Food Anal. Methods (2017) 10:330–338
DOI 10.1007/s12161-016-0584-5

Use of the Oxygen Radical Absorbance Capacity (ORAC) Assay to Predict the Capacity of Mango (*Mangifera indica* L.) By-Products to Inhibit Meat Protein Oxidation

Eva Dorta[1] · Elena María Rodríguez-Rodríguez[2] · Andrés Jiménez-Quezada[1] · Eduardo Fuentes-Lemus[1] · Hernán Speisky[3] · Eduardo Lissi[4] · Camilo López-Alarcón[1]

Received: 18 March 2016 / Accepted: 17 June 2016 / Published online: 25 June 2016

Abstract The potential activity of mango by-products to inhibit the peroxyl radical-mediated oxidation of tryptophan residues (Trp) of myofibrillar proteins (MP) was studied. Extracts of seeds, peels and pulps were studied in terms of their antioxidant activity, assessed by the oxygen radical absorbance capacity (ORAC) assay employing fluorescein and pyrogallol red as probes (ORAC-FL and ORAC-PGR, respectively). The effect of the extracts of mango by-products on the oxidation of Trp was evaluated by fluorescence spectroscopy. Data obtained employing the ORAC-FL assay showed a low discrimination of the antioxidant activity of the samples (Peel ≈ Seed > Pulp). However, employing the ORAC-PGR assay it was possible to differentiate the antioxidant activity of the samples (Seed > Peel >> Pulp). Extracts obtained from mango seed, peel, and pulp protected Trp of MP when the latter was exposed to AAPH (2,2′-azo-bis(2-amidinopropane) dihydrochloride)-derived free radicals. Such effect was in good agreement with the ORAC-PGR data, showing that this assay could be employed to predict the protection of Trp residues of MP.

Keywords Peroxyl radicals · Mango by-products · ORAC · Phenols · Tryptophan · Myofibrillar proteins

Electronic supplementary material The online version of this article (doi:10.1007/s12161-016-0584-5) contains supplementary material, which is available to authorized users.

✉ Eva Dorta
edorta@uc.cl

[1] Department of Pharmacy, Faculty of Chemistry, Pontifical Catholic University of Chile, C.P. 782 0436 Santiago, Chile

[2] Food Science and Nutrition Area, Department of Chemical Engineering and Pharmaceutical Technology, University of La Laguna, 38206 La Laguna, Santa Cruz de Tenerife, Spain

[3] Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile

[4] Faculty of Chemistry and Biology, University of Santiago de Chile, Santiago, Chile

Introduction

Interest of food chemistry researches, industries, and authorities in the utilization of by-products that are generated during the processing of polyphenolic-rich fruits has shown a significant increase during the last decades. This interest arises from the fact that such by-products, despite constituting an often excellent source of polyphenols with health potential, are continually wasted or discarded. Additionally, it should be pointed out that consumers are not only increasingly prioritizing the selection of healthy foods but also increasingly aware and concerned regarding aspects such as food quality, food safety, and environmental protection. Due to the recognized antioxidant activity of polyphenols, the use of polyphenol-rich by-products could be of interest both, to preserve the quality of foods susceptible to develop oxidative rancidity and to promote health effects upon their consumption. In this sense, research focused on the potential capacity of polyphenolic-rich by-products to act as scavengers of free radicals continues being a challenging topic.

By-products (i.e., peel and seed) of mango fruits (*Mangifera indica L.*) have been reported to be a good source of polyphenols (Ayala-Zavala et al. 2011; Jahurul et al. 2015), prompting the development of various experimental procedures to improve their extraction (Dorta et al. 2013; Ribeiro et al. 2007; Dorta et al. 2014). While mango peel contains nearly 4.5 mg of quercetin and 6 mg of gallic acid per gram of fresh weight, its seed contains between eight- and sixfold

higher amounts of such polyphenols, respectively. Besides such compounds, Dorta and coworkers have identified nearly 30 other phenolic compounds in extracts obtained from mango seed and peel (varieties Keitt, Sensation, and Gomera 3) (Dorta et al. 2014). The antioxidant activity of mango by-products has been evaluated mainly by the bleaching of stable free radicals such as $ABTS^{\bullet+}$ and DPPH (Dorta et al. 2013; Ramirez et al. 2014), and assessed through their iron-reducing capacity (FRAP, ferric reducing power potential) assay (Soong and Barlow 2004). Interestingly, employing these methodologies mango by-products have been shown to contain higher antioxidant activity than the edible parts of mango (Aziz et al. 2012; Dorta et al. 2012). Additionally, the antioxidant capacity of mango by-products has been assayed by the oxygen radical absorbance capacity (ORAC) method. Employing fluorescein as probe (ORAC-FL), Sogi et al. (2013) reported that the ORAC activity of mango seed would be at least three times higher than that of mango peel. As known, the ORAC-FL assay mainly reflects the stoichiometry of the reaction between polyphenols and peroxyl (or alkoxyl) radicals generated during the thermolysis of AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride) (Dorta et al. 2015; Lopez-Alarcon and Lissi 2006). However, there are not data regarding the actual reactivity of the mango components towards such radicals. In this regard, our laboratory has proposed the use of the pyrogallol red-based ORAC assay (ORAC-PGR) as a simple methodology to assess the reactivity of pure and mixtures of polyphenols in foods (Lopez-Alarcon and Lissi 2006; Romero et al. 2010; Alarcon et al. 2008). While, ORAC-FL index represents the total antioxidant activity of a particular sample, the ORAC-PGR index reflects mostly the rate at which the polyphenols react with AAPH-derived free radicals.

