

## Antioxidant Activity of Dietary Fruits, Vegetables, and Commercial Frozen Fruit Pulp

NEUZA MARIKO AYMOTO HASSIMOTTO, MARIA INÉS GENOVESE, AND  
FRANCO MARIA LAJOLO\*

Laboratório de Química, Bioquímica e Biologia Molecular de Alimentos, Departamento de Alimentos e Nutrição Experimental, FCF, Universidade de São Paulo, Av. Prof. Lineu Prestes 580, Bloco 14, 05508-900 São Paulo, SP, Brazil

Fruits, vegetables, and commercial frozen pulps (FP) consumed in the Brazilian diet were analyzed for antioxidant activities using two different methods, one that determines the inhibition of copper-induced peroxidation of liposome and another based on the inhibition of the co-oxidation of linoleic acid and  $\beta$ -carotene. The anthocyanin-rich samples showed the highest, concentration-dependent, antioxidant activities in both systems. In the liposome system, at both 10 and 50  $\mu$ M gallic acid equivalent (GAE) addition levels, the neutral and acidic flavonoids of red cabbage, red lettuce, black bean, mulberry, Gala apple peel, jambolao, acai FP, mulberry FP, and the acidic flavonoids of acerola FP showed the highest antioxidant activities (>85% inhibition). In the  $\beta$ -carotene bleaching system, the samples cited above plus red guava gave inhibition values >70%. On the other hand, some samples showed pro-oxidant activity in the liposome system coincident with a low antioxidant activity in the  $\beta$ -carotene system. There was no relationship between total phenolics content, vitamin C, and antioxidant activity, suggesting that the antioxidant activity is a result of a combination of different compounds having synergic and antagonistic effects.

**KEYWORDS:** Liposome oxidation;  $\beta$ -carotene bleaching; antioxidant activity; flavonoids; vitamin C; phenolic content

### INTRODUCTION

Much attention has been focused on the activity of the natural antioxidants present in fruits and vegetables, because potentially these components may reduce the level of oxidative stress. Oxidative stress has been defined as a disturbance in the equilibrium status of pro-oxidant/antioxidant systems in intact cells resulting in oxidative damage to lipids, proteins, carbohydrates, and nucleic acids, contributing to pathological dysfunction in the organism (1).

Several epidemiological studies suggest that a high intake of food rich in natural antioxidants increases the antioxidant capacity of the plasma and reduces the risk of some, but not all, cancers, heart diseases, and stroke. These properties are attributed to a variety of constituents, including vitamins, minerals, fiber, and numerous phytochemicals, including flavonoids (2). For instance, the so-called "French paradox" has been attributed in part to the routine consumption of red wine due to its abundant quantity of polyphenolics (3).

Many studies suggest that flavonoids, a family of compounds with a C<sub>6</sub>–C<sub>3</sub>–C<sub>6</sub> skeleton structure, exhibit several biological activities, including antiallergic, antiviral, antitumor, and anti-

inflammatory action and antioxidant activity, which depends mainly on the number and position of hydroxyl groups within their structure (4).

According to Halliwell and Gutteridge (5) the mechanisms of antioxidant action can include (a) suppressing reactive species formation either by inhibition of enzymes or by chelation of trace elements involved in free radical production, (b) scavenging reactive oxygen species, and (c) up-regulating or protecting antioxidant defense. Many studies have demonstrated the antioxidant activity of flavonoids, mainly for the ability in scavenging reactive oxidant species, such as superoxide anion, hydroxyl, and peroxy radicals (4). In vitro studies showed that flavonoids prevent the low-density lipoprotein (LDL) oxidation by extending the lag phase (6), probably due to the synergic effect with vitamin E present in the particle (7). The flavonoids that showed these properties in vitro were the anthocyanins delphinidin and cyanidin (8), daidzein, genistein, and equol (6) and the catechin and theaflavin from green tea (9) and grape juice (10). In general, the protection effect was related to free radical scavenging or metal chelating properties of flavonoids. Flavonoids exhibit other biological activities such as anti-inflammatory properties by inhibition of the cyclo-oxygenase and 5-lipoxygenase (11). Besides enzymatic inhibition, Liang et al. (12) showed that apigenin, genistein, and kaempferol were potent inhibitors of cyclo-oxygenase-2 transcription in rat

\* Corresponding author (telephone +55 11 3091 3647; fax +55 11 3815 4410; e-mail fmlajolo@usp.br).

macrophage. In hepatocyte cell culture, myricetin prevented the lipid oxidation and liberation of DNA oxidation product (13).

Because of the biological activities of flavonoids, natural food sources have been extensively studied. They include different plant organs such as seeds (oat, wheat, flaxseed, barley grain) (14, 15), fruits (guava, berry, apple, nectarine, grape, pineapple, banana, orange, peach, lemon, plum) (14, 16–19, 21–23), and leaves (tea, spinach, cabbage, lettuce, kale) (15, 20, 24–26).

The data of flavonoids intake from the diet are scarce. The daily intake of flavonoids for the Holland population was estimated at ~23 mg/day, 48% of the total ingestion being from tea, followed by onion (29%) and apple (7%) (27, 28). Arai et al. (29) estimated the intake of flavonoids for Japanese women as 16.7 mg/day of flavonols and flavones (46% from onion) and 47.2 mg/day of isoflavones (37% from tofu). The variation of tea, wine, or beer consumption in European countries is responsible for the great flavonoid intake variation (50 to 800 mg/day), because a cup of tea or wine contains ~200 mg of phenolic compounds (30). In a previous paper we reported the flavonoid content of vegetables and fruits consumed in Brazil and estimated the daily flavonoid intake for Brazilian women as ~70.5 mg/day, where 68% was obtained from orange, 12% from vegetables (e.g., lettuce), and 2.5% from tomato (31). This value was high when compared to Denmark (28 mg/day) and Finland (55.2 mg/day) intakes (32).

In the present study, the antioxidant activity of a series of vegetables, fruits, and commercial frozen pulps of fruits commonly consumed in Brazil was assessed. Due to the great variety of methods, two or more different methods are recommended to evaluate the antioxidant activity when the purpose is a screening. For this, two oxidation systems were used: a lipid peroxidation induced by copper in a liposome model system and a  $\beta$ -carotene bleaching system. We focused on evaluating the antioxidant activity in crude methanolic extracts to assay total antioxidant activity, and furthermore, using solid-phase extraction (SPE), we made a partial purification to evaluate the antioxidant activity mainly of the flavonoids.

