

¹Centro de Investigación en Alimentación y Desarrollo, A.C. (CIAD, AC), Hermosillo, Sonora, Mexico²Instituto Tecnológico de Sonora (ITSON), Obregon, Sonora, Mexico³Department of Cell Biology and Molecular Genetics, The University of Maryland, MD, USA⁴Department of Food Science and Technology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Antimicrobial and antioxidant properties of byproduct extracts of mango fruit

V. Vega-Vega¹, B.A. Silva-Espinoza¹, M.R. Cruz-Valenzuela¹, A.T. Bernal-Mercado¹, G.A. González-Aguilar¹, S. Ruíz-Cruz², E. Moctezuma³, MD. W. Siddiqui⁴, J.F. Ayala-Zavala^{1*}

(Received May 28, 2013)

Summary

Byproducts of fruit processing could have higher content of phenolic compounds that can act as antimicrobial and antioxidant agents. In this context, the main objective of this study was to obtain extracts from peel, seed, and unused flesh of Haden, Ataulfo and Tommy Atkins mango varieties, in order to measure their antioxidant and antimicrobial properties. The extraction was performed using different methods, such as methanolic-polar, methanolic-non-polar, ethanolic-polar, ethanolic-non-polar and water infusion. The total phenolic content of the ethanolic-non-polar extract from seed of mango Haden showed 875.06 mg/g, DPPH EC₅₀: 0.04 mg/mL, causing a 100 % inhibition of bacteria pathogens applying 25 mg/mL and inhibition of 89.78 % against *Alternaria* applying 6.25 mg/mL. The flesh always showed the lowest content and bioactivity of the tested parameters. These results demonstrate the antimicrobial and antioxidant potential uses of fruit byproducts as sources of bioactive compounds.

Introduction

Mango products are well demanded by consumers for their flavor, convenience and bioactive compounds; however, during processing considerable generation of byproducts can be an issue (KIM et al., 2007). Depending on the mango variety, the peel and seed contribute about 15-20 % and 10-25 % of the whole fruit weight, respectively (BERARDINI et al., 2005; ABDALLA et al., 2007). After industrial processing of mango, considerable amounts of peel and seeds are discarded, resulting in high economic loss to the manufacturer, as well as an impact to the environment (PURAVANKARA et al., 2000). For example, in India the mango-processing industry annually generates 3 x 10⁵ tons of seed, approximately (SOONG and BARLOW, 2004). Therefore, it is necessary to consider alternative uses for these mango byproducts, in order to avoid environmental problems and to create new revenue sources, providing greater economic returns to the agribusiness (AYALA-ZAVALA et al., 2011).

Several studies have shown that the content of phytochemical compounds is higher in peel and seeds with respect to the edible tissue of several fruits. The total phenolic contents in the peels of lemons, oranges, and grapefruits were 15 % higher than that of the pulp of these fruits (GORINSTEIN et al., 2001). Eight selected clingstone peach cultivars were studied and it was reported that the peels contained 2-2.5 times the amount of total phenolic compounds as contained in the edible product (CHANG et al., 2000). Peels from apples, peaches, pears as well as yellow and white flesh nectarines were found to contain twice the amount of total phenolic compounds as that contained in fruit pulp (GORINSTEIN et al., 2001). Several studies show that peel and seed byproducts of fresh-cut fruits have high antioxidant capacity, and in many cases, even higher than the pulp (SOONG and BARLOW, 2004; AYALA-ZAVALA et al., 2010).

Phenolic compounds are some of the most important bioactive compounds in the mango fruit, mainly found in peels and seeds (RIBEIRO et al., 2008). The antioxidant capacity is attributed mainly to some of these phenolic compounds, which also have high antimicrobial capacity (KABUKI et al., 2000). AYALA-ZAVALA et al. (2010) reported that the seed of mango var. 'Kent' presents 3,727.52 mg of Gallic Acid Equivalent (GAE) per 100 g of fresh weight (f.w), which is higher than that reported in the pulp (48.84 mg GAE/100 g f.w). This 'Kent' mango phenolic compound seed value is higher than other fruit byproducts, such as pineapple, papaya and mandarin peel (113.24, 108.88 and 352.05 mg GAE/100 g of fresh weight, respectively). Moreover, catechin, chlorogenic acid, and phloridzin, three phenolic compounds that are abundant in apple processing byproducts, exhibited varying degree of inhibitory action toward the growth of tested food pathogenic and spoilage bacteria, fungi, and yeasts (MUTHUSWAMY and RUPASINGHE, 2007). Phenolic compounds can be found in different tissues of fruits, e.g. i) peels are rich in phenolic compounds with antioxidant, antimicrobial and colorant properties, some of these are natural defenses against pathogens attack and environmental conditions; ii) the pulp poses a lower content of phenolic compounds, however, it is the main source of dietary antioxidants for humans, and finally iii) the seed can be also rich in phenolics, mainly tannins with antioxidant and antimicrobial properties that protect this tissue to perpetuate the regeneration of the species (AYALA-ZAVALA et al., 2011).

The interest of some research groups has focused on the search for natural sources of antioxidant and antimicrobials, as well as an evaluation of their properties, to preserve food quality. This interest in natural antioxidants and antimicrobial, is due to the increased potential health risks associated with consumption of synthetic antioxidants and antimicrobial compounds (SENEVIRATNE and KOTUWEGEDARA, 2009). In this context, the analysis of natural compounds with antioxidant and antimicrobial potential is getting attention. Therefore, the objective of this study was to obtain, using several methods, extracts from the peel and seed of different varieties of fresh-cut mango ('Haden', 'Ataulfo' and 'Tommy Atkins') in order to assess their antioxidant and antimicrobial properties.

Materials and methods

Sample preparation and characterization

Mangoes varieties 'Haden', 'Ataulfo' and 'Tommy Atkins' were obtained from a local store in the city of Hermosillo, Sonora, Mexico. Mangoes were selected according to color and free of physical defects in a commercial maturity stage (Tab. 1). All fruit was washed with chlorinated water (250 ppm) for 3 min and air dried at 25 °C. Fruits were weighted and the peel and seed were removed, the pulp was cut into cubes of 2 cm per side. Yield was calculated weighting the amount of peel and seed byproducts, as well as finished products and compared with the initial weight of raw material. Byproducts (peel, seed and unused pulp) were used for the extraction of phenolic compounds.