Food oxidative rancidity comprises the deterioration of both proteins and lipids. In the case of meat, particular attention has been recently placed in the oxidation of its proteins as these are easy targets of peroxyl radicals (Mercier et al. 1998; Utrera et al. 2014). Estevez and collaborators have reported that during the storage and processing of meats, carbonyl groups, an index of protein oxidation, are continually produced (Soladoye et al. 2015). Together with the formation of carbonyls, the consumption of tryptophan residues (Estevez et al. 2008a; Soladoye et al. 2015), and the occurrence of crosslinking reactions, which give place to the formation of high molecular weight meat protein aggregates, have been observed, and the latter have been related to changes in meat digestibility (Zhou et al. 2014). Meat protein oxidation is also associated with the occurrence of perferrylmyoglobin radicals, which are formed during the reaction of hydrogen peroxide and metmyoglobin (Jongberg et al. 2014). Interestingly, polyphenol-rich plant extracts have been shown to protect meat protein and meat products when these are exposed to oxidative conditions (Jongberg et al. 2014; Lara et al. 2011; Rababah et al. 2004; Rodriguez-Carpena et al. 2011). Such protection has been associated with the capacity of polyphenols to neutralize peroxyl radicals generated during lipid peroxidation and also perferrylmyoglobin radicals (Estevez et al. 2008b; Jongberg et al. 2014).

The Pica, Tommy Atkins, Kent, and Keitt are amongst the most commonly grown, processed and consumed varieties of mango within the west south Andes region of South America. In view of the potential of mango fruit by-products to serve as a source of polyphenol-rich extracts, we characterized the quality of these fruits in terms of their antioxidant capacity (employing both the ORAC-FL and ORAC-PGR assays), and their sugar and mineral composition. In addition, the potential of extracts of mango prepared from the pulp, peel, and seed, to protect meat proteins against the oxidation induced by AAPH, was assessed.

Materials and Methods

Chemicals

Pyrogallol red (PGR), fluorescein (FL), Trolox (6-hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid), and AAPH were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile high performance liquid chromatography (HPLC) grade was purchased from Merck (Darmstadt, Germany). Standards of D-(+)-glucose anhydrous, D(−)-fructose, and sucrose were purchased by Fluka (Buchs, Switzerland). All compounds were employed as received.

Fruit Characterization and Obtaining of Mango By-Products

Mango fruits (Pica, Tommy Atkins, Kent, and Keitt varieties) were obtained from chilean markets. Fruits of each variety (20–25 pieces) were bought just before the ripening stage and allowed to ripen to consumption stage in a ripening chamber at 18 °C and 80–90 % relative humidity. The mango fruits were used when they reached the consumption stage. To determine the ripening stage of mango fruits, different physical–chemical parameters such as color, texture, total soluble solids (TSS), pH, and total titratable acidity (TTA), were determined (results are shown in supplementary material, Table 1S). Fruit color was measured employing a Minolta colorimeter CR-300 (Minolta Corp., Ramsey, NJ, USA) using the Hunter Lab scale (L∗, a∗, and b∗ values). To determinate TSS, pH, and TTA, mango pulp cubes (20 g) were homogenized in 60 mL of ultrapure water for 2 min. TSS values, expressed as °Brix, were measured with an ATAGO ATC-1 (Tokyo, Japan) refractometer, sensitivity 0.1 % (Bausch & Lomb Optical Co., Rochester, NY, USA) at 23 ± 1 °C. The pH of the samples was measured using a digital pH meter (Oakton, Model 700, Eutech Instruments, Singapore), while TTA was determined by the titration of the samples until

pH 8.0 with a 0.1 mol eq NaOH/L solution. TTA of each sample were expressed as milligrams of citric acid/100 g. Firmness was measured using a firmness tester (Texture Analyzer TA-XT2; Arrow Scientific, Lane Cove, NSW, Australia) with a stainless steel spherical probe of 1/4 cm^3 diameter and a speed of 10 mm/s. Texture was reported as the force in Newton (N) needed to penetrate 10 mm (Nmm). Total of three firmness measurements per sample were made.

When mango fruits reached the optimum ripening stage, 15 units of each variety were taken, washed with tap water, and dried on filter paper. After, the peels and the seeds (shell of fibrous endocarp, testa, and embryo) of the fruits were manually separated in accordance with the practices of processing industries. Mango pulp, seed, and peel were cut into small pieces (0.5 × 1 cm), mixed (pulps, seeds, and peels of each variety separately), and freeze-dried at −40 °C/50 MPa for 5 days (Dorta et al. 2012). Freeze-dried mango pulps, peels, and seeds were ground to a fine powder (particle size between 355 and 500 μm), and stored at −20 °C until their use. Extracts of mango pulp, peels, and seeds were carried out accordingly with Dorta et al. (2012, 2014). Briefly, freeze-dried peels were added to an ethanol:water (1:1, *v*:*v*) mixture at 75 °C in a weight and solvent ratio of 1:50 (*w*:*v*), while mango pulps and seeds were added to an acetone:water (1:1, *v*:*v*) mixture at 50 °C and a weight-to-solvent volume of 1:30 (seeds) or 1:10 (pulps) (*w*:*v*). In all cases, the resulted suspensions were stirred during 60 min. After this time, the solids were separated by centrifugation (525×*g* at 4 °C for 20 min) in a Jouan CR-312 centrifuge (Thermo Electron Corporation, Madrid, Spain) and the supernatants stored at −80 °C until their use. The experimental conditions for the extraction of polyphenols from pulps, peels, and seeds were selected for ensuring the maximum efficiency of the extractive procedures.

