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Antioxidant characterization of new dietary fiber concentrates from papaya pulp and peel (*Carica papaya* L.)

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ABSTRACT

Residues of papaya pulp and peel were used to produce dietary fiber concentrates (DFCs) by treatment with ethanol and microwave drying. The fiber showed antioxidant capacity. Phenolics, carotenoids and ascorbic acid were detected. Chromatographic analysis allowed the identification of five phenolic compounds: protocatechuic acid hexoside, manghaslin, quercetin 3-O-rutinoside, caffeoyl hexoside and ferulic acid. Likewise, lutein, zeaxanthin, β-carotene and β-criptoxanthin were identified and quantified by HPLC. Finally, an analysis of digestibility showed that about 65% of the polyphenols associated to pulp and peel DFCs were potentially bioaccessible in the small intestine and that the portion of indigestible fiber had antioxidant capacity. The DFCs produced have the capacity for acting as a functional ingredient of application in the food industry, for helping to prevent the oxidation of lipids or also to meet the demand of the food market concerning the development of food with health protection capacity.

1. Introduction

In recent years, the relationship between fruit and vegetable intake and health has been the focal point of scientific investigations with the aim of identifying the specific plant components that contribute to wellbeing (Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2009). The intake of dietary fiber and antioxidant compounds through the consumption of foods rich in them like fruits and vegetables has been correlated with a lot of benefits such as low incidence of cardiovascular disease, cancer, aging and degenerative processes (Kaliora & Dedoussis, 2007). In part, some of these benefits have been attributed to the antioxidant compounds that are present in these

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Abbreviations: DFC, dietary fiber concentrate; DFCs, dietary fiber concentrates; FW, fresh weight basis; FD, freeze dried; DPPH, 2,2-diphenyl-1-picrylhydrazyl method; FRAP, ferric reducing antioxidant power; HPLC, high performance liquid chromatography

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foods, which can act in helping to reduce oxidative stress caused by free radicals that produce cell damage (Dosil-Díaz, Ruano-Ravina, Gestal-Otero, & Barros-Dios, 2008).

The biological properties of antioxidants depend on their ability to react with free radicals and develop reductive capacity, while bioavailability depends on their capacity to be released from the food matrix during digestion or colonic fermentation (Pastoriza, Delgado-Andrade, Haro, & Rufián-Henares, 2011). Polyphenols, carotenoids and vitamin C are the antioxidants that commonly have been reported to be present in tropical fruits and are also associated with antioxidant activity (Corral-Aguayo, Yahia, Carrillo-Lopez, & González-Aguilar, 2008). The determination of antioxidant capacity has been proven to be a useful tool for measuring the quality of food in relation to health (Gökmen, Serpen, & Fogliano, 2009).

Papaya is a fruit widely cultivated in the world, especially in tropical regions. Besides, it is highly appreciated due to its nutritional value, its sensory characteristics and digestive effects (Paes, da Cunha, & Viotto, 2015). In the market, there is an increase of products derived from papaya, both in the food and in the cosmetic industry. Therefore, there is an important quantity of by-products with great potential to be exploited coming from peel or pulp. The extraction of dietary fiber might be a good way to give use to these materials while generating innovative products for the food and ingredient industries.

The aim of this study was to determine the antioxidant properties and content of dietary fiber concentrates (DFCs) obtained from papaya (*Carica papaya* L.) pulp and peel and to evaluate the content of bioaccessible polyphenols in the small intestine and their antioxidant activity after an *in vitro* digestibility analysis. This study will contribute to add value to plant residues and to the finding of new sources of functional ingredients.

2. Materials and methods

2.1. Production of dietary fiber concentrates

'Formosa' papayas were purchased from a local market and were selected with a grade 4 of maturity (skin fruit with 50–75% of the surface yellow, surrounded by light green color) according to Pereira et al. (2009) as shown in Fig. 1. The fruit was separated in two fractions, pulp and peel, and subsequently they were used to produce DFCs.

The process of treatment and drying of DFCs was performed taking into account a previous study by Nieto Calvache, Soria, de Escalada Pla, and Gerschenson (2016). Briefly, the pulp and peel papaya were milled and mixed with ethanol (96 mL/100 mL) in an ethanol/sample ratio of 2.9 mL/g. During the extraction process, a Sorvall Omni Mixer equipment (USA) at 167 s^{-1} was used to homogenize the sample for 15 min at 20 °C. Subsequently, the ethanol was filtered and the residue was dried in an Ethos Plus microwave equipment (Milestone, Italy) at a temperature of 40 °C and a maximum power of 450 W. The drying was conducted until constant weight was achieved. Additionally, the water activity, a_w, was measured to assure the attainment of values below 0.6 to guarantee product stability (Muggeridge & Clay, 2001). The final moisture content of DFCs was recorded with a moisture analyzer Ohaus® MB 45 (Switzerland).

Fig. 1 – Fruit with maturation grade 4.

The dried DFCs were milled and sieved through an ASTM 40 mesh to produce DFCs with sizes below 420 microns. Then the samples were vacuum packed in Cryovac™ bags (Sealed Air Corporation, Argentina) and stored at –18 °C until their characterization.

2.2. Determination of dietary fiber

Total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) were determined in the DFCs, using the enzymatic-gravimetric method AOAC 991.43 (AOAC, 1997).

2.3. Antioxidant analysis

Antioxidant capacity was evaluated spectrophotometrically by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods. Likewise, antioxidant compounds (polyphenols, carotenoids and ascorbic acid) were determined in both DFCs. Additionally, fresh papaya pulp and peel were freeze-dried (FD) and were also analyzed, and the results were used for comparison purposes.