## MATERIALS AND METHODS

**Materials.** Fresh fruits, vegetables, and commercial frozen fruit pulps were obtained from the Central Market of São Paulo, Brazil (CEAGESP). The fruits and vegetables analyzed were as follows: star fruit (*Averrhoa carambola* L.), Gala apple (*Malus domestica* Borkh), nespera (*Eriobotrya lindleyi*), pineapple (*Ananas sativus*), wild mulberry (*Morus nigra*), red and white guava (*Psidium guajava* L.), red and rough lettuce (*Lactuca sativa* L.), green and red sweet pepper (*Capsicum annuum* L. var. *annuum*), watercress (*Nasturtium officinale* R. Br), chicory (*Chicorium intybus* L.), arugula (*Eruca sativa*), white and red cabbage (*Brassica oleracea* L.), eggplant (*Solanum gilo*), and black bean (*Phaseolus vulgaris* L.). The commercial frozen pulps (FP) analyzed were cashew (*Anacardium occidentale* L.), red guava (*P. guajava* L.), acerola (*Malpighia glabra*), graviola FP (*Annona muricata*), murici FP (*Byrsonima sericea*), acai (*Euterpe oleracea*), and mulberry (*Rubus* sp.). After the fruits and vegetables had been cleaned with tap water, edible portions were chopped, frozen under liquid nitrogen, and stored at  $-70^{\circ}\text{C}$  until the time of analyses. Commercial frozen pulps of fruits were obtained from CEAGESP and stored at  $-70^{\circ}\text{C}$ . At the time of analysis, samples were thoroughly homogenized by powdering in liquid nitrogen.

**Chemicals.** Ascorbic acid,  $\beta$ -carotene, linoleic acid, Tween 40, trichloroacetic acid, butylated hydroxytoluene, quercetin, and soybean lecithin (40% phosphatidylcholine) were purchased from Sigma Chemical Co. (St. Louis, MO). Thiobarbituric acid and rutin were obtained from Merck Chemical Co. (Darmstadt, Germany). The standards of malvidin, pelargonidin, and cyanidin were obtained from Extrasynthese (Genay, France). All other chemicals were of reagent or HPLC grade.

**Extraction.** Powdered samples (~1–2 g) were homogenized using a Brinkmann homogenizer (Polytron-Kinematica GmbH, Kriens-Luzern) for 2 min with 70% methanol, or 70% methanol plus 5% acetic acid for samples containing anthocyanins, in an ice bath. The extracts were centrifuged at 10000g for 10 min ( $4^{\circ}\text{C}$ ). The residues were re-extracted in the same conditions. The combined fractions consisted of the crude methanolic extract (CME). All extractions were done in duplicate, and the analyses were run in triplicate. All values were expressed as mean  $\pm$  standard deviation (SD).

**Solid-Phase Extraction.** For partial purification of flavonoids, an aliquot of CME was concentrated until methanol elimination on a rotary evaporator (Rotavapor RE 120, Büchi, Flawil, Sweden) at  $\leq 40^{\circ}\text{C}$  and cleaned up by SPE (33), described as follows. The extracts obtained above were passed through polyamide (CC 6, Macherey-Nagel, Germany) columns (1 g/6 mL) previously conditioned with 20 mL of methanol and 60 mL of distilled water. Impurities were washed out with 20 mL of distilled water, and retained flavonoids were further eluted with methanol (50 mL), to elute neutral flavonoids, followed by methanol/ammonia (99.5:0.5 v/v) to elute acidic flavonoids. The flow rate through the columns was controlled by means of a vacuum manifold Visiprep 24 DL (Supelco, Bellefonte, PA). For samples without anthocyanins in their composition the two eluates obtained above were kept together. These fractions were then evaporated to dryness under reduced pressure at  $40^{\circ}\text{C}$ , redissolved in methanol or methanol/acetic acid (99.5:0.5 v/v)—for samples containing anthocyanins—and analyzed for total phenolic content and antioxidant capacity.

**Ascorbic acid (AA) content** was determined according to the method of Rizzolo et al. (34). AA was extracted with metaphosphoric acid (0.3% w/v) and analyzed by reversed-phase HPLC in a Hewlett-Packard 1100 system with an autosampler and a quaternary pump coupled to a diode array detector. The column used was a  $\mu$ -Bondapak (300 mm  $\times$  3.9 mm i.d., Waters, Milford, MA), and elution (flow rate of 1.5 mL/min) was performed in isocratic condition with 0.2 M sodium acetate/acetic acid buffer (pH 4.2), monitored at 262 nm. Total AA was estimated after reduction of dehydroascorbic acid (DHA) with 10 mM dithiothreitol.

**Total phenolics** were measured in duplicate samples of each extract according to the method of Swain and Hillis (35), using the Folin–Ciocalteu reagent and gallic acid as standard. The results are expressed as gallic acid equivalents (GAE).

**In Vitro Antioxidant Assay. Liposome Oxidation Method.** The antioxidant capacity of the CME and SPE eluates was determined according to the method of Satué-Gracia et al. (8), based on the inhibition of liposome oxidation, induced by copper acetate at  $37^{\circ}\text{C}$  for 2 h. For vesicle preparation, aliquots of 1% lecithin in chloroform/methanol (2:1 v/v) were evaporated under nitrogen until dryness and then dispersed with 10 mM sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl (final concentration of phosphatidylcholine of 4 mg/mL). After vigorous agitation, the solution was sonicated for 20 min under nitrogen in the dark. For antioxidant capacity determination, 2.88 mL of the liposome solution was mixed with 90  $\mu\text{L}$  of CME or SPE eluate (final concentrations of 10 and 50  $\mu\text{M}$  GAE) and oxidized with 30  $\mu\text{L}$  of 20 mM copper acetate (final concentration of 50  $\mu\text{M}$ ). For SPE eluates from anthocyanin-rich samples, 1 and 5  $\mu\text{M}$  were also tested. The mixture was held in a water bath at  $37^{\circ}\text{C}$  for 2 h, in the dark, and the reaction was monitored by formation of thiobarbituric reactive compounds. Two aliquots of 0.5 mL were taken before and after 2 h of incubation. The oxidation was stopped by adding 0.05 mL of 2% (w/v) butylated hydroxytoluene and 1 mL of 0.345% 2-thiobarbituric acid in 15% trichloroacetic acid and chloridric acid (0.25 M) (36). The mixture was then boiled for 15 min. After cooling in an ice bath, the pink chromogen was extracted into 1.4 mL of *n*-butanol. Absorbance of the organic phase was measured at 535 nm by a UV–vis spectrophotometer (Pharmacia Biotech, Ultrospec 2000). A blank was run without addition of the samples. The antioxidant activity was calculated as the percent inhibition of liposome oxidation compared to control.