Analysis of Mineral and Sugars in Mango Pulp and By-Products

The mineral and trace elements were determined by atomic absorption spectrophotometry using a Varian SpectrAA-10 plus (Varian Iberica SL, Madrid, Spain) atomic absorption spectrometer equipped with a D2 lamp background correction system using an air–acetylene flame. The samples of dried mango pulp, peel, and seed were previously acid-digested using nitric acid, according to the procedure described by Suárez et al. (2007). Sugar determination was performed by HPLC according to the method described by Rodríguez Galdón et al. (2009). For such analysis, a Waters 2690 HPLC instrument, equipped with a differential refractive index (DRI) detector (Waters model 2414, Millford, MA, USA), was employed. A HPLC Waters Carbohydrate Analysis column (3.9 × 300 mm, kept at 25 °C) with a particle size diameter of 10 μm, coupled to a Waters Carbohydrate CarboTM 4-μm guard column was used. An acetonitrile:water (80:20, *v*/*v*) mixture was employed as mobile phase (isocratic), with a flow rate of 2 mL/min. HPLC sample peaks were identified by comparing the registered retention times with those obtained employing pure standards.

Total Phenolic Content (TP) and ORAC Determination of Mango By-Product Extracts

TP of mango extracts was determined according to the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965), using gallic acid as standard. Briefly, appropriate dilutions of the samples (1 mL) were added to 0.2 N Folin-Ciocalteu reagent (5 mL). After 5 min, sodium carbonate (75 g/L) was added. The mixtures were incubated for 1 h and the absorbance of the resulting blue color was measured at 740 nm using a Hewlett Packard 8453 spectrophotometer. Quantification was carried out through the standard curve of gallic acid, and results expressed as gallic acid equivalents (mM) per gram of dry by-product and per fruit weight.

ORAC assay was applied employing fluorescein (FL) and pyrogallol red (PGR) as probes. The consumption of FL or PGR, elicited by free radicals generated from the thermolysis of AAPH was estimated from fluorescence (F) or absorbance (A) measurements, respectively. (F) values were registered employing 493 and 515 nm as excitation and emission wavelengths, respectively. (A) values were registered at 540 nm. Fluorescence measurements were carried out using a Perkin Elmer LS-55 spectrofluorimeter (Beaconsfield, UK), while absorbance intensity was evaluated using an Agilent 8453 (Palo Alto, CA, USA) UV-visible spectrophotometer. Values of (F/F_0) or (A/A_0) were plotted as a function of the incubation time. Integration of the area under the curve (AUC) was performed up to a time such that (A/A_0) reached a value of 0.2. These areas were employed to obtain ORAC values, according to the equation:

$$\mathrm{ORAC} = \frac{(\mathrm{AUC} - \mathrm{AUC}^{\circ})}{(\mathrm{AUC}_{\mathrm{Gallic\ acid}} - \mathrm{AUC}^{\circ})}\ \mathrm{f}\ [\mathrm{Gallic\ acid}]$$

AUC	Area under curve in the presence of the tested sample, integrated between time zero and that corresponding to 80 % of the probe consumption;
AUC°	Area under curve for control experiment (in the absence of antioxidants).
$AUC_{Gallic\ acid}$	Area under curve obtained in the presence of gallic acid.
f	Dilution factor, equal to the ratio between the total volume of the AAPH-probe solution, and the added mango extract volume.
[Gallic acid]	Gallic acid molar concentration.

Obtaining Bovine Myofibrillar Proteins

Myofibrillar proteins (MP) were extracted from bovine longissimus dorsi muscle according to the method used by Zhou et al. (2014) with minor modifications. Briefly, 1 g of raw minced muscle (multiple pieces) was homogenized in 8 mL of a sodium phosphate buffer solution (20 mM) at pH 6.5 containing 0.1 M NaCl, 25 mM KCl, 2 mM $MgCl_2$, using an Ultra-Turrax homogenizer (Beijing Jingke Huarui Instrument Co. Ltd., Beijing, China) at 11,000 rpm for 1 min in an ice water bath. The extract was centrifuged at 2000×*g* for 15 min at 4 °C using a GL- 21 M centrifuge (Xiang Yi Centrifuge Instrument Co. Ltd., Changsha, China). The pellet was washed twice with 100 mL of a 50-mM KCl solution at pH 6.4 and once with 100 mL of 20 mM sodium phosphate buffer at pH 6.0. The pellet was finally suspended in 20 mM phosphate buffer containing 0.6 M NaCl (pH 6.0) and the protein concentration was adjusted to design the desired value by BCA (bicinchoninic acid) addition using bovine serum albumin (BSA) as standard (Smith et al. 1985).