For pulp and peel DFCs, the results were expressed on the basis of weight of each dietary fiber concentrate (DFC). For freeze-dried pulp and peel, the results were expressed on the basis of FD powders.

Data were, in some cases, converted to fresh peel or pulp weight basis (FW) to allow the comparison with bibliography information or between fresh tissue and DFCs.

2.3.1. DPPH method

The antioxidant capacity of DFCs was evaluated using a modification of the spectrophotometric method proposed by Brand-Williams, Cuvelier, and Berset (1995). This technique measures the ability of compounds present in a sample, to act as

complexing agent of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), through the decrease of its absorbance. Briefly, an amount of 0.2 g of DFC was mixed with 5 mL of solution of DPPH 6×10^{-5} M prepared in ethanol solution (96 mL/100 mL). Variation in absorbance was monitored every 10 minutes with constant stirring until attaining constant values (λ = 515 nm). A calibration curve was constructed with 8.8×10^{-4} M Trolox solution. Results were expressed as μmol Trolox equivalent/100 g of sample.

2.3.2. *FRAP method*

The ferric reducing antioxidant power (FRAP) was determined following the procedure used by Basanta, de Escalada Plá, Raffo, Stortz, and Rojas (2014). An amount of ≈4.5 mg of DFC was swelled in 2.0 mL of water. FRAP reagent was added and the reaction was monitored for 120 min by measuring the increase in absorbance at a wavelength (λ) of 595 nm. A calibration curve was constructed with 2 μmol/mL $FeSO_4.7H_2O$ aqueous solutions. Results were expressed as μmol Trolox equivalent/g of sample.

2.3.3. *Phenolic compounds by spectrophotometric method*

A quantity of ≈0.5500 g of DFC was hydrolyzed with 30 mL of 2 M NaOH for 24 hours at 25 °C under vacuum and protected from light. Then 5.7 mL of HCl was added in order to obtain pH < 2 and the mixture was submitted to centrifugation (Bunzel, Ralph, Marita, & Steinhart, 2000). The obtained supernatant was used to evaluate total phenolics using the Folin–Ciocalteu technique reported by Shui and Leong (2006). Gallic acid (Anedra, Buenos Aires, Argentina) was used as standard. The results were expressed as gallic acid equivalents (g/100 g).

2.3.4. *High-performance liquid chromatography (HPLC) with diode-array detector (DAD) for phenolics identification*

The extraction of phenolic compounds for HPLC analysis was carried out following the method described by Santo Domingo, Soria, Rojas, Fissore, and Gerschenson (2015). An amount of ≈0.4 g of DFC was mixed with 4 mL of methanol and then sonicated in an ultrasound bath during 45 min at 25 °C. The extracts were stored 48 h at 25 °C with constant stirring and protected from light. Then they were filtered through 0.45 μm (Millipore filters).

The methanolic extract was injected manually (injection volume: 20 μL) into a Waters 1525 chromatographic equipment (Milford, MA) with a binary HPLC pump, and equipped with photodiode array detector. An XBridge C18 column (150 mm × 4.6 mm, 5 μm) was used. Temperature of column was kept at 40 °C. The mobile phase was composed of acetic acid 0.5 mL/100 mL (A) and acetonitrile (B). The gradient program was as follows: 0% B (0 min), 20% B (10 min), 30% B (15 min), 50% B (20 min), 75% B (25 min), 100% B (30 min), 0% B (37 min), and the initial conditions were maintained for 5 minutes. The flow-rate was 0.80 mL/min. The diode-array detector was set at an acquisition range from 210 nm to 800 nm.

2.3.5. *High-performance liquid chromatography-mass spectrometry (HPLC-MS) analysis of phenolics*

HPLC-MS separation was performed by means of an Agilent 1200 Series equipment (Agilent Technologies, CA, USA). A Phenomenex Luna, C18 column (100 mm × 2.00 mm, 3 μm) was used. The mobile phases and the gradient program were the same as previously described for HPLC-DAD and the flow rate of the mobile phase was set at 0.20 mL/min The HPLC system was coupled to a Quadrupole-Time-of-Flight mass spectrometer (micrOTOF-Q II, Bruker Daltonik GmbH, Bremen, Germany), an orthogonal accelerated Q-TOF mass spectrometer, equipped with an electrospray ionization source (ESI), which was used in the negative ion mode with nitrogen as the collision gas. The spectra were acquired over a mass to charge ratio (*m/z*) ranging from 50 to 1000. The optimum values of the ESI-MS parameters were: capillary voltage, +3.5 kV; drying gas temperature, 200 °C; drying gas flow, 7.0 L/min; set nebulizer, 51 psi; and collision RF, 150 Vpp. The phenolic compounds were identified by means of the UV spectrums, the m/z, the molecular formula obtained and bibliographic data.

2.3.6. *Determination of content of carotenoids by a spectrophotometric method*

Extraction and determination of carotenoid content were adapted from the spectrophotometric method based on the mean absorption coefficients and mean absorption wavelength reported by Biehler, Mayer, Hoffmann, Krause, and Bohn (2010), with slight modifications. Briefly, 0.3335 g of DFC was mixed with 0.1665 g of calcium carbonate, 2.5 mL of methanol and 1 mL of methanolic potassium hydroxide (30 g/100 mL) in a glass tube for centrifuge. The mixture was shaken and kept on ice for 15 minutes; then it was centrifuged during 10 minutes at 2500 × g and 20 °C. The supernatant was separated and two extractions were performed on the residue with 4 mL of hexane: acetone mixture (1:1). Thereafter, the three organic extracts were combined and mixed in a separating funnel with 12 mL of saturated sodium chloride solution. The hexane phase supernatant was separated and the aqueous phase was re-extracted with 4 mL of hexane. Phases were combined and the hexane weight was recorded. Two milliliters of the hexane extract were evaporated to dryness on a Rotavap (R-124, Buchi, Switzerland) under vacuum at 30 °C. The residue was re-dissolved in 3.5 mL of acetone and the absorbance at 450 nm was measured. The results were expressed as milligrams of total carotenoids per 100 grams of DFCs.

