JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. **2010,** *58,* 331–337 **331**
DOI:10.1021/jf9024932

Evaluation of Antioxidant Activity and Genotoxicity of Alcoholic and Aqueous Beverages and Pomace Derived from Ripe Fruits of *Cyphomandra betacea* Sendt.

Roxana M. Ordóñez,[†,§,#] María Luz Cardozo,[†,§] Iris Catiana Zampini,[†,§,#] and María Inés Isla*[,†,§,#]

[†]Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Tucumán, Argentina, [§]INQUINOA-CONICET, Ayacucho 471, 4000 San Miguel de Tucumán, Argentina, and [#]Facultad de Ciencias Naturales e Instituto Miguel Lillo, Fundación Miguel Lillo, Miguel Lillo 205, (4000) San Miguel de Tucumán, Tucumán, Argentina

Cyphomandra betacea ripe fruits can be a source of value-added byproducts and products such as antioxidant supplements, ingredients for food processing or alternative medical products. The aims of the present study were to obtain different preparations of *C. betacea* fruits, such as juice, decoction, and maceration and to characterize them in terms of microbiological stability, sensorial and chemical parameters, antioxidant potential (DPPH and $ABTS^{\bullet+}$ radical scavenging, β-carotene bleaching, nitrite scavenging activities), capacity to prevent oxidative stress-induced cell death, and genotoxicity. The best antioxidant activity was found in *C. betacea* fruit maceration, probably as a consequence of the high flavonoid and anthocyanin content. Nevertheless, all preparations analyzed proved to be good as free radical scavengers (SC_{50} values between 1.88 and 44 μg/mL) and exerted protection against β-carotene oxidation. Total phenolic compounds and flavonoids showed a better correlation than anthocyanins with the free radical scavenging effect of the assayed foods. The insoluble matters (pomace) obtained after juice preparation showed antioxidant activity by quenching free radicals. Furthermore, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) reduction assay showed that *C. betacea* preparations prevent oxidative stress-induced cell death in HepG2 cells in a dose-dependent manner. *Salmonella* microsome assays show no mutagenic effect. The data presented in this study demonstrate that *C. betacea* ripe fruits, aqueous and ethanolic preparations, and pomace may be a good source of antioxidant compounds in nutraceutical or functional-food products.

KEYWORDS: *Cyphomandra betacea*; tree tomato; maceration; juice; decoction; pomace; antioxidant capacity; genotoxicity

INTRODUCTION

Cyphomandra betacea (Cav), renamed by Sendtner as *Solanum betaceum*, common name tamarillo or tree tomato, is generally believed to be native to the Andes of Peru and probably Chile, Ecuador, and Bolivia, too. It is cultivated and naturalized in Argentina, Brazil, Colombia, and Venezuela. It is widely grown in New Zealand as a commercial crop. The fruits have high contents of N, P, and K (*1*), carotenoids (*2*), anthocyanins (*3*), and phenolic compounds (*4*). Anthocyanins from tamarillo were identified as pelargonidin 3-rutinoside, cyanidin 3-rutinoside, delphinidin 3-rutinoside, pelargonidin 3-glucoside, cyanidin 3-glucoside, and delphinidin 3-glucoside in fruits from Peru (*5*), whereas pelargonidin 3-glucosylglucose, peonidin 3-glucosylglucose, malvidin 3-glucosylglucose, and delphinidin 3-rutinoside were identified in tamarillo fruits from Brazil (*3*, *6*). Six carotenoids were identified in tamarillo, also from Brazil, β-cryptoxanthin being the major one (*6*). α-Carotene, β-carotene, and β-cryptoxanthin were quantified in tamarillo collected in Australia (*7*), whereas α-carotene was not detected in fruits from the United States (*8*).

In previous works, we showed the simultaneous accumulation of an antimicrobial protein and reducing sugars during tree tomato fruit ripening (*9*, *10*). The isolated protein has inhibitory activity against hydrolytic enzymes produced by pathogenic organisms and against phytopathogenic bacteria and fungi growth (*11*). Consequently, a possible participation of the protein in the plant defense mechanism and its use in agriculture as a postharvest control agent have been proposed. According to Tene et al. (*12*), *C. betacea* mature fruit juice is used in Ecuador for curing tonsillitis, high cholesterol, and stomach pain.

Although the *C. betacea* mature fruits are daily used in salads and in the preparation of juices and jams, the available literature indicates that there are no previous studies on the functional properties of food derived from them. Increasing evidence shows

*Address correspondence to this author at INQUINOA-CONICET, Universidad Nacional de Tucumán, Ayacucho 471, 4000 San Miguel de Tucumán, Argentina (fax 54-381-4248025; e-mail misla@tucbbs.com.ar).

Published on Web 11/25/2009

that fruits and vegetables may protect against numerous chronic diseases, including cancer and cardio- and cerebrovascular, ocular, and neurological disorders (*13*, *14*). The protective effect of fruits and vegetables has generally been attributed to their antioxidant constituents, including vitamin C, α-tocopherol, carotenoids, glutathione, flavonoids, and phenolic acids (*15*). It has been hypothesized that additive and synergistic effects of these complex mixtures of phytochemicals, instead of a single component, are responsible for the health benefits derived from them (*16*).

The aim of this study was to evaluate the antioxidant potential and genotoxicity as well as microbiological stability and biochemical parameters of preparations with and without thermal treatment obtained from *C. betacea* ripe fruits.

MATERIAL AND METHODS

Plant Material. *C. betacea* Sendt. plants were grown at the Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán, Tucumán, Argentina, at 600 masl, without chemical fertilizer. The ripe fruits are egg-shaped. Although the skin is somewhat tough and unpleasant in flavor, the outer layer of the flesh is slightly firm, succulent, and soft, and the pulp surrounding the seed in two lengthwise compartments is soft, juicy, and sweet. The pulp is red-orange. The edible seeds are thin, nearly flat, circular, and larger and harder than those of the true tomato. Samples of fruits (1−4 kg) without blemish or damage were selected and cleaned to leave the samples in the same conditions in which they are consumed. The edible parts represented 65−85% of the whole fruits and had 9.0−11.0 °Brix; the moisture content was 84−88%. The samples were frozen at −20 °C until use.