Measurements Tryptophan Oxidation by Fluorescence

The consumption of tryptophan (Trp) residues induced by AAPH-derived free radicals was followed by fluorescence (excitation wavelength = 295 nm and emission wavelength = 340 nm). MP extracts (at 2 mg/mL) were incubated at 37 °C in the presence of 10 mM of AAPH in phosphate buffer (75 mM, pH 7.4). The kinetic profile of Trp consumption was followed during 2 h; each 30 min, solutions were burbled with air to ensure peroxyl radical formation from AAPH. Similar experiments were carried out in the presence of mango extracts. In the presence of such extracts, a slight decrease in the initial fluorescence intensity was observed, probably related to the capacity of the compounds present in mango samples to absorb at 295 and/or 340 nm. From the kinetic profiles of Trp consumption, the fluorescence intensity after 1 h of incubation was chosen to assess the protective effect of mango pulp and by-products on Trp consumption.

Data Expression and Analysis

Data analysis was carried out employing the Statgraphics-Plus software 5.1 (Statistical Graphics, Rockville, Md., USA). Three independent replications by each treatment were used in the statistical analysis. Grubbs' test was applied to detect outliers in the data set and analysis of variance was used to evaluate the mineral and sugar content in mango by-products and pulp extracts. Fisher's least-significant-difference test, at the 5 % significance level, was applied to assess intrapair significant differences. Simple linear correlation analysis was used to test the possible correlation between mineral content and phenol content and also mineral content and the antioxidant activity of mango by-products and pulp.

Results and Discussion

Physicochemical Characterization of Mango Fruit Varieties and Their By-Products

Table 1S (supplementary material) shows the main physicochemical characteristics of the here-studied four varieties of mango. As shown, the weight of the fruits varied between 224.7 ± 19.7 and 498.7 ± 89.5 g; Pica and Keitt being the varieties with the lowest and highest weight, respectively. In fact, the weight of Keitt fruits was 2.2 times higher than Pica variety fruits. In spite of such differences, the recovery of by-products from Pica (9.5 % for peel and 13.7 % for seed of whole fruit fresh weight) was not substantially different from that of the Keitt variety (7.1 % peel and 9.2 % seed). In terms of acceptability for the processing industry, according to Sellamuthu and coworkers (2013), the firmness and the soluble solid content are two important quality features for selecting mango fruits before their processing. In this sense, the firmness of the studied mango fruit samples, which varied between 9.0 ± 3.4 and 13.9 ± 2.5 N, was optimal for fresh cut processing. Other relevant characteristic for industrial mango processing is the TTA. The ratio of the two latter parameters (TSS/TTA) has been associated with the taste of fruits, a lower ratio representing a more sweet-sour taste. Our results showed that Keitt variety exhibited the lowest TSS/TTA ratio, followed by the Kent, Tommy Atkins, and Pica varieties. Color fruit, commonly evaluated throughout C* and h° data, is often associated with the stage of maturity of each variety (Sellamuthu et al. 2013). Obtained h° data showed a diversity of colors in the mango fruits analyzed. Kent variety showed a yellow color, while Tommy Atkins variety was characterized by a red light color. In the case of Pica and Keitt varieties, yellow-orange and light orange were observed, respectively. Regarding C* values, Pica variety showed the most intensive color, followed by Kent and Tommy Atkins varieties. Keitt was the variety with the most opaque color (lowest C* value).

Mineral and Sugar Contents of Mango By-Products

Some studies have suggested the existence of a direct association between the content of minerals and that of polyphenols in fruits such as mulberry and bananas (Sulaiman et al. 2011; Tewari et al. 2004). Additionally, the content of some minerals is relevant for the activity of antioxidant enzymes such as superoxide dismutase (Zn, Cu, and Mn), catalase (Fe), and glutathione peroxidase (Se) (Tewari et al. 2004). In our fruit samples, the mineral content of four macro (P, K, Ca, and Mg) and micro (Fe, Cu, Zn, and Mn) minerals was determined.

Results, presented in Table 2S, show that P, Ca, and Mg contents were higher in seed and peel of the four varieties compared to the content of such macrominerals in their corresponding pulps. For example, in the case of Pica variety, content of P, Ca, and Mg in peel was 1.2-, 7.9-, and 2.4-fold higher than that of the pulp. In the case of the content of microelements, the samples presented a comparable composition to that reported by Chiocchetti and coworkers (2013). In a similar way to the results obtained for the content of macroelements, mango seeds and peels showed significantly higher contents of Fe, Zn, and Mn than mango pulps. Attention should be placed onto their particularly high content of Fe and Zn, with the highest values being found in the Keitt variety; 30.5 and 15.2 mg/Kg of dry matter, respectively. While high values of Fe could be relevant by this association with child growing and development, a high content of Zn can be associated with an improved activity of several enzymes (Chiocchetti et al. 2013).

