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Effect of Roasting on Phenolic Content and Antioxidant Activities of Whole Cashew Nuts, Kernels, and Testa

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ABSTRACT: The effect of roasting on the content of phenolic compounds and antioxidant properties of cashew nuts and testa was studied. Whole cashew nuts, subjected to low-temperature (LT) and high-temperature (HT) treatments, were used to determine the antioxidant activity of products. Antioxidant activities of cashew nut, kernel, and testa phenolics extracted increased as the roasting temperature increased. The highest activity, as determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity, oxygen radical absorbance capacity (ORAC), hydroxyl radical scavenging capacity, Trolox equivalent antioxidant activity (TEAC), and reducing power, was achieved when nuts were roasted at 130 °C for 33 min. Furthermore, roasting increased the total phenolic content (TPC) in both the soluble and bound extracts from whole nut, kernel, and testa but decreased that of the proanthocyanidins (PC) except for the soluble extract of cashew kernels. In addition, cashew testa afforded a higher extract yield, TPC, and PC in both soluble and bound fractions compared to that in whole nuts and kernels. Phenolic acids, namely, syringic (the predominant one), gallic, and *p*-coumaric acids, were identified. Flavonoids, namely, (+)-catechin, (−)-epicatechin, and epigallocatechin, were also identified, and their contents increased with increasing temperature. The results so obtained suggest that HT−short time (HTST) roasting effectively enhances the antioxidant activity of cashew nuts and testa.

KEYWORDS: DPPH, ORAC, TEAC, proanthocynidins, phenolic acids, flavonoids

INTRODUCTION

Regular consumption of fruits, vegetables, grains, and nuts is considered to be beneficial to health and is known to reduce the incidences of ischemic heart disease and several types of cancer such as those of the lungs, stomach, esophagus, pancreas, and colon.[1] Studies have shown that phenolics are the major phytochemicals with health benefits in humans. Foods of plant origin, such as fruits and vegetables, tree nuts, and whole grain products, have been suggested as a natural source of antioxidants.[1]

Tree nuts have been considered to be a significant component of the Mediterranean diet. In 2003, the U.S. Food and Drug Administration recommended a qualified health claim stating that consumption of 1.5 oz (42 g) per day of most tree nuts may reduce the risk of heart disease. Because free radicals play a key role in the pathology of diseases, such as cancer, atherosclerosis, or inflammatory diseases, the supply of antioxidants via the food chain is of high importance for a healthy lifestyle.[2] In particular, nuts contain protein, unsaturated fatty acids, dietary fiber, and sterols, as well as other phytochemicals and micronutrients that may exert health benefits.[2]

Cashew (*Anacardium occidentale* L.) is one of the most important tree nuts and ranks third in international trade after hazelnuts and almonds. The processing of cashew nut is more complicated than that of other nuts. Cashew nut must be roasted or cooked in boiling water (or steam) to remove the kernel. The kernels are removed manually, followed by drying and peeling of the testa, which is a thin reddish-brown membrane that is difficult to remove. Good-quality raw cashew kernels are low in moisture content (5−6%) and are slightly off-white in color.[3]

Generally, cashew kernels are consumed as roasted nuts. Roasting is reported as one of the processing conditions that would change the constituents of edible nuts. Cashew kernels are consumed as a snack or added to confectionary and bakery products, like most other nuts. The texture, color, flavor, and appearance of cashew kernels are altered significantly during roasting. The resulting product is crisp and uniquely tasty compared to the raw kernels. The degree of roasting affects the sensory quality attributes such as aroma, color, texture, and taste of the product. Thus, selection of appropriate roasting conditions for optimum product quality is essential in the roasting operation.[4]

The biological activities of cashew nut shell liquid (CNSL) constituents have attracted much interest in the areas of antitumor activity, antimicrobial activity, inhibition of tyrosinase and xanthine oxidase, uncoupling effects of oxidative phosphorylation on liver mitochondria, and antioxidant activity.[5] Cashew apples and their juices are also reported to possess antioxidant potential and antimutagenic activity.[6] Furthermore, Kamath and Rajini[7] reported that the ethanolic extract of cashew nut testa exhibited a high antioxidant activity. The polyphenolic compounds present appear to contribute to the observed antioxidant activity of testa.[8] Very few studies have evaluated the antioxidant activity of phenolics from the edible cashew kernels.[2]

Close scrutiny of the literature shows a lack of information on the phenolic content and antioxidant activity of cashew nuts and testa (skin) subjected to different thermal processing conditions. The objective of the present study was to determine the effects of low- and high-temperature thermal processing on the content of phenolic compounds and antioxidant properties of cashew nuts and testa.

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MATERIALS AND METHODS

Materials. Raw shelled cashews with testa were obtained from Green Field Bio Plantation (Pvt.) Ltd., Colombo , Sri Lanka. Folin−Ciocalteu's reagent, gallic acid, vanillin, (+)-catechin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), fluorescein, Trolox, ethylenediaminetetraacetic acid trisodium salt (Na_3 EDTA), mono- and dibasic potassium phosphates, sodium chloride, 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), hydrogen peroxide, ascorbic acid, potassium ferricyanide, trichloroacetic acid, ferric chloride, ferrous chloride, epigallocatechin, (−)-epicatechin, syringic acid, and *p*-coumaric acid were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Hexane, methanol, ethanol, sodium hydroxide, hydrochloric acid, diethyl ether, ethyl acetate, sodium carbonate, formic acid, and acetonitrile were purchased from Fisher Scientific Ltd. (Ottawa, ON, Canada).

Methods. *Sample Preparation.* Two different processing temperatures were used in this study. For low-temperature processing (LT) raw whole cashew nuts (kernel with testa) were roasted in a forced hot-air convection oven at 70 °C for 6 h. In this, cashew kernels weighing approximately 100 g were spread in a single layer on a stainless steel wire mesh tray placed in the center of the oven during hot-air roasting. After roasting, the hot cashew kernels were cooled in a desiccator at room temperature and kept in sealed plastic bags at 4 °C until further analysis. Under industrial cashew processing operations, both small- and large-scale cashew producers use these conditions to obtain good-quality products.[9] For high-temperature processing (HT), raw whole cashew nuts were roasted in a forced air convection oven at 130 °C for 33 min. This combination of temperature and time provided the optimum roasting conditions for cashew kernels based on hedonic sensory evaluations according to Wanlapa and Jindal.[10] Raw whole cashew nuts were used as the control to compare the effect of two different roasting conditions.