* Corresponding author

Tab. 1: Physicochemical characterization of studied mango varieties 'Haden', 'Ataulfo' and 'Tommy Atkins'.

Parameters evaluated	Haden	Ataulfo	Tommy Atkins
SST (%)	12.37 ± 0.23*	14.40 ± 0.13	14.13 ± 0.16
Firmness (N)	97.66 ± 0.29	105.77 ± 0.39	109.10 ± 0.18
pH	3.42 ± 0.02	2.87 ± 0.02	3.16 ± 0.01
Acidity (% citric acid)	0.77 ± 0.03	2.07 ± 0.03	0.97 ± 0.07
Peel color			
L	52.20 ± 2.27	64.90 ± 1.72	59.48 ± 2.76
°Hue	110.74 ± 5.52	102.10 ± 1.69	101.86 ± 4.97
Chroma	44.08 ± 3.38	55.72 ± 1.44	43.49 ± 1.73
Pulp color			
L	72.42 ± 1.36	76.64 ± 1.44	75.68 ± 1.12
°Hue	94.52 ± 1.39	93.14 ± 1.02	97.52 ± 1.24
Chroma	72.78 ± 2.79	67.52 ± 2.54	57.21 ± 3.58

*Average of three determinations ± standard error

Preparation of phenolic extracts

Methanol and ethanol were used in the extraction process that has been proved to generate extracts with high antioxidant capacity (OBOH and ROCHA, 2007; MUTHUSWAMY et al., 2008; AYALA-ZAVALA et al., 2012a). Samples (10 g) were left to macerate in 100 mL of each alcohol: water (7:3) in darkness for 10 days at 25 °C. After that time, the extracts were filtered and the solvent removed using a rotary evaporator. The aqueous fraction was lyophilized and hydrolyzed (10 mL of NaOH 4M) for 4 h in the absence of light, subsequently, an acid hydrolysis was performed with HCl 4M taking every sample to pH 2. The hydrolyzed extract was subject to liquid-liquid separation by two washes with 20 mL of ethyl acetate, yielding two fractions: polar (aqueous phase) and non polar (ethyl acetate phase) (OBOH and ROCHA, 2007). Solvent was evaporated from the non polar extract and it was re-suspended in de-ionized water. Overall, methanolic polar (MPE), methanolic non polar (MNPE), ethanolic polar (EPE) and ethanolic non polar extracts (ENPE) with a concentration of 25 mg of extract of tissue per mL of water were obtained. Water infusion was used as an alternative extraction process. Lyophilized byproducts (1 g) were mixed with 40 mL of distilled water; the mixture was boiled (100 °C) for 30 min. The crude extract was centrifuged at 2,348 x g, 4 °C for 8 min. Subsequently filtered through layers of organza, the filtrate obtained (concentration 25 mg/mL) was frozen at -35 °C until further use (MUTHUSWAMY et al., 2008). All the extractions were performed by triplicate and the extract yield, total phenolic, flavonoid contents, antioxidant capacity and antimicrobial activity were measured.

Total phenolic and flavonoid contents

Total phenolic content was measured by the methods described by SINGLETON and ROSSI (1965), with some modifications. Extracts (50 µL) were mixed with 3 mL of H₂O and 250 µL of Folin-Ciocalteu's phenol reagent 1N. After 8 min of equilibration time, 750 µL of Na₂CO₃ (20 %) and 950 µL of H₂O were added to the mixture, and incubated for 30min at room temperature. After incubation, the absorbance was read at 765 nm with an UV-Vis spectrophotometer (Cary, model 50 Bio, Varian, Italy). Concentration of total phenolic compounds was calculated using a standard curve of GAE and expressed as milligrams per gram of dry weight. Flavonoid content was determined based on the method described by ZHISHEN

et al. (1999). One mL of each extract was mixed with 5 % NaNO₂, 10 % AlCl₃ and NaOH 1M and measured spectrophotometrically at 415 nm using quercetin as standard. The results were expressed as mg of quercetin equivalents (QE) per g of dry weight.

DPPH radical scavenging activity*

This assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH• (GONZÁLEZ-AGUILAR et al., 2007). A 3.9 mL aliquot of a 0.0634 mM of DPPH• solution, in methanol was added to 0.1 mL of each extract. The mixture was shaken in a vortex and kept 30 min in the dark. Absorbance was then read in an UV-VIS (Cary, model 50 Bio, Varian, Italy) spectrophotometer, at a wavelength of 515 nm. Results were expressed as EC₅₀ (concentration of the antioxidant extract required to reduce the absorbance of the radical by 50 %) in mg/mL. Analyses were performed in triplicate per each sample.

Trolox equivalent antioxidant capacity (TEAC)

This assay is based on the ability of the antioxidants to scavenge the blue-green ABTS^{•+} radical compared to the scavenging ability of the water-soluble vitamin E analogue, Trolox (RE et al., 1999). The ABTS^{•+} radical cation was generated by the interaction of 5 mL of 7 mM ABTS solution and 88 µL of 0.139 mM K₂S₂O₈ solution. After the addition of 2970 µL of ABTS solution to 30 µL of extracts (0.2 g/mL) or trolox standards (0 to 20 µM range), the absorbance was monitored exactly 1 and 6 min after the initial mixing. The percentage of absorbance inhibition at 734 nm was calculated and plotted as a function of that obtained for the extracts and the trolox standard. The final TEAC value was calculated by using a regression equation between the standard concentration and the inhibition percentage and expressed as trolox equivalents (µmol TE) per g of dry weight.