Preparation of *C. betacea* Extractive Forms. Three experimental preparations were formulated.

C. betacea Maceration (*M1*). Fresh, ripe fruits were hand cut, weighed, and macerated in ethanol 96° (1 g of tissue per 5 mL of ethanol) for 7 days with stirring (40 cycles/min) at room temperature in glass vessels in dark conditions. The extract was filtered through Whatman no. 4 filter paper and centrifuged at 4500*g* for 10 min. Then the supernatant was dried under reduced pressure at 40 °C and weighed.

C. betacea Decoction (*M2*). Ripe fruits were hand cut and boiled in water (5 g of tissue per 70 mL of distilled water) for 20 min. After cooling to 40−45 °C, the liquid was filtered and the volume adjusted to 100 mL. The aqueous extracts were lyophilized and weighed.

C. betacea Juice (*M3*). Fruits were cut and homogenized with a Waring blender (20% w/v) in distilled water. Fresh-squeezed juice was obtained after centrifugation (10 min, 4500*g*) and separated into three portions kept at −20, 4, and 20 °C, respectively, until further analysis. The remaining residue (skin, pulp, and seeds) was named pomace.

Each preparation was realized in triplicate and flushed with nitrogen to avoid possible alteration by oxidation.

Pomace (Insoluble Fractions, M4). One gram of pomace was consecutively washed with water, ethanol, and water. The washing procedure was ended after five washing cycles with 50 mL of water, four washing cycles with 50 mL of ethanol, and three washing cycles with 50 mL of water. Each washing step was followed by a centrifugation at 1500*g* for 10 min. After final washing and centrifugation, the residual precipitate was lyophilized to obtain the insoluble fraction, which was kept at 4 °C prior to antioxidant activity measurement.

Extraction and Quantification of Chemical Compounds from Fresh Fruit, Pomace, and Preparations. *Phenolic Compounds*. Samples (1 g) were extracted with 10 mL of 1% HCl in ethanol at room temperature for 60 min. After centrifugation at 2500*g* for 10 min at 4 °C, supernatant was collected. These procedures were performed several times, until no phenolic compounds were detected in the liquid extractions. Supernatants were pooled and adjusted to pH 3. The solution was increased to a volume of 50 mL with ethanol (*17*).

Carotenoid. The extraction was carried out according to the method of Lee and Castle (*18*). Samples (1 g) were extracted with 10 mL of hexane/acetone/ethanol (50:25:25, v/v/v). After centrifugation at 3000*g* for 10 min at 4 °C, the top hexane layer containing the color was recovered and adjusted to 25 mL with hexane. Total carotenoid content was calculated according to the method of De Ritter and Purcell (*19*) using an extinction coefficient of β-carotene of $E^{1\%} = 2505$.

Determination of Total Phenolic Compounds and Flavonoids. Samples (0.2 mL of different dilutions) were oxidized with 0.1 mL of 0.2 N Folin−Ciocalteu reagent (*20*), and then the reaction was neutralized with 0.8 mL of 15.9% sodium carbonate. Absorbance of the resulting blue color was measured at 765 nm after incubation at 50 °C for 5 min. Results were expressed in milligrrams of gallic acid equivalents per gram of fresh fruit (mg of GAE/g of FW) and milligrams per milliliter (mg/mL) of different preparations.

Total flavone and flavonol content was measured by a spectrophotometric assay based on aluminum chloride complex formation (*21*). Results were expressed in milligrams of quercetin equivalents per gram of fresh fruit and milligrams per milliliter of different preparations.

Total flavanone and dihydroflavanone content was estimated using the colorimetric method from DAB9 modified for propolis (*21*). Results were expressed in milligrams of naringenin equivalents per gram of fresh fruit and milligrams per milliliter of different preparations.

Vanillin Assay for Proanthocyanidins. Aliquots (250 μL) of fruit preparations were added to 1.75 mL of vanillin reagent, and the tubes were incubated in a water bath at 20 °C for exactly 15 min. Optical density was recorded at 490 nm against methanol. Catechin was used as standard (*22*, *23*).

Anthocyanins. Fresh fruits (3 g) were extracted with 25 mL of 1% HCl in methanol for 2 h. The solution obtained was then filtered with Whatman no. 1 filter paper, and the remaining solids were extracted exhaustively with 100 mL of 1% HCl in methanol at room temperature. All acidic−methanolic extracts obtained were combined and vacuum-concentrated to evaporation of methanol until reaching 5 mL in a rotary evaporator. Samples were increased to 25 mL with water.

Determination of Total Anthocyanin Content. Total anthocyanin content of all samples was measured using the pH differential method described by Giusti and Wrolstad (*24*). Samples were dissolved in potassium chloride (0.025 M, pH 1.0) or sodium acetate (0.4 M, pH 4.5) buffer. Optical density was recorded at 510 and 700 nm. The absorbance (A) of the diluted sample was then calculated as follows:

$$A = (A_{510\text{nm}} - A_{700\text{nm}})_{\text{pH 1.0}} - (A_{510\text{nm}} - A_{700\text{nm}})_{\text{pH 4.5}}$$

The anthocyanin pigment concentration in the original sample was calculated according to the formula

$$\text{anthocyanin content (mg/L)} = \frac{A \times \text{MW} \times \text{DF} \times 1000}{\varepsilon \times L}$$

where cyanidin-3-glucoside molecular weight (MW = 449.2) and molar absorptivity (ε = 26,900) constants were used.