**$\beta$ -Carotene Bleaching Method.** The antioxidant activity of the CME and SPE eluates was determined according to the  $\beta$ -carotene bleaching method, following a modification of the procedure described by Marco

**Table 1.** Antioxidant Activity of Crude Methanolic Extracts and Solid-Phase Extraction Eluates of Fresh Vegetables Analyzed by the Liposome Oxidation and  $\beta$ -Carotene Bleaching Methods

vegetable	liposome method				$\beta$ -carotene bleaching method			
	crude methanolic extract		SPE eluate		crude methanolic extract		SPE eluate	
	10 $\mu$ M <sup>a</sup>	50 $\mu$ M <sup>a</sup>	10 $\mu$ M <sup>a</sup>	50 $\mu$ M <sup>a</sup>	10 $\mu$ M <sup>a</sup>	50 $\mu$ M <sup>a</sup>	10 $\mu$ M <sup>a</sup>	50 $\mu$ M <sup>a</sup>
red lettuce	17.5 $\pm$ 4.1	93.0 $\pm$ 2.2	89.1 $\pm$ 1.5 <sup>(1)</sup> 77.0 $\pm$ 0.9 <sup>(2)</sup>	88.0 $\pm$ 1.0 <sup>(1)</sup> 70.4 $\pm$ 3.0 <sup>(2)</sup>	26.8 $\pm$ 2.1	47.3 $\pm$ 1.5	37.8 $\pm$ 1.9 <sup>(1)</sup> 26.0 $\pm$ 3.8 <sup>(2)</sup>	54.0 $\pm$ 1.8 <sup>(1)</sup> 46.4 $\pm$ 2.7 <sup>(2)</sup>
rough lettuce	-7.4 $\pm$ 3.6	-20.9 $\pm$ 2.1	-12.7 $\pm$ 2.8	-33.6 $\pm$ 5.5	35.2 $\pm$ 3.2	75.9 $\pm$ 4.6	6.6 $\pm$ 1.9	41.5 $\pm$ 1.6
green sweet pepper	0.8 $\pm$ 2.7	14.8 $\pm$ 1.9	0.1 $\pm$ 2.8	20.1 $\pm$ 1.4	18.3 $\pm$ 4.6	31.4 $\pm$ 6.1	42.0 $\pm$ 2.9	38.3 $\pm$ 7.3
red sweet pepper	11.3 $\pm$ 2.7	16.8 $\pm$ 2.1	29.5 $\pm$ 3.8	52.4 $\pm$ 2.9	29.9 $\pm$ 2.8	49.2 $\pm$ 2.7	50.2 $\pm$ 2.6	52.7 $\pm$ 2.3
watercress	-1.6 $\pm$ 3.8	-0.7 $\pm$ 3.8	-18.6 $\pm$ 2.8	-38.5 $\pm$ 2.7	48.8 $\pm$ 4.3	73.5 $\pm$ 5.8	5.6 $\pm$ 1.8	45.9 $\pm$ 4.1
wild chicory	-11.2 $\pm$ 2.7	-20.0 $\pm$ 2.6	-22.5 $\pm$ 2.6	-49.7 $\pm$ 3.9	27.8 $\pm$ 3.2	66.8 $\pm$ 3.4	5.9 $\pm$ 1.7	14.5 $\pm$ 4.0
arugula	9.4 $\pm$ 1.0	21.4 $\pm$ 2.3	-5.5 $\pm$ 1.3	-2.3 $\pm$ 1.6	41.2 $\pm$ 5.3	69.5 $\pm$ 3.0	10.5 $\pm$ 1.8	33.6 $\pm$ 3.3
white cabbage	24.5 $\pm$ 2.1	48.2 $\pm$ 2.2	-3.4 $\pm$ 0.8	9.1 $\pm$ 1.9	34.5 $\pm$ 4.5	65.9 $\pm$ 2.3	15.0 $\pm$ 2.6	46.5 $\pm$ 3.4
red cabbage	89.4 $\pm$ 0.8	96.8 $\pm$ 1.0	92.3 $\pm$ 0.7 <sup>(1)</sup> 89.1 $\pm$ 1.2 <sup>(2)</sup>	100.0 $\pm$ 2.0 <sup>(1)</sup> 91.8 $\pm$ 0.9 <sup>(2)</sup>	41.5 $\pm$ 2.0	57.8 $\pm$ 1.7	64.1 $\pm$ 2.8 <sup>(1)</sup> 72.4 $\pm$ 1.6 <sup>(2)</sup>	69.9 $\pm$ 2.4 <sup>(1)</sup> 80.3 $\pm$ 1.8 <sup>(2)</sup>
eggplant	-3.1 $\pm$ 2.5	-3.4 $\pm$ 3.0	-8.2 $\pm$ 1.2	-17.9 $\pm$ 1.5	28.4 $\pm$ 3.2	48.4 $\pm$ 6.0	19.9 $\pm$ 2.9	33.0 $\pm$ 3.0
black bean, cotyledon	37.3 $\pm$ 3.1	73.5 $\pm$ 1.9	14.0 $\pm$ 1.0 <sup>(1)</sup> 2.2 $\pm$ 3.6 <sup>(2)</sup>	24.7 $\pm$ 0.8 <sup>(1)</sup> 12.0 $\pm$ 1.2 <sup>(2)</sup>	71.6 $\pm$ 1.3	79.9 $\pm$ 0.7	17.6 $\pm$ 4.6 <sup>(1)</sup> 27.0 $\pm$ 5.3 <sup>(2)</sup>	32.0 $\pm$ 2.8 <sup>(1)</sup> 36.3 $\pm$ 5.9 <sup>(2)</sup>
black bean, whole	88.7 $\pm$ 1.2	97.1 $\pm$ 3.1	92.1 $\pm$ 1.7 <sup>(1)</sup> 93.9 $\pm$ 2.1 <sup>(2)</sup>	86.4 $\pm$ 0.7 <sup>(1)</sup> 92.7 $\pm$ 2.4 <sup>(2)</sup>	66.3 $\pm$ 2.8	75.5 $\pm$ 1.7	48.7 $\pm$ 1.5 <sup>(1)</sup> 73.1 $\pm$ 1.1 <sup>(2)</sup>	60.0 $\pm$ 2.1 <sup>(1)</sup> 73.5 $\pm$ 2.1 <sup>(2)</sup>
black bean, seed coat	nd	nd	87.5 $\pm$ 0.7 <sup>(1)</sup> 90.7 $\pm$ 0.8 <sup>(2)</sup>	82.2 $\pm$ 1.4 <sup>(1)</sup> 83.7 $\pm$ 1.7 <sup>(2)</sup>	nd	nd	59.4 $\pm$ 6.3 <sup>(1)</sup> 70.5 $\pm$ 2.4 <sup>(2)</sup>	67.9 $\pm$ 6.8 <sup>(1)</sup> 73.1 $\pm$ 1.0 <sup>(2)</sup>

<sup>a</sup> Expressed as GAE: (1) acidic flavonoids; (2) neutral flavonoids; nd, not determined; values are the mean  $\pm$  SD (triplicate).