Antifungal assay

The antifungal potential of byproducts extracts was tested against *Alternaria alternata* using the central inoculation method, the extracts were diluted in the agar (AYALA-ZAVALA et al., 2012b). For this 6.25 mg/mL of each extract were added to Petri dishes containing potato dextrose agar, and then inoculated in the center with the fungi. The efficiency of the treatments was evaluated at 5 days of incubation at 25 °C. Controls were pure agar inoculated with the fungus and without exposure to any extract, no effect of the solvent on the fungal growth was observed. Mycelial area was recorded (cm²) in triplicate by the digital analysis of the image of every plate using the UTHSCSA ImageTool version 3.0 software. Results were expressed as inhibition percentage of mycelial growth of *A. alternata* at day 5.

Antibacterial assay

The antibacterial ability of every extract against *Salmonella enterica* subsp. *enterica* serovar. Choleraesuis ATCC 14028, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* O157:H7 ATCC 43890 and *Staphylococcus aureus* ATCC 65384, was assessed. A loopful (~20 µL) of the bacterium was transferred to a tube containing 10 mL of tryptic soy broth and incubated overnight at 37 °C. Initial inoculums of 1 x 10⁸ CFU/mL were placed in tubes with 1 mL of Mueller Hinton broth and then 1mL of extract was added (25 mg/mL), including a control tube without the extract. The tubes were incubated at 37 °C by 24 h and the CFU of each bacteria in presence and absence of extracts were determined on Mueller Hinton agar. Results were expressed as a percentage of growth inhibition compared with controls.

Statistical analysis

The experimental design consisted of a 3x3x5 factorial arrangement: 3 = variety ('Haden', 'Ataulfo', 'Tommy Atkins'), 3 = tissue (peel, seed, pulp) and 5 = extracts (MPE, MNPE, EPE, ENPE, water infusion) for the evaluation of the total phenolics, flavonoids, and antioxidant capacity. For the evaluation of the antimicrobial activity a factorial arrangements of 3x2x5: 3 = variety ('Haden', 'Ataulfo', 'Tommy Atkins'), 2 = tissue (peel, seed), and 5 = extracts (MPE, MNPE, EPE, ENPE, water infusion) were used, respectively. ANOVA was performed ($p \leq 0.05$) to estimate significant differences between treatments and Tukey-Kramer was the mean test used for comparison ($p \leq 0.05$) using the NCSS software (2007).

Results and discussion

Yield of fresh-cut mango byproducts

Fresh-cut mango byproducts, composed of peel, seed and useless pulp, represented 40 % of the total weight of the fruit, while fresh-cut produce in the form of cubes yielded 60 % of the total weight. Amongst byproduct, seed yields from 11-12.33 %, peel 8.90-13 % and unused pulp 15.3-59 % (Tab. 2). Authors like BERARDINI et al. (2005) reported that industrial processing of mango yields of 33-85 % of pulp, 7-24 % of peel and 9-40 % of seed, which depends on the variety of mango and the type of product to be manufactured with the pulp. Although the range is wide, this is indicative of the large percentage of byproducts that minimum processing of mango generates, which usually have no further use.

Previous studies demonstrated that the minimal processing of several kinds of fruits produced high amounts of byproducts. The processed fruits were apples (*Malus domestica* var. Golden Delicious), mandarins (*Citrus reticulata*), papayas (*Carica papaya* var. Maradol), pineapples (*Ananas comosus* var. Premium cayenne) and mangos (*Mangifera indica* var. Kent). Processing of apples produced 10.91 % of pulp and seed (core) byproducts and 89.09 % of apple slices. Mandarins processing produced 16.05 % of peels and 83.95 % of wedges. Processing of papayas produced 6.51 % of seeds, 8.47 % of peels, 32.06 % of unusable pulp (due to the lack of shape uniformity in a cube) and 52.96 % of cubes. Pineapples processing pro-

duced 9.12 % of core, 13.48 % of peels, 14.49 % of pulp, 14.87 % of top and 48.04 % of slices. Mangos 'Kent' produced 13.5 % of seeds, 11 % of peels, 17.94 % unusable pulp and 57.56 % of cubes (AYALA-ZAVALA et al., 2010). It has to be highlighted that regarding the considerable amounts of fruit byproducts of the minimal processing, it can be considered the possibility of creating alternative uses to give added value to this wasted material.

Antioxidant capacity of the fresh-cut mango byproducts extracts

Fig. 1 shows the content of phenolic compounds (total phenolics and flavonoids) from fresh-cut mango byproducts extracts and Fig. 2 shows the antioxidant capacity (DPPH and TEAC) of the extracts obtained from such tissues. A significant effect of the three evaluated mango varieties was found ($p \leq 0.05$). Regarding the global effect of the variety (including all the tissues and the extractions): Haden and Ataulfo had the highest total phenolics (168 and 145 mg GAE/g, respectively), flavonoids (58 and 91 mg QE/g), antioxidant capacity expressed by DPPH EC_{50} (0.8456 and 0.5406 mg/mL), and TEAC values (45.93 and 45.88 mmol TE/g), followed by Tommy Atkins (phenolics = 128 mg GAE/g, flavonoids = 56 mg QE/g, DPPH EC_{50} = 1.23 mg/mL, and TEAC = 37.5 mmol TE/g). No significant differences between Haden and Ataulfo were found ($p \geq 0.05$), but between Ataulfo and Haden with Tommy Atkins, the antioxidant capacity was significantly different ($p \leq 0.05$).

