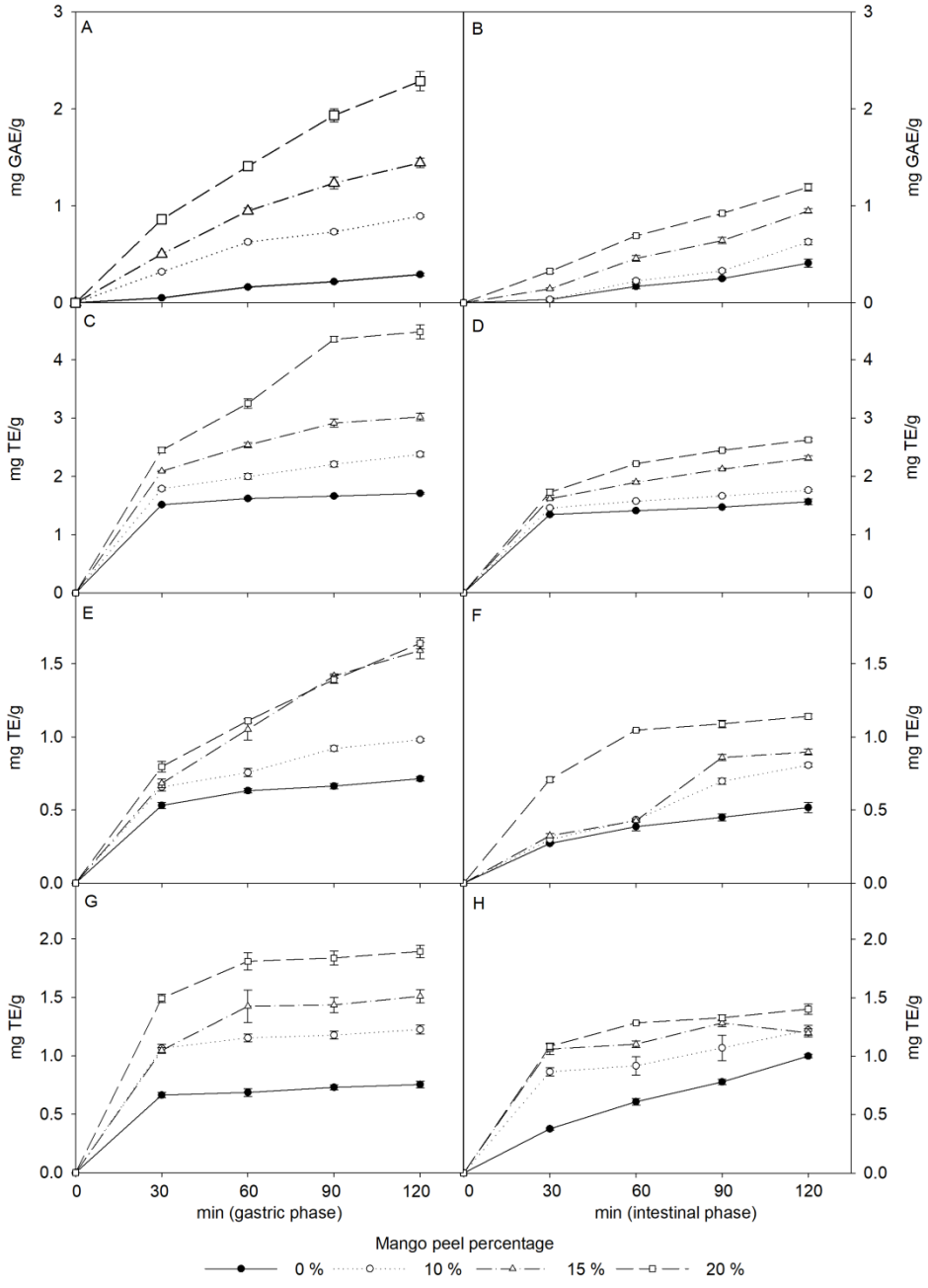

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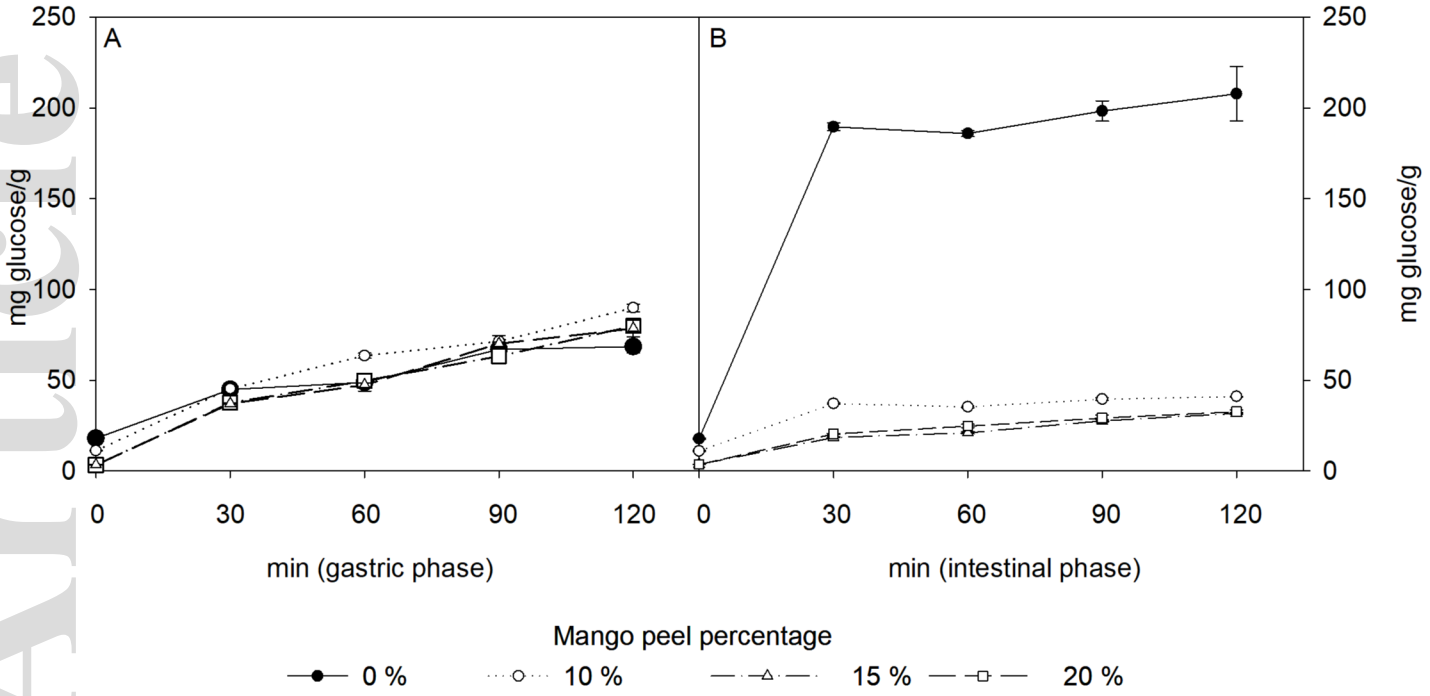

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