2.3.7. *High-performance liquid chromatography (HPLC) of carotenoids*

The extraction procedure proposed by Rodriguez-Amaya and Kimura (2004) was selected for the studied samples. This method involves a primary extraction with acetone and a phase change to petroleum ether. Briefly, 0.5 g of DFC was weighted and rehydrated with 2 mL of water for 30 min. Then 5 mL of acetone was added and after 15 min, the solvent was filtered through 0.45 μm (Millipore filters). At least 2 more extractions with 10 mL of acetone were performed until all visual color was removed. One third of the extract was poured over 5 mL of petroleum ether in a separatory funnel and gently, 50 mL of water was added without shaking. Water was discarded and this procedure was repeated 3 times. Then samples were dried with anhydrous sodium sulfate and evaporated in a Rotavap system (Buchi, Switzerland). Dry samples were re-suspended in 2 mL of acetone : methanol (50 : 50) and with butyl hydroxyl toluene (0.05%). The vials were then closed after flushing the surface with N_2 in order to minimize carotenoid degradation. All procedures were done under yellow light and 0.5% butylated hydroxyanisole (BHA) was added to the solvents.

The quantification of major carotenoids was performed by high-performance liquid chromatography. An XBridge C18 column (100 mm × 2.1 mm, 3.5 μm) was used. The HPLC equipment was a Waters 2576 (Milford, MA) with a photodiode array detector, the flow rate was 1 mL/min and column temperature was 25 °C. Elution gradient started with 93% MeOH, 7% water and, in 30 min, 100% MetOH was reached, using a convex model curve. Total run time was 60 min for each sample including re-equilibration. Characteristic UV/Vis spectra were determined and identification was done at 450 nm.

Calibration curves for lutein, zeaxanthin, β-criptoxanthin and β-carotene were performed with commercially analytical standards (Extrasynthese, Lyon Nord, France). Range of concentrations used were 0.03–5 μg/mL ($R^2 = 0.989$), 0.06–3 μg/mL ($R^2 = 0.999$), 0.2–2 μg/mL ($R^2 = 0.984$), 0.4–4 μg/mL ($R^2 = 0.987$), respectively. The injection volume was 20 μL for each DFC sample or standard solution. To check for reproducibility, one random solution of each standard was injected each day of analysis. The results were expressed as mg/100 g.

2.3.8. *Ascorbic acid determination by a spectrophotometric method*

The extraction and determination of ascorbic acid were performed according to the method proposed by De'Nobili et al. (2013). Briefly, an amount of 0.1000 g of DFC fractions was mixed with 6 mL of oxalic acid for 1.5 hours at 5 °C. The extract was centrifuged at 2500 × g at 6 °C for 30 min. Ascorbic acid concentration was evaluated in the supernatant using the spectrophotometric method of 2,6-dichlorophenol indophenol. Results were expressed as mg/100 g.

2.4. In vitro *digestibility assay*

The simulated gastrointestinal procedure included the steps of gastric and intestinal digestion and was performed according to Saura-Calixto, Serrano, and Goñi (2007) with some modifications. Briefly, 1.2 g of DFC was mixed with 30 mL of pepsin-HCl solution containing 0.3 g/100 mL pepsin (Merck, 0.7 FIP-U/mg) in 0.04 N HCl, followed by incubation at 37 °C with orbital shaking at 2 s^{-1} for 2 h. Subsequently, the pH of the systems was adjusted to 7.5–8.0 with 2 N NaOH and finally, 40 mL of intestinal solution (0.05 M KH_2PO_4) containing 0.6 g/100 mL of bile salts and 0.3 g/100 mL of pancreatin (Parafarm, Argentina) were added, followed by incubation at 37 °C with orbital shaking at 2 s^{-1} for another 2 h. Solutions were centrifuged (15 min 3000 × g) and the supernatant was removed.

The insoluble indigestible fraction was washed twice with 5 mL distilled water, and all supernatants were combined. These solutions were transferred to dialysis tubes (12000–14000 molecular weight cut off) and dialyzed against water for 48 h at 37 °C (water flow 7 L/h) to eliminate residues of bile salts and others.

The soluble indigestible fraction of the dialysis was frozen whereas the insoluble indigestible fraction was freeze-dried and stored at –18 °C until its characterization.

Phenolic compounds content as well as antioxidant capacity determined by FRAP method were evaluated on the soluble and insoluble indigestible fractions by the spectrophotometric techniques described above. Polyphenols accessible in the small intestine were calculated as the difference between polyphenol content in the original sample and polyphenols associated with the indigestible fraction (soluble and insoluble) according to Saura-Calixto et al. (2007).

2.5. *Statistical analysis*

Unpaired t test was performed to compare the results between pulp DFC and peel DFC and, when corresponded, between freeze-dried pulp and freeze-dried peel. Besides, one-way ANOVA with Tukey's *post hoc* test was conducted to compare the result of *in vitro* digestibility assay. GraphPad Prism version 5.00 for Windows (San Diego, CA, USA) was used for all analysis.

3. Results and discussion

It has been demonstrated that the treatment of plant materials such as fruits by products with ethanol 96 mL/100 mL, followed by a dehydration process, produces ingredients concentrated in cell wall polymers, which can be composed of soluble and insoluble dietary fiber (de Escalada Pla et al., 2012; de Escalada Pla, Uribe, Fissore, Gerschenson, & Rojas, 2010; Nieto Calvache et al., 2015).