The raw whole and roasted whole nuts were peeled manually to remove the testa. Raw and roasted whole cashew nuts, kernels, and recovered testa were ground separately using a coffee bean grinder (model CBG5 series, Black & Decker, Canada Inc., Brockville, ON, Canada) to obtain a fine powder which passed through mesh 16 (sieve opening 1 mm, Tylor test sieve, Mentor, OH). Each sample was defatted by blending with hexane (1:5, w/v, 5 min, three times) in a Waring blender (model 33BL73, Waring Products Division Dynamics Co. of America, New Hartford, CT) at ambient temperature (20 °C). Defatted samples were vacuum packed in polythene pouches and stored at −20 °C until used for extraction of phenolics.

Extraction of Soluble Phenolic Compounds. Preliminary studies showed that heating of ground samples under reflux conditions with 80% (v/v) ethanol afforded high total phenolic content and antioxidant activity. Therefore, refluxing conditions were used to extract soluble phenolic compounds.[11] Defatted meal (6 g) was mixed with 100 mL of 80% (v/v) ethanol and then placed in a thermostated water bath at 60 °C for 40 min. After centrifugation of the resulting slurry for 5 min at 4000*g* (IEC Centra MP4, International Equipment Co., Needham Heights, MA), the supernatant was collected and extraction was repeated two more times. Combined supernatants were evaporated in vacuo at 40 °C (Buchi, Flawil, Switzerland) and lyophilized for 72 h at −46 °C and 34×10^{-3} mbar (Freezone, model 77530, Labconco Co., Kansas City, MO). Residues of samples were air-dried for 12 h and stored at −20 °C until used to extract bound phenolic compounds within a week.

Extraction of Bound Phenolic Compounds. The sample residue obtained after the extraction of soluble phenolics, as explained above, was mixed with 50 mL of 4 M NaOH and hydrolyzed at room temperature for 4 h with stirring under a stream of nitrogen. The resulting slurry was acidified to pH 2 with 6 M HCl and extracted five times with hexane. Insoluble bound phenolic compounds were extracted five times with diethyl ether/ethyl acetate (1:1, v/v) and subsequently desolventized to dryness at room temperature using a rotary evaporator. Phenolic compounds were reconstituted in 6 mL of HPLC grade methanol and stored at −20 °C until used for further analysis within 2 weeks.

Determination of Total Phenolic Content (TPC). The contents of total phenolics of the extracts were determined according to the Folin−Ciocalteu reagent assay described by Singleton and Rossi[12] with some modifications as explained previously.[13] The crude extracts of soluble phenolic compounds were dissolved in methanol to obtain a concentration of 0.2 mg/mL. Folin−Ciocalteu's reagent (0.5 mL) was added to centrifuge tubes containing 0.5 mL of extracts. The contents were mixed thoroughly, and 1 mL of a saturated solution of sodium carbonate was added to each tube to neutralize the reaction. The volume was adjusted to 10 mL with distilled water, and the contents were thoroughly mixed by vortexing. Tubes were allowed to stand at ambient temperature in the dark for 35 min followed by centrifugation at 4000*g* for 10 min. The absorbance of the resulting blue color supernatant was read at 725 nm (model HP 8452A diode array spectrophotometer, Agilent Technologies, Palo Alto, CA) using appropriate blanks. The content of total phenolics in each extract was determined using a standard curve prepared for gallic acid and expressed as milligrams of gallic acid equivalents (GAE) per gram of defatted meal.

Determination of Proanthocyanidin Content (PC). Proanthocyanidin content of crude phenolic extracts of cashew was determined colorimetrically as described by Price et al.[14] To 1 mL of methanolic solution of the extract was added 5 mL of 0.5% vanillin−HCl reagent followed by incubation for 20 min at room temperature. A separate blank for each sample was read with 4% HCl in methanol. The absorbance was read at 500 nm, and the content of proanthocyanidins was expressed as micromoles of catechin equivalents (CE) per gram of defatted meal.

Determination of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Capacity Using Electron Paramagnetic Resonance (EPR) Spectrometry. The DPPH radical scavenging assay described by Shahidi et al.[11] was adapted with slight modifications. Two milliliters of DPPH in methanol (0.18 mM solution) was added to 500 μL of extracts dissolved in methanol. The contents were mixed well, and after 10 min, the mixture was passed through the capillary tubing that guides the sample through the sample cavity of a Bruker E-scan EPR spectrometer (Bruker E-scan, Bruker Biospin Co., Billercia, MA). The spectrum was recorded using the appropriate software (E-Scan analyzer, Bruker Biospin Co.). The parameters were set as follows: 5.02×10^2 receiver gain, 1.86 G modulation amplitude, 2.621 s sweep time, 8 scans, 100.000 G sweep width, 3495.258 G center field, 5.12 ms time constant, 9.795 GHz microwave frequency, 86.00 kHz modulation frequency, and 1.86 G modulation amplitude. DPPH radical scavenging capacities of the extracts were calculated by using the following equation: DPPH radical scavenging capacity (%) = 100 − [EPR signal intensity for the medium containing the additive/EPR signal intensity for the control medium] × 100.

Determination of Oxygen Radical Absorbance Capacity (ORAC). The ORAC was determined using a Fluostar Optima plate reader (BMG Labtech, Durham, NC) equipped with an incubator and two injector pumps with fluorecsein as the probe and AAPH as the radical generator. The reaction was carried out in 75 mM phosphate buffer (pH 7.0) using a final reaction mixture of 200 μL in a 96-well Costar 2650 black plate. Fluorescein (120 μL; 64 nM final concentration) was injected into the wells containing the extract using the injector pump. The mixture was incubated for 20 min at 37 °C in the built-in incubator, and subsequently APPH solution (60 μL; 29 mM final concentration), equilibrated at 37 °C, was rapidly injected into the wells. The plate was shaken for 4 s after each addition. To optimize the signal amplification to obtain maximum sensitivity, a gain adjustment was performed at the beginning by manually pipetting 200 μL of fluorescein into a designated well.

