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Effect of Processing on the Phytochemical Profiles and Antioxidant Activity of Corn for Production of Masa, Tortillas, and Tortilla Chips

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The phytochemical profiles (total phenolics, anthocyanins, ferulic acid, carotenoids) and antioxidant activities of five types of corn (white, yellow, high carotenoid, blue, and red) processed into masa, tortillas, and tortilla chips were studied. The nixtamalization process significantly ($p < 0.05$) reduced total phenolics and antioxidant activities when compared to raw grains. Nixtamalized grains exhibited higher concentration of free phenolics and soluble conjugated ferulic acid and had lower concentrations of bound phenolics and ferulic acid than unprocessed grains. Among processed products, there was little difference in the phytochemical contents and antioxidant activities. Among types of corn, the highest concentrations of total phenolics, ferulic acid, and antioxidant activity were observed in the high-carotenoid genotype followed by the regular yellow counterpart. The white corn contained the lowest amount of total phenolics and antioxidant activity. The pigmented blue corn had the highest anthocyanin concentration followed by the red counterpart. These findings suggest that lime-cooking significantly reduced the phytochemical content of nixtamalized products but released phenolics and ferulic acid.

KEYWORDS: Nixtamalization; corn; masa; tortilla; tortilla chip; phenolic; antioxidant; ferulic acid; carotenoids; anthocyanins; thermal processing

INTRODUCTION

Table tortillas are the staple food of Mexico and Central America, and corn and tortilla chips have become the most important salted snack food in the world. Sales of these snacks in the United States in 2004 totaled more than $5 billion (*1*). Table tortillas and tortilla chips are obtained after a thermal−alkaline treatment, or nixtamalization process, of the corn kernels. This treatment causes profound changes in the structure, chemical composition, and nutritional value of the foods. One of the changes is the partial removal of the pericarp or bran due to alkali treatment, nixtamal washing, and handling (*2*), so that the finished products are considered as semi-whole grain foods. This is important because whole grain consumption has been associated with the prevention of cardiovascular disease (CVD), type 2 diabetes, and some cancers (*3*, *4*). In addition, the new USDA Dietary Guidelines for Americans emphasizes the need for consumption of whole grains. The phytochemicals present in whole grains are mainly in bound form (*5*−*7*). Ferulic acid is one of the important phytochemicals in corn and other cereal grains and is present mainly in bound form in corn (*5*−*9*). A previous study by our laboratory demonstrated that thermal processing significantly increased the free phytochemical content and antioxidant activity of sweet corn (*8*).

Corn is the most produced cereal in the world with a global production that exceeds 721 million metric tons (*10*). Corn has a higher antioxidant capacity when compared to wheat, oat, and rice (*5*−*7*). Table tortillas and fried tortilla chips are obtained from either fresh masa or dry masa flour. Both products are obtained after lime-cooking and steeping followed by nixtamal washing, which aims to remove excess lime and adhered pericarp tissue. The cooking liquor, called nejayote, is rich in solids including important phytochemicals that leach during cooking (*11*). Lime-cooking increases calcium content, improves niacin bioavailability, removes most of the pericarp, and significantly reduces mycotoxins present in raw kernels (*12*). In Mexico, most of the dietary calcium is provided by tortillas and related products (*12*, *13*). The nixtamalization process affects pericarp removal and functional properties of the cooked grain via starch and protein modifications. Nutritionally, lime-cooking also affects the quality of protein, the amount of resistant starch, and concentrations of anthocyanins, vitamins, minerals, and phytic acid. However, little is known about the effect of nixtamalization, tortilla baking, and tortilla chip frying on the fate of phytochemicals and antioxidant activity of nixtamalized products, masa, tortillas, and tortilla chips (*12*, *14*−*17*).

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The objective of this study was to examine the effect of nixtamalization, tortilla baking, and tortilla chip frying on the fate and content of total phenolics, anthocyanins, ferulic acids (free, soluble-conjugated, and insoluble-bound), carotenoid content, and the lipophilic and hydrophilic antioxidant activity of five contrasting corn genotypes. Whole grains, masa, table tortillas, and tortilla chips were studied for all five corn genotypes.

MATERIALS AND METHODS

Chemicals and Reagents. Folin−Ciocalteu reagent, hydrochloric acid, ferulic acid, lutein, ascorbic acid, dichlorofluorescin diacetate, α-tocopherol, and gallic acid were obtained from Sigma Chemical Co. (St. Louis, MO). Zeaxanthin and β-cryptoxanthin were purchased from Indofine Chemical Co. Inc. (Hillsborough, NJ). Randomly methylated β-cyclodextrin was from Cyclodextrin Technologies (High Springs, FL). 2,2′-Azobis(amidinopropane) was purchased from Wako Chemicals (Richmond, VA). Sodium hydroxide, potassium hydroxide, hexane, magnesium carbonate, and acetonitrile were obtained from Fisher Scientific (Pittsburgh, PA). Ethyl acetate, trifluoroacetic acid, and ethanol were purchased from Mallinckrodt (Paris, KY). All reagents used were of analytical grade.

Corn Types. The five contrasting types of corn used in the present study were white, yellow, high-carotenoid, blue, and red corns. White (Asgrow 902W) and commercial yellow corns, which are preferred and commonly used by the tortilla industry, were used as controls. The whole grain corn was provided by the Texas Agriculture Experiment Station corn-breeding program led by Dr. Javier Betran (College Station, TX). The corn samples were milled to a fine powder through a 60-mesh screen. All samples were stored at −20 °C until use. The moisture content was determined in all samples to express the results on a dry weight basis.