In other context, sugars are commonly present in fruits and their by-products, and these compounds are particularly abundant in mango fruits. Due to the ability of some sugars to act as reductive agents, upon reaching a critical concentration (Magalhaes et al. 2010), these compounds could be capable of inducing the reduction of the Folin-Ciocalteu (FC) reagent. Such reduction would be evidenced by an increase in the visible absorption of the oxidized FC derivative, implying the possibility that a false positive interference by some sugars in the FC assay could have occurred (Magalhaes et al. 2010). For such reason, we decided to evaluate the content of sugars in mango pulp and mango by-products. The content of sugars (fructose, glucose, and sucrose) of the studied samples is shown in Table 3S. Sucrose content was significantly higher than the content of glucose and fructose, the total content of sugars, following the order: Pulp > peel > seed. Nonetheless, the total content of sugars in all the analyzed samples was significantly lower than the minimal concentration that has been demonstrated to be required to induce some interference in the FC assay (Magalhaes et al. 2010). In fact, considering the highest value of sucrose in mango fruits (59.7 % of dry matter) which means a concentration of 0.17 mM in the working solutions, a negligible interference of this sugar in the TP of mango pulp, seed, and peel would be expected (Magalhaes et al. 2010).

Total Phenolics of Mango By-Products and Their Antioxidant Activity (Assessed by the ORAC Assays)

The content of TP, assessed by the Folin-Ciocalteu assay, and the antioxidant activity (assessed by the ORAC-FL and ORAC-PGR methodologies) of mango pulp, seed, and peel were determined. As shown in Table 1, the TP content of mango peel was significantly higher than that of seed and pulp. For example, TP in the Keitt and Pica peels were nearly 3.5- and 6.9-fold higher than the TP in the corresponding pulp, respectively. Interestingly, the average values of TP obtained in seeds (0.003 mmol GA/g) were in the same order than that of the corresponding mango pulps (0.005 mmol GA/g), meaning that mango seeds have a similar contribution of phenolic compounds than edible parts of mango fruits.

Regarding the antioxidant activity of the samples, assessed by the ORAC-FL and PGR assays, typical results (depicted in Fig. 1) showed that, as expected, lower concentrations of mango extracts were necessary for protecting the FL probe compared to those required to protect the PGR probe. This difference could be related to a lower reactivity of FL than PGR towards AAPH-derived free radicals. From results similar to those contained in Fig. 1, the areas under the curve (AUC) of the kinetic profiles were determined, and thereby, the ORAC indexes of the samples estimated. While the ORAC-FL values ranged from 1.0 to 2.75 mmol GA/g, the ORAC-PGR values ranged from 0.018 to 0.369×10^{-3} mmol GA/g (Table 1). For analysis simplicity, we estimated the average of the ORAC-FL and ORAC-PGR index for mango pulps and mango by-products. Thus, the average values of the ORAC-FL index of mango pulps, seeds, and peels were 1.25, 2.00, and 2.12 mmol GA/g, respectively, implying the following order: Peel ≈ Seed > Pulp. Employing the ORAC-PGR index, the antioxidant activity of mango pulps, seeds, and peels was 0.043, 0.297, and 0.196×10^{-3} mmol GA/g, respectively, implying the order: Seed > Peel >> Pulp. These results show that employing ORAC-FL assay a low discrimination between mango by-products was observed. In fact, seed and peel showed almost the same antioxidant capacity; and both materials showed a slightly higher (≈1.6 times) antioxidant capacity than mango pulp. Contrarily, when ORAC-PGR was employed as assay, it was possible to discriminate the antioxidant capacity of both by-products. Additionally, the ORAC-PGR index of mango seed was 6.9 times higher than pulp. These results could be associated with the fact that the ORAC-FL assay mostly represents the stoichiometric of the reaction between polyphenols and the AAPH-derived peroxyl (or alkoxyl) radicals (Dorta et al. 2015; Lopez-Alarcon and Lissi 2006). By contrast, the ORAC-PGR assay would mostly represent the reactivity of the under-assay polyphenols towards AAPH-derived peroxyl radicals (Dorta et al. 2015).

As mentioned above, some investigators have suggested that in some particular fruits, the content of certain minerals and the total polyphenol content could be directly associated (Sulaiman et al. 2011). Thus, a simple linear correlation analysis was used to estimate the possible correlation between both parameters, as well as, between ORAC values and the mineral contents. In all cases, the p values obtained for each of the attempted correlations were always higher than 0.01, showing the absence of significant correlations between the studied parameters.

Table 1 Total phenolic content (TP) and antioxidant capacity measured as oxygen radical absorbance capacity (ORAC) of mango pulp, seed, and peel extracts