Table 1. Extract Yield, Total Phenolic Content, and Proanthocyanidin Content of Cashew Nut Kernel and Testa from Different Roasting Temperatures[a]

	soluble phenolics			bound phenolics		
processing condition	whole	kernel	testa	whole	kernel	testa
			Extract Yield (g/100 g of Defatted Meal)			
raw	27.2 ± 1.10 a	23.1 ± 1.20 a	42.9 ± 0.90 a	5.72 ± 0.02 a	0.18 ± 0.01 a	7.32 ± 0.15 a
LT treated	26.3 ± 1.20 a	25.3 ± 0.40 b	43.9 ± 1.10 a	0.62 ± 0.01 b	0.75 ± 0.02 a	8.98 ± 0.03 b
HT treated	27.0 ± 2.40 a	25.1 ± 0.30 b	44.2 ± 1.40 a	0.34 ± 0.01 c	3.59 ± 0.10 a	9.63 ± 0.12 c
			Total Phenolic Content (GAE mg/g of Defatted Meal)			
raw	07.01 ± 1.20 a	01.14 ± 0.43 a	269.05 ± 9.77 a	0.06 ± 0.01 a	0.028 ± 0.01 a	1.36 ± 0.10 a
LT treated	08.88 ± 0.19 a	04.89 ± 0.84 b	308.51 ± 9.35 a	0.16 ± 0.01 b	0.082 ± 0.01 b	4.26 ± 0.15 b
HT treated	30.24 ± 3.97 b	05.28 ± 1.00 b	347.99 ± 6.88 b	0.18 ± 0.01 b	0.089 ± 0.01 b	4.53 ± 0.12 b
			Proanthocyanidin Content (CE mg/g of Defatted Meal)			
raw	2.58 ± 0.04 a	0.11 ± 0.01 a	23.89 ± 0.50 a	0.03 ± 0.01 a	0.01 ± 0.01 a	0.31 ± 0.01 a
LT treated	1.53 ± 0.02 b	0.12 ± 0.01 a	22.64 ± 0.36 b	0.03 ± 0.01 a	0.01 ± 0.01 a	0.29 ± 0.01 b
HT treated	1.50 ± 0.01 b	0.13 ± 0.01 a	22.57 ± 0.45 b	0.03 ± 0.01 b	0.01 ± 0.01 b	0.21 ± 0.04 c

[a] Data are expressed as the mean ± SD ($n = 3$). Means ± SD followed by the same letter within a column are not significantly different ($p > 0.05$). GAE, gallic acid equivalents; CE, catechin equivalents; LT, low temperature; HT, high temperature.

Fluorescence was determined and recorded every minute for 60 min, and the antioxidant activity of the extracts was calculated as Trolox equivalents using a standard curve prepared with 1−10 μM.[13]

Determination of Hydroxyl Radical Scavenging Capacity. Hydroxyl radicals were generated via the Fe^{2+}-catalyzed Fenton reaction and spin-trapped with DMPO. The resultant 2-hydroxy-5,5-dimethyl-1-pyrrolidinyloxy (DMPO−OH) adduct was detected using a Bruker E-scan EPR. Cashew extracts were dissolved in deionized water and diluted to obtain various concentrations (1.33−13.2 mg/mL final concentrations). Extracts (100 μL) were mixed with 100 μL of 10 mM H_2O_2, 200 μL of 17.6 mM DMPO, and 100 μL of 1 mM ferrous sulfate. After 1 min, the mixtures were introduced into the EPR spectrometer, and the spectrum was recorded. Hydroxyl radical scavenging capacities of the extracts were calculated by using the following equation: hydroxyl radical scavenging capacity (%) = 100 − [EPR signal intensity for the medium containing the additive/EPR signal intensity for the control medium] × 100.

Determination of Trolox Equivalent Antioxidant Activity (TEAC). The TEAC assay was performed using a modified version of the method described by Chanrasekara and Shahidi.[13] The TEAC assay is based on the scavenging of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) radical anion ($ABTS^{\bullet-}$). A solution of $ABTS^{\bullet-}$ was prepared in 100 mM phosphate buffered saline (pH 7.4, 0.15 M sodium chloride) (PBS) by mixing the AAPH with 2.0 mM ABTS solution. The solution was heated for 16 min at 60 °C, covered in tin foil to protect it from light, and used within 2 h as the absorbance of the radical itself depletes with time. Extracts were dissolved in PBS at a concentration of 1 mg/mL and diluted accordingly to have them fit in the range of values in the Trolox standard curve. For measuring antioxidant capacity, 40 μL of the sample was mixed with 1.96 mL of the $ABTS^{\bullet-}$ solution. The absorbance of the above mixture was read at 734 nm at 0 and 6 min. The decrease in absorption at 734 nm after 6 min of addition of cashew extract was used for calculating the TEAC values. A standard curve was prepared by measuring the reduction in the absorbance of the $ABTS^{\bullet-}$ solution at different concentrations of Trolox. Appropriate blank measurements (decrease in absorption at 734 nm due to solvent without any compound added) were carried out and the values recorded. TEAC values were expressed as micromoles of Trolox equivalents per gram of defatted material.

Determination of Reducing Power. The reducing power of cashew extracts was determined using the method explained by Chandrasekara and Shahidi.[13] The assay medium contained 2.5 mL of extract (2 mg/mL) in 0.2 M PBS (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. After 20 min of incubation at 50 °C, 2.5 mL of 10% trichloroacetic acid (TCA) was added followed by centrifugation at 1750*g* for 10 min. The supernatant (2.5 mL) was transferred into a tube containing 2.5 mL of deionized water and 0.5 mL of 0.1% $FeCl_3$. The absorbance was measured at 700 nm, and the results were expressed as ascorbic acid equivalents using appropriate standard curves.