(37). An aliquot (20  $\mu$ L) of  $\beta$ -carotene chloroform solution (2 mg/mL) was added to a flask containing 0.4 mL of linoleic acid, 1.0 mL of chloroform, and 0.4 mL of Tween 40 and mixed. The chloroform was evaporated to dryness under nitrogen. After this, oxygenated distilled water (100 mL) was added and the mixture shaken. Aliquots (100  $\mu$ L) of 70% methanol (as control) or plant extracts (at 10 and 50  $\mu$ M GAE) were added to 2.9 mL of the  $\beta$ -carotene solution in a cuvette and mixed well. For eluates from anthocyanin-rich samples, 1 and 5  $\mu$ M were also tested. The absorbance of the solution at 470 nm was measured before and after 2 h of incubation in a water bath at 50  $^{\circ}$ C using the spectrophotometer Hewlett-Packard 8453. Antioxidant activity was calculated as percent inhibition relative to the control.

## RESULTS AND DISCUSSION

When the antioxidant activity of biological material is to be evaluated, one of the major problems is the choice of the method of analysis because usually they are specific for only one property. Many chemical analyses can be based on the ability of inhibiting the oxidation of a target substrate initiated by a free radical, for example, superoxide anion, hydroxyl, and peroxy radicals. Usually, the target can be a lipid, LDL, liposome, or DNA. Other methods evaluate the metal chelating properties and the ability of scavenging synthetic radicals such as the  $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazin radical (DPPH) and the 2,2'-azinobis(3-ethylbenzothiazolin)6-sulfonic acid (ABTS cation).

With the purpose of making a screening of the antioxidant activity of edible plants important in the Brazilian diet, two oxidant systems were used: the liposome oxidation method and the  $\beta$ -carotene bleaching method. The liposome method targets the scavenging of lipid peroxy radicals as well as the chelation of copper. The liposome system was chosen because it allows the protection of a substrate by an antioxidant to be investigated, in a model biological membrane, and also allows the analysis of water- and lipid-soluble antioxidants in the same system (38). However, it has a disadvantage, because the TBA reagent reacts with sugars (39) and, therefore, the crude methanolic extract (CME) of fruits and frozen pulps could not be analyzed. For this reason, the antioxidant efficacy of the samples was also evaluated on the basis of their abilities to inhibit the  $\beta$ -carotene bleaching, caused by free radicals generated during peroxidation of linoleic acid.

Besides CME extracts, the total antioxidant activity was also evaluated after solid phase extraction (SPE eluates) to evaluate the antioxidant activity of phenolic compounds, mainly flavonoids. In SPE, the neutral flavonoids are eluted in methanol, whereas acidic flavonoids are eluted in methanol/ammonia. As the purpose of this work was to evaluate the antioxidant activity due to total flavonoids, the two eluted fractions were analyzed together, with the exception of samples containing anthocyanins, for which the eluates were analyzed separately.

The values of antioxidant activity are classified as high (>70% inhibition), intermediate (40–70% inhibition), and low (<40% inhibition), at equimolar concentrations (10 or 50  $\mu$ M gallic acid equivalents).

**Vegetables.** Table 1 summarizes the antioxidant activity of the CME and SPE eluates of 11 vegetables. In the liposome oxidation method, at 10  $\mu$ M GAE addition level, the CME of red cabbage and black bean had the highest activity. At 50  $\mu$ M GAE addition level, red cabbage, black bean, and red lettuce showed the highest antioxidant activities (up to 90% inhibition) followed by white cabbage, with intermediate activity (40–70% inhibition), and arugula and red and green sweet peppers, with low activities (<40% inhibition). Rough lettuce and wild chicory had concentration-dependent pro-oxidant activities. Watercress and eggplant presented low pro-oxidant activities at both concentrations tested.

On the other hand, in the  $\beta$ -carotene bleaching system, the highest antioxidant activities (at 50  $\mu$ M) were shown by CME of rough lettuce, watercress, and arugula (>70% inhibition), followed by wild chicory, white cabbage, red cabbage, sweet red pepper, and red lettuce, with intermediate activities (40–70% inhibition), and sweet green pepper (<40% inhibition), with low activity.

In the  $\beta$ -carotene bleaching method a decrease in antioxidant activity was observed after SPE. The SPE eluates of rough lettuce, watercress, wild chicory, arugula, white cabbage, and eggplant had a dramatic decrease as compared with the respective CME. These results were in accordance with those obtained in the liposome system, because the pro-oxidant activity of SPE eluates was higher than that of the CME. In this way, the antioxidant components could have been washed out in the polyamide column or the flavonoids purification could have been

**Table 2.** Antioxidant Activity of Crude Methanolic Extracts and Solid-Phase Extraction Eluates of Fresh Fruits and Commercial Frozen Pulp Analyzed by the Liposome Oxidation and  $\beta$ -Carotene Bleaching Methods