Variety		TP		ORAC-FL		ORAC-PGR	
		(mmol GA/g)	(mmol GA/fruit)*	(mmol GA/g)	(mmol GA/fruit)*	(mmol GA/g)#	(mmol GA/fruit) #*
Pulp	Pica	0.008 ± 0.002	1.38 ± 0.34	1.33 ± 0.08	229.3 ± 13.8	0.108 ± 0.009	18.60 ± 1.60
	Tommy	0.003 ± 0.001	0.82 ± 0.30	1.00 ± 0.17	273.1 ± 46.4	0.027 ± 0.009	7.37 ± 2.51
	Kent	0.003 ± 0.001	1.10 ± 0.36	1.00 ± 0.08	367.3 ± 29.4	0.018 ± 0.009	6.61 ± 3.31
	Keitt	0.004 ± 0.002	1.67 ± 0.84	1.67 ± 0.50	697.4 ± 208.8	0.018 ± 0.009	7.52 ± 3.76
Seed	Pica	0.003 ± 0.001	0.093 ± 0.031	2.75 ± 0.08	84.90 ± 2.60	0.315 ± 0.018	9.73 ± 0.55
	Tommy	0.002 ± 0.001	0.068 ± 0.034	1.75 ± 0.17	59.32 ± 5.77	0.333 ± 0.045	11.29 ± 1.52
	Kent	0.003 ± 0.002	0.124 ± 0.083	1.92 ± 0.25	79.30 ± 10.32	0.171 ± 0.009	7.06 ± 0.37
	Keitt	0.003 ± 0.001	0.137 ± 0.046	1.58 ± 0.08	72.21 ± 3.65	0.369 ± 0.036	16.86 ± 1.65
Peel	Pica	0.055 ± 0.010	1.177 ± 0.214	2.08 ± 0.17	44.51 ± 3.64	0.090 ± 0.018	1.926 ± 0.385
	Tommy	0.022 ± 0.002	0.521 ± 0.048	2.00 ± 0.08	47.40 ± 1.89	0.162 ± 0.036	3.840 ± 0.853
	Kent	0.043 ± 0.009	1.360 ± 0.288	2.42 ± 0.17	76.71 ± 5.39	0.180 ± 0.009	5.71 ± 0.28
	Keitt	0.014 ± 0.003	0.496 ± 0.106	2.00 ± 0.08	70.8 ± 2.83	0.351 ± 0.009	12.42 ± 0.32

Values represent the mean ± standard deviation of the least three determinations

*Values are expressed as gallic acid equivalents per gram of dry by-product or per total gram of by-products per fruit

Values were amplified ten times

On the other hand, when TP and the ORAC activity is expressed in function of the total weight of pulp, seed, and peel contained in each mango fruit, it is evidenced that a high fraction of the antioxidant activity is present in the non-edible materials of mango (Table 1). Such characteristic depends on the probe employed in the ORAC assay. For example, in the case of Kent variety, its ORAC-FL values of pulp, seed, and peel were 367.3 ± 29.9, 79.30 ± 10.32, and 76.71 ± 5.39 mmol GA/g, respectively. These values mean that close to 40 % of the ORAC-FL activity in this variety is present in its by-products. Contrarily, the ORAC-PGR index of the same mango fruit (Kent variety) showed a similar antioxidant activity for pulp, seed, and peel; 6.61, 7.06, and 5.71 × 10^{-3} mmol GA/g, respectively. The latter means that employing ORAC-PGR assay, the mango by-products (seed plus peel) contained a higher antioxidant activity than mango pulp.

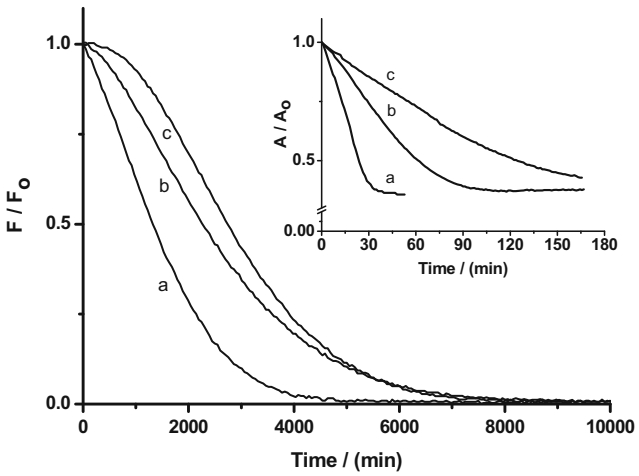

Fig. 1 Kinetic profiles of FL (70 nM) consumption mediated by AAPH (10 mM) derived free radicals in the absence (**a**) and presence of a mango "Keitt" seed (**b**, 30 μg/mL) peel (**c**, 100 μg/mL). *Inset*: kinetic profiles of PGR (5 μM) consumption mediated by AAPH (10 mM) derived free radicals in the absence (**a**) and presence of a mango "Kent" seed (**b**, 0.75 mg/mL) and peel (**c**, 3.3 mg/mL)

Consumption of Trp Residues in a Preparation of Myofibrillar Proteins Elicited by AAPH-Derived Free Radicals

Figure 1S (supplementary material) shows the fluorescence spectrum of a myofibrillar preparation (0.13 mg/mL) in the presence of AAPH (10 mM), at zero time and after 60 min of incubation at 37 °C. As mentioned in the experimental section, to ensure the production of peroxyl radicals from the thermolysis of AAPH, during the incubation the samples were slightly bubbled with air, for 30 s, every 30 min. In the presence of AAPH, the initial maximum fluorescence intensity (181 FU) was reached at 340 nm (with an excitation wavelength of 295 nm). After 1 h of incubation, a significant decrease in the intensity of such band was evidenced (Fig. 1S). From this type of experiments, the kinetic of Trp consumption elicited by AAPH-derived free radicals was evaluated. The consumption of Trp residues was selected to follow the oxidation of myofibrillar proteins, since the intrinsic fluorescence of this amino acid has been used to monitor physicochemical changes in proteins (Estevez et al. 2008a). In fact, the evaluation of its fluorescence intensity has been suggested as an index of protein oxidation (Estevez et al. 2008a). As Fig. 2