Lime-Cooking and Production of Masa, Tortillas, and Tortilla Chips. The optimum lime-cooking time for each type of corn was determined according to preliminary cooking trials in which 100 g samples contained in nylon bags were lime-cooked for 0, 15, 30, and 45 min (*2*, *13*). Linear regression equations were calculated to predict optimum cooking. The optimum nixtamal moisture or water uptake was the cooking time sufficient to increase nixtamal moisture to 48%.

Three kilograms of each type of corn was lime-cooked with 9 L of water containing 30 g of lime. The cooking times for the white, yellow, high-carotenoid, blue, and red corns were 32.7, 24.1, 52.5, 53.5, and 41.1 min, respectively. The cooked kernels were allowed to steep for 12 h. Resulting nixtamals were washed with tap water and subsequently stone-ground using a commercial mill into a coarse masa suitable for the production of tortilla chips (*18*). Water was used during grinding to increase the masa moisture to approximately 56% and to cool the temperature generated during milling. Resulting masas were continuously formed into tortilla disks and baked on a triple-pass gas-fired baking oven operating at an average temperature of 280 °C for 60 s. After baking, the resulting tortillas were allowed to equilibrate to room temperature for at least 30 min and fried at 175 °C for 1 min using a Hobart fryer (*19*). Chips were blotted between paper towels to remove excess oil and then allowed to cool for 20 min. Samples of raw corn kernels, masas, tortillas, and tortilla chips were dried at 60 °C and ground to pass though a 60-mesh screen. The samples were stored at −20 °C until use. The moisture content was determined in all samples to express the results on a dry weight basis.

Extraction of Free Phenolics. Free phenolic compounds were extracted using the method previously reported by our laboratory (*5*−*8*). Briefly, 5 g of corn was blended with 20 mL of 80% chilled ethanol for 10 min and then centrifuged at 2500*g* for 10 min. The supernatant was removed, and the extraction was repeated. The supernatant was evaporated to 5 mL under vacuum at 45 °C and reconstituted with water to a final volume of 10 mL. The extracts were stored at −40 °C until used.

Extraction of Bound Phenolics. Bound phenolic compounds of corn and their products were extracted according to the method reported previously (*5*−*7*). For each raw corn or corn product, 1 g of sample was extracted twice with 80% ethanol and the supernatant discarded. The residue of the extraction was digested with 20 mL of 2 M sodium hydroxide at room temperature; the O_2 was removed with nitrogen gas, and the sample was shaken for 1 h. The mixture was neutralized with 4 mL of hydrochloric acid and extracted with hexane to remove lipids. The final solution was extracted five times with ethyl acetate. The ethyl acetate fraction was evaporated to dryness. Phenolic compounds were reconstituted in 10 mL of distilled water. The extracts were stored at −40 °C until used.

Extraction of Soluble-Conjugated Ferulic Acid. Soluble-conjugated ferulic acid was extracted using the method reported previously by our laboratory (*5*, *6*). Briefly, soluble conjugated ferulic acid was extracted from a 0.5 mL aliquot of free phenolic extract. The free phenolic extract was digested with 2 M NaOH for 1 h under nitrogen gas. The solution was neutralized with HCl and then was extracted five times with ethyl acetate. The ethyl acetate was evaporated to dryness at 35 °C under nitrogen gas. Ferulic acid was recovered in aqueous 20% (v/v) acetonitrile and quantified via HPLC (*7*).

Extraction of Carotenoids. Carotenoids of samples were extracted using the method reported previously from our laboratory (*6*, *7*). Briefly, a 1 g sample was mixed with 0.06 g of magnesium carbonate and extracted with 3 mL of methanol/tetrahydrofuran (1:1, v/v) solution at 75 °C for 5 min. The organic phase was removed after centrifugation at 2500*g* for 6 min. The extraction was repeated twice. The resulting organic fractions were dried with anhydrous sodium sulfate and evaporated to dryness under nitrogen gas at 35 °C. The residue was dissolved in 0.5 mL of methanol/tetrahydrofuran (1:1, v/v) for RP-HPLC analysis (*6*, *7*).

Determination of Total Phenolics. The total phenolic content of sample extracts was determined using the Folin−Ciocalteu colorimetric method described previously by Singleton et al. (*20*) and modified in our laboratory (*8*). Briefly, the appropriate dilutions of extracts were oxidized with the Folin−Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. After 90 min, absorbance was measured at 760 nm using an MRX II Dynex Microplate Reader (Dynex Technologies, Inc., Chantilly, VA). Gallic acid was used as standard, and the total phenolic content was expressed as mean (micrograms of gallic acid equivalents per 100 g of dry weight of sample) ± SD for triplicates.

Anthocyanin Analysis. Total anthocyanin was determined using the spectrophotometric method adapted from that of Abdel-Aal and Huel (*21*). A 0.5 g corn sample was weighed in a 50 mL centrifuge tube, and 10 mL of chilled, acidified methanol (95% methanol and 1 N HCl 85:15, v/v) was added. The tube was flushed with nitrogen gas, agitated for 30 min, and then centrifuged at 3000*g* for 10 min, and the supernatant was collected. Absorbance of the solution was measured immediately at 535 and 700 nm. Anthocyanin content was calculated by

$$C = [(A_{535\text{nm}} - A_{700\text{nm}})/\epsilon] \times (\text{total vol of extract}) \times \text{MW} \times (1/\text{sample wt})$$

where C is the concentration of total anthocyanin (mg of cyanidin 3-glucoside equivalents per g of sample), ϵ is the molar absorptivity (cyanidin-3-glucoside = 25965/cm/M), and MW is the molecular weight of cyaniding-3-glucoside, 449.2 (*21*).

Analysis of Ferulic Acid. Ferulic acid was determined using the method reported previously from our laboratory (*5*, *6*). Briefly, ferulic acid in sample extracts was quantified using RP-HPLC equipped with a 250 mm × 4.6 mm, 3 μm Supelcosil LC-18-DB column with isocratic elution of 20% (v/v) acetonitrile in water adjusted to pH 2 with trifluoroacetic acid at a flow rate of 0.6 mL/min. Detection was at a wavelength of 280 nm with a Waters 484 UV−visible detector (Waters Corp., Milford, MA). Detector signals were acquired and integrated by Waters Millenium software. Peak identification of free ferulic acid in sample extracts was based on retention time and chromatography of an authentic ferulic acid standard. Ferulic acid concentrations in samples were calculated by extrapolation on a calibration curve. Twenty microliter injections were used for all analyses.

Carotenoid Analysis. Carotenoids were quantified according to a previously reported method (*6*, *7*) using RP-HPLC employing a 250

× 4.6 mm, 3 μm YMC Carotenoid C30 column (Waters Corp.). Mobile phases used were solvent A [methanol/water (95:5, v/v)] and solvent B [(methyl *tert*-butyl ether (MTBE)]. Isocratic elution was performed with 75% solvent A and 25% solvent B, delivered by two Waters 510 HPLC pumps at a flow rate of 1.9 mL/min. A Waters 2487 dual-wavelength absorbance detector was used for analyte detection at 450 nm (*6*, *7*). Carotenoid concentrations were extrapolated from pure carotenoid standard curves. Lutein, zeaxanthin, β-cryptoxanthin, and β-carotene were used as standards. All analyses were performed using 20 μL injections, and peak heights were used for all calculations.

Antioxidant Activity. Hydrophilic and lipophilic antioxidant capacities were measured using the peroxy radical scavenging capacity (PSC) assay (*22*). This assay is based on the degree of inhibition of dichlorofluorescin oxidation by antioxidants that scavenge peroxyl radicals, generated from the thermal degradation of 2,2-azobis-(amidinopropane). Just prior to use in the reaction, 80 μL of 2.48 mM dichlorofluorescin diacetate was hydrolyzed with 900 μL of 1.0 mM KOH for 3−5 min to remove the diacetate and then diluted to 6 mL total volume with 75 mM phosphate buffer (pH 7.4). 2,2′-Azobis-(amidinopropane) (200 mM) was prepared fresh in 75 mM phosphate buffer. Aliquots of 100 μL of corn extracts diluted in 75 mM phosphate buffer (pH 7.4) were transferred into 96-plate wells, and 100 μL of dichlorofluorescin was added. The 96-well plate was loaded into the plate holder for the Fluoroskan Ascent fluorescent spectrophotometer (Thermo Labsystems, Franklin, MA), and the solution was mixed by shaking at 1200 rpm for 20 s. The reaction was then initiated by adding 50 μL of 2,2′-azobis(amidinopropane) from the autodispenser on the equipment. The reaction was carried out at 37 °C, and the fluorescence was measured at 485 nm excitation and 538 nm emission with the fluorescent spectrophotometer. The areas under the average fluorescence−reaction time kinetic curve (AUC) for both control and samples were integrated and used for calculating antioxidant activity with the equation

$$\text{PSC unit} = 1 - (\text{SA}/\text{CA})$$

where SA is the AUC for the sample or standard dilution and CA is the AUC for the control reaction using buffer alone. The median effective concentration (EC_{50}) was determined from the dose−response curve of concentration. Results were expressed as micromoles of vitamin C equivalents per 100 g of dry weight of sample. The lipophilic PSC assay was performed by first solubilizing lipophilic compounds with 12% (v/v) randomly methylated β-cyclodextrin in 50% (v/v) acetone in water. The fluorescent dye was prepared by hydrolyzing 11 μL of 2.48 mM dichlorofluorescin diacetate with 1.0 mL of 1.0 mM KOH and then diluted to a total volume of 8 mL with 75 mM phosphate buffer (pH 7.4). The reaction mix of the lipo-PSC assay contained 100 μL of appropriate dilutions of extracts in 12% randomly methylated β-cyclodextrin, 100 μL of dichlorofluorescin, and 50 μL of 200 mM 2,2′-azobis(amidinopropane). The reaction of the lipo-PSC assay was completed in 45 min, and the AUC was calculated as described above. Results obtained for lipophilic antioxidant activity for sample extracts were expressed as micromoles of vitamin E equivalents per 100 g of dry sample weight.

Statistical Analysis. Data were analyzed and reported as mean ± SD in triplicate. ANOVA was used, and differences among treatments were determined using Fisher's pairwise comparison tests run on Minitab release 12 software (State College, PA).

RESULTS

Phenolic Content. The total phenolic content of white, yellow, red, blue, and high-carotenoid raw corns, masa, tortillas, and tortilla chips is presented in **Table 1**. The range of total phenolic content of raw corn was from 243.8 ± 4.6 to 320.1 ± 7.6 mg/100 g of dry weight of sample. The range of total phenolic content for masa was from 125.3 ± 2.8 to 198.3 ± 1.4 mg/100 g. The range of total phenolic content for tortillas was from 136.5 ± 2.9 to 207.3 ± 1.3 mg of gallic acid/100 g of dry weight of sample, and the range for tortilla chips was from 111.7 ± 1.9 to 155.0 ± 1.9 mg of gallic acid/100 g of dry weight of sample. There was a significant reduction in phenolic content when corn was nixtamalized or lime-cooked. However, the difference between masa and tortillas was not significant ($p > 0.05$), indicating that the main losses were incurred during lime-cooking and steeping. The high-carotenoid raw corn contained the highest phenolics, whereas the white and red corns contained the lowest. The differences between them were statistically different ($p < 0.05$). Free phenolic content was not greatly affected during the process of tortilla baking and chip frying. Most of the phenolics in corn were bound. Bound phenolics of masa, tortillas, and chips were approximately half of the total phenolic compound in the raw corn. The content of bound phenolics of chips was statistically lower ($p < 0.05$) than the bound phenolic content of the other nixtamalized products masa and tortillas.

Table 1. Total Phenolic Content of Raw Corns and Their Nixtamalized Corn Products (Masa, Tortilla, and Chips) (Mean ± SD, $n = 3$)

type of corn	sample/product	total phenolics[a] (mg of gallic acid equiv/100 g of dry wt) free	bound	total
white	corn	34.7 ± 0.4 b	226.0 ± 6.3 b	260.7 ± 6.1 c
	masa	39.1 ± 0.9 ab	126.1 ± 3.1 d	165.2 ± 4.0 f
	tortilla	47.2 ± 1.8 a	119.0 ± 6.2 d	166.2 ± 6.2f
	chips	46.3 ± 2.7 a	97.3 ± 3.3 e	143.6 ± 2.1 g
yellow	corn	43.6 ± 1.8 ab	242.2 ± 13.1 b	285.8 ± 14.0 b
	masa	41.5 ± 0.6 ab	140.7 ± 2.6 d	182.2 ± 2.4 f
	tortilla	51.1 ± 1.3 a	132.2 ± 1.6 d	183.3 ± 1.4 f
	chips	43.1 ± 0.9 ab	102.1 ± 2.0 e	145.2 ± 1.2 g
red	corn	38.2 ± 0.4 ab	205.6 ± 4.5 c	243.8 ± 4.6 d
	masa	28.0 ± 0.8 c	97.2 ± 3.6 e	125.3 ± 2.8 h
	tortilla	30.5 ± 0.7 bc	106.0 ± 3.6 e	136.5 ± 2.9 g
	chips	26.4 ± 0.8 c	85.3 ± 1.8 f	111.7 ± 1.9 i
blue	corn	45.5 ± 0.5 a	220.7 ± 0.5 b	266.2 ± 0.7 c
	masa	30.3 ± 1.1 bc	128.2 ± 1.7 d	158.5 ± 1.2 g
	tortilla	39.1 ± 1.5 ab	122.7 ± 0.6 de	161.8 ± 2.1 g
	chips	41.4 ± 1.6 ab	95.5 ± 1.1 e	136.9 ± 1.2 g
high carotenoid	corn	50.0 ± 2.5 a	270.1 ± 9.4 a	320.1 ± 7.6 a
	masa	40.3 ± 1.0 ab	158.0 ± 0.8 d	198.3 ± 1.4 e
	tortilla	53.0 ± 1.3 a	154.5 ± 0.6 d	207.5 ± 1.3 e
	chips	46.4 ± 0.7 a	108.6 ± 2.2 e	155.0 ± 1.9 g

[a] Values with no letters in common are significantly different ($p < 0.05$).

Ferulic Acid. Lime-cooking, tortilla baking, and tortilla chip frying increased the amount of free and soluble conjugated ferulic acid (**Table 2**). The high-carotenoid corn contained the highest amount of total ferulic acid ($p < 0.05$). Lime-cooking caused a significant increase in the amount of both free and soluble conjugated ferulic acid. Bound ferulic acid content was significantly decreased in all corn types. Approximately 94−98% of the total ferulic acids were in bound form in raw kernels. Among nixtamalized products, tortillas generally had the highest concentration of free and conjugated ferulic acid.

Anthocyanin Content. The anthocyanin content of corn and processed samples is presented in **Table 3**. Most anthocyanins were lost during lime-cooking by leaching into the steep solution or nejayote. There were significant differences in anthocyanin content among types of corn; blue corn contained the highest amount (36.9 mg of cyanidin-3-glucoside equiv/100 g, dry weight basis), followed by red corn (9.75 mg of cyanidin-3-glucoside equiv/100 g, dry weight basis). The anthocyanin amounts of the remaining types of corn were low (0.57−4.63 mg of cyanidin-3-glucoside equiv/100 g, dry weight basis). Lime-cooked blue and red corn kernels lost approximately 80 and 23% of the anthocyanins present in their respective raw

Table 2. Ferulic Acid Content and Percentage Contribution of Raw Corns and Their Nixtamalized Corn Products (Masa, Tortilla, and Chips) (Mean ± SD, $n = 3$)

		ferulic acid content[a] (μg of ferulic acid/100 g of dry wt of sample)			
type of corn	sample/ product	free	soluble conjugated	bound	total
white	corn	495 ± 18 f (0.41)[b]	756 ± 73 d (0.63)	119201 ± 11173 d (98.96)	120453 b
	masa	7558 ± 757 e (17.90)	14201 ± 1305 c (33.63)	20470 ± 1617 d (48.47)	42229 d
	tortilla	9988 ± 495 d (11.73)	23267 ± 1832 b (27.32)	51905 ± 3582 c (60.95)	85160 c
	chips	6643 ± 832 e (10.94)	15989 ± 1000 c (26.34)	38070 ± 4772 cb (62.72)	60702 d
yellow	corn	645 ± 14 f (0.63)	1474 ± 102 d (1.43)	100849 ± 5034 b (97.94)	102968 c
	masa	12596 ± 964 c (16.17)	20747 ± 1331 cb (26.64)	44527 ± 3083 cd (57.18)	77870 cd
	tortilla	17462 ± 668 b (15.28)	27827 ± 2500 b (24.34)	69025 ± 3850 c (60.38)	114314 bc
	chips	13237 ± 551 c (14.67)	25860 ± 1861 b (28.66)	51142 ± 2377 c (56.67)	90239 c
red	corn	588 ± 21 f (0.45)	1259 ± 48 d (0.97)	128450 ± 11553 b (98.58)	130297 b
	masa	6224 ± 364 e (12.49)	20747 ± 1331 cb (41.63)	22865 ± 3303 d (45.88)	49836 d
	tortilla	8202 ± 455 ed (11.11)	15588 ± 720 c (21.11)	50036 ± 7934 c (67.78)	73826 cd
	chips	8083 ± 572 ed (10.72)	29391 ± 2084 b (39.0)	37890 ± 4177 cd (50.28)	75364 cd
blue	corn	683 ± 47 f (0.53)	1451 ± 27 d (1.12)	127851 ± 8094 b (98.35)	129985 b
	masa	10220 ± 1370 ed (19.17)	19760 ± 1145 cb (37.07)	23330 ± 1917 d (43.76)	53310 d
	tortilla	17587 ± 1259 b (17.35)	31746 ± 2519 b (31.32)	52030 ± 1499 c (51.33)	101363 c
	chips	14360 ± 965 c (16.77)	28163 ± 2548 b (32.88)	43129 ± 2172 cd (50.35)	85652 c
high carotenoid	corn	970 ± 82 f (0.64)	1965 ± 33 d (1.28)	150077 ± 12419 a (98.08)	153012 a
	masa	11170 ± 267 ed (14.65)	24450 ± 654 b (32.08)	40610 ± 3798 cb (53.27)	76230 cd
	tortilla	21566 ± 505 a (15.79)	37743 ± 1295 a (27.63)	77307 ± 6570 c (56.58)	136616 b
	chips	13963 ± 747 c (13.10)	29931 ± 2169 c (28.08)	62714 ± 3322 c (58.82)	106608 c

[a] Values with no letters in common are significantly different ($p < 0.05$). [b] Percent contribution to total.

Table 3. Anthocyanin Content of White, Yellow, Red, Blue, and High-Carotenoid Corns and Their Nixtamalized Corn Products (Masa, Tortilla, and Chips) (Mean ± SD, $n = 3$)

	anthocyanin content[a] (mg of cyanidin-3-glucoside equiv/100 g of dry wt of sample)			
type of corn	corn	masa	tortilla	chips
white	1.33 ± 0.02 e	0.28 ± 0.01 f	0.48 ± 0.01 f	0.51 ± 0.01 f
yellow	0.57 ± 0.01 f	0.31 ± 0.01 f	0.29 ± 0.00 f	0.36 ± 0.01 f
red	9.75 ± 0.44 b	2.21 ± 0.07 e	2.08 ± 0.05 e	2.41 ± 0.04 e
blue	36.87 ± 0.71 a	2.63 ± 0.12 e	3.81 ± 0.11 d	3.29 ± 0.10 d
high carotenoid	4.63 ± 0.06 c	0.56 ± 0.01 f	0.68 ± 0.02 f	0.97 ± 0.02 f

[a] Values with no letters in common are significantly different ($p < 0.05$).

kernels. Differences among masa, tortillas, and tortilla chips were not significant ($p > 0.05$).

Carotenoids. The carotenoid content of raw corns and their nixtamalized products is presented in **Table 4**. Lutein concentration was highest in yellow corn (406.2 ± 4.9 μg of lutein/100 g, dry weight basis) and lowest in the blue and white corns (5.17 ± 0.49 and 5.73 ± 0.18 μg of lutein/100 g, dry weight basis, respectively). Lime-cooking significantly decreased the lutein content in yellow, red, and high-carotenoid corns. Further processing into tortillas and tortilla chips did not significantly affect ($p > 0.05$) lutein concentration, except for yellow corn chips. No impact of lime-cooking or of further processing was observed with white and blue corns (**Table 4**). Zeaxanthin content was highest in yellow corn (353.2 ± 23.1 μg/100 g, dry weight basis) and high-carotenoid corn (322.3 ± 10.7 μg/100 g, dry weight basis). As expected, white corn contained low levels of zeaxanthin, 6.01 ± 0.06 μg/100 g, dry weight basis. Amounts of zeaxanthin were similar among masa, tortillas, and chips for white, red, blue, and high-carotenoid corns. Levels of zeaxanthin declined in yellow corn after lime-cooking as well as after baking; frying further reduced the levels of zeaxanthin.

High-carotenoid, yellow, and red corns contained the highest amounts of β-cryptoxanthin (23.11 ± 1.01, 19.10 ± 1.23, and 13.10 ± 1.82 μg/100 g, dry weight basis, respectively). High-carotenoid and yellow kernels contained the highest amounts of β-carotene. The main β-carotene losses occurred during lime-cooking. Interestingly, only the high-carotenoid tortilla chips contained significant amounts of β-carotene. For the remaining types of corn, the amount of β-carotene in tortilla chips was not detectable. As expected, the white corn kernels contained the lowest amounts of β-carotene.

Antioxidant Activity. Figures 1 and **2** show the hydrophilic and lipophilic antioxidant activities of free and bound phenolics of the raw corns and their nixtamalized products as micromoles of vitamin C or E per 100 g of sample, respectively. It was clear that bound phenolics were the primary contributors of antioxidant activity, consistent with the previous observations for total phenolics and ferulic acid content (*5*−*7*). The hydrophilic antioxidant activity of free phenolics significantly increased ($p > 0.05$) after nixtamalization. The range of antioxidant activity for raw grains was from 41.5 ± 2.9 to 49.6 ± 4.40 μmol of vitamin C equiv/100 g. High-carotenoid corn contained the highest antioxidant capacity. The values for masa, tortillas, and chips varied from 68.0 to 114.1 μmol of vitamin C/100 g. The hydrophilic antioxidant activity in the bound form, however, decreased after lime-cooking. The activity decreased approximately 30% in masa and more than 50% in tortilla chips, when compared to raw corn. The hydrophilic antioxidant activity represented ~98% of the total antioxidant activity. The free lipophilic antioxidant activity also increased when products were lime-cooked and processed into tortillas and tortilla chips.

The values of lipophilic antioxidant activity in free form for the high-carotenoid raw corn, masa, tortillas, and tortilla chips were 1.3 ± 0.04, 1.9 ± 0.1, 2.8 ± 0.2, and 2.3 ± 0.1 μmol of vitamin E equiv/100 g. The loss of lipophilic antioxidant activity during lime-cooking for the bound phenolics was ~75%. High-carotenoid corn contained the highest antioxidant capacity expressed as micromoles of vitamin E equivalants per 100 g. Values of lipophilic antioxidant activity in bound form for raw corn, masa, tortillas, and chips were 26.2 ± 0.4, 6.1 ± 0.5, 7.5 ± 0.5, and 4.5 ± 0.1 μmol of vitamin E equiv/100 g, respectively.

Table 4. Carotenoid Content of White, Yellow, Red, Blue, and High-Carotenoid Corns and Their Nixtamalized Products

type or corn	sample/ product	carotenoid content[a] (μg/100 g of dry wt of sample)			
		lutein	zeaxanthin	β-cryptoxanthin	β-carotene
white	corn	5.73 ± 0.18 f	6.01 ± 0.06 f	1.27 ± 0.06 d	4.92 ± 0.18 e
	masa	8.16 ± 0.15 f	7.76 ± 0.23 f	1.48 ± 0.01 d	nd
	tortilla	8.61 ± 0.27 f	7.50 ± 0.43 f	1.57 ± 0.03 d	nd
	chips	0.82 ± 0.07 f	9.80 ± 0.31 f	0.08 ± 0.00 d	nd
yellow	corn	406.2 ± 4.9 a	353.2 ± 23.1 a	19.1 ± 1.2 b	33.6 ± 1.2 b
	masa	129.2 ± 14.3 c	132.4 ± 11.3 b	3.00 ± 0.18 d	20.7 ± 0.9 c
	tortilla	108.2 ± 7.5 c	101.6 ± 10.1 c	1.81 ± 0.14 d	11.1 ± 0.8 d
	chips	77.3 ± 7.2 d	64.9 ± 5.5 d	0.11 ± 0.01 d	nd
red	corn	121.7 ± 12.1 c	111.9 ± 9.2 c	13.1 ± 1.8 c	20.2 ± 1.9 c
	masa	42.6 ± 3.2 e	44.9 ± 3.9 d	1.42 ± 0.14 d	7.02 ± 0.91 e
	tortilla	41.4 ± 4.1 e	42.3 ± 1.1 e	1.32 ± 0.10 d	6.39 ± 0.11 e
	chips	40.9 ± 2.5 e	30.2 ± 2.1 e	1.78 ± 0.09 d	nd
blue	corn	5.17 ± 0.49 f	14.3 ± 1.0 f	3.41 ± 0.39 d	23.1 ± 2.1 c
	masa	4.71 ± 0.35 f	8.71 ± 0.10 f	1.00 ± 0.06 d	12.5 ± 1.2 d
	tortilla	4.53 ± 0.02 f	8.81 ± 0.90 f	1.22 ± 0.09 d	12.1 ± 0.4 d
	chips	4.48 ± 0.28 f	8.78 ± 0.71 f	0.96 ± 0.08 d	nd
high carotenoid	corn	245.6 ± 9.4 a	322.3 ± 10.7 a	23.1 ± 1.0 a	45.8 ± 3.9 a
	masa	74.9 ± 7.2 d	112.6 ± 14.4 c	11.5 ± 1.0 c	25.9 ± 1.7 c
	tortilla	72.5 ± 4.8 d	105.3 ± 6.6 c	12.4 ± 0.4 c	14.6 ± 1.0 d
	chips	61.1 ± 3.4 d	92.5 ± 1.4 c	4.71 ± 0.20 d	8.39 ± 0.21 e

[a] Values with no letters in common are significantly different ($p < 0.05$). nd, not determined.

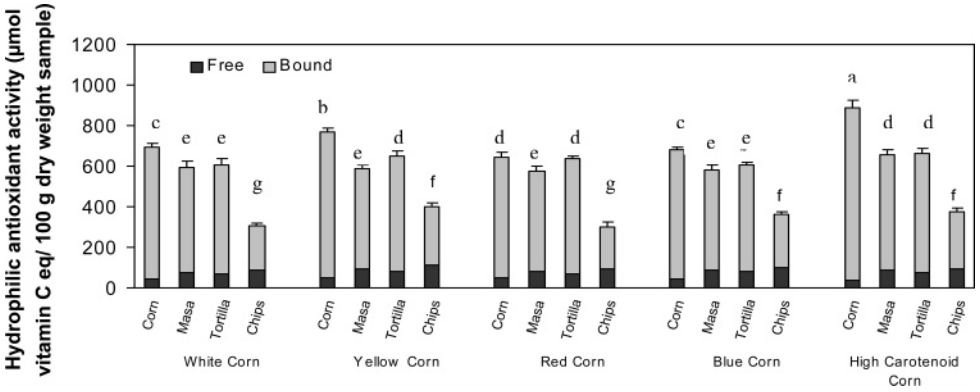

Figure 1. Hydrophilic antioxidant activity of raw corns and their nixtamalized corn products (masa, tortilla, and chips) expressed in micromoles of vitamin C equivalents per 100 g of dry weight of sample. Values with no letters in common are significantly different ($p < 0.05$).

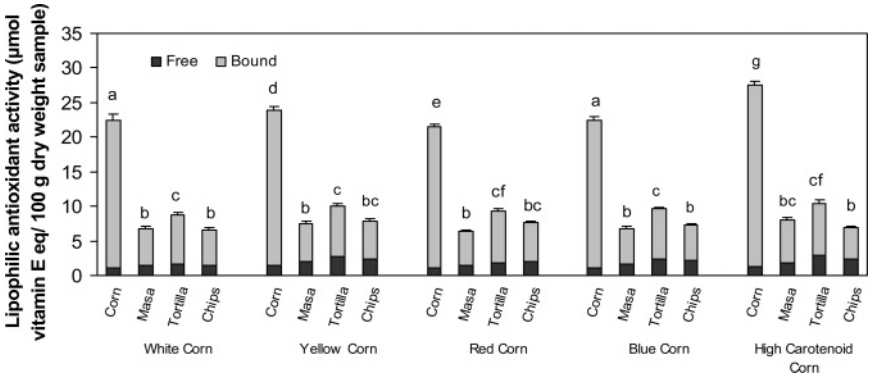

Figure 2. Lipophilic antioxidant activity of raw corns and their nixtamalized corn products (masa, tortilla, and chips) expressed in micromoles of vitamin E per 100 g of dry weight of sample. Values with no letters in common are significantly different ($p < 0.05$).

DISCUSSION

Phenolics. Several studies have investigated phenolic composition of cereals, especially wheat, barley, and corn (*5*−*7*, *9*, *15*, *23*). These studies demonstrated that most of the phenolics were bound or attached to cell wall structures. Our results in the present study agree with previous investigations and confirmed that >80% of total phenolics were in bound form. **Table 1** clearly shows that lime-cooking caused important losses of phenolic compounds that leached into the steep liquor or nejayote. It is well-known that nixtamalization modifies proteins and enhances calcium uptake (*12*, *16*) and that the calcium hydroxide hydrolyzes fiber components located in the pericarp,

aleurone cell wall, and endosperm cell walls. Most of the ferulic acid is associated with the corn bran or pericarp (*24*). Gonzalez et al. (*24*) found that the alkaline treatment of corn partially hydrolyzes the hemicelullose and lignin fractions rich in phenolics. The chemical hydrolysis releases and solubilizes phenolics that leach into the cooking liquor. Baking of masa to obtain tortillas and frying of tortillas to yield chips had a slight effect on the phenolic concentration and composition. Only the free phenolics slightly increased when masa was transformed into tortillas and chips. The study of Dewanto et al. (*8*) showed there were significant increases in the content of total free phenolics following thermal treatment with time, which is consistent with the data of this work. Unlike free phenolic content, the concentration of bound phenolics decreased when raw corn was processed into tortillas and chips.

Ferulic Acid Content of Corn and Nixtamalization Products. Ferulic acid is the most common phenolic acid found in the Gramineae family. It has been confirmed that ferulic acid occurs mostly as an ester, linked to the cell wall, and it is mainly located in the aleurone layer and pericarp tissue (*25*, *26*). Potential health benefits of ferulic acid are attributed to the antioxidant activity, primarily the prevention of the oxidation of key proteins and neuron damage (*27*).

Additionally, ferulic acid inhibits lipid oxidation and protects cells from oxidative damage. Ferulic acid is one of the major phenolics ingested by humans. It is well-known that ferulic acid is absorbed in the duodenum or small intestine (*17*). Adom et al. (*5*) compared concentrations of ferulic acid in corn, wheat, oats, and rice and reported that corn had the highest amount (906 μmol/100 g of grain). Dewanto et al. (*8*) found that the bound ferulic acid in sweet corn was liberated in higher amounts by prolonged processing, a tendency that agrees with results obtained in this work. We found, among all corn genotypes studied, that approximately 97% of the ferulic acid was in bound form, in agreement with other authors (*5*−*7*, *15*, *25*, *29*). High amounts of bound ferulic acid in raw kernels were lost during lime-cooking. The bound ferulic acid was concentrated in the aleurone layer and pericarp tissue removed during cooking and steeping. The increase of free ferulic acid can be justified by the fact that heat processing and lime-cooking liberate *trans*-ferulic acid from bound ester glycosides mainly associated with cell walls (*16*). Tortillas and tortilla chips contained higher amounts of bound ferulic acid than the masa, indicating that the heat processes of baking and frying complexed ferulic acid.

Anthocyanin Content. The anthocyanins in corn are mainly located in the aleurone layer and pericarp (*28*). Salinas-Moreno et al. (*28*) found that during the nixtamalization of blue corn a majority of anthocyanins were degraded. The synergistic effect of the alkaline pH (approximately 10) and temperature caused important structure transformation or modifications. Cortes et al. (*26*) compared the anthocyanins of corn cooked with different amounts of lime and concluded that increasing concentrations of calcium hydroxide increased anthocyanin losses. **Table 3** clearly shows that anthocyanins losses were higher in the blue and red corn genotypes. Blue corn products contained the highest anthocyanin content. Our results showed that losses varied according to the type of corn; the average losses of anthocyanins during sequential steps of lime-cooking, tortilla baking, and tortilla chip frying were 77, 73, and 70%, respectively, consistent with data from Salinas et al. (*28*), who found losses from 73 to 100% during nixtamalization. Results clearly showed that lime-cooking had the highest negative effect on anthocyanin losses. Further processing into tortillas and chips did not significantly affect anthocyanin concentrations ($p > 0.05$).

Carotenoid Content. Corn contains higher amounts of lutein, zeaxanthin, and other carotenoids when compared to wheat, oats, and rice (*30*). Lutein and zeaxanthin have been found to be major carotenoids of cereal grains and byproducts (*14*, *31*, *32*). This agrees with the results found in this study. The carotenoid profile reported by Kurilich et al. (*31*) for 44 sweet and dent corns indicated large differences according to genotype. These authors reported from 0 to 200 μg/100 g of dry weight for lutein. Our data showed mean values of the raw corns and their nixtamalized products from 0.82 to 246 μg/100 g of dry weight for lutein, from 4.92 to 45.8 μg/100 g of dry weight for β-cryptoxanthin, from 6.01 to 322 μg/100 g of dry weight for zeaxanthin, and from 0.07 to 23.1 μg/100 g of dry weight for β-carotene. The carotenoid content of raw corns and their nixtamalized products showed that yellow corn had the greatest total level, followed closely by high-carotenoid corn. The loss of carotenoids through the sequential processing steps is clear and significant. It is well-known that carotenoids are highly sensitive to light, heat, air, and pH (*30*, *32*). Among carotenoids, lutein has the best heat stability (*32*). In our study, lutein was the main carotenoid in terms of concentration in raw kernels, processed tortillas, and chips. Lime-cooking was the processing step that most affected the concentration of carotenoids. During this step approximately 50% of the carotenoids were lost into the cooking liquor. The carotenoid concentrations in masa, tortillas, and chips were similar. Scott et al. (*14*) compared carotenoids in fresh, frozen, and canned yellow and white corns and found that there was a large difference in the contents of carotenoids between types of corns (702 μg/100 g for yellow compared with 35.5 μg/100 g of dry weight for white). Processing the corn either maintained or in some instances slightly increased carotenoid concentration. These findings support the premise that calcium hydroxide was responsible for the observed carotenoid losses, and this can be justified by the fact that the alkaline treatment used during the process causes the nejayote solution to have a high concentration of organic matter, including part of the endosperm, germ, and carotenoids (*11*).

Antioxidant Activity. Few studies have investigated the antioxidant activity of foods exerted by intrinsic hydrophilic and lipophilic chemical components. Most antioxidant research has focused on determining the antioxidant activity of water-soluble food extracts, in spite of the role of some lipid components on the prevention of some stress-oxidative diseases (*7*, *33*). There are limited data on processing effects; data from the USDA on 100 different sorts of foods showed that the cooking process can increase or decrease the antioxidant activity of foods depending on the nature and molecular structure of the antioxidant compound (*33*). The sequential heat treatments of lime-cooking, tortilla baking, and tortilla chip frying affected the antioxidant activity. Deep-fat frying had the most detrimental effect on antioxidant capacity because tortilla chips contained <50% of the antioxidant capacity of the raw grain. Baking masa into tortillas slightly increased the antioxidant capacity, likely due to increased soluble phenolics from bound phytochemicals.

In summary, the effects of lime-cooking, baking, and frying on total phenolics, anthocyanins, ferulic acids, carotenoids, and antioxidant activities were studied in five contrasting types of corn. The nixtamalization process significantly influenced and reduced total phenolics and antioxidant activities. Lime-cooking was the most critical in terms of incurred losses. The best overall phytochemical profile was observed in the high-carotenoid genotype followed by yellow, blue, and red corns. White corn had the lowest amounts of anthocyanins, carotenoids, and

antioxidant capacity. Unfortunately, most industrially manufactured nixtamalized products are obtained from white corn. The utilization of yellow and pigmented corns instead of white corn should provide nutritionally better products. More research is needed to determine the health implications of yellow and pigmented corns processed into tortillas and chips.

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