Ascorbic Acid Content Determinations. Ascorbic acid content was measured according to the method of Mukherjee and Choudhuri (*25*). Briefly, 10 g of each sample was extracted with 10 mL of 2% trichloroacetic acid. Then, 50 μL of 10% thiourea (in methanol 70%) was added to each tube. The mixture was boiled for 15 min in a water bath. Five milliliters of sulfuric acid 80% (v/v) was added at 0 °C. Absorbance at 530 nm was measured inmediately.

Total Sugar and Reducing Sugar Determinations. Glucose (*26*), fructose (*27*), and sucrose (*28*) were measured in all preparations. Total neutral sugars were also determined by Dubois et al. (*29*).

Protein Determinations. Protein concentration was determined according to the method of Lowry et al. (*30*) using bovine serum albumin as standard.

Antioxidant Activity. *DPPH Free Radical Scavenging Activity*. DPPH solution (1.5 mL of 300 μM in 96% ethanol) was incubated with the samples (2.5−50 μg of phenolic compounds). The reaction mixture was shaken. Absorbance at 515 nm was measured for 20 min at 20 °C. BHT was used as reference compound (*31*). The percentage of free radical scavenging activity (RSA) was calculated according to the method of Ordóñez et al. (*32*). SC_{50} values (sample concentration required to scavenge 50% of DPPH free radicals) were calculated. To avoid the interference of compounds (such as carotenoids) with maximal absorption in the same wavelength range as DPPH, extract absorbance controls were carried out.

Total Antioxidant Capacity Assay. The antioxidant capacity assay was carried out by using the improved ABTS radical cation ($ABTS^{\bullet+}$) method as described by Re et al. (*33*). $ABTS^{\bullet+}$ was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature (23 °C) for 12−16 h before use. $ABTS^{\bullet+}$ solution (1 mL; absorbance of 0.7 ± 0.02 at 734 nm) was added to 2.5−10 μg of phenolic compounds of each preparation (M1, M2, and M3) and mixed thoroughly. The reactive mixture was allowed to stand at room temperature, and the absorbance reading was taken at 30 °C exactly 1 min after initial mixing. Then, it was taken six times at 1 min intervals. Results were expressed in terms of percentage of residual scavenging activity (%RSA).

Antioxidant Activity of the Insoluble Fraction (Pomace, M4). Antioxidant activities of the insoluble fractions obtained from *C. betacea* preparations were measured using the ABTS and DPPH methods as described Serpen et al. (*34*). Lyophilized fractions were powdered and sieved prior to measurement. A 10 mg portion of the powdered sample was transferred to an Eppendorf, and the reaction was started by adding 1 mL of ABTS reagent or 1.5 mL of DPPH reagent. The mixture was vortexed for 2 min to facilitate the surface reaction between the insoluble matter and the ABTS or DPPH reagent. Following centrifugation at 10000*g* for 5 min, the absorbance of the optically clear supernatant was measured. All measurements were performed exactly 6 or 20 min after the insoluble matter had been mixed with the ABTS or DPPH reagent, respectively.

β-Carotene−Linoleic Acid Assay. The antioxidant activity of *C. betacea* fruit was determined according to the method of Ordóñez et al. (*32*). One milliliter of 0.2 mg/mL β-carotene dissolved in chloroform was added to round-bottom flasks (50 mL) containing 0.02 mL of linoleic acid and 0.2 mL of Tween 20. Each mixture was then dosed in 0.2 mL of different sample dilutions or positive (BHT) or negative (water and ethanol) control. After evaporation to dryness under vacuum at room temperature, oxygenated distilled water (25 mL) was added. The mixture was shaken for 2 min and then subjected to thermal autoxidation at 50 °C for 60 min. The absorbance of the solution was monitored at 470 nm on a spectrophotometer (Beckman DU-650) by taking measurements at 10 min intervals, and the rate of bleaching of β-carotene was calculated by fitting linear regression to data over time. All samples were assayed in triplicate. Antioxidant activity (AA) was calculated as percent inhibition relative to control.

Nitrite Scavenging Measurement. The nitrite scavenging capacity of different preparations was determined according to the method of Gray and Dugan (*35*) with some modifications. One milliliter of sample was added into 1 mL of a nitrite solution (1 ppm) and increased to 10 mL, pH was adjusted to 1.2 using 0.1 N HCl or to 4.2 and 6.0 using a 0.2 M citric acid buffer. The reaction mixture was incubated at 37 °C in a water bath for 1 h. A 1 mL aliquot combined with 5 mL of 2% acetic acid and 0.4 mL of Griess reagent (1:1 solution of 1% sulfanilic acid in 30% acetic acid and 1% of naphthylamine in 30% acetic acid) was vortexed and kept at room temperature for 15 min. The amount of residual nitrite in the reaction mixture was measured spectrophotometrically and determined at 520 nm.

Analysis of H_2O_2-Induced HepG2 Cell Death. To test the protective effect of *C. betacea* preparations in HepG2 cells, the cells were plated onto poly-L-lysine coated 12-well plates (Corning 3513, NY) at a density of 1.5×10^5 cells/well and pretreated with different concentrations of sample (25−100 μg/mL) in a serum-free MEM medium during 30 min at 37 °C and 5% CO_2 with a fully humidified atmosphere. After pretreatment, 100 μM freshly prepared H_2O_2 was incubated with cells for 24 h at the same conditions.

Cells were incubated with MTT (0.5 mg/mL) in a serum-free MEM medium during 4 h at 37 °C. The medium was removed carefully by aspiration, and formazan crystals were solubilized in 0.2 mL of acid-isopranol (0.04 N HCl in isopropanol). The extent of the reduction of MTT was determined by measurement of the absorbance at 550 nm in a microplate reader (model 550, Bio-Rad).

Characterization and Storage Studies of Fruit Preparations. Preparations were stored at room (25 ± 2 °C) and refrigeration (4 °C) temperatures during 3 months and were analyzed for color, pH changes, microbiological parameters, and sensory qualities at regular, 30 day intervals.