The treatment applied rendered 2.56 g of pulp DFC and 8.8 g of peel DFC from 100 g of the respective tissue (Table 1). Dehydration step assisted by microwaves reduced moisture content to 8.7 g/100 g for pulp DFC and 6.6 g/100 g for peel DFC. These fractions were mainly composed by dietary fiber presenting values of ≈60 and 54 g of TDF in 100 g of pulp and peel DFC, respectively. Both DFCs were composed mainly by IDF (Table 1) and pulp DFC presented the higher ($p < 0.05$) SDF content which constituted the 33% of the TDF. The ratio IDF/SDF showed values of 2.0 and 6.1 for pulp and peel DFC, respectively. Martínez et al. (2012) used co-products from canned and juice extraction of mango (*Mangifera indica* L., cv. Tommy Atkins and Haden), guava (*Psidium guajava* L., cv. Red) and passion fruit (*Passiflora edulis* L., cv. Flovicarpa) to obtain fiber concentrates and observed that the ratio between IDF/SDF took values of 1.5, 5.2, and 1.3, respectively.

3.1. *Evaluation of antioxidant capacity*

The ability of plant materials for exerting antioxidant activity is a topic of global interest and as such has been extensively studied in recent years. Many researchers have used the techniques of DPPH and FRAP for measuring antioxidant capacity in materials such as fresh fruits and vegetables, emphasizing the differences observed between the pulp, peel and seeds (Martínez et al., 2012; Rufino, Fernandes, Alves, & de Brito, 2009). Moreover, the inclusion of natural ingredients such as dietary fiber in the development of new food products has shown that in addition to nutritional supplementation, many times they can also exert a protective effect against oxidative rancidity in food products (Tseng & Zhao, 2013).

Results obtained in this research showed that both DFCs had antioxidant activity as measured through DPPH and FRAP methods (Table 1). It can be also observed that by DPPH method, peel DFC had an antioxidant capacity almost five times greater ($p < 0.05$) than pulp DFC (54.86 vs 12 μmol trolox/100 g). Also, the antioxidant capacity of peel DFC evaluated by FRAP assay was

Table 1 – Antioxidant properties, dietary fiber content and yield of the dietary fiber concentrate (DFC) from pulp or peel and freeze-dried samples.

	Pulp DFC	Peel DFC	Freeze-dried pulp	Freeze-dried peel
DPPH (μmol trolox/100g)[1]	12 ± 1[a]	54.86 ± 0.08[b]	450 ± 5[A]	485 ± 4[B]
FRAP (μmol trolox/g)[1]	10.2 ± 0.6[a]	25 ± 1[b]	19 ± 1[A]	26.4 ± 0.9[B]
Phenolic compounds content (g/100g)[1]	*0.47 ± 0.03[a]	*0.99 ± 0.04[a]	0.53 ± 0.01[A]	1.18 ± 0.05[B]
Ascorbic Acid (mg/100g)[1]	4.7 ± 0.6[a]	14 ± 1[b]	419 ± 11[A]	32 ± 3[B]
Total carotenoids (mg/100g)[1]	5.10 ± 0.03[a]	8.1 ± 0.8[b]	14.0 ± 0.2[A]	12.5 ± 0.1[B]
Total dietary fiber (g/100g)[1]	59.8 ± 0.5[a]	53.8 ± 0.3[b]	–	–
Insoluble dietary fiber (g/100)[1]	39.9 ± 0.5[a]	46.24 ± 0.06[b]	–	–
Soluble dietary fiber (g/100g)[1]	19.93 ± 0.01[a]	7.5 ± 0.2[b]	–	–
Yield (g/100g of fresh tissue)[2]	*2.56	*8.8	10.1 ± 0.1	19.9 ± 0.9

* Retrieved from Nieto Calvache et al. (2016).
[1] For pulp and peel DFC, the results are expressed on the basis of weight of each DFC. For freeze-dried (FD) pulp and peel, the results are expressed on the basis of FD powders.
[2] Fresh tissue means fresh pulp or fresh peel.
DPPH and FRAP mean, respectively: 2,2-diphenyl-1-picrylhydrazyl and ferric reducing antioxidant power.
Different lowercase letters in a row mean significant differences ($p < 0.05$).
Different capital letters in a row mean significant differences ($p < 0.05$).

twice ($p < 0.05$) that found for pulp DFC (25 vs. 10.2 μmol trolox/g). Accordingly, the freeze-dried pulp and peel also showed the same trend for DPPH and FRAP assays as can be observed in Table 1. It is noteworthy that both DFCs retained part of the original antioxidant activity after the production treatments. It can be observed that ferric reducing power was better preserved in the DFCs production procedure. Contreras-Calderón, Calderón-Jaimes, Guerra-Hernández, and García-Villanova (2011) found values for FRAP assay of 3.71 μmol trolox/g FW in Mountain papaya (*Carica pentagona*) samples. In this work, the conversion of FRAP values for peel and pulp DFCs to fresh peel and pulp weight basis rendered values of 2.2 and 0.26 μmol trolox/g FW respectively, which is in the order of previously cited data. Compounds which may be contributing to the antioxidant activity of DFCs are reported below.

3.2. *Spectrophotometric analysis of antioxidants compounds*

The phenolic compounds found in DFCs from peel were twice ($p < 0.05$) that found in the pulp DFC (0.99 vs 0.47 g/100 g). The same trend was observed for the freeze-dried fractions (Table 1). For comparing purposes, the yield values (Table 1) were used to refer data to fresh pulp or fresh peel basis. It is interesting to remark that about 22% and more than 37% of polyphenols found in the fresh samples of papaya pulp and peel respectively, remained attached to the corresponding DFCs.