A significant effect ($p \leq 0.05$) of the evaluated tissues on the antioxidant properties of the extracts was observed, disregarding the effect of the variety and extraction. The seed had the highest values of the evaluated parameters (phenolics = 346 mg GAE/g, flavonoids = 126.9 mg QE/g, DPPH EC_{50} = 0.307 mg/mL, and TEAC = 112 mmol TE/g), followed by the peel (phenolics = 91 mg GAE/g, flavonoids = 78 mg QE/g, DPPH EC_{50} = 1.438 mg/mL, and TEAC = 16 mmol TE/g), and finally the pulp (phenolics = 4.42 mg GAE/g, flavonoids = 1.9 mg QE/g, DPPH < 50 % inhibition at mg/mL, and TEAC = 0.25 mmol TE/g). Also, a significant effect of the different extraction solvents on the antioxidant status of the extracts was observed ($p \leq 0.05$), overall ENPE showed higher values (phenolics = 230 mg GAE/g, flavonoids = 46 mg QE/g, DPPH EC_{50} = 0.716 mg/mL, and TEAC = 77 mmol TE/g), and MNPE (phenolics = 186 mg GAE/g, flavonoids = 107 mg QE/g, DPPH EC_{50} = 0.51 mg/mL, and TEAC = 64 mmol TE/g), followed by MPE (phenolics = 143 mg GAE/g, flavonoids = 93 mg QE/g, DPPH EC_{50} = 1 mg/mL, and TEAC = 22 mmol TE/g), EPE (phenolics = 109 mg GAE/g, flavonoids = 50 mg QE/g, DPPH EC_{50} = 0.72 mg/mL, and TEAC = 37 mmol TE/g), and water infusion (phenolics = 67.8 mg GAE/g, flavonoids = 47 mg QE/g, DPPH EC_{50} = 1.41 mg/mL, and TEAC = 13 mmol TE/g). In the triple interaction among factors (variety-tissue-solvent of extraction), the ENPE of mango seed variety Haden showed the highest values (phenolics = 875 mg GAE/g, flavonoids = 164 mg QE/g, DPPH EC_{50} = 0.04 mg/mL, and TEAC = 272 mmol TE/g), followed by the same extract of the variety Tommy Atkins (phenolics = 746 mg GAE/g, flavonoids = 112 mg QE/g, DPPH EC_{50} = 0.095 mg/mL, and TEAC = 232 mmol TE/g), and the MNPE of the same tissue of the variety Ataulfo (phenolics = 424 mg GAE/g, flavonoids = 261 mg QE/g, DPPH EC_{50} = 0.133 mg/mL, and TEAC = 168 mmol TE/g). In general, there was a positive relation between phenolic content and antioxidant capacity.

Comparing the results of this study with previous knowledge the antioxidant content and activity of extracts from mango byproducts are high. MATSUSAKA and KAWABATA (2010) reported the total phenolic content and the antioxidant capacity of the non-edible parts of some tropical fruits, resulting that the mango seed and mango peel possess the major content of phenolics (153 and 123.80 mg/g dw respectively) compared to avocado, starfruit, canistel, passion fruit,

Tab. 2: Fresh-cut mango and byproducts yield.

Variety and part of the fruit	Yield (%)
Haden	
Peel	13.0 ± 0.58*
Seed	11.0 ± 1.15
Unused pulp	15.33 ± 0.58
Final product (cubes)	60.67 ± 1.15
Ataulfo	
Peel	8.90 ± 0.09
Seed	11.83 ± 0.08
Unused pulp	22.27 ± 1.13
Final product (cubes)	57.00 ± 1.15
Tommy Atkins	
Peel	11.67 ± 0.58
Seed	12.33 ± 0.29
Unused pulp	17.00 ± 1.50
Final product (cubes)	59.00 ± 2.29

*Average of three determinations ± standard error

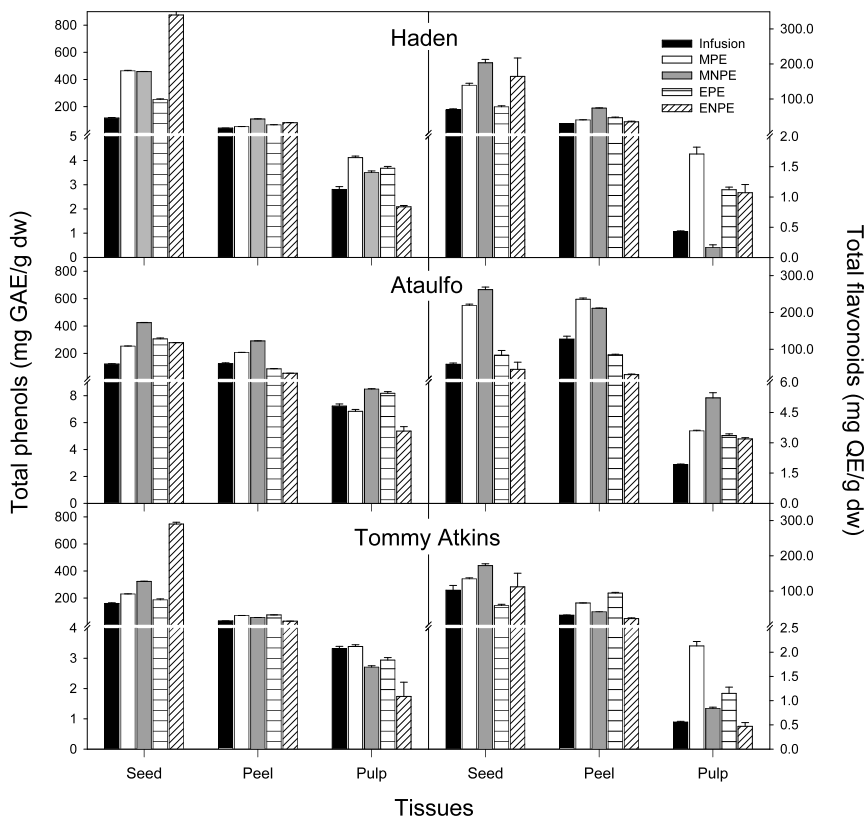


Fig. 1: Total phenolics and flavonoids from fresh-cut mango byproducts extracts. *Average of three determinations ± standard error, PME: Polar methanolic extract; NPME: Non polar methanolic extract; PEE: Polar ethanolic extract; NPEE: Non polar ethanolic extract.