Microbiological Controls. Dilutions of each preparation were made with sterile water or ethanol. One milliliter of the corresponding dilution was added to a plate with MH-agar (Britania), and incubated, and total aerobic plate count (APC) was determined as the number of colony-forming units (CFU) per milliliter of sample (*36*). Each experiment was made in triplicate.

Sensory Evaluation. An internal panel of seven members, which was selected and trained following the guidelines of the ISO (1993) standard (*37*), evaluated the products' quality.

The assessors (two men and five women; mean age = 34 years, range 23−49 years) were selected on the basis of their willingness to participate and their normal sense of smell and taste. Prior to data collection, two training sessions familiarized the assessors with the differences in sensory attributes and the use of rating scales. During the training sessions, two reference solutions with concentrations representing mild and strong levels of a tastant (sweetness, 10 and 20 g/L sucrose; sourness, 0.7 and 1.2 g/L citric acid; bitterness, 0.4 and 0.7 g/L caffeine; and astringency, 0.5 and 1.0 g/L aluminum sulfate) were used to introduce taste qualities to the panelists.

The evaluations were held on two consecutive afternoons starting the day after the training sessions. Six samples (unsweetened, each 40 mL in a 120 mL glass beaker, marked with three-digit random codes) were presented to assessors seated in individual booths at the sensory laboratory, University National of Tucumán, illuminated with red light. Juices were served at room temperature (21 °C) because the odors and flavors associated with the juice products were magnified and differentiable at that temperature, which facilitated the characterization and comparison of each juice sample. The order of samples was randomized for each assessor and with a replication.

The color, odor, taste, and overall acceptability of the products were rated by a hedonic scale consisting of 5 points, where 5 = excellent, 4 = very good, 3 = good, 2 = acceptable, and 1 = unacceptable (see **Table 5**). There was a 10 min break between the ratings of each set of samples. Water was provided to wash the oral cavity after testing each sample. The Ethical Committee concluded that all samples were acceptable for human testing in the concentrations and quantities to be served.

Ames Test. To examine genotoxic effects of different *C. betacea* preparations on *Salmonella typhimurium* strains, TA98 and TA100, the samples were added to overnight-cultured *S. typhimurium* strains and S9 mix (0.5 mL) or 0.1 M phosphate buffer, pH 7 (0.5 mL) instead of S9 mix (*38*). The plates were incubated at 37 °C for 48 h, and the number of colonies was counted. Mutagenic effects were assayed with and without metabolic activation (S9 mix fraction).

All experiments were performed in triplicate with at least two replicates. The mutagens used as positive controls were 4-nitro *o*-phenylenediamine (NPD, 5 μg/plate), which is a direct acting mutagen, and isoquinoline (IQ, 0.1 μg/plate for TA 98 and 0.5 μg/plate for TA100), which required S9 mix for metabolic activation.

Statistical Analysis. All measurements were replicated three times, and data were analyzed by ANOVA. Correlation coefficients (*r*) were also calculated.

RESULTS AND DISCUSSION

In a previous work it was reported that *C. betacea* fruits accumulate reducing sugars during fruit ripening (*10*). Mwithiga et al. (*39*) confirmed these results and reported that juice yield and soluble solids increase, too. From this point of view, the ripe tree tomato is a fruit with prominent economic potential and nutritional interest. During this study, functional properties of *C. betacea* preparations (maceration, decoction, juice) and pomace were analyzed. The liquid preparations obtained had pH values around 4−5 and a pleasant flavor, although taste was slightly astringent for M1 and M2. **Table 1** shows the sugar content of each sample. M3 displayed the highest value of reducing sugars (as fructose). Samples had low sucrose content; this result was in agreement with the value reported for mature fresh fruit (*9*). Total protein concentrations were similar in the three preparations and had values from 7.10 to 11.91 mg/mL.

Total phenolic, flavonoid, and anthocyanin contents in fresh fruit, pomace, and different preparations are shown in **Table 2**. M3 had the highest amount of phenolic compounds, with 2.05 mg of GAE/g of FW, followed by M2 and M1. M1 also presented a higher content of flavonoids and anthocyanins than M2 and M3. A highly linear relationship between phenolic and flavonoid content was verified (r^2 0.8867, $n = 35$). Among the different preparation forms, fresh fruit and maceration had the highest flavonoid content values (principally flavanone), which are superior to those of other tropical fruits (*40*). On the other hand, anthocyanin content in all preparations was lower when compared with that of other berrylike fruits such as raspberries, blackberries, red currants, gooseberries, and Cornelian cherries (*41*). Total carotenoids of tree tomatoes were higher than those of most tropical fruits, making them functionally interesting (*42*, *43*). The total carotenoid content found in fresh fruit, 30.6 μg/g of FW, was in the range of those previously found by Rodríguez-Amaya et al. (*2*) (31−59 μg/g of FW) and de Rosso and Mercadante (*6*) (44 μg/g of FW) in tamarillo harvested in Brazil. Lower results found by researchers from other countries for tree tomatoes (*7*, *8*) may be due to differences in extraction method and quantification techniques. Unfortunately, some of the examined preparations showed small detectable amounts of carotenoids, probably due to the high polarity of the solvents chosen for extractions. **Table 2** shows that M1 preparation was able to retain 30% of total ascorbic acid content, whereas M2 and M3 showed no significant values. When fruits were processed to obtain juice, decoctions, or maceration, substantial waste material was generated. Nevertheless, pomace was able to retain 45% of ascorbic acid, 20% of the carotene content, phenolic compounds, and flavonoids, and 30% of anthocyanins. These results suggest that pomace should not be thrown away during home cooking or industrial processing because it constitutes a good source of natural products and a potential raw material, useful to formulate nutraceutical products. Samples kept at 4 °C for 3 months did not show any microbiological alterations. M2 and M3 (aqueous extracts) at room temperature had a shelf life of 7 days, whereas M1 (ethanolic extract) was even more stable.

The content of anthocyanin, flavones, and flavanone in ethanolic maceration was higher than in the aqueous extracts, so these compounds can exert antibacterial effect (**Table 3**).