The ascorbic acid content was determined both in the freeze-dried powders as in DFCs. The ascorbic acid found in the freeze-dried pulp was 419 mg/100 g. Wall (2006) found values between 45.3 and 55.6 mg/100 g FW in papayas harvested at different locations in Hawaii. Valente, Albuquerque, Sanches-Silva, and Costa (2011) reported values of ascorbic acid of 64.2 and 77.1 mg/100 g edible portion, in papaya fruits variety Formosa and Sunrise respectively. It is important to state that ascorbic acid is a water-soluble vitamin present in exotic fruits such as papaya and its content in great part depends on the variety of fruit. In the present research, the conversion of data from Table 1 to fresh pulp weight basis rendered values of 42.3 mg/100 g FW, which is in the order of previously cited data of the literature.

Although the process of extraction and drying produced a remarkable loss of ascorbic acid in the DFCs with respect to the fresh samples, the peel DFC preserved a higher content of ascorbic acid ($p < 0.05$) than pulp DFC (14 vs 4.7 mg/100 g). The conversion of these values to FW basis gave a value of 1.2 and 0.1 mg/100 g FW, for peel and pulp DFCs respectively. It can be concluded that the ascorbic acid content in the pulp (42.3 mg/100 g FW) was almost completely lost due to the DFC production process. Nevertheless, around 19% of ascorbic acid present in the original peel (6.4 mg/100 g FW) could be detected in the corresponding DFC. Possibly, a more reducing environment showed by DFC peel (Table 1) could exert a protective effect against ascorbic acid loss.

Also, it was found that the freeze-dried samples of pulp and peel (Table 1) had a content of total carotenoids of 14.0 and 12.5 mg/100 g respectively. The conversion of these values to FW basis gave a value of 1.4 and 2.48 mg/100 g FW. Researchers like Gayosso-García Sancho, Yahia, and González-Aguilar (2011) studied the content of carotenoids in papayas harvested at different stages of ripening, finding values between 0.92 and 3.27 mg/100 g FW, with a higher concentration of carotenoids in advanced stages of ripening. These data are in the order of those previously reported for our samples. Moreover, when the content of carotenoids in DFC of pulp and peel was studied, values of 5.10 and 8.1 mg/100 g, respectively, were found. These data converted to FW basis rendered values of 0.13 and 0.71 mg/100 FW for pulp and peel. As a consequence, about 29% of the initial carotenes of the peel remained in the DFC despite the process applied; meanwhile, pulp DFC kept about 9% of them. Carotenoid degradation by heat treatment was reported in studies realized by de Ancos, Cano, Hernandez, and Monreal (1999) who observed a loss of 57% of the total carotenoid content in papaya puree treated 45 seconds by microwave heating at 475 W. Therefore, carotene losses can be attributed to the extraction and drying stresses involved in the production of DFCs.

3.3. *Identification of phenolic compounds by HPLC MS*

Phenolics are characterized by having one or more aromatic rings with hydroxyl groups. Phenolic compounds can be

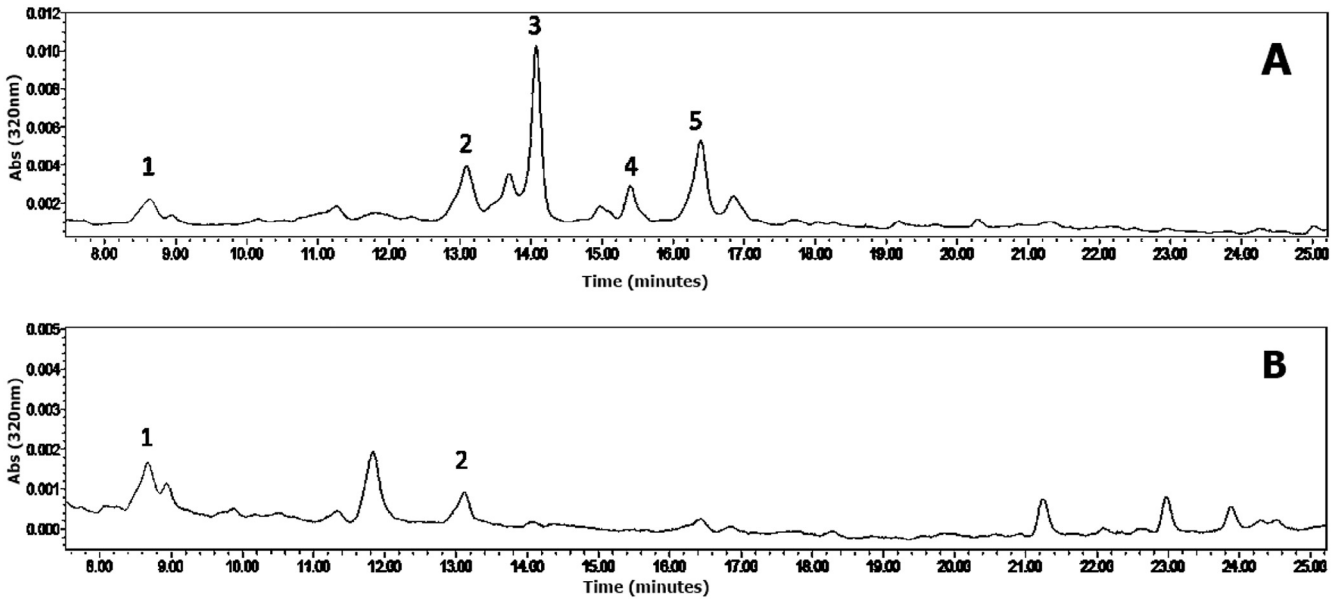

Fig. 2 – Polyphenols peaks identified by high-performance liquid chromatography-mass spectrometry analysis in the dietary fiber concentrate from peel (A) or pulp (B).

classified as phenolic acids, flavonoids, stilbenes, coumarins and tannins (Liu, 2004). The introduction of these compounds in human diet has been associated with health benefits such as reduced risk of different chronic diseases (Liu, 2004), protection against the proliferation of cancer cells, reduced vascularization, protection of neurons against oxidative stress, stimulation of vasodilation and enhancement of insulin secretion (Palafox-Carlos et al., 2009).