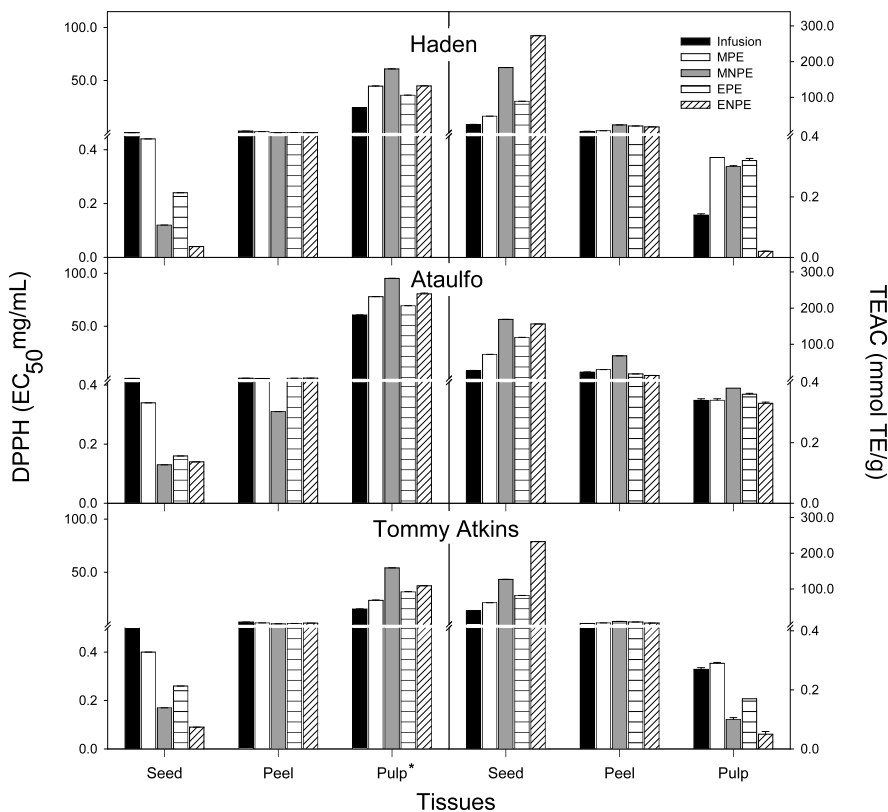


Fig. 2: Antioxidant capacity from fresh-cut mango byproducts extracts. *Average of three determinations ± standard error, **Reported as inhibition % at the concentration 25 mg/mL, because the EC₅₀ was not found

kiwifruit, papaya and kiwano. Also the mango byproducts, seed (4188 TEAC $\mu\text{mol/g dw}$) and peel (1846 TEAC $\mu\text{mol/g dw}$) showed higher antioxidant potencial by the DPPH and ABTS radical scavenging assays than the avocado seed (462 TEAC $\mu\text{mol/g dw}$), passionfruit peel (75.4 TEAC $\mu\text{mol/g dw}$), papaya peel (58.4 TEAC $\mu\text{mol/g de}$) and kiwano peel (14.2 TEAC $\mu\text{mol/g dw}$). In the other hand, the mango seed possess major phenolic content than apple peel (1144 mg GAE/100g dw), kiwifruit peel (820 mg GAE/100g dw) and pink grapefruit peel (2335 mg GAE/100g dw) (WIINGAARD et al., 2009). The phenolics content found in these studies for mango byproducts were similar to that obtained during the infusion in our study, nevertheless the ENPE yield major phenolic contents than the byproducts of some tropical fruits. These results confirm the findings from previous studies that showed that the extraction of phenolics compounds with hydro-alcoholic mixtures yield higher values (BUCIC-KOLIC et al., 2009). The differences may be attributed to the process and the solvent used in the extraction, the variety of the fruits and the antioxidant compounds characteristics of each byproduct extract. With this in mind, the mango byproducts have shown to be a potent source of phenolic compounds with high antioxidant capacity.

Antimicrobial activity of the fresh-cut mango byproducts extracts

Fig. 3 shows the antifungal activity of the extracts from fresh-cut mango byproducts varieties Haden, Ataulfo and Tommy Atkins against *A. alternata* at a concentration of 6.25 mg/mL. A significant global effect ($p \leq 0.05$) of the evaluated variety (including all the tissues and the extractions) on the antifungal activity of the obtained extracts was found. Ataulfo and Haden varieties had the highest antifungal activity (37.5 and 34 %, respectively), showing no significant differences between them ($p \geq 0.05$), followed by Tommy Atkins (33 %), which showed no significant difference as between tissues was observed, disregarding the effect of the variety and extraction compared to Haden ($p \geq 0.05$). A significant difference ($p \leq 0.05$), with the highest antifungal activity values found in the peel (33 % inhibition), followed by the seed (37 %). On the other hand, there was a significant difference ($p \leq 0.05$) in antifungal activity depending on the type of extraction solvent used for this study. The MPE showed the highest antifungal capacity (42 %), followed by MNPE (36 %), ENPE (36 %), EPE (31 %), and finally water infusion (30 %). All the interactions were significant ($p \leq 0.05$), with the ENPE obtained from the seed of the variety Haden showing the highest inhibition (89.78 %) of the mycelial growth of *A. alternata*. The water infusion and the MPE obtained from the seed of the variety Tommy Atkins had antifungal values of 89.69 and 87.34 %, respectively, and no difference ($p \geq 0.05$) was found between them.

Fig. 4 and 5 show the inhibition of bacterial strains by the presence of the bioactive extracts. A significant effect ($p \leq 0.05$) of the evaluated varieties (including all the tissues and the extractions) was observed against food pathogens survival. The extracts from the Haden variety showed higher values of inhibition percent of bacterial strains (*E. coli* 0157:H7 = 48 %, *S. Choleraesuis* = 47.6 %, *L. monocytogenes* = 48.5 %, and *S. aureus* = 48 %), followed by Ataulfo (*E. coli* 0157:H7 = 49 %, *S. Choleraesuis* = 47 %, *L. monocytogenes* = 47.8 %, and *S. aureus* = 47.8 %), and Tommy Atkins (*E. coli* 0157:H7 = 47.5 %, *S. Choleraesuis* = 46 %, *L. monocytogenes* = 45 %, and *S. aureus* = 46 %), all presenting significant differences ($p \leq 0.05$) amongst them. Similarly, a significant effect ($p \leq 0.05$) of the extracted tissue and extraction solvent was found. Amongst the different tissues, disregarding the effect of the variety and extraction, the extracted peel showed higher values of inhibition (*E. coli* 0157:H7 = 47.7 %, *S. Choleraesuis* = 48 %, *L. monocytogenes* = 47 %, and *S. aureus* = 47.6 %), followed by the seed (*E. coli* 0157:H7