Analysis of Phenolic Compounds by High-Performance Liquid Chromatography (HPLC). The reversed phase HPLC (RP-HPLC) analysis of phenolics was carried out using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a G1311A quaternary pump, a G1379A degasser, a G1329A ALS automatic sampler, a G1330B ALS Therm, a G1316A Colcom column compartment, a G1315B diode array detector (DAD), and a system controller linked to Chem Station Data handling system (Agilent Technologies). For analytical work, dilute solutions of freeze-dried crude extracts (10 mg/mL) were passed through a 0.45 μm polytetrafluoroethylene (PTFE) membrane syringe filter (Whatman Inc., Florham Park, NJ), and 10 μL aliquots were injected onto a Superlcosil LC-18 column (4.6 × 250 mm, 5 μm; Merck, Darmstadt, Germany). A gradient profile using two solvents was applied at room temperature (25 °C), with solvent A (0.05% aqueous formic acid) and solvent B (methanol/acetonitrile 5:95, v/v) and a flow rate of 0.6 mL/min. Compounds of interest were detected on the basis of their characteristic UV−vis spectra (spectral range of 254−520 nm) and retention times. To confirm the identity of phenolic compounds, HPLC−mass spectrometry (MS) analysis was performed under the HPLC analytical conditions explained above using an Agilent 1100 series capillary liquid chromatography−mass selective detector (LC-MSD) ion trap system in electrospray ionization (ESI) negative ion mode. Complete system control and data evaluation were achieved with Agilent LC-MSD trap software (Agilent Technologies). An external standard method with authentic compounds was used for quantification of identified compounds.

Statistical Analysis. Results were expressed as the mean ± standard deviation (SD) of at least three independent experiments. Differences were estimated by the analysis of variance (ANOVA) followed by Tukey's "Honest Significant Difference" test. Differences were considered to be significant at $p \leq 0.05$. Correlation analysis was performed between phenolic contents and antioxidant activity of soluble and bound

extracts using Pearson correlation. All statistical analyses were performed using the free statistical software SPSS 13.0 version (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The yields of extracts of raw and LT- and HT-roasted whole cashew nut, cashew nut kernel, and cashew nut testa are shown in Table 1. The highest yields of the soluble and insoluble bound phenolic extracts of 44.2 ± 1.4 and 9.63 ± 0.1 g/100 g of defatted meal were afforded by the HT-processed cashew testa, respectively, whereas the lowest values of 23.1 ± 1.2 and 0.18 ± 0.001 g/100 g of defatted meal, respectively, were observed for raw cashew kernels. Higher yields were obtained for both soluble and bound extracts of the cashew nut testa. These results are in agreement with those of hazelnut kernel and byproducts.[11] To the best of our knowledge this is the first study that determines the soluble and insoluble bound phenolic contents and corresponding antioxidant activities of thermally processed cashew nuts and their testa as a byproduct.

Total Phenolic Contents (TPC). The contents of total phenolic compounds of soluble and insoluble bound cashew extracts obtained with different processing conditions are shown in Table 1. The data were expressed as milligrams of gallic acid equivalents (GAE) per gram of defatted meal. The phenolic contents of soluble and insoluble bound extracts of raw and LT- and HT-treated cashew nuts and testa ranged from 1.14 ± 0.43 to 348.99 ± 6.88 and from 0.03 ± 0.01 to 4.53 ± 0.12, respectively.

The results of this study showed that the contribution of bound phenolic fraction to the total phenolic content of the cashew nuts and testa was not prominent, and the values ranged from 0.5 to 2% and from 0.6 to 1.7% for raw and HT-treated samples, respectively. In contrast to the results obtained in this study, Yang et al.[1] reported a high contribution (72%) of insoluble bound form to the total phenolic content of cashew nut kernels. The exact reason for the observed difference is unknown, but the type of samples used and cultivar as well as cultivation practices might have contributed to these variations.

Raw cashew kernel showed the least TPC, whereas HT-treated testa showed the highest. In general, raw as well as LT- and HT-treated cashew testa showed higher TPC than that of kernel (Table 1). It is reported that the outer layers such as peels, shells, and hulls or skin of plant materials contain higher phenolic content, thus acting as defense substances against pathogens, parasites, and predators, as well as contributing to the color of plants.[15]

In this study, HT-treated soluble extracts of cashew nuts and testa showed a significant ($p \leq 0.05$) increase, which ranged from 29 to 372% in TPC compared with their raw counterparts. Furthermore, HT-treated bound extracts also showed significantly ($p \leq 0.05$) higher phenolic contents than their raw bound extract counterparts that ranged from 173 to 234%. The TPC of soluble extracts of LT-treated cashew nuts and testa ranged from 4.89 ± 0.84 to 308.52 ± 9.53 mg of GAE/g of defatted meal. The soluble extracts of LT-treated kernel and testa showed significantly ($p \leq 0.05$) higher TPC that ranged from 14 to 344% compared to their raw counterparts. In addition, bound extracts of LT-treated cashew nuts and testa showed significantly ($p \leq 0.05$) higher TPC compared to raw counterparts and the increment ranged from 153 to 223%.

According to the results obtained in the present study, thermal processing increased the TPC of cashew nuts and their testa. These results are in agreement with similar studies conducted using other types of nuts such as peanuts and hazelnuts.[16,17] Yu et al.[16] showed that roasting (175 °C for 5 min) increased the TPC of peanut skin by 40% compared to the raw peanut skin. According to Locatelli et al.[17] high roasting conditions (180 °C, 20 min) brought about higher TPC of the soluble extract than did medium roasting (180 °C, 10 min) of hazelnut skin. Talcott et al.[18] found that the TPC of peanuts (testa removed) increased or decreased depending on the cultivar upon roasting at 175 °C for 10 min.