sample	liposome method		$\beta$ -carotene bleaching method			
	SPE eluate		crude methanolic extract		SPE eluate	
	10 $\mu$ M <sup>a</sup>	50 $\mu$ M <sup>a</sup>	10 $\mu$ M <sup>a</sup>	50 $\mu$ M <sup>a</sup>	10 $\mu$ M <sup>a</sup>	50 $\mu$ M <sup>a</sup>
fruits						
star fruit	-5 $\pm$ 3	-23.8 $\pm$ 4.2	23.2 $\pm$ 4.3	52.8 $\pm$ 1.0	14.9 $\pm$ 3.9	53.8 $\pm$ 3.5
Gala apple, pulp	-13 $\pm$ 1	-21.4 $\pm$ 2.1	9.3 $\pm$ 2.0	33.7 $\pm$ 3.1	0.62 $\pm$ 2.6	23.5 $\pm$ 2.3
Gala apple, peel	87 $\pm$ 1 <sup>(1)</sup>	90.2 $\pm$ 1.0 <sup>(1)</sup>	67.6 $\pm$ 2.6	79.6 $\pm$ 4.5	58.9 $\pm$ 1.8 <sup>(1)</sup>	68 $\pm$ 1.1 <sup>(1)</sup>
	90 $\pm$ 2 <sup>(2)</sup>	92.0 $\pm$ 3.5 <sup>(2)</sup>			62.7 $\pm$ 3.0 <sup>(2)</sup>	75.7 $\pm$ 2.6 <sup>(2)</sup>
nespera	-10 $\pm$ 2	-39.8 $\pm$ 4.5	9.3 $\pm$ 2.4	34.1 $\pm$ 3.5	1.0 $\pm$ 4.1	18.9 $\pm$ 3.0
pineapple	12 $\pm$ 2	45.6 $\pm$ 4.5	40.6 $\pm$ 3.0	49.1 $\pm$ 2.9	51.0 $\pm$ 3.0	58.1 $\pm$ 3.4
mulberry	37 $\pm$ 2 <sup>(1,a)</sup>	81.3 $\pm$ 0.8 <sup>(1,b)</sup>	53.2 $\pm$ 2.1	74.6 $\pm$ 2.6	67.2 $\pm$ 1.9 <sup>(1)</sup>	78.0 $\pm$ 3.0 <sup>(1)</sup>
	36 $\pm$ 5 <sup>(2,a)</sup>	91.6 $\pm$ 0.5 <sup>(2,b)</sup>			68.7 $\pm$ 2.8 <sup>(2)</sup>	80.8 $\pm$ 1.3 <sup>(2)</sup>
jambolao	90 $\pm$ 2 <sup>(1)</sup>	96.9 $\pm$ 2.6 <sup>(1)</sup>	16.9 $\pm$ 1.6	41.7 $\pm$ 1.7	59.9 $\pm$ 2.5 <sup>(1)</sup>	66.8 $\pm$ 2.9 <sup>(1)</sup>
	88 $\pm$ 2 <sup>(2)</sup>	89.8 $\pm$ 2.4 <sup>(2)</sup>			68.8 $\pm$ 4.5 <sup>(2)</sup>	73.3 $\pm$ 4.2 <sup>(2)</sup>
white guava, pulp	26 $\pm$ 2	49.1 $\pm$ 1.8	11.9 $\pm$ 1.2	14.6 $\pm$ 1.2	42.5 $\pm$ 2.5	58.6 $\pm$ 4.0
white guava, peel	1 $\pm$ 2	16.5 $\pm$ 0.7	16.8 $\pm$ 2.3	23.2 $\pm$ 1.6	44.1 $\pm$ 2.7	59.2 $\pm$ 3.8
red guava, pulp	25 $\pm$ 2	53.5 $\pm$ 4.3	36.9 $\pm$ 2.8	52.8 $\pm$ 4.7	45.5 $\pm$ 2.6	68.2 $\pm$ 2.8
red guava, peel	3 $\pm$ 2	22.9 $\pm$ 1.6	11.9 $\pm$ 1.4	29.7 $\pm$ 2.1	42.1 $\pm$ 3.6	69.1 $\pm$ 3.9
frozen pulps						
red guava	24 $\pm$ 2	57.5 $\pm$ 2.9	30.4 $\pm$ 4.3	38.3 $\pm$ 3.8	48.4 $\pm$ 4.7	63.9 $\pm$ 5.7
cashew	28 $\pm$ 3	54.5 $\pm$ 1.1	25.3 $\pm$ 2.8	44.5 $\pm$ 4.7	42.8 $\pm$ 3.5	59.1 $\pm$ 2.0
acerola	-1 $\pm$ 2 <sup>(1)</sup>	25.4 $\pm$ 2.4 <sup>(1)</sup>	-4.9 $\pm$ 1.2	8.1 $\pm$ 0.9	17.0 $\pm$ 2.0 <sup>(1)</sup>	45.9 $\pm$ 3.9 <sup>(1)</sup>
	18.2 $\pm$ 0.9 <sup>(2)</sup>	89.4 $\pm$ 1.8 <sup>(2)</sup>			30.9 $\pm$ 2.2 <sup>(2)</sup>	61.1 $\pm$ 3.2 <sup>(2)</sup>
graviola	3.5 $\pm$ 0.8	16.3 $\pm$ 2.3	24.7 $\pm$ 3.6	50.3 $\pm$ 3.8	36.7 $\pm$ 2.9	55.2 $\pm$ 4.5
murici	-9.9 $\pm$ 1.2	-1.1 $\pm$ 0.8	14.4 $\pm$ 1.4	42.6 $\pm$ 3.6	-1.0 $\pm$ 2.2	19.0 $\pm$ 1.8
acai	6 $\pm$ 2 <sup>(1,c)</sup>	87 $\pm$ 1 <sup>(1,d)</sup>	57.2 $\pm$ 2.3	73.3 $\pm$ 3.4	65.7 $\pm$ 3.2 <sup>(1)</sup>	78 $\pm$ 3.3 <sup>(1)</sup>
	7 $\pm$ 2 <sup>(2,c)</sup>	86 $\pm$ 2 <sup>(2,d)</sup>			67.3 $\pm$ 2.1 <sup>(2)</sup>	78.1 $\pm$ 1.9 <sup>(2)</sup>
mulberry	90 $\pm$ 1 <sup>(1,b)</sup>	91 $\pm$ 1 <sup>(1,c)</sup>	12.2 $\pm$ 1.1	30.2 $\pm$ 1.9	65.7 $\pm$ 2.1 <sup>(1)</sup>	79.3 $\pm$ 1.0 <sup>(1)</sup>
	89 $\pm$ 2 <sup>(2,b)</sup>	88 $\pm$ 1 <sup>(2,c)</sup>			59.7 $\pm$ 5.6 <sup>(2)</sup>	71.9 $\pm$ 3.7 <sup>(2)</sup>

<sup>a</sup> Expressed as GAE: (1) acidic flavonoids; (2) neutral flavonoids; (a) 0.1  $\mu$ M; (b) 1  $\mu$ M; (c) 5  $\mu$ M; (d) 10  $\mu$ M; values are the mean  $\pm$  SD (triplicate).