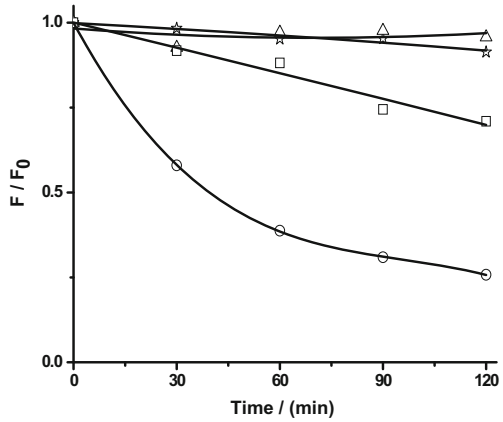

Fig. 2 Kinetic profile of the fluorescence consumption from myofibrillar protein extract (0.13 mg/mL). In the presence of AAPH (10 mM) (*circle*); in the presence of both AAPH (10 mM) and mango seed (*square*). Control experiments myofibrillar prepareation in the absence of AAPH (*triangle*) and in the presence of mango seed extract (*star*)

shows, in the absence of mango by-products, the presence of AAPH induced a clear decrease in the fluorescence of Trp residues; the initial consumption rate being 0.18 μM/min. This value was estimated considering only the Trp residues present in myosin and actin, which gave a Trp concentration of 75 μM. After 90 min of incubation, a very slow rate of fluorescence consumption was registered, evidencing a plateau in the kinetic profiles. In fact, after 120 min of incubation, the fluorescence reached 75 % of the initial intensity. This result means that not all the Trp residues were consumed at the same rate. This behavior is not surprising, since the protein preparation contains a complex mixture of proteins, including actin and myosin, as was evidenced by SDS-PAGE experiments (data not shown). When aliquots of mango seed extracts were added to a solution containing MP and AAPH, a clear decrease in the initial consumption rate of Trp residues was evidenced. In fact, in the presence of mango seed an initial consumption rate of 0.025 μM/min was estimated, which is 7.2-fold lower than the control experiment value. From these results, the percentage of Trp protection, elicited by the addition of mango by-products was estimated after 60 min of incubation. The results show a linear dependence with the concentration of mango by-products until 80 μg/mL (as example, Fig. 2S shows results for mango seed and peel). Figure 3 presents the results as protection percentage (%) of the MP preparation by the mango by-products. Such results were obtained at different total phenolic contents of mango extracts; mango seed and peel extracts were added at 0.2 and 1.0 μg of gallic acid equivalents/milliliter, respectively, while the effect of mango pulp was determined at a final concentration of 4.1 mM GA equivalents/milliliter. However, independently of the higher concentration of mango peel and pulp employed, these showed a lower protection than that afforded by mango seed; the following order was determined:

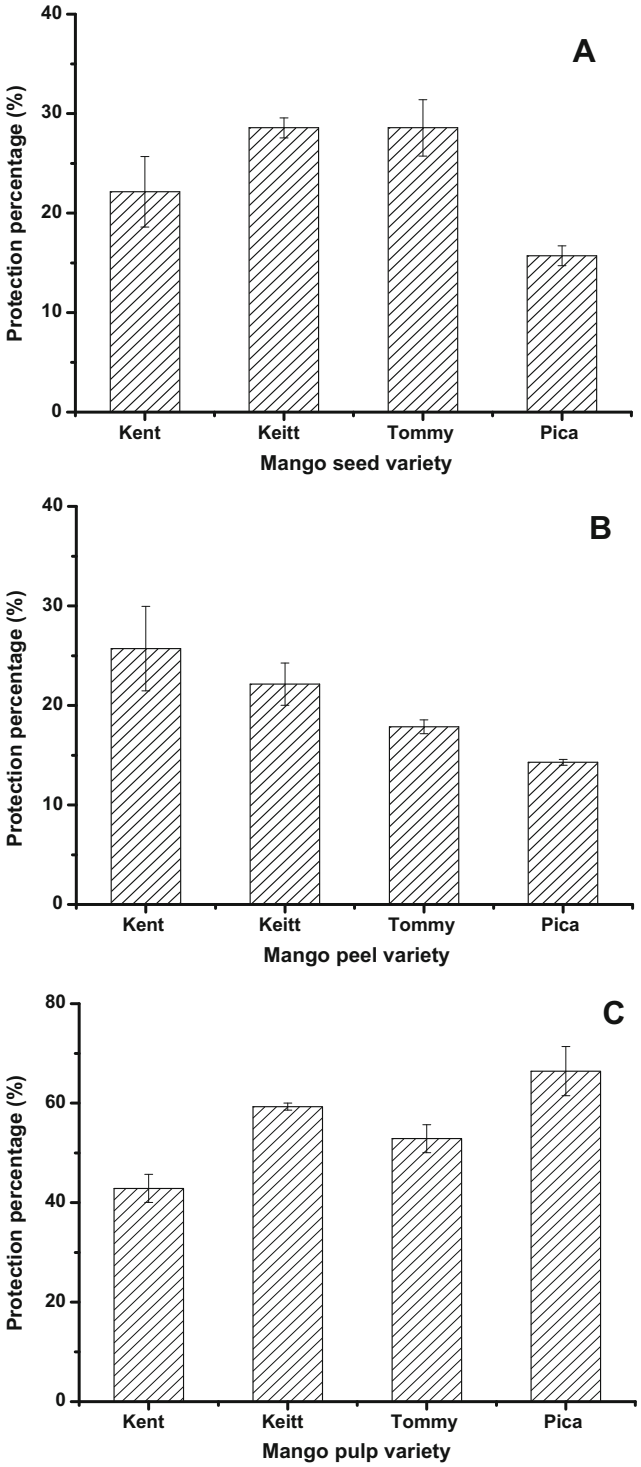

Fig. 3 Effect of mango by-products on the Trp-related fluorescence of MP extract induced by peroxyl radicals. MP (0.13 mg/mL) were incubated with AAPH (10 mM) and mango extracts in phosphate buffer pH 7.4, 37 °C. Graphic A in the presence of mango seed extract (8.3 μg/mL). Graphic B in the presence of mango peel (27 μg/mL). Graphic C in the presence of mango pulp (0.8 mg/mL). Results are expressed as percentage (%) of protection. Fluorescence (excitation wavelength = 295 nm and emission wavelength = 340 nm) was determined after 1 h incubation

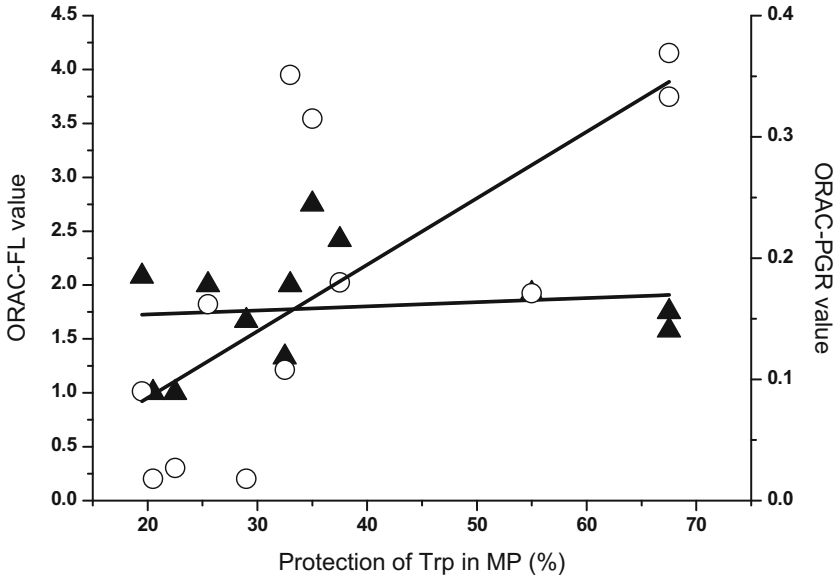

Fig. 4 Dependence of the ORAC-FL (*triangle*) and ORAC-PGR (*circle*) of mango pulp, seed, and peel with their activity to protect Trp residues in MP. The protection of Trp (%) elicited by mango pulp and by-products was normalized by the final concentration of mango extracts

Seed > Peel >> Pulp. This rank order is in agreement with that obtained employing the ORAC-PGR assay, but it does not according with ORAC-FL and TP data, implying that to reflect the antioxidant activity of complex mixtures towards MP, the ORAC-PGR assay could be considered as a better index. In fact, when ORAC indexes are plotted as function of the Trp protection (%, normalized by the final concentration of mango extracts, Fig. 4), it is evidenced a better behavior in the case of the ORAC-PGR. The linear correlation between ORAC-PGR and Trp protection showed a slope of 0.050 and a $R^2 = 0.44$ ($p = 0.01$). While the dependence of the ORAC-FL values with Trp protection showed a slope of 0.0038 and $R^2 = 0.08$ ($p = 0.7$).

Conclusions

The antioxidant activity, assessed by the ORAC-FL and ORAC-PGR assays, of mango pulp and mango by-products do not correlate with the total phenolic and mineral content of the samples. Mango seed, peel, and also pulp were all found to be effective in protecting Trp residues of myofibrillar proteins when they were exposed to AAPH-derived free radicals. Such actions were in agreement with the antioxidant activity displayed by mango by-products as evaluated by the ORAC-PGR assay, implying that this assay could be consider as a simple method to predict the protection of myofibrillar proteins when they are exposed to peroxyl radicals. The latter could indicate that the protective effect of mango by-products on the oxidation of myofibrillar proteins is overall directly associated with the reactivity of polyphenols towards peroxyl radicals. Mango pulp and mango by-products could be consider as a good alternative for inhibiting the oxidation of myofibrillar proteins induced by peroxyl radicals. However, new research regarding the potential application of mango by-products to protect food meat and meat processing products are clearly necessary.

Compliance with Ethical Standards

Funding This study was funded by FONDECYT (grants numbers n° 3140307 and 1141142).

Conflict of Interest Eva Dorta declares that she has no conflict of interest. Elena María Rodríguez-Rodríguez declares that she has no conflict of interest. Andrés Jiménez-Quezada declares that he has no conflict of interest. Eduardo Fuentes-Lemus declares that he has no conflict of interest. Hernán Speisky declares that he has no conflict of interest. Eduardo Lissi declares that he has no conflict of interest. Camilo López-Alarcón declares that he has no conflict of interest.

Ethical Approval Not applicable.

Informed Consent Not applicable.

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