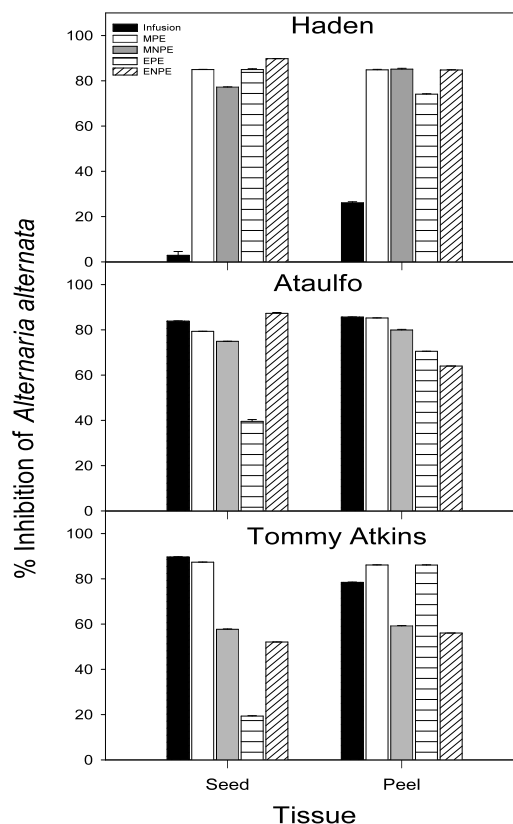


Fig. 3: Inhibition percentage of mycelial growth of *Alternaria alternata* at day 5. *Concentration 6.25 mg/mL. **Average of three determinations \pm standard error.

= 48.6 %, *S. Choleraesuis* = 46.6 %, *L. monocytogenes* = 46.9 %, and *S. aureus* = 47 %). Similarly, a significant effect ($p \leq 0.05$) of the extraction solvent was observed (disregarding the effect of the variety and tissues), no differences were found ($p \geq 0.05$) in methanolic (*E. coli* 0157:H7 = 50 %, *S. Choleraesuis* = 49 %, *L. monocytogenes* = 50 %, and *S. aureus* = 50 %) and ethanolic (*E. coli* 0157:H7 = 49.9 %, *S. Choleraesuis* = 50 %, *L. monocytogenes* = 50 %, and *S. aureus* = 50 %) extracts, but significant differences were found ($p \leq 0.05$) with the water infusion extraction method (*E. coli* 0157:H7 = 42 %, *S. Choleraesuis* = 34 %, *L. monocytogenes* = 37 %, and *S. aureus* = 37 %).

Considering the factors interactions, all were significant ($p \leq 0.05$). The extracts that showed 100 % inhibition of all food pathogens were: Haden (seed MNPE and EPE and peel MPE); Ataulfo and Tommy Atkins (seed and peel MNPE, MPE, ENPE, EPE), while the water infusions of all tissues exhibited the lowest inhibition. In general, the most sensitive bacterial strains to the presence of bioactive extracts were the gram positives: *L. monocytogenes* and *S. aureus*, with 100 % inhibition values obtained with the EPE, ENPE, MPE, and MNPE as compared against gram negative strains of *E. coli* O157:H7 and *S. choleraesuis*. Similar results have been reported by KABUKI et al. (2000), showing that extracts from mango seed are more effective against gram-positive bacteria than gram-negative bacteria. Gallotannins (tannins, penta-, hexa-, and hepta-O-galloylglucose) extracted from mango kernels inhibited the proliferation of Gram-positive food spoilage bacteria and reduced the growth of Gram-negative *E. coli*, although the growth of lactic acid bacteria was not inhibited (ENGELS et al., 2009). This effect could be attributed to the differences in structure of cell envelope, including cytoplasmic membrane and cell wall components of gram-positive bacteria, as compared to gram-negative species (HUGO and RUSSELL, 1987).

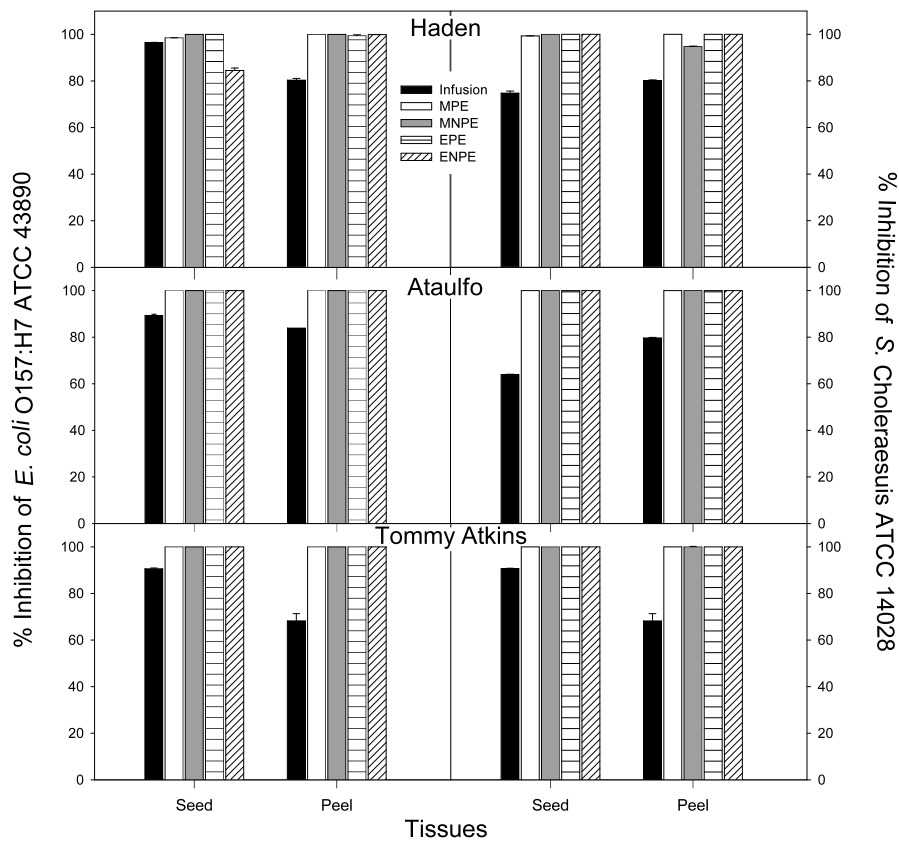


Fig. 4: Percentage of inhibition of gram negative bacterial strains exposed to bioactive extracts from fresh-cut mango byproducts. *Concentration 25 mg/mL. **Average of three determinations ± standard error.