**Table 3.** Comparative Antioxidant Activity of Purified Compounds Evaluated by the Liposome System and the  $\beta$ -Carotene Bleaching Method

compound	liposome oxidation method		$\beta$ -carotene bleaching method	
	10 $\mu$ M	50 $\mu$ M	10 $\mu$ M	50 $\mu$ M
BHT	9.5 $\pm$ 3.1	68.8 $\pm$ 3.9	33.7 $\pm$ 2.8	77.6 $\pm$ 0.4
ascorbic acid	-5.5 $\pm$ 1.9	-17.5 $\pm$ 2.7	-5.13 $\pm$ 2.9	-11.7 $\pm$ 1.9
ascorbic acid without Cu <sup>2+</sup>	wo <sup>a</sup>	wo		
quercetin	-3.56 $\pm$ 1.22	36.8 $\pm$ 1.9	22.5 $\pm$ 2.9	49.2 $\pm$ 3.4
rutin	-17.1 $\pm$ 2.0	-40.9 $\pm$ 2.0	1.2 $\pm$ 2.8	12.7 $\pm$ 2.3
rutin without Cu <sup>2+</sup>	wo	wo		
cyanidin	27.6 $\pm$ 2.3	84.8 $\pm$ 3.9	53.9 $\pm$ 3.5	67.8 $\pm$ 4.5
pelargonidin	27.3 $\pm$ 2.1	86.8 $\pm$ 1.7	52.8 $\pm$ 2.0	68.2 $\pm$ 3.9
malvidin	95.9 $\pm$ 1.4	98.5 $\pm$ 1.8	65.1 $\pm$ 2.1	71.7 $\pm$ 2.7

<sup>a</sup> Without oxidation.

responsible for the enhancement of the pro-oxidant activity. Among flavonoids, the flavonol rutin is the main component present in these leaves (31) and, in our liposome system, rutin showed high pro-oxidant activity at 10 and 50  $\mu$ M addition levels (Table 3), similar to the range of rutin concentration derived from these samples. However, the liposome oxidation was not induced by rutin without copper ion, showing that its pro-oxidant activity was copper dependent. The pro-oxidant activity was previously observed for tea extract (26) by the total oxygen radical absorbance assay (ORAC) induced by copper. Cao et al. (40) demonstrated that flavonoids might present antioxidant or pro-oxidant activities depending on the radical-generating system. They observed that when copper was the initiator in ORAC, kaempferol, quercetin, and myricetin, which presented hydroxyl and peroxyl radical scavenging activities, behave like anti- or pro-oxidants depending on their concentra-

tion. In the first case, flavonoids would act by absorbing reactive species produced by the reactions among Cu<sup>2+</sup>, H<sub>2</sub>O<sub>2</sub>, and O<sub>2</sub>, and in the second case, they would produce reactive species through the direct reaction with Cu<sup>2+</sup> and O<sub>2</sub>. In this way, in the  $\beta$ -carotene system, where the generation of radicals does not depend on the presence of copper, the CME acted as an antioxidant. However, in the liposome system, where the generation of radicals is copper dependent, the reaction among copper and flavonoids resulted in pro-oxidant activity.

In cruciferous vegetables, such as cabbage (red and white), watercress, broccoli, and cauliflower, besides flavonoids, the antioxidant activity has also been associated with glucosinolates, because they are present in high quantities. However, Plumb et al. (41) showed that the glucosinolates are unlikely to account for the antioxidant effects of extracts from cruciferous vegetables, because the isolated glucosinolates exhibited only weak antioxidant properties compared to the effects of whole extracts, in five different oxidation systems.

The CME and SPE eluates of the seed, coat, and whole black bean showed strong antioxidant activity (Table 1). The CME of cotyledons and whole black beans produced strong inhibition of liposome oxidation. However, after SPE, the eluates of cotyledons exhibited a lower antioxidant activity. On the contrary, the neutral and acidic flavonoids of whole beans and seed coat had strong activity, at both concentrations tested. Similar results were also reported by Tsuda et al. (42, 43) and attributed to the presence of the pigment pelargonidin 3-O- $\beta$ -D-glucoside in the seed coat of black bean.

**Fruits.** At 10 and 50  $\mu$ M GAE addition levels, the neutral and acidic flavonoids eluates of Gala apple peel, wild mulberry, and jambolao had the highest antioxidant activities (>86% inhibition at the 10  $\mu$ M GAE addition level) against liposome oxidation (Table 2). Pineapple and the pulp and peel of white and red guava had antioxidant activities lower than 60%, at the

**Table 4.** Phenolic Content, Total Ascorbic Acid Content, and Recovery of Phenolics after Solid-Phase Extraction of Vegetables, Fruits, and Frozen Fruit Pulp

sample	phenolic content (mg/100 g of FW)	recovery of phenolic after SPE (%)	total AA <sup>a</sup> (mg/100 g of FW)
fruits			
star fruit	126 ± 10	19 ± 3	37.4 ± 0.2
Gala apple, pulp	82 ± 3	29 ± 3	nd <sup>b</sup>
Gala apple, peel	309 ± 5	35 ± 1	5.9 ± 0.1
nespera	112 ± 2	59.8 ± 0.4	3.8 ± 0.1
pineapple	67.2 ± 0.6	10.7 ± 0.6	22.4 ± 0.9
wild mulberry	373 ± 11	77.4 ± 2.9	10.5 ± 0.7
jambolao	583 ± 16	11.0 ± 0.4	22.9 ± 0.6
white guava, pulp	160 ± 15	5.4 ± 0.5	136.4 ± 0.2
white guava, peel	428 ± 19	11.4 ± 0.03	268.9 ± 0.3
red guava, pulp	124 ± 4	13.5 ± 0.3	56 ± 2
red guava, peel	420 ± 14	12.0 ± 0.9	198 ± 1
commercial frozen pulp			
red guava	119 ± 4	13.2 ± 0.9	49.9 ± 0.3
cashew	234 ± 8	6.8 ± 0.2	195.0 ± 4.0
acerola	861 ± 62	7.0 ± 0.1	885 ± 33
graviola	120 ± 8	12.6 ± 0.2	nd
murici	67 ± 3	23.5 ± 0.5	nd
acai	328 ± 9	55.2 ± 2.3	nd
mulberry	225 ± 19	49.5 ± 1.0	nd
vegetables			
red lettuce	170 ± 1	90.3 ± 3.0	12.2 ± 0.7
rough lettuce	53 ± 9	29.7 ± 0.5	9.3 ± 0.2
green sweet pepper	119 ± 10	6.6 ± 0.2	92 ± 2
red sweet pepper	131 ± 12	6.8 ± 0.4	105 ± 1
watercress	168 ± 6	62.7 ± 0.8	57.2 ± 0.6
wild chicory	102.7 ± 0.1	75 ± 5	11.9 ± 0.9
arugula	90 ± 4	23.5 ± 2.0	48.6 ± 0.3
white cabbage	40 ± 5	17 ± 3	16.6 ± 0.2
red cabbage	178 ± 14	47 ± 4	37.3 ± 0.6
eggplant	45 ± 2	11.0 ± 0.4	22.0 ± 0.8
black bean, cotyledon	43 ± 2	78 ± 7	
black bean, seed coat	2971 ± 82	28 ± 1	
black bean, whole	213 ± 20	56.3 ± 0.8	

<sup>a</sup> Difference between ascorbic acid content and total ascorbic acid. <sup>b</sup> Not detected.