Peaks corresponding to phenolic compounds observed at wavelength of 320 nm for pulp and peel DFCs are shown in Fig. 2. Analyses of the peaks identified by HPLC-MS are shown in Table 2. Peak 1 was found in both DFCs (peel and pulp). According to its mass to charge ratio (m/z) of 315.07, corresponding to its deprotonated ion, and the molecular formula proposed ($C_{13}H_{15}O_9$), this compound was identified as protocatechuic acid hexoside. This phenolic compound is classified as a phenolic acid derived from hydroxybenzoic acids (Liu, 2004). This compound was also found by Rivera-Pastrana, Yahia, and González-Aguilar (2010) in samples of the exocarp and the mesocarp of the papaya variety Maradol. Meanwhile, Canini, Alesiani, D'Arcangelo, and Tagliatesta (2007) reported this compound in leaves of *Carica papaya* L. Two peaks (2 and 3) observed correspond to the flavonols family of quercetins (Liu, 2004). The compound corresponding to peak 2 was identified as manghaslin with a value of m/z of 755.20 and molecular formula $C_{33}H_{39}O_{20}$; this phenolic compound was present in both DFCs obtained. This compound was identified in methanolic extracts of papaya as reported by Brasil et al. (2014). Also, this compound was identified on samples of mountain papaya (*Vasconcellea pubescens*) in the studies made by Simirgiotis, Caligari, and Schmeda-Hirschmann (2009). The peak 3 corresponds to a compound with a value of m/z of 609.14 and with a molecular formula $C_{27}H_{29}O_{16}$, which was identified as quercetin 3-O-rutinoside and was only found in the DFC from peel. Rivera-Pastrana et al. (2010) also identified this compound in the exocarp of Maradol papaya fruit. Peaks 4 and 5 that correspond to phenolic acids derived from hydroxycinnamic acid (Liu, 2004) were found. In peak 4, a compound with m/z of 341.09 and molecular formula $C_{15}H_{17}O_9$ was tentatively identified as caffeoyl hexoside and was only found in DFC from peel. This compound was also identified by Sulaiman, George, and Balachandran (2013) in fruits like *Garcinia gummi-gutta* and *Terminalia chebula*. Peak 5 was identified only in the DFC from peel and presented a value of m/z of 193.05 and a molecular

Table 2 – Phenolic compounds found in the dietary fiber concentrates from papaya pulp and peel by high-performance liquid chromatography-mass spectrometry.

Compound number	Molecular formula	Retention time	m/z experimental	Proposed compound	Proposed classification	Additional details
1	$C_{13}H_{15}O_9$	8.6	315.07173	Protocatechuic acid hexoside	Phenolic acid	Derived from hydroxybenzoic acid
2	$C_{33}H_{39}O_{20}$	13.1	755.20405	Manghaslin	Flavonoid	Flavonol family
3	$C_{27}H_{29}O_{16}$	14.1	609.14730	Quercetin 3-O-rutinoside		
4	$C_{15}H_{17}O_9$	15.4	341.09009	Caffeoyl hexoside	Phenolic acid	Derived from hydroxycinnamic acid
5	$C_{10}H_9O_4$	16.4	193.05098	Ferulic acid		

m/z, mass to charge ratio.

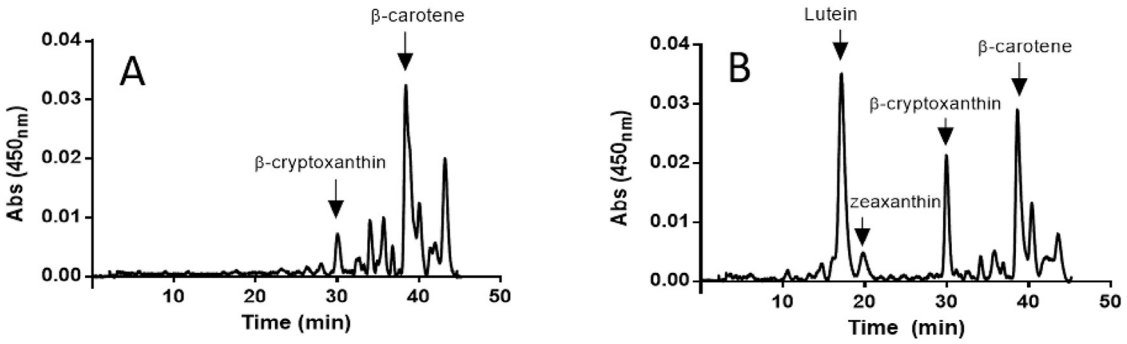

Fig. 3 – Carotenoids quantified by high-performance liquid chromatography in the dietary fiber concentrate from pulp (A) or peel (B).

formula $C_{10}H_9O_4$, which corresponded to ferulic acid which was also identified in papaya peel (Maradol variety) by Gayosso-García Sancho et al. (2011) and by Rivera-Pastrana et al. (2010).