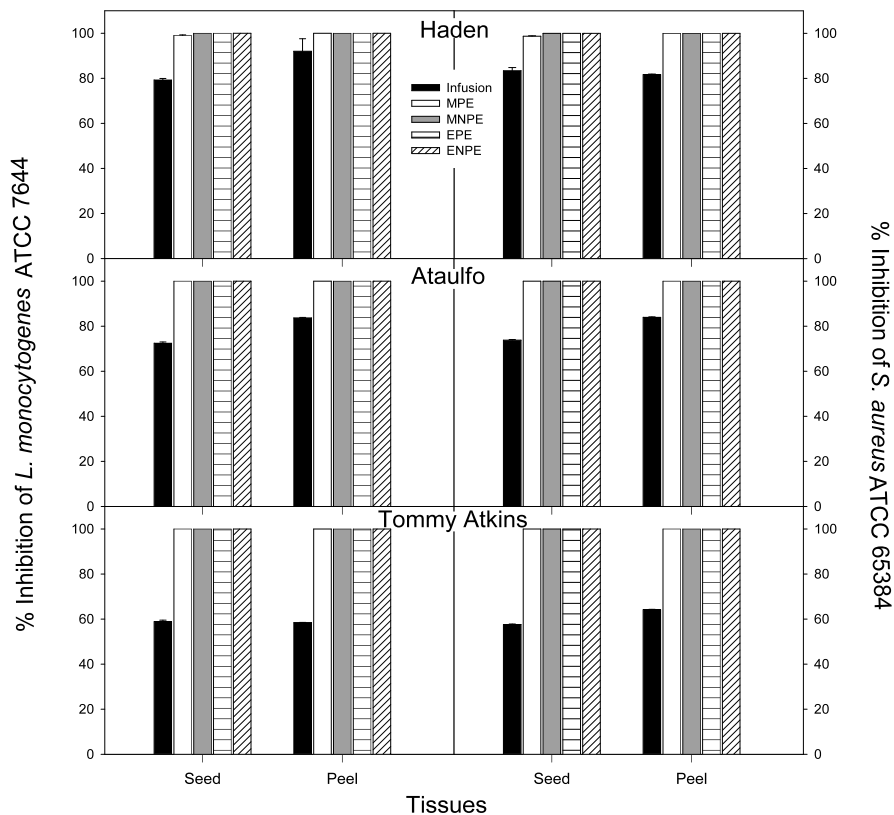


Fig. 5: Percentage of inhibition of gram positive bacterial strains exposed to bioactive extracts from fresh-cut mango byproducts. *Concentration 25 mg/mL. **Average of three determinations ± standard error.

Considering the total phenolic content of the obtained extracts, it can be related that these compounds could be responsible of their antimicrobial activity. The antimicrobial mechanisms of these compounds are not well understood; however, it has been suggested that phenolic compounds will cause changes in the membrane through its interaction with the carboxyl groups of the hydrophilic amino acids of the protein in the cell membrane, thus altering its permeability (RAYBAUDI-MASSILIA et al., 2009). Phenolic compounds will cause an alteration of pH and electrical potential, causing the release of protons to the outside. Thus, there will be a coagulation of cytoplasmic content in the bacteria, accompanied by a loss of normal cell metabolism, leading to cell death (RAYBAUDI-MASSILIA et al., 2009). Although, the antimicrobial activity of the extracts is attributed to phenolic compounds it is recommended to identify the profile of compounds in the different tissues, consider that other natural compounds with antimicrobial activity could be found in mango tissues, e.g. terpenoids (NEGI et al., 2002).

Conclusions

The present study demonstrates a significantly higher total antioxidant capacity, phenolic content and antimicrobial activity of mango byproducts than of the edible portions. The NPEE obtained from the seed and peel, of mango var. 'Ataulfo' and 'Haden' showed the highest antioxidant and antimicrobial activity. This study opens the possibilities of the potential applications of the obtained extracts as antioxidants and antimicrobial agents.

Acknowledgements

Authors thank the financial support of the Mexican council for science and technology CONACYT and the national program for the improvement of the professorate PROMEP.