The AA of *C. betacea* fruit preparations was tested using assays based on different reaction mechanisms. The highest AA was recorded for M1. The bleaching of the DPPH solution increases simultaneously with increasing amounts of phenolic compounds in a given volume of solution (SC_{50} for the samples was around 5−37 μg/mL). Considering these SC_{50} values, the activity of *C. betacea* samples was similar to or higher than that of natural antioxidants such as ascorbic acid (SC_{50} value of 10 μg/mL) or quercetin (SC_{50} value of 20 μg/mL) and more potent than that of BHT (SC_{50} value of 36 μg/mL; **Figure 1**).

Given the fact that all liquid preparations showed ABTS scavenging activity (SC_{50} values of 1.88−3.75 μg/mL; **Figure 2**) and β-carotene bleaching inhibitory capacity (IC_{50} = 25−75 μg/mL; **Figure 3**) similar to those of commercial antioxidants used in the food industry, we may state that *C. betacea* preparations could be a good source of antioxidant compounds. The NO scavenging activity showed SC_{25} values of 5−12.5 μg/mL (**Figure 4**).

Correlations between AA and phenolic compounds, flavonoids, and anthocyanins were calculated. The r^2 values indicated that the phenolic compounds and flavonoids contributed to AA ($r^2 = 0.99$, $n = 35$) more than anthocyanins ($r^2 = 0.75$, $n = 35$). Similar results were found in several berry species (*41*) and in tropical fruits (*40*). Our results must be interpreted with caution because AA could be the result of synergies (or antagonisms) between different, still unknown, components. Additional studies are necessary to isolate the phenolic compounds responsible for the antioxidant activity.

The soluble preparations derived from *C. betacea* fruits have higher antioxidant activity than the insoluble fraction (**Table 4**),

Table 1. Analysis of Carbohydrate (Total and Reducing Sugars, Glucose, Fructose, and Sucrose) and Protein Contents from the Different Preparations of *C. betacea* Mature Fruits

	mg/g of FW[a]		
chemical compound	M1	M2	M3
total sugars	480.50 ± 2.5	620.11 ± 2.5	84.60 ± 1.0
reducing sugars	52.20 ± 1.5	55.50 ± 1.5	84.83 ± 1.5
glucose	5.95 ± 0.5	10.44 ± 0.5	0.18 ± 0.5
fructose	31.88 ± 1.2	35.31 ± 1.2	78.31 ± 1.5
sucrose	18.82 ± 0.8	26.01 ± 0.8	22.10 ± 0.5
protein	7.10 ± 0.25	8.10 ± 0.20	11.91 ± 0.20

[a] Results are expressed as mg/g of fresh weight: mean for triplicate determination ± standard errors.

Table 2. Total Phenolic, Anthocyanin, Flavone, Flavanone, Ascorbic Acid, and Total Carotene Contents in the Fresh Fruit, M1, M2, M3, and M4 (Pomace)

	concentration[a]					
preparation	phenolic compounds (mg/g of FW)	anthocyanin (mg/g of FW)	flavone (mg/g of FW)	flavanone (mg/g of FW)	ascorbic acid (mg/g of FW)	total carotene (μg/g of FW)
fruit	3.24 ± 0.50	3.47 ± 0.25	0.95 ± 0.05	2.10 ± 0.50	1.53 ± 0.5	30.6 ± 2.00
M1	1.39 ± 0.20	2.87 ± 0.20	0.63 ± 0.02	1.36 ± 0.25	0.48 ± 0.02	nd
M2	1.83 ± 0.25	1.35 ± 0.25	0.18 ± 0.05	0.77 ± 0.05	0.26 ± 0.01	nd
M3	2.05 ± 0.50	0.89 ± 0.05	0.16 ± 0.01	0.55 ± 0.01	0.15 ± 0.05	nd
pomace (M4)	0.72 ± 0.05	1.05 ± 0.05	0.28 ± 0.05	0.45 ± 0.02	0.13 ± 0.01	2.35 ± 0.50

[a] Mean for triplicate determination ± standard errors. nd, not determined.

Table 3. Determination of Total Viable Counts of Aerobic Bacteria in Each *C. betacea* Preparation

	total viable counts[a] (CFU/mL)					
	4 °C			25 °C		
storage time (days)	M1	M2	M3	M1	M2	M3
7	nd	nd	nd	nd	$8 \times 10^1 \pm 5$	$5 \times 10^1 \pm 5$
30	nd	$<1 \times 10^1 \pm 2$	$<1 \times 10^1 \pm 2$	nd	$1.7 \times 10^2 \pm 10$	$6 \times 10^2 \pm 50$
90	nd	$2 \times 10^1 \pm 2$	$1.5 \times 10^1 \pm 2$	$1 \times 10^1 \pm 1$	$1.5 \times 10^3 \pm 80$	$1.4 \times 10^3 \pm 95$

[a] Each value is the mean ± SD of two replicate experiments with three samples analyzed per replicate ($n = 3$). nd, not detected.