In the present study, roasting at low and high temperatures resulted in higher TPC compared to raw cashew nuts and testa. This could be attributed to the liberation of cashew phenolics during roasting, which could be more soluble in ethanol; in this study ethanolic extracts were used to assess TPC with Folin–Ciocalteu reagent. Jeong et al.[19] showed that the content of phenolic compounds of defatted sesame meal extract submitted to different roasting temperatures increased, probably due to the release of bound phenolic compounds. On the other hand, during heat treatment, reaction between reducing sugars and amino acids, known as the Maillard reaction, can take place, thus leading to the formation of a variety of byproducts, intermediates, and brown pigments (melanoidins), which may contribute to the TPC, flavor, antioxidative activity, and color of food. The reaction is favored by low water activity during roasting of nuts, pulses, and seeds. The intermediate Maillard reaction products (MRPs) as well as the resultant melanoidins have high antioxidant activities, which are related to the presence of reductone-type structures.[20] Thus, in addition to phenolics, other compounds such as MRPs present in the extracts of roasted samples could interfere with the determination of TPC by Folin–Ciocalteu's assay, giving higher values compared to the raw samples in the present study.[21] Due to the fact that cashew nuts and their skins contain protein and sugars,[22,23] formation of MRPs in cashew skins and kernel during roasting is possible. It appears that roasting conditions as well as type of nut affect the TPC of the extracts of cashew skins. Monagas et al.[24] reported that the TPC values of roasted (145 °C, 30 min) peanut, hazelnut, and almond skins were 371, 315, and 134 mg of GAE/g of sample, respectively. In the present study HT-treated cashew skins showed 348 mg of GAE/g of defatted meal, which is in the range of the values reported by Monogas et al.[24]

Proanthocyanidins Content (PC). The proanthocyanidins, also known as condensed tannins, are flavan-3-ol oligomers/polymers, occurring in a wide variety of foods including berries, red wines, and nuts.[25] Venkatachalam and Sathe[23] reported that cashew contained 40 mg of tannins/100 g of edible nut.

PCs of different cashew extracts obtained from raw and samples roasted under different conditions are shown in Table 1. The PC of soluble and insoluble bound extracts ranged from 0.11 ± 0.001 to 23.89 ± 0.51 mg catechin equivalents (CE)/g of defatted meal and from 0.0016 ± 0.0001 to 0.3077 ± 0.0003 mg CE/g of defatted meal, respectively. In general, roasting significantly ($p \leq 0.05$) decreased the content of PC of soluble and bound extracts of cashew nuts as well as their testa, and this reduction ranged from 6 to 42% and from 7 to 34% for soluble and bound extracts, respectively.

The results of the present work demonstrated that heat processing decreased the content of assayable tannins ,and this may partly be due to the degradation of tannins. Tan et al.[26]

Table 2. Free Radical Scavenging Capacities of Extracts of Cashew Nut Kernel and Testa from Different Roasting Temperatures[a]

	soluble phenolics			bound phenolics		
processing condition	whole	kernel	testa	whole	kernel	testa
			DPPH Scavenging Activity (GAE mg/g of Defatted Meal)			
raw	65.35 ± 2.24 a	3.17 ± 0.15 a	179.29 ± 1.14 a	5.07 ± 0.32 a	0.13 ± 0.01 a	81.16 ± 5.38 a
LT treated	65.61 ± 1.10 a	36.92 ± 1.50 b	640.51 ± 38.20 b	4.95 ± 0.38 a	0.27 ± 0.04 b	73.32 ± 3.04 a
HT treated	74.86 ± 6.51 a	58.14 ± 2.84 c	708.49 ± 6.32 c	4.68 ± 0.45 a	0.12 ± 0.00 a	33.07 ± 1.65 b
			ORAC Activity (TE μmol/g of Defatted Meal)			
raw	14089 ± 1651 a	3207 ± 209 a	54171 ± 2900 a	0.002 ± 0.001 a	0.012 ± 0.001 a	0.026 ± 0.003 a
LT treated	14796 ± 366 a	3925 ± 173 a	62159 ± 1591 b	0.001 ± 0.001 a	0.025 ± 0.002 b	0.023 ± 0.001 a
HT treated	15207 ± 904 a	4136 ± 536 b	74088 ± 2956 c	0.016 ± 0.006 b	0.020 ± 0.002 c	0.046 ± 0.002 b
			OH Radical Scavenging (CE μmol/g of Defatted Meal)			
raw	19.69 ± 0.25 a	23.70 ± 0.88 a	1091.52 ± 71.7 a	68.75 ± 0.17 a	6.89 ± 0.00 a	679.14 ± 5.53 a
LT treated	18.50 ± 1.21 a	46.54 ± 3.56 b	1090.64 ± 72.7 a	68.97 ± 0.15 a	6.92 ± 0.02 a	684.67 ± 1.78 a
HT treated	18.73 ± 0.79 a	44.99 ± 1.17 b	1021.41 ± 91.7 a	69.44 ± 1.09 a	6.90 ± 0.02 a	684.24 ± 13.65 a

[a] Data are expressed as the mean ± SD ($n = 3$). Means ± SD followed by the same letter within a column are not significantly different ($p > 0.05$). GAE, gallic acid equivalents; CE, catechin equivalents; TE, Trolox equivalents; LT, low temperature; HT, high temperature.

reported that dry heat treatment of winged beans reduced the tannins levels by 56−75%. In accordance with the results of the present study, Sze-Tao et al.[27] also showed that tannins content of thermally processed (204 °C, 5 min) walnuts decreased by 14% compared to that of their unroasted counterparts. Furthermore, Gentile et al.[28] showed that roasting of pistachios decreased their proanthocyanidin content by 12% compared to the raw nuts.

DPPH Radical Scavenging Capacity. DPPH is a synthetic organic radical frequently used to evaluate antiradical properties of bioactive compounds and food extracts. It is more stable than common natural radicals and unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. The assay is based on the measurement of the reducing ability of antioxidants toward DPPH, which can be monitored by measuring the decrease in the absorption intensity of the EPR signal or absorption at 517 nm.

DPPH radical scavenging activity of raw and roasted cashew nuts and testa extracts is presented in Table 2. DPPH radical scavenging activity of soluble and insoluble bound extracts of raw cashew nuts and testa ranged from 3.17 ± 0.15 to 179.3 ± 1.14 mg of GAE/g of defatted meal and from 0.13 ± 0.01 to 81.16 ± 5.38 mg of GAE/g of defatted meal, respectively. The DPPH radical scavenging activity of soluble phenolic extracts of kernel and testa significantly ($p \leq 0.05$) increased with increasing roasting temperature,whereas bound extracts generally showed a decrease. The soluble extracts of HT-treated testa showed a higher DPPH radical scavenging activity than that of LT-treated testa. However, according to Locatelli et al.[17] there was no significant ($p \leq 0.05$) difference between the soluble extracts of medium- and high-temperature-treated hazelnut skin. This could be due to the varietal differences of nuts, the content of phenolics therein, and processing conditions employed.