50  $\mu$ M GAE addition level. On the other hand, star fruit, nespera, and Gala apple pulps showed high pro-oxidant activities at both concentrations. The abilities to inhibit liposome oxidation of guava pulps and the respective commercial frozen pulp did not differ. Red and white guava pulps had 2 and 3 times the activity of their respective peels. However, for crude acetone/water extracts, Jiménez-Scrig et al. (19) showed that the peel fraction was more effective as antioxidant than the respective deseeded pulp, by three different methods (free radical DPPH scavenging, ferric reducing antioxidant power assay, and inhibition of copper-catalyzed in vitro human LDL oxidation). These differences are probably the results of different compositions among the extracts.

The peel of Gala apple had an antioxidant activity higher than that of the pulp. A similar result was previously found by Wolfe et al. (44), using the total oxyradical scavenging assay, showing higher antioxidant activity for acetone extracts of apple peel when compared to the pulp or whole fruit. As observed, the phenolic contents of Gala apple and guava peels were significantly higher than those of the respective pulps, for both CME and SPE eluates fraction (Table 4). The phenolic content of Gala apple peel was 4 times higher than that of the pulp and 3 times higher than that of guava varieties. The higher phenolic content of apple (44) and guava peels (19) has been previously reported and is related to the protection of the plant against UV light (4).

In the  $\beta$ -carotene bleaching method, the CME (at 50  $\mu$ M GAE) of Gala apple peel and mulberry had the highest activities (>70% inhibition). Star fruit, pineapple, jambolao, and red guava pulp showed intermediate activities (40–70% inhibition), and the other fruits had activities below 40% inhibition. For SPE eluates, the fraction of acidic flavonoids of Gala apple peel and neutral and acidic flavonoids of mulberry and jambolao showed the highest antioxidant activities (>70%). Guava fruit showed intermediate activity without difference among parts. Red guava FP had activity similar to that of the fruit. In general, the SPE eluates of fruits showed more efficacy as antioxidants than the respective CME against  $\beta$ -carotene bleaching, with the exception of the eluates from Gala apple pulp and nespera. The neutral and acidic flavonoids of wild mulberry showed high antioxidant activities also at the concentrations of 1  $\mu$ M GAE.

**Commercial Frozen Pulp.** Among the frozen fruit pulps (Table 2), acidic and neutral flavonoids of mulberry and acai showed the highest antioxidant activities, at the 10  $\mu$ M GAE addition level, by both methods. Besides, the mulberry FP inhibited completely the liposome oxidation at the concentration of 1  $\mu$ M.

The CME of acerola FP showed low antioxidant activity as compared with the fraction obtained by SPE, perhaps due to the high content of ascorbic acid present in it. In the liposome system, AA showed pro-oxidant activity at 10 and 50  $\mu$ M in both systems (Table 3). This pro-oxidant behavior of AA has also been described by others (45) and is due to the formation of the ascorbyl radical during the oxidation reaction. Similar to rutin, the pro-oxidant activity of ascorbic acid in the liposome system was copper dependent, because in the absence of the transition metal, AA did not initiate the oxidation (Table 3).

According to the results obtained, it appears that an important criteria for predicting a high antioxidant activity of fruits and vegetables is their anthocyanin content. The anthocyanin-rich samples, including acerola FP, acai FP, mulberry FP, wild mulberry, jambolao, red lettuce, red cabbage, and black bean (whole and seed coat), showed the highest liposome antioxidant activities for both CME and SPE eluates. The main anthocyanins present in these samples were cyanidin derivatives as shown by the UV-vis spectra obtained by DAD coupled to HPLC (31). Results from the literature report the presence of cyanidin 3,5-diglucoside and cyanidin 3-sophoroside in red cabbage, the latter found in various acylated forms (46); cyanidin 3-arabinoxide and cyanidin 3-arabinoxylarabinoxide in fruits of *Euterpes oleraceae* (acai) (47); and cyanidin 3-galactoside (48) in the peel of apple. In mulberry (*Morus* sp., cv. Mavromouria) cyanidin 3-glucorutinoside, cyanidin 3-sophoroside, and pelargonidin 3-glucoside were identified (49).

Moreover, all of the anthocyanin-containing samples were more effective in inhibiting the liposome oxidation, compared to the  $\beta$ -carotene bleaching, but for the other samples, the inverse occurred. Usually, both systems evaluate the lipidic peroxidation; however, in the liposome oxidation system the abilities to chelate transition metal ions and also the ability of the antioxidant to interact with the interface of the membrane are also involved. The Cu<sup>2+</sup> ion chelation occurs mainly through the catechol structure in flavones, flavonols (50), and anthocyanidins (8). However, besides the direct interaction of ions with the flavonoids, the partition coefficient has great importance because it determines the accessibility and interaction with lipid peroxyl radicals within the liposome.

The efficiency of anthocyanins as antioxidant components can be observed in Table 3. In both oxidation systems, the anthocyanidins cyanidin, pelargonidin, and malvidin were better

antioxidants than the flavonol quercetin and the respective glucoside, rutin (quercetin 3-glucorhamnoside), and the synthetic antioxidant BHT. When the antioxidant activities of anthocyanidins in the liposome system are compared, a higher activity seems to be related to a lower polarity, similar to that reported by Heinonen et al. (22). At the 10  $\mu$ M addition level, malvidin was the best inhibitor among anthocyanidins, due to the lowest polarity conferred by the substitution of the 3',5'-dihydroxyl groups of the B-ring with methoxyl groups and better affinity for the interface of the liposome (8). On the other hand, these differences in polarity were not reflected in the antioxidant activity measured by the  $\beta$ -carotene bleaching method, at least when the end products of the oxidation were measured.