Other studies performed in fruits have determined that compounds derived from hydroxycinnamic acid are generally more abundant than those derived from hydroxybenzoic acids (Castillo Muñoz, Fernández González, Gómez Alonso, García Romero, & Hermosín Gutiérrez, 2009). Derivatives of hydroxybenzoic acid, such as the protocatechuic acid, can be found as part of complex structures such as lignin, while the derivatives of hydroxycinnamic acid such as ferulic acid, are mainly linked to structural components of cell wall (cellulose, lignin and protein) through ester linkages (Liu, 2004).

3.4. *Quantification of carotenoids by spectrophotometry and HPLC*

Carotenoids are natural pigments found in fruits and vegetables and they are responsible for their yellow, orange and red characteristic colors (Perera & Yen, 2007). Recent studies showed that diets high in β-carotene and α-carotene are associated with a reduction of the incidence of type 2 diabetes in generally healthy men and women (Sluijs et al., 2015).

By an HPLC analysis, some of the major carotenoids (Fig. 3) present in the DFCs were quantified (Table 3). In pulp DFC, amounts of 1.0 and 1.33 mg/100 g or 0.026 and 0.034 mg/100 g FW of β-cryptoxanthin and β-carotene, respectively, were found. Furthermore, in peel DFC, 0.54 and 0.11 mg/100 g or 0.047 and 0.0097 mg/100 g FW of lutein and zeaxanthin, respectively, and 2.0 and 1.5 mg/100 g or 0.176 and 0.132 mg/100 g FW of β-cryptoxanthin and β-carotene, respectively, were found. As observed, carotenoid content which remained associated with the DFCs after the steps of ethanolic treatment and drying is quite interesting. Wall (2006) quantified the carotenoids from papayas harvested in different cultivars, reporting values of β-carotene ranging between 0.08 and 0.410 mg/100 g FW, values between 0.2884 and 1.0344 mg/100 g FW of β-cryptoxanthin, and values between 0.0933 and 0.3176 mg/100 g FW of lutein.

Table 3 – Carotenoids identified by high-performance liquid chromatography in the dietary fiber concentrate (DFC) from pulp and peel.

	Pulp DFC	Peel DFC	Retention time (min)
Lutein (mg/100g)[a]	ND	0.54 ± 0.04	17
Zeaxanthin (mg/100g)[a]	ND	0.11 ± 0.01	19
β-criptoxanthin (mg/100g)[a]	1.0 ± 0.1	2.0 ± 0.1	29
β-carotene (mg/100g)[a]	1.33 ± 0.05	1.5 ± 0.2	38

[a] For pulp and peel DFC, the results are expressed on the basis of weight of each DFC.
ND, not detected.

According to the analysis of antioxidant compounds, a great part of the antioxidant capacity observed in the DFCs (pulp and peel) can be explained by the presence of ascorbic acid, carotenoids and phenolic compounds, which make their contribution in different degrees depending on the type of sample (pulp or peel). According to Gardner, White, McPhail, and Duthie (2000), polyphenols are the main dietary antioxidants and also have higher antioxidant capacity *in vitro* than vitamins and carotenoids.

According to the results observed above, the next trends can be highlighted: i) the antioxidant activity of peel DFC was always higher ($p < 0.05$); ii) also, phenolic compounds, ascorbic acid and carotenoids were always greater in peel DFC ($p < 0.05$) than in pulp DFC; iii) the occurrence of different phenolic compounds and carotenoids was diverse according to the tissue used for DFC production; iv) although pulp showed higher values of ascorbic acid content, the loss due to production process was higher in pulp DFC.

3.5. In vitro *digestibility of DFCs and bioaccesibility of polyphenols in the small intestine*

Phenolic compounds were released from the dietary fiber matrix by digestion *in vitro*. Then the amount of phenolic compounds potentially bioaccessible in the small intestine was determined.

The results (Table 4) show that in DFCs from pulp and peel, about 0.31 and 0.63 g/100 g of phenolic compounds passed through the dialysis membrane corresponding to 66% and 64%, respectively, of the polyphenols present in the original sample (Fig. 4). These quantities of phenolics are denominated as potentially bioaccessible in the small intestine. Saura-Calixto et al. (2007) studied polyphenols potentially bioaccessible in the small intestine of typical fruits consumed in the Spanish diet, and found that 1.157 g/100 g dry sample of polyphenols of the fruits are bioaccessible in the small intestine; moreover, they proposed

Table 4 – *In vitro* digestibility of the dietary fiber concentrate (DFC) from pulp or peel and bioaccesibility of polyphenols in the small intestine.

	Soluble indigestible fraction		Insoluble indigestible fraction		Polyphenols bioaccesible in the small intestine[1]	
	Pulp DFC	Peel DFC	Pulp DFC	Peel DFC	Pulp DFC	Peel DFC
Total polyphenols (g/100g DFC)	0.112 ± 0.004[a]	0.209 ± 0.006[b]	0.049 ± 0.002[c]	0.155 ± 0.004[d]	0.31	0.63
FRAP (μmol trolox/g DFC)	1.54 ± 0.07[a]	4.10 ± 0.08[b]	0.17 ± 0.01[c]	0.77 ± 0.02[d]		

[1] Phenolic compounds in the DFCs (Table 1), minus polyphenols associated with the indigestible fractions (soluble plus insoluble).
FRAP, ferric reducing antioxidant power.
Different letters in a row mean significant differences ($p < 0.05$).

that the bioaccessible and bioavailable polyphenols are potentially susceptible to absorption through the gut barrier.