The DPPH radical scavenging activity of soluble extracts of cashew nuts and byproducts highly correlated with TPC ($r^2 = 0.943$; $p < 0.0001$) and PC ($r^2 = 0.966$; $p < 0.0001$). Furthermore, DPPH radical scavenging activity of bound phenolic extracts positively and significantly correlated with their corresponding TPC ($r^2 = 0.999$; $p < 0.0001$) and PC ($r^2 = 0.997$; $p < 0.0001$). This study clearly demonstrated that roasting of cashew has a significant effect on the DPPH radical scavenging activity of the extracts of nuts and their testa, and this could be attributed to their phenolic contents as well as MRPs present.

Oxygen Radical Absorbance Capacity. The present study showed that ORAC values of soluble and insoluble bound phenolic fractions of cashew extracts were different and depended on whether raw or roasted nuts were considered (Table 2). The ORAC of soluble extracts of HT-treated cashew kernels and testa showed significantly ($p \leq 0.05$) higher values compared to their raw counterparts. The ORAC values obtained in the present study showed the same trend as TPC and PC. There was a strong positive relationship between TPC and ORAC ($r^2 = 0.977$; $p < 0.0001$) as well as PC and ORAC ($r^2 = 0.972$; $p < 0.0001$) in soluble extracts of cashew nuts and testa. According to Monogas et al.[33] ORAC values of roasted peanut, hazelnut, and almond skins were 13.3, 14.5, and 4.03 mmol of TE/g of sample. However, in the present study ORAC of HT-treated cashew testa showed a higher value (74 mmol of TE/g of defatted meal) than those reported in other studies for roasted nut skins. Davis et al.[29] reported a 14% higher ORAC value for whole roasted blanched peanuts than for the raw sample, whereas the findings of the present study showed an 8% higher ORAC value for HT-treated whole cashew nuts compared to their raw counterpart.

Hydroxyl Radical Scavenging Capacity. The hydroxyl radical is generated through Fenton reactions in the presence of Fe^{2+} and H_2O_2 and may be spin-trapped with DMPO due to the very short life of the radical. The DMPO adduct, a relatively stable free radical, can easily be detected using EPR spectroscopy.

The hydroxyl radical scavenging capacities of cashew extracts obtained under different processing conditions are shown in Table 2. The hydroxyl radical scavenging capacity of soluble and insoluble bound samples ranged from 18.5 ± 1.21 to 109.15 ± 7.17 mg of CE/g of defatted meal and from 6.89 ± 0.0 to 684.67 ± 1.78 mg of CE/g of defatted meal, respectively. Interestingly, roasting did not change the hydroxyl radical scavenging capacity of whole cashew nut and testa significantly ($p \leq 0.05$) except for the soluble extract of cashew kernel, which showed a 2-fold

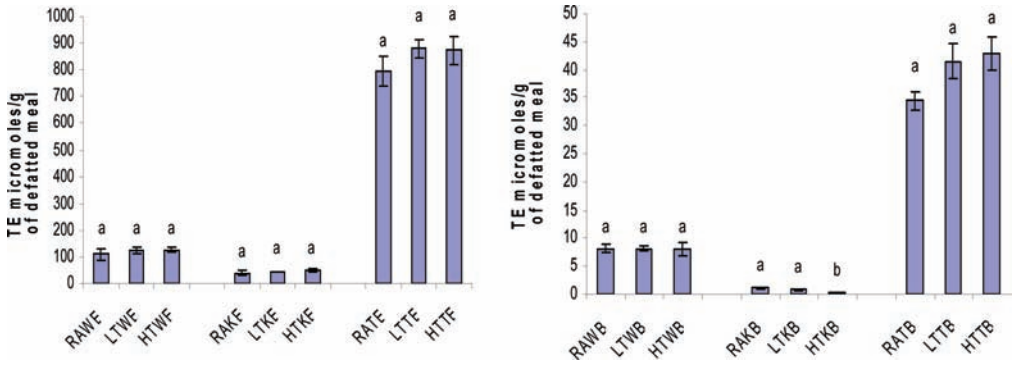

Figure 1. Trolox equivalent antioxidant activity of soluble and bound extracts of different cashew products. Data are expressed as the mean ± SD (n = 3). Means ± SD followed by the same letter,on bars are not significantly different ($p > 0.05$). TE, Trolox equivalents; RAWE, raw whole nut soluble; RAKE, raw kernel soluble; RATE, raw testa soluble; LTWE, low-temperature whole soluble; LTKE, low-temperature kernel soluble; LTTE, low-temperature testa soluble; HTWE, high-temperature whole soluble; HTKE, high-temperature kernel soluble; HTTE, high-temperature testa soluble; RAWB, raw whole nut bound; RAKB, raw kernel bound; RATB, raw testa bound; LTWB, low-temperature whole bound; LTKB, low-temperature kernel bound; LTTB, low-temperature testa bound; HTWB, high-temperature whole bound; HTKB, high-temperature kernel bound; HTTB, high-temperature testa bound.

increase compared to its raw counterpart. The present analysis showed that extracts of whole cashew nut and testa contained a higher amount of flavonoids such as (+)-catechin, (−)-epicatechin, and epigallocatechin, which may have prooxidative effects at high concentrations, especially in the presence of transition metal ions.[30] Thus, although the content of phenolic compounds of roasted cashew extracts increased, as determined by Folin−Ciocalteu's assay and HPLC analysis, all phenolics present may not contribute to hydroxyl radical scavenging activity in the extracts. However, as cashew kernel contained a comparatively low content of flavonoids, this effect may not be prominent and may exert high hydroxyl radical scavenging capacity as observed in the present study. To the best of our knowledge this is the first study that showed the effect of roasting on the hydroxyl radical scavenging activity of cashew nuts and testa.