In this way, the antioxidant activity of phenolic compounds depends on several factors: the oxidation system, degree of glycosylation, partition coefficient, concentration, and parameter measured. Also, the rules determining the behavior of a class of flavonoids in a system do not seem to apply to another class (e.g., anthocyanidins, flavonols, and phenolic acids in the liposome system) (22, 51).

**Total Phenols and Ascorbic Acid Contents.** The total phenolic contents of the edible plant materials investigated varied from 40 to 861 mg of GAE/100 g of fresh weight (FW) (Table 4). Among all of the crude methanolic extract of fresh fruits analyzed, jambolao had the highest phenolic content (583 mg of GAE/100 g of FW) and pineapple the lowest (67 mg of GAE/100 g of FW). The highest recovery of phenolics after SPE was obtained for mulberry (77.4%), corresponding to 298 mg of GAE/100 g of FW.

Acerola FP had the highest total phenolic content (861 mg of GAE/100 g of FW) among CME from commercial FP; however, this value was probably due to the high content of ascorbic acid (885 mg/100 g of FW) present. Ascorbate in amounts  $>5 \mu$ g gives a positive Folin reaction, but this level of concentration was not present in the CME analyzed, except for acerola FP. Thus, acai FP had the highest phenolic content among commercial frozen pulps (328 mg of GAE/100 g of FW) and the highest recovery after SPE (55.2%, corresponding to 181.3 mg of GAE/100 g of FW). The commercial frozen pulp of red guava had a phenolic content similar to that of the fresh fruit.

Among CME of vegetables, red cabbage and red lettuce showed the highest values (178 and 169 mg of GAE/100 g of FW, respectively). Red lettuce and red cabbage presented phenolic contents 3 and 4 times higher, respectively, than the green equivalents. These results are in accordance with Hertog et al. (27) and DuPont et al. (52), who attributed the large variation of flavonoid content in leafy vegetables (lettuce, cabbage, and endive) to differences among varieties, portion of vegetable, season, and agronomic conditions, mainly because flavonoid formation is light-dependent. The highest recovery of phenolics after SPE (Table 4) was obtained for red lettuce (90.3%). According to Arabbi et al. (31), cyanidin contributed 28–31% of the total flavonoid content of red lettuce, whereas quercetin represented the main flavonoid, contributing 56–67% of the total ( $\sim 67$  mg/100 g of FW).

Quercetin was the most abundant flavonoid in the samples analyzed in our laboratory by Arabbi et al. (31), which were the same used here. Catechins were present in apple, graviola, and murici. Other flavonoids were also found, such as kaempferol in watercress, arugula, and chicory; luteolin in lettuce, sweet pepper, arugula, and acai; and cyanidin in red fruits and vegetables. The highest flavonoid contents were present in wild mulberry (274 mg/100 g of FW), the peel of Gala apple

(94 mg/100 g of FW), red lettuce (67 mg/100 g of FW), arugula (41 mg/100 g of FW), murici FP (39 mg/100 g of FW), and red cabbage (34 mg/100 g of FW). The anthocyanins were predominant over other flavonoids in wild mulberry (260 mg/100 g of FW), acai FP (36 mg/100 g of FW), and red cabbage (34 mg/100 of FW) (31).

Although there are some reports (17, 45) showing a high correlation between antioxidant activity and phenolic content, other authors (21) have found a low correlation. In the present study no linear correlation was observed between the total phenolic content and antioxidant activity ( $R^2 < 0.1$ ). On the basis of the data from Arabbi et al. (31) the calculated linear correlation between flavonoid content and antioxidant activity was  $<0.1$ . This could be explained by differences in the phenolic composition among plant extracts and variation in the response of different phenolic compounds to the Folin–Ciocalteu reagent (14). In addition, absolute values of antioxidant capacities can vary largely according to the methodology used.

Vitamin C is the generic term for all compounds exhibiting biological activity of L-ascorbic acid (AA), such as L-dehydroascorbic acid (DHA), usually present in  $<10\%$  of the total pool of vitamin (53). The concentration of vitamin C of fresh fruits and vegetables (Table 4), which varied from 3.8 to 885 mg/100 g of FW, was in accordance with the "Food Composition and Nutrition Tables" (54) except for white cabbage, which presented a lower concentration. All commercial FPs showed lower contents of AA when compared to the respective fresh fruits. This was expected because AA is very susceptible to chemical and enzymatic oxidation during the processing, cooking, and storage procedures. Although in Brazil the pulps are not subjected to bleaching or pasteurization, during depulping and freezing some vitamin C losses are expected to occur. All fruit peels (Gala apple and guava) had higher levels of vitamin C than the respective pulps. Usually, peel tissues have more AA to protect the fruit from outside stress caused by light and oxidation (53). Among vegetables, total AA is only composed by DHA, with the exception of the sweet pepper varieties. The rapid oxidation of vitamin C is observed in conditions favorable to water loss or, alternatively, water stress, by affecting free radical scavenging systems in the plant tissue (55). This was observed in broccoli, spinach, and lettuce, in which refrigeration with high humidity caused a great retention of total AA compared to low-humidity storage (56).

Vitamin C is the most abundant hydrosoluble antioxidant in plants. However, in the  $\beta$ -carotene and liposome systems, AA showed pro-oxidant activity (Table 3) and, in this way, may have influenced the values of antioxidant activity obtained for CME, mainly for acerola FP. This may explain why, despite the high amount of anthocyanin in acerola FP, the antioxidant activity was low. Other authors have previously reported a low contribution of vitamin C (0.35–8.6% of the total antioxidant activity) in fruits (17) and  $<15\%$  for fresh fruits and fruit juices (23). In the present work, the correlation between antioxidant activity and vitamin C using the liposome and  $\beta$ -carotene bleaching methods was not possible to establish because the vitamin C was pro-oxidant in both systems (Table 3). The negative influence of vitamin C was reported by Kalt et al. (45), who showed that the ascorbate content and the antioxidant capacity were negatively correlated ( $R = -0.80$ ) for strawberry, raspberry, and high- and lowbush blueberry.

**Conclusion.** The antioxidant activity of vegetables and fruits varied largely, in both systems tested, but anthocyanin-rich samples exhibited the most potent concentration-dependent antioxidant activities, including red lettuce, red cabbage, black

bean, Gala apple peel, mulberry, Jambolao, and acai FP. However, the antioxidant activity was not correlated with phenolics or vitamin C contents.

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