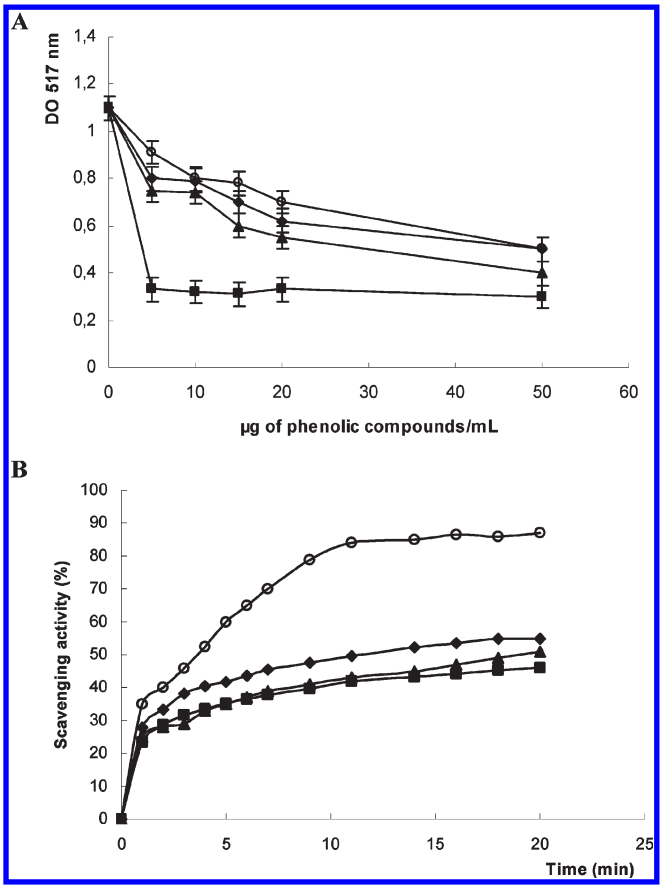

Figure 1. Scavenging activity of DPPH radical (**A**) Different concentrations of *C. betacea* extractive solution (time contact between extracts and DPPH = 20 min). SC_{50} values: maceration, 5 μg/mL; decoction, 22 μg/mL; juice, 44 μg/mL. (**B**) DPPH bleaching kinetics in the presence of SC_{50} of each extractive solution: (■) maceration; (▲) decoction; (◆) juice. BHT was used as positive control (○). The curves obtained are statistically different.

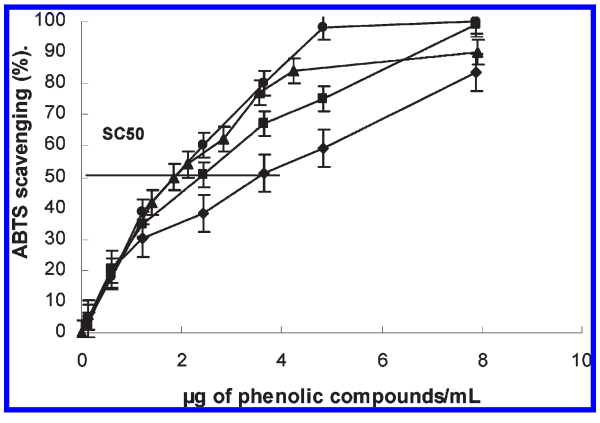

Figure 2. Scavenging activity of ABTS radical with increasing quantities of phenolic compounds (■), maceration, (▲) decoction, and (◆) juice at 6 min. SC_{50} was determined in each sample: M1, 1.88 μg/mL; M2, 2.21 μg/mL; M3, 3.75 μg/mL. BHT was used as control (●).

but functional groups bound to the insoluble components are still reactive toward radicals as confirmed by using ABTS and DPPH (TEAC values = 12.24 μmol of Trolox/g of fresh weight). The insoluble matters remain in the gastrointestinal tract for a long time and may help in quenching the free radicals that are continuously formed in the intestine and that could be involved in the etiology of degenerative disease, such as colon cancer.

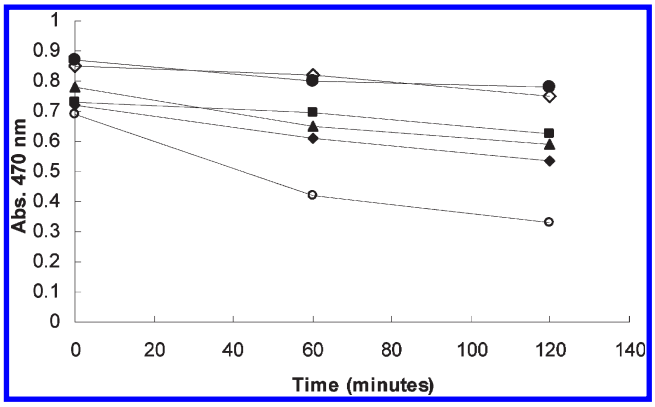

Figure 3. β-Carotene bleaching assay of *C. betacea* preparations. The β-carotene oxidation induced by heat was monitored until 120 min. The maximum concentration used was 75 μg of phenolic compounds/mL: (■) maceration; (▲) decoction; (◆) juice; (○) negative control; (●) 100 μg/mL BHT; (◇) 100 μg/mL ascorbic acid.

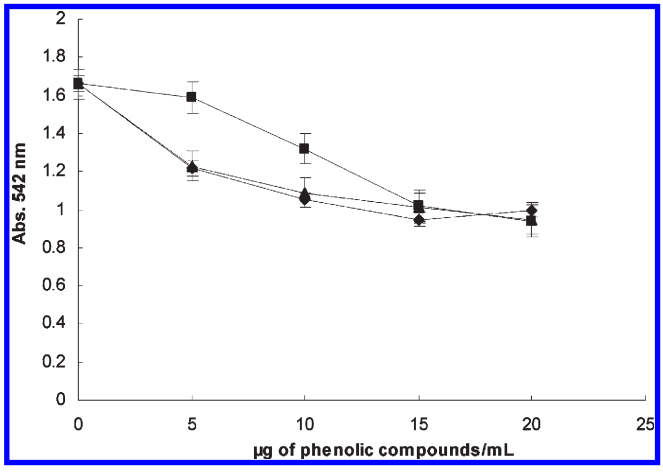

Figure 4. Effect of the extract concentration (as phenolic compounds equivalents) on NO scavenging: (■) maceration; (▲) decoction; (◆) juice. SC_{25} was determined in each sample.

Table 4. Antioxidant Activities of *C. betacea* Preparations (Soluble Fractions) and Insoluble Fractions (Pomace) by ABTS Scavenging Method[a]

extractive solution	TEAC value (μmol of Trolox/g of fresh weight)
M1	178.70 ± 10.00
M2	127.60 ± 10.00
M3	34.00 ± 5.00
pomace (M4)	12.24 ± 5.00

[a] Results are expressed in terms of Trolox equivalent antioxidant capacity (TEAC, μmol of Trolox equivalents/g of dry weight of flour). Mean for triplicate determination ± standard errors. nd ,not determined.