Trolox Equivalent Antioxidant Activity. The ABTS solution is oxidized by an oxidizing agent, leading to the formation of $ABTS^{\bullet-}$, which is intensely colored. The antioxidative capacity of test compounds is assessed by measuring their ability to reduce the ABTS radical anion to its nonradical form. $ABTS^{\bullet-}$ can be generated chemically by oxidizing $ABTS^{2-}$ using ferrylmyoglobin, magnesium oxide, AAPH, and potassium persulfate or through enzymatic reactions. However, the use of oxidizing agents in the assay medium allows the antioxidant compounds to directly react with them, thus leading to erroneous estimations.

The TEAC values of tested cashew samples are summarized in Figure 1. The soluble extracts of LT-treated cashew testa yielded the highest TEAC value of 880 ± 33 μmol of TE/g of defatted meal, whereas that of raw kernel had the least value of 38.9 ± 1.0 μmol of TE/g of defatted meal. Consistent with the results obtained for other antioxidant assays employed in this study, the soluble extracts showed high TEAC values for whole cashew, kernel, and testa, which were 15, 57, and 21 times higher than these of their bound counterparts, respectively. Pellegrini et al.[31] reported that TEAC values of soluble extracts of hazelnuts, pistachios, almonds, and walnuts were 1.3, 1.5, 2.2, and 7 times higher than those of their bound extracts, respectively. The analyses in the present study are also in agreement with TEAC values of soluble extracts reported for cashew nuts thermally processed at 150 °C for 60 min.[32] In the present study, TEAC of HT-treated cashew kernels showed a 1.3 times higher value compared to the raw kernels. Acar et al.[32] also reported a 1.6 times increase in TEAC values of HT-treated cashew kernels compared to that of its unroasted counterparts. In addition, Yu et al.[16] reported that roasting increased the TEAC value of peanut skin compared to its raw counterpart due to the increase of phenolic content during thermal processing. The present analysis showed a direct strong relationship between TPC and TEAC (r^2 = 0.991; $p < 0.0001$) as well as PC and TEAC (r^2 = 0.993; $p < 0.0001$) of cashew extracts, further confirming the findings of others.[16,19]

Reducing Power. The reducing power of an extract serves as a good indicator of its antioxidative activity.[33] Ascorbic acid was used as a standard in this study, and the results were expressed as micromoles of ascorbic acid equivalents (AAE) per gram of defatted meal. Figure 2 depicts the reducing power of different cashew extracts examined in this study. Among the soluble extracts HT-treated cashew testa had the highest reducing power of 2394 ± 120 μmol of AAE/g of defatted meal, whereas raw kernel showed the least at 9.5 ± 0.23 μmol of AAE/g. Insoluble bound extracts of cashew kernels and byproduct showed a lesser reducing power compared to their soluble counterparts. The reducing power of the extracts followed a trend similar to that of TPC reported in this study. Furthermore, the correlation analysis showed a strong positive association between reducing power and TPC (r^2 = 0.972; $p < 0.0001$) and reducing power and PC (r^2 = 0.963; $p < 0.0001$) in this study. Thus, phenolics present in the extracts demonstrated a substantial reducing power due to their ability to donate electrons or to terminate radical chain reactions by converting free radicals to stable products. In addition, enhancement in the reducing power by roasting could be due to the formation of new reductones during heat treatment.

Phenolic Compounds in Soluble Cashew Extracts. Major phenolic acids and flavonoids identified in soluble extracts of raw and roasted cashew nuts and testa are presented in Table 3. On the basis of the results obtained for TPC, the contribution of bound phenolics was <1%. Therefore, no attempts were made to identify individual compounds in this fraction. In general, the predominant phenolic acids identified in cashew whole nuts and testa were syringic, gallic, and p-coumaric acids. However, this work showed that only trace amounts of syringic and p-coumaric

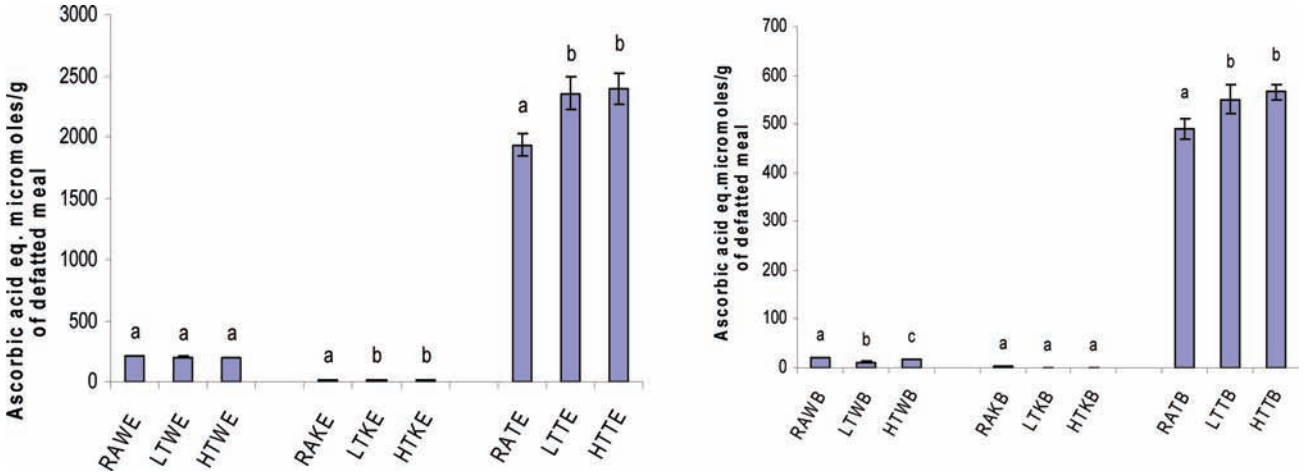

Figure 2. Reducing power of soluble and bound extracts of different cashew products. Data are expressed as the mean ± SD (n = 3). Means ± SD followed by the same letter on bars are not significantly different (p > 0.05). RAWE, raw whole nut soluble; RAKE, raw kernel soluble; RATE, raw testa soluble; LTWE, low-temperature whole soluble; LTKE, low-temperature kernel soluble; LTTE, low-temperature testa soluble; HTWE, high-temperature whole soluble; HTKE, high-temperature kernel soluble; HTTE, high-temperature testa soluble; RAWB, raw whole nut bound; RAKB, raw kernel bound; RATB, raw testa bound; LTWB, low-temperature whole bound; LTKB, low-temperature kernel bound; LTTB, low-temperature testa bound; HTWB, high-temperature whole bound; HTKB, high-temperature kernel bound; HTTB, high-temperature testa bound.