References

- ABDALLA, A.E.M., DARWISH, S.M., AYAD, E.H.E., EL-HAMAHMY, R.M., 2007: Egyptian mango byproduct I. Compositional quality of mango seed kernel. *Food Chem.* 103, 1134-1140.
- AYALA-ZAVALA, J.F., ROSAS-DOMÍNGUEZ, C., VEGA-VEGA, V., GONZÁLEZ AGUILAR, G.A., 2010: Antioxidant enrichment and antimicrobial protection of fresh cut fruits using their own byproducts: looking for integral exploitation. *J. Food Sci.* 75, 175-181.
- AYALA-ZAVALA, J.F., VEGA-VEGA, V., ROSAS-DOMÍNGUEZ, C., PALAFOX-CARLOS, H., VILLA-RODRIGUEZ, J.A., SIDDIQUI, M.W., DÁVILA-AVIÑA, J.E. GONZÁLEZ-AGUILAR, G.A., 2011: Agro-industrial potential of exotic fruit byproducts as a source of food additives. *Food Res. Int.* 44, 1866-1874.
- AYALA-ZAVALA, J.F., PEREZ-CARLON, J.J., ESQUEDA, M., GONZALEZ-AGUILAR, G.A., LEYVA, J.M., CRUZ-VALENZUELA, M.R., MOCTEZUMA, E., 2012a: Polar fractionation affects the antioxidant properties of methanolic extracts from species of genus *Phellinus* Quel. (Higher Basidiomycetes). *Int. J. Med. Mushrooms.* 14, 563-573.
- AYALA-ZAVALA, J.F., SILVA-ESPINOZA, B.A., CRUZ-VALENZUELA, M.R., VILLEGAS-OCHOA, M.A., ESQUEDA, M., GONZALEZ-AGUILAR, G.A., CALDERON-LOPEZ, Y., 2012b: Antioxidant and antifungal potential of methanol extracts of *Phellinus* spp. from Sonora, Mexico. *Rev. Iberoam. Micol.* 29, 132-138.
- BERARDINI, N., KNÖDLER, M., SCHIEBER, A. CARLE, R., 2005: Utilization of mango peels as a source of pectin and polyphenolics. *Innov. Food Sci. Emerg. Technol.* 6, 442-452.
- BUCIC-KOJIC, A., PLANINIC, M., TOMAS, S., JAKOBEK, L., SERUGA, M., 2009: Influence of solvent and temperature on extraction of phenolic compounds from grape seed, antioxidant activity and colour of extract. *Int. J. Food Sci. Tech.* 44, 2394-2401.
- CHANG, S., TAN, C., FRANKEL, E.N., BARRETT, D.M., 2000: Low-density lipoprotein antioxidant activity of phenolic compounds and polyphenol oxidase activity in selected Clingstone peach cultivars. *J. Agr. Food Chem.* 48, 147-151.
- ENGELS, C., KNÖDLER, M., ZHAO, Y.Y., CARLE, R., GANZLE, M.G., SCHIEBER, A., 2009: Antimicrobial activity of gallotannins isolated from mango (*Mangifera indica* L.) kernels. *J. Agr. Food Chem.* 57, 7712-7718.
- GONZÁLEZ-AGUILAR, G.A., VILLEGAS-OCHOA, M.A., MARTÍNEZ-TÉLLEZ, M.A., GARDEA, A.A., AYALA-ZAVALA, J.F., 2007: Improving antioxidant capacity of fresh-cut mangoes treated with UV-C. *J. Food Sci.* 72, 197-202.
- GORINSTEIN, S., MARTIN-BELLOSO, O., PARK, Y.S., HARUENKIT, R., LOJEK, A., CIZ, M., CASPI, A., LIBMAN, I., TRAKHTENBERG, S., 2001: Comparison of some biochemical characteristics of different citrus fruits. *Food Chem.* 74, 309-316.
- HUGO, W.B., RUSSELL, A.D., 1987: In *Pharmaceutical microbiology*. 4th ed. Oxford, Blackwell Scientific Publications.
- KABUKI, T., NAKAJIMA, H., ARAI, M., UEDA, S., KUWABARA, Y., DOSAKO, S., 2000: Characterization of novel antimicrobial compounds from mango (*Mangifera indica* L.) kernel seeds. *Food Chem.* 71, 61-66.
- KIM, Y., BRECHT, J.K., TALCOTT, S.T., 2007: Antioxidant phytochemical and fruit quality changes in mango (*Mangifera indica* L.) following hot water immersion and controlled atmosphere storage. *Food Chem.* 105, 1327-1334.
- MUTHUSWAMY, S., RUPASINGHE, H.P.V., 2007: Fruit phenolics as natural antimicrobial agents: Selective antimicrobial activity of catechin, chlorogenic acid and phloridzin. *J. Food Agric. Environ.* 5, 81-85.
- MUTHUSWAMY, S., RUPASINGHE, H.P.V., STRATTON, G.W., 2008: Antimicrobial effect of cinnamon bark extract on *Escherichia coli* O157: H7, *Listeria innocua* and fresh-cut apple slices. *J. Food Safety.* 28, 534-549.
- NCSS, 2007: Number Cruncher Statistical Systems. Statistical program for windows, Hintze JL, EUA.
- NEGI, P.S., JOHN, K.S., RAO, U.J.S.P., 2002: Antimicrobial activity of mango sap. *Eur. Food Res. Technol.* 214, 327-330.
- OBOH, G., ROCHA, J.B.T., 2007: Distribution and antioxidant activity of polyphenols in ripe and unripe tree pepper (*Capsicum pubescens*). *J. Food Biochem.* 31, 456-473.
- PURAVANKARA, D., BOGHRA, V., SHARMA, R.S., 2000: Effect of antioxidant principles isolated from mango (*Mangifera indica* L) seed kernels on oxidative stability of Buffalo Ghee (Butter Fat). *J. Sci. Food Agric.* 80, 522-526.
- RAYBAUDI-MASSILIA, R.M., MOSQUEDA-MELGAR, J., SOLIVA-FORTUNY, R., MARTIN-BELLOSO, O., 2009: Control of pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. *Compr. Rev. Food Sci. F.* 8, 157-180.
- RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M., RICE-EVANS, C., 1999: Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad. Biol. Med.* 26, 1231-1237.
- RIBEIRO, S.M.R., BARBOSA, L.C.A., QUEIROZ, J.H., KNÖDLER, M., SCHIEBER, A., 2008: Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. *Food Chem.* 110, 620-626.
- SENEVIRATNE, K.N., KOTUWEGEDARA, R.T., 2009: Antioxidant activities of the phenolic extracts of seed oils and seed hulls of five plant species. *Food Sci. Technol. Int.*, 15, 419-425.
- SINGLETON, V.L., ROSSI, J.A., 1965: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.* 16, 144-158.
- SOONG, Y.Y., BARLOW, P.J., 2004: Antioxidant activity and phenolic content of selected fruit seeds. *Food Chem.* 88, 411-417.
- ZHISHEN, J., MENGCHENG, T., JIANMING, W., 1999: The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64, 555-559.

Address of the corresponding author:
E-mail: jayala@ciad.mx