Oxidative stress-induced cell death was examined by determination of MTT reduction after incubation of HepG2 cells with H_2O_2 as oxidant agent for 24 h. Hydrogen peroxide (100 μM) caused a significant decrease in cell viability (50%). The treatment of HepG2 cells with maceration, decoction, and juice blocked oxidative stress in a dose-dependent manner. The cell viability increased to 100% with 50 μg/mL for maceration and decoction and 100 μg/mL for *C. betacea* juice.

The preparations derived from *C. betacea* fruits showed mutagenicity relation (MR) values below 2, which indicate that they do not exert mutagenic effects in the assay using *S. typhimurium* TA 98 and TA 100 with and without metabolic activation.

Table 5. Sensorial Evaluation[a] of Preparations during Storage at 4 °C

storage (months)		juice	maceration	decoction
0	color	3.00	3.0	2.7
	odor	3.08	2.9	2.85
	taste	2.89 (sour)	2.7 (sour)	2.80 (sour)
	OA[c]	3.20	2.90	2.79
1	color	3.00	3.00	2.56
	odor	3.05	2.97	2.80
	taste	2.70 (sour)	2.79 (sour)	2.40 (sour)
	OA	3.1	3.00	2.60
2	color	2.8	2.80	2.70
	odor	3.00	2.80	2.75
	taste	2.90 (sour)	2.64 (sour)	2.70 (sour)
	OA	2.85	2.60	2.65
3	color	2.90	2.70	2.65
	odor	3.00	2.75	2.70
	taste	2.70 (sour)	2.75 (sour)	2.70 (sour)
	OA	2.90	2.50	2.60

[a] Scoring method: 5, excellent; 4, very good; 3, good; 2, acceptable; 1, unacceptable. [b] Sour: The basic taste sensation on the tongue simulated by acid. [c] OA, overall acceptance.

This study revealed that maceration has a great potential to be used in functional food, because it presented a remarkable content of bioactive compounds and a significant antioxidant activity. M3 and M2 antioxidant activity is also substantial. Data obtained for decoction indicate that compounds responsible for AA are thermostable; therefore, fruit could be used to prepare jams and jellies. We also found that pomace was able to retain bioactive compounds and should also be taken in consideration as a possible antioxidant ingredient for the nutraceutical or functional-food market.

LITERATURE CITED

(1) Clark, C. J.; Richardson, A. C. Biomass and mineral nutrient partitioning in a developing tamarillo (*Cyphomandra betacea*) crop. *Sci. Hortic.* **2002**, *94*, 41–51.

(2) Rodríguez-Amaya, D. B.; Bobbio, P. A.; Bobbio, F. A. Carotenoid composition and vitamin A value of the Brazilian fruits *Cyphomandra betacea*. *Food Chem.* **1983**, *12*, 61–65.

(3) Bobbio, F. O.; Bobbio, P. A.; Rodríguez-Amaya, D. B. Anthocyanins of the Brazilian fruit *Cyphomandra betaceae*. *Food Chem.* **1983**, *12*, 189–195.

(4) Schmeda-Hirschmann, G.; Feresin, G.; Tapia, A.; Hilgert, N.; Theoduloz, C. Proximate composition and free radical scavenging activity of edible fruits from the Argentinian yungas. *J. Sci. Food Agric.* **2005**, *85*, 1357–1364.

(5) Wrolstad, R. E.; Heatherbell, D. A. Identification of anthocyanins and distribution of flavonoids in tamarillo fruit (*Cyphomandra betaceae* (Cav.) Sendt.). *J. Sci. Food Agric.* **1974**, *25*, 1221–1228.

(6) De Rosso, V.; Mercadante, A. HPLC-PDA-MS/MS of anthocyanins and carotenoids from dovyalis and tamarillo fruits. *J. Agric. Food Chem.* **2007**, *55*, 9135–9141.

(7) Wills, R. B. H.; Lim, J. S. K.; Greenfield, H. Composition of Australian foods. Tropical and sub-tropical fruit. *Food Technol. Aust.* **1986**, *38*, 118–123.

(8) Homnava, A.; Rogers, W.; Eitenmiller, R. R. Provitamin A activity of specialty fruit marketed in the United States. *J. Food Compos. Anal.* **1990**, *3*, 119–133.

(9) Ordóñez, R. M.; Vattuone, M. A.; Isla, M. I. Changes in carbohydrate content and enzymes related activity during *Cyphomandra betacea* Sendt. fruits maturation. *Postharvest Biol. Technol.* **2005**, *35*, 293–301.

(10) Ordóñez, R. M.; Ordóñez, A. A. L.; Nieva Moreno, M. I.; Sayago, J. E.; Isla, M. I. Antimicrobial activity of glycosidase inhibitory protein isolated from *Cyphomandra betacea* Sendt fruits. *Peptides* **2006**, *27*, 1187–1191.

(11) Isla, M. I.; Ordóñez, R. M.; Nieva Moreno, M. I.; Vattuone, M. A.; Sampietro, A. R. Inhibition of hydrolytic enzyme activities and plant pathogen growth by invertase inhibitors. *J. Enzyme Inhib.* **2002**, *17*, 37–43.

(12) Tene, V.; Malagón, O.; Vita Finzi, P.; Vidari, G.; Armijos, Ch.; Zaragoza, T. An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador. *J. Ethnopharmacol.* **2007**, *111*, 63–81.

(13) Block, G.; Patterson, B.; Subhar, A. Fruit, vegetables and cancer prevention: a review of epidemiological evidence. *Nutr. Cancer* **1992**, *18*, 1–29.

(14) Youdim, K. A.; Joseph, J. A. A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: multiplicity of effects. *Free Radical Biol. Med.* **2001**, *30*, 583–594.