Table 3. Major Soluble Phenolic Compounds Identified from Cashew Nut Kernel and Testa from Different Roasting Temperatures[a]

processing condition	gallic acid	syringic	*p*-coumaric	(+)-catechin	(−)-epicatechin	epigallocatechin
			Cashew Whole (mg/g of dDefatted Meal)			
raw	0.108 ± 0.005 a	0.613 ± 0.001 a	0.099 ± 0.012 a	11.733 ± 0.254 a	7.429 ± 0.140 a	4.459 ± 0.123 a
LT treated	0.098 ± 0.000 b	0.483 ± 0.011 b	0.073 ± 0.002 b	9.608 ± 0.153 ab	6.083 ± 0.044 b	4.208 ± 0.087 b
HT treated	0.251 ± 0.000 c	0.867 ± 0.001 c	0.112 ± 0.002 a	15.646 ± 0.276 c	8.368 ± 0.001 c	6.544 ± 0.023 c
			Cashew Kernel (mg/g of Defatted Meal)			
raw	0.215 ± 0.002 a	tr	tr	0.702 ± 0.018 a	0.095 ± 0.007 a	1.640 ± 0.019 a
LT treated	0.037 ± 0.001 b	tr	tr	1.888 ± 0.007 b	0.257 ± 0.002 b	0.504 ± 0.008 b
HT treated	0.065 ± 0.002 c	tr	tr	2.912 ± 0.064 c	0.437 ± 0.009 c	0.481 ± 0.000 b
			Cashew Testa (mg/g of Defatted Meal)			
raw	0.361 ± 0.005 a	2.507 ± 0.009 a	0.252 ± 0.000 a	47.289 ± 3.760 a	28.291 ± 0.081 a	2.005 ± 0.061 a
LT treated	0.437 ± 0.001 b	2.800 ± 0.009 b	0.337 ± 0.001 b	45.235 ± 2.444 a	28.292 ± 0.086 a	2.251 ± 0.104 a
HT treated	0.974 ± 0.030 c	5.705 ± 0.000 c	0.693 ± 0.043 c	109.012 ± 0.932	77.045 ± 2.144 b	4.065 ± 0.159 b

[a] Data are expressed as the mean ± SD (n = 3). Means ± SD followed by the same letter within a column are not significantly different (p > 0.05). LT, low-temperature; HT, high-temperature; tr, trace.

acids were present in cashew kernels, whereas testa was a rich source of all three phenolic acids identified. The contents of syringic, gallic, and *p*-coumaric acids were 2.507, 0.361, and 0.252 mg/g of defatted raw testa meal, respectively. Thermal processing affected the content of phenolic acids present in cashew soluble extracts. Thus, HT-treated cashew kernels had a significantly ($p \leq 0.05$) lesser content of gallic acid compared to the raw kernel. Conversely, testa of HT-treated cashew nuts had about 3 times higher gallic acid content compared to its raw counterpart, suggesting liberation of gallic acid during heat processing. Pillai et al.[34] reported that cashew nut testa contained a considerable amount of hydrolyzable tannins. Thus, it is possible that roasting may yield gallic acid from hydrolyzable tannins present, leading to a higher content of it in the HT-treated testa as shown in the present study. Earlier, Shahidi et al.[11] reported five phenolic acids, namely, gallic, caffeic, *p*-coumaric, ferulic, and sinapic acids, in hazelnut kernel and its byproduct. In almond and its byproduct, Wijeratne et al.[35] showed the presence of caffeic, *p*-coumaric, ferulic, and sinapic acids. Nevertheless, the results of the present study showed that cashew nuts had syringic acid as a predominant phenolic acid in the samples tested. Senter et al.[36] reported the presence of 0.23 μg of syringic acid/g of extract of pine nuts. Walnuts also contained a considerable amount (34 mg/100 g kernel) of syringic acid.[37]

Major flavonoids identified in the present study were catechin, followed by epicatechin and epigallocatechin. The contents of catechin, epicatechin, and epigallocatechin in defatted meals of raw cashew nut kernel and testa were 0.70, 0.09, and 1.64 mg/g and 47.28, 28.29, and 2.0 mg/g, respectively. These results suggest that cashew testa, which is a byproduct of cashew

processing, has a significant importance due to its high content of polyphenolic compounds, including flavonoids. It is well established that flavonoids are effective natural antioxidants. In agreement with the present results, Mathew and Parpia[38] previously reported the presence of catechin and epicatechin as predominant polyphenolics in cashew testa. In general, HT-treated testa had a higher flavonoid content, which showed a 2−4-fold increase when compared to that in the raw testa. The results obtained in the HPLC analysis suggest liberation and isomerization of such compounds during heat treatment of cashew nuts and testa. This further lends support to the significant ($p \leq 0.05$) decrease in tannin content in HT-treated cashew testa compared to raw testa (Table 1). Furthermore, Yu et al.[39] showed that roasting decreased proanthocyanidin (trimers and tetramers) content of peanut skin and increased its monomers content when compared to the raw skin.

The results of the present study indicate that cashew nut kernels and testa contain phenolic compounds that are responsible for a wide array of antioxidant activities. In addition, phenolic extracts from cashew nut kernels and testa were evaluated for their antioxidant activities in several food and biological model systems as communicated elsewhere.[40] The contribution of bound fraction is insignificant ($p \leq 0.05$) compared to the soluble phenolic fraction of cashew nuts and testa. The HT-treated cashew nuts and testa showed a higher phenolic content and antioxidant activity than LT-treated samples. Overall, the findings of this study suggest the notion that thermal processing enhances the antioxidant value of cashew kernels. Furthermore, it is noteworthy that cashew testa, a byproduct with high phenolic content exhibiting excellent antioxidant properties, can be utilized as a health-promoting and disease-preventing ingredient.

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