The *in vitro* digestion assay also showed that for every 100 g of DFC of pulp and peel digested, approximately 0.112 g and 0.209 g polyphenols, respectively, remain associated with the soluble indigestible fraction while 0.049 g and 0.155 g of polyphenols, respectively, remain associated with the insoluble indigestible fraction. It is evident that a significant amount of polyphenols (Table 4) remained in the indigestible fractions (soluble and insoluble) and would reach the large intestine wherein another amount of phenolic compounds could be released by fermentation processes performed by the bacterial flora of the environment exerting some antioxidant activity (Palafox-Carlos et al., 2009).

The antioxidant capacities evaluated by FRAP technique (Table 4) of the indigestible fractions from peel DFC were 4.10 (soluble) and 0.77 (insoluble) μmol Trolox/g and were higher ($p < 0.05$) than those found in the pulp DFC (1.54 and 0.17 μmol trolox/g for soluble and insoluble indigestible, respectively). The same trend in antioxidant capacity was observed for DFCs and freeze-dried pulp and peel (Table 1) as was discussed above.

4. Conclusions

A treatment with ethanol followed by microwave drying allowed the production of dietary fiber concentrates (DFCs) from papaya pulp and peel. The analysis of DFCs showed the presence of ascorbic acid and interesting quantities of phytochemicals such as phenolic compounds, of which were identified the

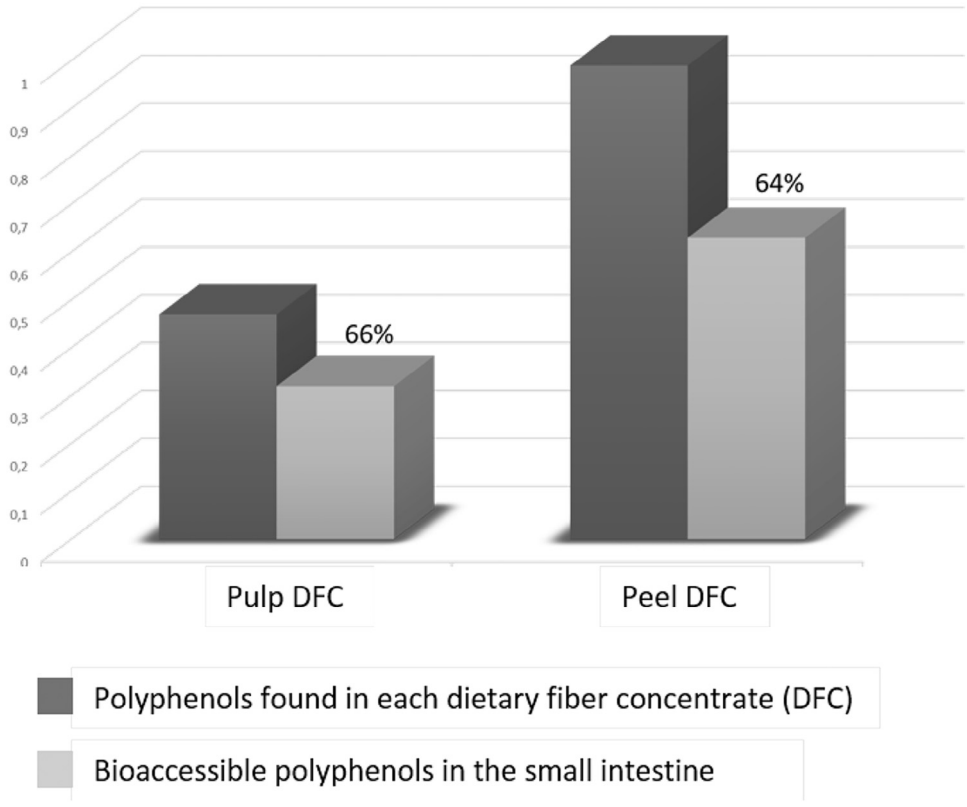

Fig. 4 – Phenolic compounds in the dietary fiber concentrates and their *in vitro* bioaccessibility in the small intestine.

protocatechuic acid hexoside, manghaslin, quercetin 3-O-rutinoside, caffeoyl hexoside and ferulic acid. Carotenoids such as lutein, zeaxhantin, β-criptoxanthin and β-carotene in the DFC samples were identified and quantified. The greatest quantity of phenolic compounds, carotenoids and ascorbic acid were detected in the peel DFC. Also in this fraction were identified phenolic compounds and carotenoids that were not present in the pulp fact that might explain the higher antioxidant capacity observed in peel DFC as well as in the indigestible fractions (soluble and insoluble) obtained after digestibility assay. The fractions obtained in the digestibility assay were only analyzed in this stage of the research for FRAP antioxidant capacity and polyphenol content; the results obtained are promising in relation to health issues and the studies will be deepened in future research.

Although fresh papaya from other varieties has been previously studied and their antioxidant capacity and composition have been determined, no data have been reported, according to our knowledge, about the antioxidant capacity of DFCs obtained from papaya of Formosa variety. Additionally, the novelty of this work lies in the fact that results showed that DFCs could represent not only a source of dietary fiber but also of associated compounds (phenolics, carotenoids, ascorbic acid) with antioxidant activity in spite of the process applied for DFC production. Moreover, it was observed after *in vitro* digestibility assays that these compounds could exert their antioxidant function although they were associated to dietary fiber.

In conclusion, the DFCs produced in this work, may be considered as a material capable of producing multiple benefits. At first, it can be mentioned the benefits already known of dietary fiber as a nutrient for the body which gives to DFCs the capacity for acting as a functional ingredient of application in the food industry. Moreover, the presence of phenolics, carotenoids and ascorbic acid in the obtained fractions enhances their nutritional value and can provide additional functionality to DFCs, for example helping to prevent the oxidation of lipids in foods with high fat content or also to meet the demand of the food market concerning the development of food with health protection capacity.

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