(15) Sies, H.; Stahl, W. Vitamins E and C, β-carotene and carotenoids as antioxidant. *Am. J. Clin. Nutr.* **1995**, *62*, 1315–1321.

(16) Aruoma, O. I.; Bahorun, T. Neuroprotection of bioactive components in medicinal and food plant extracts. *Mutat. Res.* **2003**, *544*, 203–215.

(17) Cheel, J.; Theoduloz, C.; Rodríguez, J.; Caligari, P.; Schmeda-Hirschmann, G. Free radical scavenging activity and phenolic content in achenes and thalamus from *Fragaria chiloensis* ssp. *chiloensis*, *F. vesca* and *F.* × *ananassa* cv. Chandler. *Food Chem.* **2007**, *102*, 36–44.

(18) Lee, H. S.; Castle, W. S. Seasonal changes of carotenoid pigments and color in Hamlin, Earlygold, and Budd Blood orange juices. *J. Agric. Food Chem.* **2001**, *49*, 877–882.

(19) De Ritter, E.; Purcell, A. E. Carotenoid analytical methods. In *Carotenoids as Colorants and Vitamin A Precursors: Technological and Nutritional Applications*; Bauernfeind, J. C., Ed.; Academic Press: New York, 1981; pp 815−882.

(20) Singleton, V.; Orthofer, R.; Lamuela-Raventos, R. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin−Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178.

(21) Popova, M.; Silici, S.; Kaftanoglu, O.; Bankova, V. Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. *Phytomedicine* **2005**, *12*, 221–228.

(22) Broadhurst, R. B.; Jones, W. T. Analysis of condensed tannins using acidified vainillin. *J. Sci. Food Agric.* **1978**, *29*, 788–794.

(23) Hagerman, A.; Harvey-Mueller, I.; Makkar, H. P. S. In *Quantification of Tannins in Tree Foliage*; FAO/IAEA: Vienna, Austria, 2000; pp 21−24.

(24) Giusti, M. M.; Wrolstad, R. E. Unit F1.2.1-13. Anthocyanins. Characterization and measurement with UV−visible spectroscopy. In *Current Protocols in Food Analytical Chemistry*; Wrolstad, R. E., Ed.; Wiley: New York, 2001.

(25) Mukherjee, P.; Choudhuri, M. A. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiol. Plant.* **1983**, *58*, 166–170.

(26) Jorgensen, O. S.; Andersen, B. An improved glucose-oxidase-peroxidase coupled assay for the β-fructofuranosidase activity. *Anal. Chem.* **1973**, *53*, 141.

(27) Ameyama, M. Ezymatic assay of D-fructose dehydrogenase. *Methods Enzymol.* **1982**, *89*, Part D, 20−29.

(28) Cardini, C. E.; Leloir, L. F.; Chiriboga, J. The biosynthesis of sucrose. *J. Biol. Chem.* **1955**, *214*, 149–155.

(29) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, P. Colorimetric methods for determination of sugar and related substances. *Anal. Chem.* **1956**, *28*, 350–356.

(30) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.

(31) Yamaguchi, T.; Takamura, H.; Matoba, T.; Terao, Y. HPLC method for evaluation of the free radical-scavenging activity of food by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 1201–1204.

(32) Ordoñez, A. A.; Gomez, D.; Vattuone, M. A.; Isla, M. I. Antioxidant activity of *Sechium edule* (Jacq) Swartz. *Food Chem.* **2006**, *97*, 452–458.

(33) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improvet ABTS radical cation decoloration assay. *Free Radical Biol. Med.* **1999**, *26*, 1231–1237.
(34) Serpen, A.; Capuano, E.; Fogliano, V.; Gokmen, V. A New procedure to measure the antioxidant activity of insoluble food components. *J. Agric. Food Chem.* **2007**, *55*, 7676–7681.
(35) Gray, J. I.; Dugan, L. R. Inhibition of *N*-nitrosamine formation in model food system. *J. Food Sci.* **1975**, *40*, 981–984.
(36) AOAC. *Official Methods of Analysis of AOAC International*; Gaithersburg, MD, 1997; ISSN 1080-0344.
(37) ISO. *ISO Standard 8586-1. Sensory Analysis—General Guidance for the Selection, Training, and Monitoring of Assessors. Part 1—Selected Assessors*, 1st ed.; International Organization for Standardization: Geneva, Switzerland, 1993.
(38) Maron, D. M.; Ames, B. N. Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.* **1983**, *113*, 173–215.
(39) Mwithiga, G.; Mukolwe, M. I.; Shitanda, D.; Karanja, P. N. Evaluation of the effect of ripening on the sensory quality and properties of tamarillo (*Cyphomandra betaceae*) fruits. *J. Food Eng.* **2007**, *79*, 117–123.
(40) Lim, Y. Y.; Lim, T. T.; Tee, J. J. Antioxidant properties of several tropical fruits: a comparative study. *Food Chem.* **2007**, *103*, 1003–1008.
(41) Pantedilis, G. E.; Vasilakakis, M.; Manganais, G. A.; Dianantidis, G. Antioxidant capacity phenol, anthocyanins and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. *Food Chem.* **2007**, *102*, 777–783.
(42) Breithaupt, D. E.; Bamedi, A. Carotenoids esters in vegetables and fruits: a screening with emphasis on β-cryptoxanthin esters. *J. Agric. Food Chem.* **2001**, *49*, 2064–2070.
(43) Kimura, M.; Rodríguez-Amaya, D. B.; Yokoyama, S. M. Cultivar differences and geographic effects on the carotenoid composition and vitamin A value of papaya. *LWT−Food Sci. Technol.* **1991**, *24*, 415–418.

Received for review July 21, 2009. Revised manuscript received November 6, 2009. Accepted November 09, 2009. This research was supported by grants from Consejo de Investigación de la Universidad Nacional de Tucumán, Argentina (CIUNT), Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina (CONICET), and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT).