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Potential of Chilean Native Corn (*Zea mays* L.) Accessions as Natural Sources of Phenolic Antioxidants and in Vitro Bioactivity for Hyperglycemia and Hypertension Management

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ABSTRACT: Thirty-three Chilean corn accessions were screened for the first time regarding their phenolic profiles, total phenolic contents (TPC), antioxidant capacity (DPPH and ABTS), and in vitro inhibition against key enzymes relevant for hyperglycemia (α-amylase and α-glucosidase) and hypertension (angiotensin I-converting enzyme, ACE-I) in both free and cell wall-bound fractions. TPC varied from 132.2 to 262.5 mg of gallic acid equivalents/100g dry weight (DW), and around 88% of TPC and antioxidant capacity were found in the bound form. Vanillin, vanillic, protocatechuic, ferulic, and *p*-coumaric acids were detected by HPLC in free fractions, whereas ferulic and *p*-coumaric acids were found in the bound form. Pisankalla accession (red kernel) had the highest ferulic acid content (269.5 mg/100g DW). No α-amylase and ACE-I inhibition were found; however, all free fractions inhibited α-glucosidase (10.8−72.5%). Principal component analysis revealed that darker samples (free fraction) showed higher TPC and antioxidant capacity, while α-glucosidase inhibition was related to yellow-colored samples.

KEYWORDS: *Zea mays L., phenolic phytochemicals, in vitro functionality, hyperglycemia*

INTRODUCTION

Chile is an important center of biological diversity and includes a wide variety of phytogenetic native resources such as potatoes, several Andean corn races, wild berries, and other important food crops.[1] Corn represents a good example of Chilean crop diversity. More than 900 native corn accessions have been identified corresponding to 23 local races or landraces which are defined as material that has been cultivated for hundreds of years under traditional agriculture conditions.[1] Most of these accessions are fully pigmented or multicolored, and representative samples of corn germplasm are currently preserved under strict storage and manipulation conditions in germplasm banks across Chile.[1] The majority of these native accessions are produced in a very low scale or are no longer cultivated due to their apparent low commercial value compared to that of yellow varieties. Therefore, studies conducted to reevaluate them by investigating their phytochemical composition and potential health-linked functional properties are important and necessary.

Several studies stated that corn is not only an important source of macro and micronutrients but also a rich source of several functional phytochemicals such as carotenoids, anthocyanins, flavonoids, and a variety of hydroxycinnamic acid derivatives.[2,3] Phenolic antioxidants in cereals such as corn are either in free or bound forms, and the contribution of the bound fraction to the total phenolic content is generally high.[4] The free phenolic fraction is overall composed of flavonoids and phenolic acids, whereas the bound phenolic fraction is ester-linked to cell wall polymers and consists mainly of ferulic acid and other derivatives.[5]

The interest in phenolic compounds is that they have been associated with various health-linked functional properties such as in vitro and in vivo antioxidant activities related to their ability to scavenge free radicals, break radical chain reactions, and chelate trace metals.[6] Moreover, several epidemiological and experimental studies have shown that phenolic compounds may play a role in the prevention of oxidation-linked chronic diseases such as cardiovascular diseases, cancer, diabetes, and neurodegenerative dysfunctions.[7,8] In regard to corn-linked phenolics bioactivity, some studies have reported that specific corn varieties such as blue-colored corn rich in anthocyanins show interesting health-related properties such as antioxidant capacity,[9] antimicrobial and antimutagenic effects,[10] prevention of obesity and amelioration of hyperglycemia in mice.[11] Therefore, diet-linked phenolic compounds such as grain sources may constitute key functional bioactive elements for redox homeostasis regulation and prevention of oxidative-linked chronic diseases.

Obesity is prevalent in Chile, contributing to the potential to increase chronic diseases. According to a recent national health survey conducted by the Ministry of Health,[12] 39% of the Chilean population over 15 years-old is overweight, whereas the prevalence of obesity has increased to 30.9% especially among schoolchildren from specific southern regions.[13] Obesity and increased weight increase the risk of insulin resistance and other metabolic alterations named metabolic syndrome, which in turn raises the prevalence of type 2 diabetes and cardiovascular diseases (CVD). The increase in metabolic syndrome has already been observed in adults from Talca city in Chile;[14]

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Table 1. Identification of Native Corn Accessions

no.	race	accession	geographical distribution of race (region of Chile)[a]	no.	race	accession	geographical distribution of race (region of Chile)[a]
1	Camelia	CHZM 07098	III, IV, V, VI, VII, VIII, IX, X and XIII	18	Negrito Chileno	CHZM 02013	II and XIII
2		CHZM 03012		19		CHZM 13113	
3		CHZM 07047		20	Diente de Caballo	CHZM 13010	III, IV, V, VI, VIII, IX and XIII
4		CHZM 07088		21		CHZM 13054	
5		CHZM 07089		22		CHZM 13075	
6	Cristalino Chileno	CHZM 13005	III, IV, V, VII, VIII and XIII	23	Capio Chileno	CHZM 01042	I and II
7		CHZM 13007		24		CHZM 01052	
8		CHZM 07065		25	Maíz de Rulo	CHZM 07091	VI and VII
9		CHZM 07076		26		CHZM SI	
10		CHZM 07102		27	Harinoso Tarapaqueño	CHZM 01053	I and II
11	Curagua	CHZM 05023	I, II, V, VII, VIII and XIII	28		CHZM 01059	
12		CHZM 05048		29	Choclero	CHZM SI	I, II, III, IV, V, VI, VII, VIII and XIII
13		CHZM 05079		30	Marcame	CHZM 02009	II
14		CHZM 13134		31	Polulo	CHZM 01030	I
15	Morocho Blanco	CHZM 03017	II, III, V, VI, VII, X and XIII	32	Pisankalla	CHZM 13104	II, V, VI, VII, VIII, IX and XIII
16		CHZM SI		33	Amarillo Nuble	CHZM 08085	VI, VII, VIII, IX and XIII
17		CHZM 05086					

[a]Regions I, II, III, and IV = North zone; V, XIII, VI, VII, and VIII = Central zone; IX, X = South zone; and XI and XII = Austral zone.

therefore, the risk of developing type 2 diabetes among the Chilean population is imminent. Hyperglycemia, one of the main components of metabolic syndrome, is characterized by an abnormal postprandial increase of blood pressure and has been related to the onset of type 2 diabetes and associated oxidation-linked vascular complications.[15,16] The rapid increase in blood glucose levels due to the hydrolysis of starch by pancreatic α-amylase and absorption of glucose released in the small intestine by α-glucosidase may be controlled by the inhibition of these enzymes involved in carbohydrate digestion. The consumption of natural inhibitors such as phenolic compounds from diet constituents could be an effective therapy for managing postprandial hyperglycemia with minimal side effects.[17] Further, one of the main macrovascular complications of diabetes is hypertension. Angiotensin I-converting enzyme (ACE) is an important enzyme involved in maintaining vascular tension. Control of hypertension via the modulation of ACE by phenolic-based dietary antihypertensive ingredients may be an important strategy to manage this risk factor.[18,19]

Efforts conducted to study Chilean corn native accessions are currently focused on the agronomic field, and no data exist regarding their phenolic phytochemical composition and derived health-related functional properties. This knowledge would allow for identifying potential corn accessions as natural sources of phenolic bioactives with the potential application to the design of functional foods as a part of current strategies to counteract rising diet-linked obesity and associated chronic diseases. Therefore, the objective of the current research was to study the potential health-linked functionality of 33 Chilean native corn accessions corresponding to 14 local races or landraces, through the screening of their phenolic profiles in both the free and cell wall-bound fractions, their total phenolic contents, the antioxidant capacity by the DPPH and ABTS methods, and the in vitro inhibitory activities against key enzymes relevant to hyperglycemia and hypertension management. Further, a possible relationship of the studied in vitro functional properties with specific phenolic profiles and corn color was also explored through a multivariate PCA (principal component analysis) tool. PCA is an unsupervised exploratory tool that allows one to visualize main variations between samples, ease sample clustering, and to reveal the underlying relationships between samples and variables. PCA reduces the data dimensionality and allows their visualization retaining as much as possible the variability present in the original data.[20]

MATERIALS AND METHODS

Samples. Representative samples (400 g) of mature seeds from several corn accessions (33) corresponding to 14 local races or landraces of native germoplasm were supplied by the germoplasm bank of the National Institute of Agronomic Research (INIA) located at La Platina in Santiago, Chile. This material was kept under low temperature and proper humidity conditions (−5 °C and 40−45% of relative humidity) within camera-like incubators at INIA.

Subsamples (100 g) from each accession were milled to a fine powder (500 μm) by using a laboratory grinder (IKA Universal mill M20, Staufen, Germany) provided with cold water recirculation (10 °C). Milled samples were stored at −20 °C until analysis. Table 1 summarizes sample classification, accession identification, and the common races' geographical distribution in Chile according to information provided by INIA.

Chemical and Reagents. All chemicals and solvents employed were of high performance liquid chromatography (HPLC) or analytical grade. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, 6-hydroxy-2,5,7,8-tetrathylchroman-2-carboxylic acid (Trolox), and 2,2′-azino-di-[3-etilbenzotiazolin sulfonate] (ABTS) were purchased from Sigma Chemical Co. (St Louis, MO). The Folin−Ciocalteu reagent, methanol, ethanol, acetone, glacial acetic acid, and ethyl acetate were of analytical grade and purchased from Merck (Darmstadt, Germany). Porcine pancreatic α-amylase (EC 3.2.1.1), baker's yeast α-glucosidase (EC 3.2.1.20), and lung rabbit angiotensin-converting enzyme (EC 3.4.15.1) were purchased from Sigma Chemical Co. (St. Louis, MO).

Color Determination. Seed corn color was characterized in whole grains by measuring the CIELAB parameters using a Minolta model CR-200b Chroma meter (Minolta Camera Co., Japan) with a D-65 light source and 0° observer angle as proposed by Yang et al.[21] The instrument was calibrated with a standard white plate. The measured parameters were L^* (lightness), a^* (redness), and b^* (yellowness), and measurements were replicated at least 10 times.

Extract Preparation. In a previous study, the conditions for the extraction of both free and bound phenolic compounds from corn were optimized by applying a response surface methodology (data not shown). The Cristalino Chileno accession CHZM 13005 (sample 6) was used for all experiments, and response variables corresponded to

the total phenolic content determined by the Folin−Ciocalteu method and the antioxidant capacity by the 2,2-diphenyl-2-picrylhydrazyl radical (DPPH•) inhibition assay. These methods will be further described in the sections below.

Extraction of Free Phenolic Compounds. Free phenolic compound extraction was optimized based on conditions proposed by Lopez-Martinez et al.,[2] Dewanto et al.,[22] and Adom and Liu.[23] Evaluated factors influencing the free phenolic extraction were the solvent type (ethanol, methanol and acetone), solvent concentration in water, and the extraction time. After the experimental trials and statistical optimization process, the best conditions for extracting the highest total phenolics contents and obtaining the highest antioxidant capacity were those using the acetone solvent (69:31 v/v in water) for 63 min of agitation. Therefore, the extraction of the free phenolic fraction was performed by following the next procedure. A total of 5 g of powdered (flour) sample was extracted by the addition of 20 mL of acetone/water (69:31 v/v) under agitation at 230 rpm in an orbital shaker at room temperature for 63 min, and the homogenate was then centrifuged at 6037*g* (HERMLE Z326K, Wehingen, Germany) and the supernatant removed. A second extraction was performed on the residue under the same solvent conditions for 30 min. Both supernatants were pooled, then vacuum-evaporated to dryness at 40 °C, and reconstituted in 10 mL with distilled water. The extracts were frozen at −20 °C until analysis.

Extraction of Bound Phenolic Compounds. Extraction of bound phenolic compounds was optimized based on conditions stated by Adom and Liu,[23] De la Parra et al.,[24] and Lopez-Martinez et al.[2] In this case, evaluated factors influencing the extraction of the bound phenolic compounds were the alkali concentration and the time of alkaline hydrolysis. Sodium hydroxide was used. After the experimental trials and statistical analysis, the best conditions for extracting the highest total phenolic contents and obtaining the highest antioxidant capacity were the use of 3 N sodium hydroxide for 88 min. Therefore, the extraction of the bound phenolic fraction was performed by following the next procedure.

Briefly, 0.5 g of powdered (flour) sample was mixed with 2 mL of acetone/water (69:31 v/v), and the experiment was conducted under the same conditions as those applied for the extraction of free phenolic compounds. Supernatants were discarded, and the residue obtained after the second extraction was recovered and suspended in 20 mL of 3 N sodium hydroxide. Alkaline hydrolysis was performed under agitation at 230 rpm in an orbital shaker at room temperature for 88 min. The mixture was neutralized to a pH of ∼2.5 with concentrated hydrochloric acid and then extracted seven times with 10 mL of ethyl acetate. The ethyl acetate fractions were mixed and vacuum-evaporated to dryness at 40 °C. The residue was finally reconstituted in 10 mL of distilled water and stored at −20 °C until analysis.

Total Phenolic Contents. The total phenolics contents (TPC) were determined in both the free and bound phenolic fractions according to the Folin−Ciocalteu method modified by Shetty et al.[25] Briefly, 0.5 mL of corn extract was transferred into a test tube and mixed with 1 mL of 95% ethanol and 5 mL of distilled water. To each sample, 0.5 mL of Folin−Ciocalteu reagent (1 N) was added and mixed. After 5 min, 1 mL of 5% Na_2CO_3 was added to the reaction mixture and allowed to stand for 60 min in a dark place. The absorbance was read at 725 nm. The standard curve was established using various concentrations of gallic acid in 95% ethanol, and results were expressed as mg of gallic acid equivalents (GAE) per 100 g of sample in dry weight (DW).

Antioxidant Capacity by 2,2-Diphenyl-2-picrylhydrazyl Radical (DPPH•) Inhibition Assay. The DPPH scavenging activity of both phenolic fractions was determined by an assay reported by Perez-Jimenez and Saura-Calixto.[26] To 3.9 mL of 60 μM DPPH in methanol, 100 μL of each sample extract (from the free or bound phenolic fraction) was added, and the decrease in the absorbance was monitored after 25 min at 515 nm. The absorbance of a control (methanol instead of sample extract) was also recorded after 25 min at the same wavelength. The percentage of inhibition was calculated, and the antioxidant capacity was expressed as μmol Trolox equivalents/100 g sample DW by using a standard curve of Trolox (hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid) solution at different concentrations (200−900 μM Trolox).

Antioxidant Capacity by the 2-2′s-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) Radical Cation (ABTS•+) Inhibition Assay. The ABTS antioxidant capacity assay was developed according to Perez-Jimenez and Saura-Calixto.[26] A sample aliquot (from the free or bound phenolic fraction) of 100 μL was allowed to react with 3.9 mL of ABTS reagent (10 mL of a solution of potassium persulfate containing 66 mg in 100 mL of distilled water mixed with 38.4 mg of ABTS•+ and stirred overnight, after which the solution was diluted with methanol up to a final absorbance of 0.7 ± 0.02 at 734 nm). The mixture was incubated at room temperature for 6 min. The absorbance was recorded at 734 nm, and a control was also run under the same conditions. The percentage of inhibition was calculated, and the antioxidant capacity was expressed as μmol Trolox equivalents/100 g sample DW using a standard curve of a Trolox solution at different concentrations (200−1000 μM Trolox).

Phenolic Acid Profiles by High Performance Liquid Chromatography (HPLC) Analysis. Corn sample extracts from the free and bound fractions were filtered (pore size 0.2 μm), and the HPLC analysis was performed according to Hu and Xu[27] with further modifications as follows. A HPLC system series 200 with a UV/vis detector (PerkinElmer Inc., Shelton, CT, USA) equipped with a binary pump, an autosampler, and controlled by the TotalChrom software (Perkin-Elmer Inc., Shelton, CT, USA) was used. The analytical column was a HibarLiChrospher 100 RP-18 (5 μm) (Merck, Darmstadt, Germany). The injection volume was 25 μL, the flow rate was 1 mL/min, and the eluates were monitored at 280 nm at 25 °C. The mobile phase was composed of solvent A [water/acetic acid (90:10) pH 2.31] and B (100% acetonitrile). Initial conditions were 95% A and 5% B, and a linear gradient of solvent B was used: from 5 to 8% for 3 min, then from 8 to 12% for 5 min, from 12 to 15% for 5 min, from 15 to 20% for 7 min, from 20 to 100% for 5 min, and then returned to initial conditions by 5 min (total run time 30 min). Detected phenolic compounds in sample extracts corresponded mainly to phenolic acids and were identified by comparison of their retention times with those of pure standards. Spiking of pure standards was applied when necessary to better confirm peak identity. Quantification of phenolic acids was performed using the corresponding calibration curves ($r = 0.9990$) of pure standards (vanillin, protocatechuic, vanillic, *p*-coumaric, and ferulic acids) diluted in methanol, and results were expressed as mg per 100 g sample DW.

α-Amylase Inhibition Assay. The α-amylase inhibitory activity was determined by an assay based on the Worthington Enzyme Manual.[28] A total of 500 μL of each corn sample extract and 500 μL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing α-amylase solution (0.5 mg/mL) was incubated at 25 °C for 10 min. After preincubation, 500 μL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube at timed intervals. The reaction mixtures were then incubated at 25 °C for 10 min. The reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 10 min and cooled to room temperature. The reaction mixture was then diluted after adding 15 mL of distilled water, and absorbance was measured at 540 nm. The absorbance of sample blanks (buffer instead of enzyme solution) and a control (buffer in place of sample extract) were recorded as well. The final extract absorbance (A_{540} extract) was obtained by subtracting its corresponding sample blank reading. The α-amylase inhibitory activity was calculated according to the equation below:

$$\%\ \text{inhibition} = \frac{A_{540}(\text{control}) - A_{540}(\text{extract})}{A_{540}(\text{control})} \times 100$$

α-Glucosidase Inhibition Assay. A modified version of the assay described by the Worthington Enzyme Manual[29] was used. A volume of 50 μL of sample extract diluted with 50 μL of 0.1 M potassium phosphate buffer (pH 6.9) and 100 μL of 0.1 M potassium phosphate buffer (pH 6.9) containing α-glucosidase solution (1.0 U/mL) was incubated in 96-well plates at 25 °C for 10 min. After preincubation, 50 μL of 5 mM *p*-nitro-phenyl-α-D-glucopyranoside solution in 0.1 M

potassium phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 5 min. Before and after incubation, absorbance readings (A_{405} extract) were recorded at 405 nm by a microplate reader and compared to a control which had 50 μL of buffer solution in place of the extract (A_{405} control). The α-glucosidase inhibitory activity was expressed as a percentage of inhibition and was calculated as follows:

$$\%\ \text{inhibition} = \frac{\Delta A_{405}(\text{control}) - \Delta A_{405}(\text{extract})}{\Delta A_{405}(\text{control})} \times 100$$

Angiotensin I-Converting Enzyme (ACE) Inhibition Assay. ACE inhibition was performed based on the method developed by Kwon et al.[30] with some modifications. A volume of 50 μL of sample extract was incubated with 200 μL of 0.1 M NaCl-borate buffer (0.3 M NaCl, pH 8.3) containing 2 mU of ACE solution at 25 °C for 10 min. After preincubation, 100 μL of a 5.0 mM substrate (hippuryl-histidyl-leucine) solution was added to the reaction mixture. Test solutions were incubated at 37 °C for 1 h. The reaction was stopped with 150 μL of 0.5 N HCl. The hippuric acid formed was detected by a high-performance liquid chromatography (HPLC) method. A volume of 25 μL of sample was injected using a HPLC system series 200 with a UV/vis detector (PerkinElmer Inc., Shelton, CT, USA) equipped with a binary pump and an auto sampler and controlled by TotalChrom software (Perkin-Elmer Inc., Shelton, CT, USA). The solvents used for the gradient were (A) 10 mM phosphoric acid, pH 2.5, and (B) 100% acetonitrile. The acetonitrile concentration was increased to 60% for the first 6 min and to 100% for 4 min, then decreased to 0% for the next 5 min, and then returned to initial conditions for 5 min (total run time, 20 min). The analytical column used was HibarLiChrospher 100 RP-18 (5 μm) (Merck, Darmstadt, Germany) at a flow rate of 1 mL/min at 25 °C. The absorbance was recorded at 228 nm. Sample blanks (buffer in place of enzyme and substrate), a control (distilled water instead of sample extract), and a blank (buffer instead sample) were used to calculate the % inhibition, according to the next equation:

$$\%\ \text{inhibition} = \frac{(area_{\text{control}} - (area_{\text{sample}} - area_{\text{sample blank}}))}{(area_{\text{control}} - area_{\text{blank}})} \times 100$$

Statistical Analysis. Extractions from both the free and bound phenolic fractions were run in duplicate, and all analyses were carried out in triplicate. Results were expressed as the mean ± standard deviation. Data were subjected to one-way analysis of variance (ANOVA) or the Kruskal–Wallis test ($p < 0.001$) when necessary. Pearson linear correlations ($p < 0.05$) were calculated using the Statgraphics Centurion XVI (StatPoint Inc., Rockville, MD, USA), and further relationships among all studied variables were explored through the multivariate principal component analysis (PCA) tool using SIMCA-P (Umetrics, Umeå, Sweden).

RESULTS AND DISCUSSION

Color Measurement. The color of 33 native corn accessions corresponding to 14 local races was evaluated according to their color ((*L**), (*a**), and (*b**)) parameters, and the results are shown in Table 2. In addition, the pictures of evaluated corn races are shown in Figure 1. The importance of objectively characterizing the color of kernels is that it helps to better explore potential correlations between the color of corn accessions and their phenolic composition and in vitro functionality.

Significant differences in the color measurements were observed among all analyzed corn races ($p < 0.001$), and color differences were also found among accessions from the same race. Overall, a sample's color parameters were related to their visually perceived color, and this was more evident in homogeneously colored accessions (not multicolored). Sample 27 had the highest *L** value (76.3 ± 7.6), whereas sample 18 showed the lowest one (26.4 ± 4.7), corresponding to lighter and darker kernels, respectively. The (*a**) parameter with coordinate variability between red (positive) and green (negative) color was high for sample 5 (42.3 ± 5.1), while the lowest value was for sample 27 (0.4 ± 0.5). Sample 5 from the Camelia race showed a close to red-colored pericarp (Figure 1). The (*b**) parameter varies from yellow (positive) and blue (negative), and high (*b**) values were observed in yellow-colored accessions, with sample 20 showing the highest value (39.5 ± 5.7). High standard deviations and related coefficients of variations (data not shown) in measured color parameters were especially observed in accessions belonging to Marcame, Curagua, and Capio Chileno races, and this may be related to their natural multipigmented appearance (Figure 1).

Table 2. Color Measurements of Native Corn Accessions[a]

no.	*L**	*a**	*b**
1	52.0 ± 11.0	36.3 ± 4.0	21.1 ± 3.1
2	40.7 ± 7.4	37.3 ± 5.2	14.5 ± 2.8
3	60.1 ± 5.4	10.4 ± 3.9	36.8 ± 8.1
4	41.4 ± 4.8	34.7 ± 5.2	24.6 ± 3.5
5	49.4 ± 6.9	42.3 ± 5.1	23.8 ± 3.5
6	34.2 ± 2.7	23.3 ± 2.8	18.6 ± 3.0
7	40.4 ± 8.8	19.7 ± 4.1	25.4 ± 5.4
8	48.5 ± 5.8	15.3 ± 5.3	30.4 ± 6.0
9	48.8 ± 6.5	18.8 ± 3.5	31.4 ± 5.7
10	50.4 ± 6.7	17.2 ± 3.3	26.4 ± 3.1
11	48.8 ± 5.6	19.9 ± 3.0	22.7 ± 2.0
12	31.6 ± 9.1	15.0 ± 6.0	12.9 ± 9.1
13	42.0 ± 7.2	18.9 ± 5.3	20.0 ± 4.3
14	46.5 ± 6.8	13.1 ± 2.2	20.5 ± 2.3
15	55.7 ± 15.5	10.1 ± 10.6	21.5 ± 6.3
16	61.8 ± 1.6	8.2 ± 1.0	26.6 ± 1.5
17	66.7 ± 4.5	1.7 ± 0.6	20.8 ± 1.5
18	26.4 ± 4.7	11.1 ± 1.9	4.6 ± 2.5
19	36.0 ± 5.8	17.3 ± 3.0	13.0 ± 8.2
20	60.1 ± 9.0	10.1 ± 2.8	39.5 ± 5.7
21	61.6 ± 6.1	9.2 ± 3.3	37.3 ± 6.7
22	58.6 ± 4.5	13.2 ± 3.1	34.1 ± 4.6
23	72.1 ± 5.5	5.0 ± 3.2	33.3 ± 3.8
24	38.3 ± 7.9	22.3 ± 5.8	17.8 ± 4.6
25	52.9 ± 10.0	14.9 ± 4.9	30.8 ± 4.9
26	51.4 ± 5.5	17.1 ± 5.0	35.1 ± 5.4
27	76.3 ± 7.6	0.35 ± 0.5	22.5 ± 1.9
28	73.4 ± 5.6	1.2 ± 2.1	36.9 ± 4.5
29	57.9 ± 4.1	9.5 ± 1.6	36.0 ± 4.2
30	65.8 ± 8.3	5.4 ± 3.3	26.2 ± 4.3
31	62.2 ± 3.2	5.2 ± 1.0	32.3 ± 3.3
32	35.3 ± 5.2	22.7 ± 2.3	13.9 ± 1.3
33	55.9 ± 6.9	9.0 ± 1.6	37.2 ± 4.6
mean	51.6	15.6	25.7
min	26.5	0.35	4.6
max	76.3	42.3	39.5
accessions	**	**	**
race	**	**	**

[a]Results are expressed as the mean ± SD; $n = 10$. **Significant at $p < 0.001$.

Total Phenolic Contents (TPC). The major phenolic compounds in cereals are mostly attached to the cell wall material, whereas free phenolic forms are less abundant.[31] Total phenolic contents of whole grains have been generally underestimated by several studies due to the fact that cell wall-linked phenolic forms are not quantified. Various hydrolytic

Figure 1. Races of Chilean native corn.

procedures have been described to extract phenolic compounds bound to the cell wall. Most of these procedures are based on alkaline hydrolysis,[2,24] although other procedures based on enzymatic hydrolysis have been also reported.[4] In the current study, both free and bound phenolic fractions were quantified. Alkaline hydrolysis was applied to release the bound forms, and hydrolysis conditions were optimized (data not shown) based on reported literature.[2,23,24]

Table 3 shows the TPC found in both free and bound phenolic extracts from the evaluated corn accessions. The final total phenolic contents calculated as the sum of phenolic levels linked to the free and bound fractions were also included. Significant differences were observed in the TPC from the free and bound fractions of different races and even among accessions from the same race ($p < 0.001$). The geographical distribution of accessions from the same race is sometimes variable (Table 1), which could explain observed differences in TPC; however, further studies are needed to better determine how the environmental factors affect TPC among studied corn accessions.

All bound fraction extracts showed higher TPC (from 94.7 to 244.4 mg GAE/100 g DW; samples 24 and 32, respectively) than free fractions (9.2 to 37.5 mg GAE/100 g DW; samples 17 and 24, respectively). These data clearly show that most of the phenolic compounds in corn cereals are in the bound form and corresponded to an average of 88% with respect to the final total phenolic content in sample corns (free and bound). Similarly, Montilla et al.[4] and Žilić et al.[3] found that the bound phenolic fraction represented in general more than 80% of the total phenolic content in Mexican yellow corn samples and in different colored corn genotypes.

Total phenolic contents (free and bound) ranged from 132.2 to 262.5 mg GAE/100 g DW, and the orange to red sample 32 (Pisankalla accession) had the highest content. The current results are higher than those reported by Cabrera-Soto et al.[32] (90.5−92.2 mg GAE/100 g DW in two yellow corn samples) and in agreement with those obtained by De la Parra et al.[24] who reported ranges of 243.8−285.8 mg GAE/100 g DW in white, red, and purple Mexican samples. Lopez-Martinez et al.[2] found values of 170 to 617 mg GAE/100 g mainly in black and purple Mexican corn varieties. Such differences might be related to the cultivar influence and the geographical origin. Conversely, Montilla et al.[4] and Žilić et al.[3] found higher TPC values (311.0−611.7 and 522.7−1052.8 mg GAE/100 g DW, respectively) than those reported in the current study when red to dark blue colored corn kernels were analyzed. Both authors applied different extraction conditions to obtain high amounts of anthocyanins in the free soluble phenolic fraction, which in turn increased the total phenolic content in such purple varieties. According to Montilla et al.,[4] the TPC in the free soluble fraction varied from 68.1 to 309.7 mg GAE/100 g DW, contents higher than those obtained in the current work where less colored samples were analyzed, and anthocyanins were not extracted under the applied extraction conditions.

A negative correlation was found between the free TPC and the L^* (lightness) color parameter ($r = -0.44$, $p < 0.05$) likely indicating that darker kernels would be associated with higher TPC. However, the low correlation value may be attributed to the high variability in measured color parameters within each accession sample as explained above. Other studies showed stronger positive correlations between TPC and grain color especially when evaluating more homogeneous and darker kernels than those analyzed in the current work.[3]

The importance of knowing the phenolic content in both free and bound forms is that they may show different health-linked functional properties. The fraction bound to dietary fiber seems to be released by bacteria enzymes at the large intestine level, thus promoting important biological effects.[33] In the case of the soluble phenolic fraction, it has been stated that it remains soluble in the digestive media from the small intestine and is likely exerting a potential antioxidant effect.[34]

Antioxidant Capacity by the DPPH and ABTS Methods. The ABTS and DPPH (both based on electron transfer) methods were used to evaluate the antioxidant capacity of free and bound phenolic extracts from corn accessions (Table 3). Despite the fact that both radicals are foreign to biological systems, antioxidant capacity by the ABTS assay has shown a good correlation to that by the oxygen radical absorbance capacity (ORAC) method (which uses more physiologically relevant peroxyl radicals),[35] and this correlation was mainly observed in cereal grain samples.[36] Therefore, a better understanding of antioxidant capacity in corn accessions would be possible by considering both assays.

The antioxidant capacity measured by the DPPH and ABTS methods exhibited significant differences ($p < 0.001$) among evaluated corn races and even among accessions from the same race. Although results from both methods were quantitatively different, similar trends were observed, and all bound phenolic extracts exhibited higher antioxidant capacities than free forms as will be further discussed.

The antioxidant capacity based on the DPPH free radical inhibition varied from 471.1 ± 27.2 to 923.0 ± 32.4 μmol

Table 3. Total Phenolic Contents and Antioxidant Capacity by the DPPH and ABTS Methods[a]

	total phenolics (mg GAE/100 g DW)			DPPH (μmol Trolox equiv/100 g DW)			ABTS (μmol Trolox equiv/100 g DW)		
no.	free	bound	total	free	bound	total	free	bound	total
1	21.0 ± 1.4	182.2 ± 2.0	203.2 ± 1.0	65.2 ± 3.5	908.1 ± 28.0	973.3 ± 25.1	206.4 ± 3.1	1517.2 ± 3.1	1723.5 ± 2.1
2	23.2 ± 0.7	173.1 ± 9.1	196.4 ± 9.5	48.5 ± 3.4	796.9 ± 25.2	845.4 ± 24.2	95.1 ± 1.6	1636.2 ± 60.5	1731.2 ± 61.4
3	20.0 ± 0.9	194.7 ± 5.4	214.8 ± 4.9	62.4 ± 2.8	789.1 ± 14.7	851.4 ± 12.5	146.0 ± 7.5	1541.7 ± 65.1	1687.7 ± 60.3
4	18.3 ± 0.5	165.0 ± 1.7	183.2 ± 2.0	66.7 ± 2.6	650.6 ± 10.0	717.4 ± 11.1	128.1 ± 3.1	1420.9 ± 4.9	1548.9 ± 3.6
5	21.7 ± 3.1	175.4 ± 7.4	197.1 ± 10.5	58.6 ± 4.9	770.7 ± 29.0	829.3 ± 26.8	94.2 ± 1.9	1604.5 ± 9.1	1698.8 ± 9.4
6	25.1 ± 0.2	182.8 ± 1.8	207.9 ± 1.8	103.5 ± 3.6	703.9 ± 15.1	807.4 ± 17.1	203.0 ± 7.3	1384.6 ± 13.3	1587.6 ± 19.6
7	20.7 ± 0.9	188.0 ± 2.5	208.7 ± 3.4	68.0 ± 4.0	602.5 ± 18.1	670.5 ± 19.6	140.0 ± 6.7	1390.4 ± 12.1	1530.4 ± 6.8
8	19.4 ± 1.6	130.2 ± 3.3	149.5 ± 4.8	67.6 ± 1.7	829.7 ± 12.6	897.3 ± 13.8	179.9 ± 6.5	1607.8 ± 7.3	1787.6 ± 5.3
9	21.7 ± 0.4	175.5 ± 4.0	197.2 ± 4.3	71.2 ± 1.8	702.0 ± 10.5	773.1 ± 10.5	148.2 ± 3.2	1611.7 ± 30.2	1759.8 ± 27.3
10	12.5 ± 1.4	182.5 ± 0.7	194.9 ± 1.4	38.0 ± 3.1	771.0 ± 30.8	808.7 ± 31.0	82.0 ± 5.3	1696.4 ± 14.4	1778.4 ± 19.3
11	23.7 ± 0.2	130.3 ± 2.2	154.1 ± 2.3	74.5 ± 1.9	769.2 ± 29.8	843.7 ± 31.3	144.2 ± 5.4	1466.1 ± 15.4	1610.3 ± 20.7
12	34.1 ± 0.4	188.7 ± 3.2	222.8 ± 3.0	115.5 ± 3.0	690.9 ± 20.1	806.4 ± 18.9	164.2 ± 0.3	1381.1 ± 5.9	1545.3 ± 6.1
13	20.6 ± 2.6	163.7 ± 4.0	184.3 ± 1.7	78.9 ± 1.0	795.0 ± 27.7	873.9 ± 27.8	192.0 ± 8.5	1657.7 ± 24.7	1849.7 ± 32.6
14	21.3 ± 1.4	171.9 ± 2.7	193.2 ± 3.9	40.6 ± 1.2	823.0 ± 25.3	863.6 ± 25.4	76.9 ± 2.1	1610.4 ± 14.2	1687.3 ± 15.1
15	14.1 ± 0.8	140.5 ± 3.1	154.6 ± 3.1	56.8 ± 2.1	923.0 ± 32.4	979.8 ± 31.0	153.9 ± 4.4	1515.7 ± 26.6	1669.6 ± 30.0
16	22.3 ± 1.6	184.0 ± 9.8	206.3 ± 8.7	90.2 ± 3.8	821.8 ± 24.9	912.0 ± 23.2	127.3 ± 5.4	1429.9 ± 20.4	1557.2 ± 20.5
17	9.2 ± 0.7	169.8 ± 7.7	179.0 ± 7.3	31.9 ± 3.2	719.1 ± 19.6	751.0 ± 19.6	93.2 ± 3.1	1326.1 ± 35.1	1419.3 ± 36.1
18	32.9 ± 1.2	171.6 ± 1.9	204.5 ± 1.5	168.3 ± 2.8	483.6 ± 26.6	651.9 ± 24.7	330.2 ± 5.5	1095.7 ± 62.6	1425.9 ± 65.9
19	25.7 ± 0.6	116.0 ± 4.1	141.7 ± 4.5	112.3 ± 8.8	540.9 ± 8.3	653.2 ± 12.4	121.8 ± 3.4	1184.9 ± 18.5	1306.7 ± 20.8
20	15.1 ± 0.5	194.0 ± 1.9	209.1 ± 2.0	57.4 ± 3.0	785.1 ± 13.4	842.5 ± 13.4	158.1 ± 6.7	1385.0 ± 7.5	1543.2 ± 8.6
21	21.5 ± 3.2	147.9 ± 6.0	169.3 ± 3.0	52.3 ± 1.8	800.1 ± 31.1	852.4 ± 32.3	94.8 ± 1.9	1619.5 ± 40.9	1714.3 ± 39.8
22	21.1 ± 0.2	143.0 ± 8.1	164.1 ± 8.3	67.7 ± 1.5	736.7 ± 28.1	804.5 ± 28.9	147.2 ± 0.5	1628.7 ± 12.8	1775.9 ± 13.0
23	24.1 ± 0.9	126.6 ± 1.7	150.7 ± 2.2	86.3 ± 1.5	599.3 ± 17.2	685.6 ± 18.8	190.6 ± 9.2	1284.5 ± 24.3	1475.1 ± 33.2
24	37.5 ± 2.6	94.7 ± 4.0	132.2 ± 6.3	171.1 ± 3.4	718.0 ± 21.8	889.2 ± 23.8	343.8 ± 13.6	1355.0 ± 7.4	1698.9 ± 19.6
25	20.2 ± 1.1	151.7 ± 1.9	171.9 ± 1.4	53.5 ± 1.7	613.8 ± 26.9	667.3 ± 26.2	90.5 ± 4.1	1401.4 ± 20.3	1491.9 ± 16.8
26	23.8 ± 0.7	189.3 ± 5.7	213.1 ± 5.8	100.0 ± 4.9	637.4 ± 31.3	737.4 ± 27.5	216.0 ± 7.4	1413.3 ± 76.9	1630.8 ± 83.2
27	21.6 ± 1.5	154.3 ± 1.6	175.9 ± 2.5	86.2 ± 1.4	729.4 ± 43.6	815.6 ± 43.5	196.2 ± 5.6	1252.0 ± 52.4	1448.1 ± 57.0
28	22.6 ± 0.6	135.3 ± 4.5	158.0 ± 4.3	72.8 ± 2.3	667.9 ± 26.9	740.7 ± 28.0	150.4 ± 2.3	1297.8 ± 21.6	1448.2 ± 19.9
29	20.9 ± 1.1	142.5 ± 8.1	163.4 ± 9.1	56.4 ± 6.0	699.6 ± 27.3	756.1 ± 32.3	126.8 ± 8.6	1550.7 ± 8.4	1677.4 ± 15.7
30	17.0 ± 1.3	151.1 ± 2.6	174.1 ± 2.0	98.0 ± 1.8	471.1 ± 27.2	569.1 ± 26.6	198.5 ± 9.5	1146.9 ± 5.3	1345.4 ± 8.6
31	21.5 ± 0.3	196.7 ± 1.3	218.2 ± 1.4	65.2 ± 2.6	794.4 ± 47.9	859.6 ± 47.2	131.2 ± 5.7	1451.5 ± 40.7	1582.7 ± 35.0
32	18.1 ± 0.7	244.5 ± 5.8	262.5 ± 5.5	63.3 ± 7.5	899.2 ± 40.7	962.5 ± 47.8	153.9 ± 0.3	1486.4 ± 29.8	1640.3 ± 29.6
33	17.0 ± 1.3	155.4 ± 7.9	172.3 ± 7.2	49.8 ± 2.3	715.6 ± 23.6	765.4 ± 22.9	87.5 ± 1.7	1465.6 ± 8.5	1553.0 ± 7.8
mean	21.7	164.0	185.7	75.7	726.0	801.7	154.9	73.2	1596.8
min	9.2	94.7	132.2	31.9	471.1	569.1	76.9	1095.7	1306.7
max	37.5	244.5	262.5	171.1	923.0	979.8	343.8	1696.4	1849.7
accessions	*	*	*	*	*	*	*	*	*
race	*	*	*	*	*	*	*	*	*

[a]Mean ± SD. *Significant at $p < 0.001$.

Trolox equivalents/100 g DW in the bound phenolic fraction and was significantly higher than values obtained in the free fraction (31.9 ± 3.2 to 171.1 ± 3.4 μmol Trolox equivalents/100 g DW). Sample 15 (Morocho Blanco, multicolored accession) showed the highest antioxidant capacity in the bound form, whereas sample 24 (Capio Chileno accession with orange to red kernels) had the highest value among free phenolic extracts followed by sample 18 (168.3 ± 2.7 μmol Trolox equivalents/100 g DW, Negrito Chileno purple corn accession).

In the case of the antioxidant capacity measured by the ABTS free radical inhibition, the bound phenolic fraction showed higher antioxidant capacity (from 1095.7 ± 62.6 to 1696.4 ± 14.4 μmol Trolox equivalents/100 g DW) than the free fraction (77.0 ± 2.1 to 343.8 ± 13.6 μmol Trolox equivalents/100 g DW). Samples 10 (Cristalino Chileno accession with yellow to orange kernels) and 24 (Capio Chileno accession) had the highest antioxidant capacity in the bound and free forms, respectively. The free fraction from sample 24 also exhibited the highest antioxidant capacity when the DPPH method was applied, and this may be related to its high free TPC as stated above. Sample 18 (Negrito Chileno accession) also showed the second highest antioxidant capacity among free phenolic fractions.

Higher antioxidant capacity results obtained in all bound phenolic fractions compared to free forms may be attributable to their higher bound TPC. Adom and Liu[23] pointed out that bound fractions highly contributed to the total antioxidant capacity measured by the total oxyradical scavenging capacity when yellow corn samples were analyzed. Lopez-Martinez et al. made a similar observation[2] when Mexican red, purple, black, red, and some yellow corn kernels were evaluated with the same antioxidant methods.

Pearson correlation analysis revealed that free TPC significantly contributed to the antioxidant capacity of the free fraction ($r = 0.65$ and 0.86, $p < 0.05$, for the DPPH and ABTS results, respectively). Further statistical analysis considering only results from clear to yellow-colored accessions showed that bound TPC were also directly proportional to the bound DPPH antioxidant capacity data ($r = 0.74$; $p < 0.05$).

ABTS and DPPH radical inhibition results significantly correlated to each other in both free and bound fractions ($r = 0.86$ and 0.71,

respectively, $p < 0.05$) indicating that in general, samples with higher antioxidant capacity by the DPPH method also showed higher responses by the ABTS method. However, all ABTS results were consistently higher than those obtained by the DPPH assay. This may be related to the fact that the $ABTS^{\bullet+}$ free radical is more sensitive to phenolic-containing compounds than $DPPH^{\bullet}$.[37] Also, Floegel et al.[35] found out that the ABTS assay may be more useful than the DPPH assay for detecting antioxidant capacity in a variety of plant foods.

A lack of clarity in correlation between the kernel color and the antioxidant capacity measured by the two methods was found when the statistical analysis was applied considering all data. This might be related to the high variability of seed color patterns shown by each evaluated accession. However, when Pearson correlation analysis was only performed on data from clear to yellow-colored accessions, a significant negative correlation between the L^* parameter and the ABTS results from the bound fraction was found ($r = -0.62$, $p < 0.05$). This likely indicates that darker yellow grains would be associated with higher antioxidant capacities in such fractions. In this sense, Zilic et al.[3] used the same methods and concluded that darker colored corn has more antioxidant capacity. Lopez-Martinez et al.[2] also observed that pigmented black, purple, red, and blue corn overall exhibited higher $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging activity than nonpigmented samples and that this response was linked to higher anthocyanin contents in such colored corn varieties.

Phenolic Acid Profiles Analyzed by HPLC. Phenolic acids were quantified by HPLC, and the results are shown in Table 4. In addition, typical HPLC chromatograms obtained in free and bound fractions are shown in Figure 2, and Pearson correlations were determined to explore potential correlations between specific phenolic profiles and the evaluated antioxidant capacity.

Both free and bound phenolic extracts had different phenolic acid profiles, and the most abundant phenolic compounds were detected in the bound fraction. Hydroxybenzoic derivatives such as vanillin (0.12 to 0.96 mg/100g DW) and vanillic acid (0.36 to 2.46 mg/100 g DW) were detected in almost all free fractions, whereas only seven accessions (two from Cristalino Chileno and two from Morocho Blanco races) were characterized by the presence of protocatechuic acid (0.38 to 1.05 mg/100 g DW). Moreover, *p*-coumaric and ferulic acids were also found in all free fractions. Contents varied from 0.26 to 1.80 and from 0.26 to 1.00 mg/100 g DW for *p*-coumaric and ferulic acids, respectively. Comparable ranges of ferulic (0.41−0.89 mg/100 g DW), vanillic (0.24−0.32 mg/100 g DW), and protocatechuic acids (0.13−0.60 mg/100 g DW) were also detected by Hu and Xu[27] in the free phenolic fraction of several colored mature waxy corn samples cultivated in China. The same authors also reported the presence of *p*-coumaric acid in evaluated corn free fractions; however, the found levels (0.02−0.05 mg/100 g DW) were lower than those reported here. According to the Pearson correlation analysis, vanillic acid contents significantly contributed to the antioxidant capacity measured in the free fraction among red to purple corn accessions when evaluated by the two methods ($r = 0.59$ and $r = 0.77$, $p < 0.05$; for the DPPH and ABTS results, respectively).

Total free phenolic acid contents obtained by calculating the sum of all detected free phenolic acids ranged from 0.91 to 4.3 mg/100 g DW. These values are significantly lower than the ranges found for the free TPC analyzed by the Folin−Ciocalteu method (9.2 to 37.5 mg GAE/100 g DW) likely indicating the presence of other free soluble phenolic compounds not detected by the applied HPLC analysis. In cereals, soluble phenolic compounds not only exist in free form but also in conjugated forms linked to soluble carbohydrates by ester (esterified) and ether (etherified) bonds.[38] Cabrera-Soto et al.[32] determined free, glycosylated, and esterified phenolic compounds among the soluble phenolic compounds in Mexican corn grains. It is most likely that only free phenolic acids were detected in the current work.

Major phenolic compounds in bound fractions were hydroxycinnamic acids such as *p*-coumaric and ferulic acids. Ferulic acid was the most dominant phenolic acid detected in all bound forms. Bound ferulic acid contents ranged from 126.6 to 268.5 mg/100 g DW; meanwhile, *p*-coumaric acid levels varied from 10.6 to 36.4 mg/100 g DW. In addition, bound ferulic acid contents were highly correlated to the antioxidant capacity measured by the DPPH and ABTS methods ($r = 0.72$ and 0.71, $p < 0.05$; respectively). Correlation coefficients increased when statistical analysis was performed just considering data from red to purple corn kernels ($r = 0.78$ and 0.82, $p < 0.05$; for DPPH and ABTS results, respectively). This indicates that higher antioxidant capacities observed in all bound fractions were mainly associated with their high ferulic acid contents and that this correlation was more evident in darker accessions. It has been reported that hydroxycinnamic acids such as ferulic acid derived from the bound fraction of millet cereal showed effective in vitro and in vivo antioxidant capacities.[39] Moreover, Bauer et al.[40] found that ferulic acid extracted from corn fiber was an effective antioxidant on fish oil-containing emulsions.

By calculation of the total hydroxycinnamic acid contents (free and bound), we found that values ranged from 127.03 to 269.47 mg/100 g DW for ferulic acid and from 11.2 to 37.9 mg/100 g DW for *p*-coumaric acid, respectively. Approximately 76−91% of the total ferulic and *p*-coumaric acid contents was in the bound form. Sample 32 (Pisankalla accession) showed the highest total ferulic and *p*-coumaric acid levels among evaluated corn accessions. This sample also showed the highest TPC (free and bound) as stated above.

Several studies reported that most abundant phenolic compounds in cereals belong to the class of hydroxycinnamic acids and that the main one is ferulic acid, followed by diferulic acids and other minor compounds such as *p*-coumaric and sinapic and caffeic acids, together with hydroxybenzoic acid derivatives.[23,41,42] Around 95% of cereal phenolic compounds are linked to cell wall polysaccharides, mainly to arabinoxylans present within cell walls of the aleuronic layer with considerable contribution to the stability and digestibility of dietary fibers.[43,44] In the current work, bound ferulic acid quantified by HPLC overall contributed to around 90% of the total phenolic acids contents (free and bound), and the total ferulic acid amounts found here were higher than those reported by De la Parra et al.[24] (102−153 mg/100 g DW) and Lopez-Martínez et al.[2] (140−164 mg/100 g DW), and comparable to those found by Zilic et al.[3] (155.6−452.1 mg/100 g DW) and Montilla et al.[4] (132.9−298.4 mg/100 g DW) when several pigmented and purple corn varieties, respectively, were evaluated. Differences could be related to the cultivar type, origin, and other agroclimatic conditions. In this sense, further studies would be needed to better explore the effect of geographical origin and growing climatic conditions on found differences among phenolic acid contents from the studied corn accessions.

Table 4. Phenolic Acid Profiles Detected by HPLC (mg/100 g of Dry Weight)[a]

	vanillic acid			vanillin			protocatechuic acid			*p*-coumaric acid			ferulic acid			total phenolic acids		
no.	free	bound	total	free	bound	total	free	bound	total	free	bound	total	free	bound	total	free	bound	total
1	1.24 ± 0.05	ND	1.24 ± 0.05	0.28 ± 0.01	ND	0.28 ± 0.01	ND	ND	ND	1.22 ± 0.03	20.54 ± 0.65	21.76 ± 0.62	0.57 ± 0.01	182.7 ± 9.7	183.2 ± 9.7	3.32 ± 0.05	203.21 ± 10.33	206.53 ± 10.32
2	0.71 ± 0.02	ND	0.71 ± 0.02	0.30 ± 0.02	ND	0.30 ± 0.02	ND	ND	ND	0.85 ± 0.02	26.51 ± 0.45	27.36 ± 0.46	0.59 ± 0.03	256.8 ± 5.1	257.3 ± 5.1	2.45 ± 0.06	283.27 ± 4.84	285.72 ± 4.83
3	0.69 ± 0.02	ND	0.69 ± 0.02	0.26 ± 0.01	ND	0.26 ± 0.01	ND	ND	ND	0.66 ± 0.03	17.18 ± 0.81	17.73 ± 0.80	0.37 ± 0.02	209.9 ± 2.0	210.2 ± 2.1	1.65 ± 0.81	227.11 ± 2.44	228.76 ± 2.76
4	0.92 ± 0.03	ND	0.92 ± 0.03	0.20 ± 0.02	ND	0.20 ± 0.02	ND	ND	ND	0.68 ± 0.04	17.29 ± 0.50	17.97 ± 0.52	0.33 ± 0.01	149.7 ± 0.5	150.1 ± 0.5	2.13 ± 0.08	167.03 ± 0.94	169.16 ± 1.01
5	0.65 ± 0.03	ND	0.65 ± 0.03	0.25 ± 0.01	ND	0.25 ± 0.01	ND	ND	ND	0.70 ± 0.04	16.64 ± 0.67	17.23 ± 0.74	0.38 ± 0.03	177.2 ± 3.2	177.5 ± 3.2	1.66 ± 0.82	193.83 ± 3.56	195.49 ± 3.43
6	0.77 ± 0.01	ND	0.77 ± 0.01	0.37 ± 0.01	ND	0.37 ± 0.01	0.42 ± 0.03	ND	0.42 ± 0.03	0.70 ± 0.01	20.55 ± 0.41	21.25 ± 0.41	0.50 ± 0.01	200.4 ± 6.2	200.9 ± 6.2	2.77 ± 0.03	220.93 ± 6.26	223.70 ± 6.28
7	0.68 ± 0.01	ND	0.68 ± 0.01	0.34 ± 0.00	ND	0.34 ± 0.00	0.48 ± 0.02	ND	0.48 ± 0.02	0.56 ± 0.00	20.58 ± 0.28	21.05 ± 0.37	0.49 ± 0.00	188.6 ± 2.7	189.0 ± 2.7	2.14 ± 1.05	209.14 ± 2.91	211.28 ± 3.46
8	0.78 ± 0.03	ND	0.78 ± 0.03	0.23 ± 0.01	ND	0.23 ± 0.01	ND	ND	ND	0.66 ± 0.01	13.84 ± 0.81	14.50 ± 0.82	0.46 ± 0.02	186.6 ± 4.1	187.0 ± 4.1	2.13 ± 0.04	200.39 ± 4.36	202.52 ± 4.37
9	0.89 ± 0.08	ND	0.89 ± 0.08	0.20 ± 0.05	ND	0.20 ± 0.05	ND	ND	ND	0.88 ± 0.04	16.96 ± 0.46	17.84 ± 0.49	0.41 ± 0.01	202.8 ± 7.6	203.2 ± 7.6	2.38 ± 0.08	219.76 ± 8.00	222.14 ± 8.06
10	0.70 ± 0.01	ND	0.70 ± 0.01	0.31 ± 0.02	ND	0.31 ± 0.02	ND	ND	ND	0.60 ± 0.02	18.35 ± 0.61	18.95 ± 0.62	0.72 ± 0.01	205.6 ± 6.8	206.3 ± 6.8	2.33 ± 0.05	223.97 ± 7.46	226.30 ± 7.46
11	1.04 ± 0.08	ND	1.04 ± 0.08	0.40 ± 0.01	ND	0.40 ± 0.01	ND	ND	ND	1.45 ± 0.02	26.02 ± 0.91	27.47 ± 0.92	0.64 ± 0.04	200.6 ± 6.1	201.2 ± 6.2	3.53 ± 0.15	226.63 ± 6.91	230.15 ± 7.00
12	1.14 ± 0.08	ND	1.14 ± 0.08	0.40 ± 0.02	ND	0.40 ± 0.02	ND	ND	ND	1.30 ± 0.07	26.19 ± 0.75	27.49 ± 0.81	0.55 ± 0.03	189.1 ± 1.3	189.7 ± 1.3	3.40 ± 0.20	215.29 ± 1.90	218.69 ± 2.09
13	1.57 ± 0.15	ND	1.57 ± 0.15	0.21 ± 0.01	ND	0.21 ± 0.01	ND	ND	ND	1.80 ± 0.19	28.13 ± 0.12	29.93 ± 0.19	0.35 ± 0.03	207.4 ± 2.3	207.7 ± 2.3	3.93 ± 0.35	235.51 ± 2.41	239.44 ± 2.32
14	0.66 ± 0.05	ND	0.66 ± 0.05	0.38 ± 0.03	ND	0.38 ± 0.03	ND	ND	ND	1.43 ± 0.12	28.41 ± 0.06	29.81 ± 0.09	0.39 ± 0.03	212.2 ± 3.5	212.6 ± 3.5	2.86 ± 0.20	240.58 ± 3.47	243.44 ± 3.67
15	0.49 ± 0.01	ND	0.49 ± 0.01	0.26 ± 0.00	ND	0.26 ± 0.00	ND	ND	ND	0.35 ± 0.01	15.46 ± 0.75	15.76 ± 0.73	0.37 ± 0.02	222.3 ± 5.4	222.6 ± 5.4	1.23 ± 0.60	237.74 ± 6.12	238.96 ± 5.91
16	1.54 ± 0.02	ND	1.54 ± 0.02	0.21 ± 0.01	ND	0.21 ± 0.01	0.86 ± 0.02	ND	0.86 ± 0.02	1.50 ± 0.05	32.44 ± 1.59	33.94 ± 1.55	0.32 ± 0.02	178.8 ± 9.1	179.1 ± 9.1	4.47 ± 0.11	211.25 ± 10.67	215.72 ± 10.57
17	0.36 ± 0.02	ND	0.36 ± 0.02	0.28 ± 0.01	ND	0.28 ± 0.01	0.38 ± 0.02	ND	0.38 ± 0.02	0.35 ± 0.00	14.33 ± 0.33	14.68 ± 0.33	0.26 ± 0.02	148.2 ± 4.3	148.5 ± 4.3	1.65 ± 0.03	162.56 ± 4.57	164.21 ± 4.59
18	1.12 ± 0.03	ND	1.12 ± 0.03	0.96 ± 0.04	ND	0.96 ± 0.04	1.03 ± 0.02	ND	1.03 ± 0.02	0.64 ± 0.01	17.85 ± 0.58	18.38 ± 0.67	0.59 ± 0.02	128.3 ± 8.5	128.9 ± 8.4	3.66 ± 1.79	146.15 ± 8.83	149.81 ± 8.29
19	0.53 ± 0.01	ND	0.53 ± 0.01	0.35 ± 0.03	ND	0.35 ± 0.03	ND	ND	ND	1.73 ± 0.16	17.45 ± 0.47	19.18 ± 0.32	0.42 ± 0.03	126.6 ± 0.9	127.0 ± 0.9	3.03 ± 0.19	144.06 ± 1.37	147.08 ± 1.21
20	1.05 ± 0.02	ND	1.05 ± 0.02	0.38 ± 0.03	ND	0.38 ± 0.03	ND	ND	ND	0.99 ± 0.04	14.68 ± 0.55	15.68 ± 0.57	0.34 ± 0.01	165.7 ± 4.9	166.0 ± 4.9	2.77 ± 0.06	180.37 ± 5.45	183.13 ± 5.48
21	0.84 ± 0.02	ND	0.84 ± 0.02	0.47 ± 0.01	ND	0.47 ± 0.01	ND	ND	ND	0.82 ± 0.01	16.34 ± 0.19	17.16 ± 0.19	0.52 ± 0.02	203.4 ± 1.8	203.9 ± 1.8	2.66 ± 0.02	219.69 ± 1.93	222.35 ± 1.93
22	0.89 ± 0.01	ND	0.89 ± 0.01	0.25 ± 0.00	ND	0.25 ± 0.00	ND	ND	ND	0.92 ± 0.05	21.35 ± 0.10	22.26 ± 0.10	0.49 ± 0.03	172.5 ± 2.5	173.0 ± 2.6	2.54 ± 0.09	193.81 ± 2.53	196.35 ± 2.62
23	ND	ND	ND	0.23 ± 0.01	ND	0.23 ± 0.01	ND	ND	ND	0.31 ± 0.01	16.01 ± 0.75	16.32 ± 0.76	0.38 ± 0.01	133.1 ± 11.2	133.4 ± 11.2	0.91 ± 0.03	149.08 ± 11.91	149.99 ± 11.91
24	2.46 ± 0.12	ND	2.46 ± 0.12	0.36 ± 0.01	ND	0.36 ± 0.01	ND	ND	ND	0.87 ± 0.05	13.07 ± 0.57	13.94 ± 0.57	ND	165.8 ± 1.1	165.8 ± 1.1	3.69 ± 0.17	178.88 ± 0.56	182.57 ± 0.57
25	0.77 ± 0.03	ND	0.77 ± 0.03	0.26 ± 0.01	ND	0.26 ± 0.01	ND	ND	ND	0.57 ± 0.02	15.26 ± 0.46	15.74 ± 0.57	0.42 ± 0.01	138.0 ± 3.8	138.4 ± 3.8	1.69 ± 0.83	153.24 ± 3.78	154.93 ± 3.46
26	0.48 ± 0.01	ND	0.48 ± 0.01	ND	ND	ND	1.00 ± 0.07	ND	1.00 ± 0.07	0.75 ± 0.01	17.88 ± 0.38	18.63 ± 0.37	0.45 ± 0.01	187.3 ± 5.1	187.7 ± 5.1	2.73 ± 0.06	205.16 ± 5.44	207.89 ± 5.50
27	ND	ND	ND	0.31 ± 0.01	ND	0.31 ± 0.01	ND	ND	ND	0.57 ± 0.01	10.64 ± 0.61	11.21 ± 0.61	0.42 ± 0.00	160.1 ± 13.1	160.5 ± 13.1	1.31 ± 0.03	170.72 ± 13.67	172.03 ± 13.67
28	0.41 ± 0.02	ND	0.41 ± 0.02	0.12 ± 0.01	ND	0.12 ± 0.01	ND	ND	ND	0.26 ± 0.02	14.81 ± 0.48	15.07 ± 0.50	0.27 ± 0.01	136.3 ± 0.5	136.6 ± 0.5	1.06 ± 0.04	151.15 ± 0.38	152.22 ± 0.38
29	1.39 ± 0.06	ND	1.39 ± 0.06	0.30 ± 0.03	ND	0.30 ± 0.03	ND	ND	ND	1.09 ± 0.06	23.12 ± 1.17	24.21 ± 1.23	0.52 ± 0.03	202.2 ± 11.6	202.8 ± 11.6	3.30 ± 0.18	225.36 ± 12.75	228.66 ± 12.93
30	1.26 ± 0.03	ND	1.26 ± 0.03	0.22 ± 0.00	ND	0.22 ± 0.00	ND	ND	ND	0.38 ± 0.01	15.27 ± 0.30	15.65 ± 0.30	0.73 ± 0.01	129.4 ± 1.4	130.1 ± 1.4	2.58 ± 0.02	144.63 ± 1.16	147.22 ± 1.18
31	0.86 ± 0.03	ND	0.86 ± 0.03	0.43 ± 0.03	ND	0.43 ± 0.03	ND	ND	ND	0.85 ± 0.02	31.56 ± 0.48	32.41 ± 0.49	0.46 ± 0.03	168.4 ± 2.8	168.9 ± 2.8	2.60 ± 0.06	199.97 ± 3.25	202.57 ± 3.30
32	1.07 ± 0.03	ND	1.07 ± 0.03	0.58 ± 0.01	ND	0.58 ± 0.01	ND	ND	ND	1.53 ± 0.01	36.37 ± 1.66	37.90 ± 1.64	1.00 ± 0.02	268.5 ± 7.5	269.5 ± 7.5	4.19 ± 0.03	304.83 ± 9.16	309.02 ± 9.15
33	1.47 ± 0.04	ND	1.47 ± 0.04	0.19 ± 0.01	ND	0.19 ± 0.01	1.05 ± 0.02	ND	1.05 ± 0.02	1.09 ± 0.01	19.78 ± 0.78	20.87 ± 0.77	0.55 ± 0.01	186.7 ± 6.1	187.2 ± 6.1	4.40 ± 0.05	206.47 ± 6.90	210.86 ± 6.86
mean	0.90			0.31			0.13			0.89	20.03	20.89	0.46	181.54	182.0	3.06	201.57	204.63
min	ND			ND			ND			0.26	10.64	11.21	ND	126.1	127.0	0.91	144.06	147.08
max	2.46			0.96			1.05			1.80	36.37	37.90	1.00	268.5	269.5	4.19	304.83	309.02
accessions	*			*			*			*	*	*	*	*	*	*	*	*
race	*			*			*			*	*	*	*	*	*	*	*	*

[a]Mean ± SD; ND = not detected. *Significant at $p < 0.001$.

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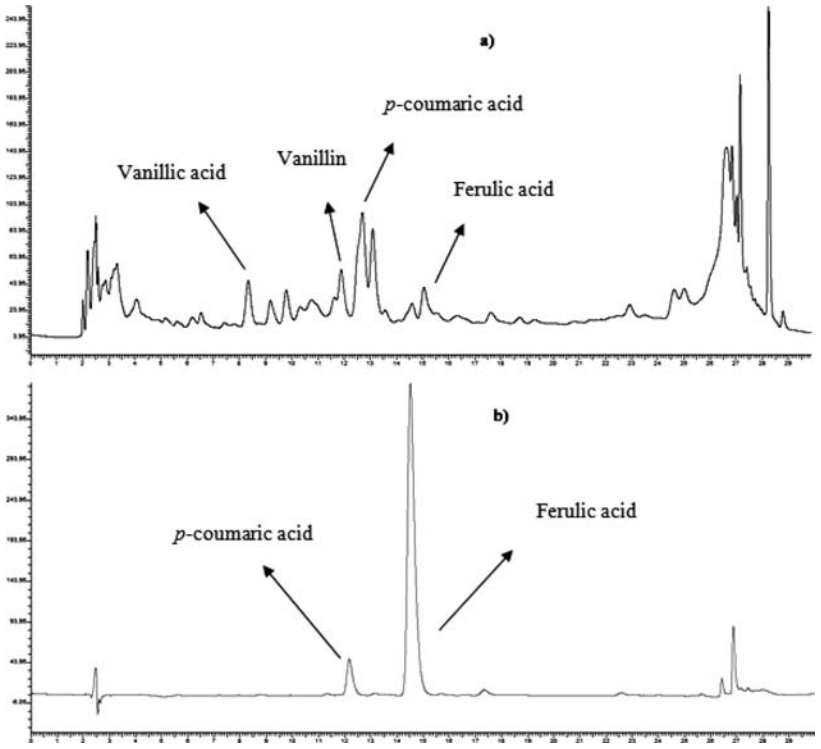

Figure 2. HPLC phenolic acid profiles (Pisankalla, CHZM 13104): (a) free phenolic fraction and (b) bound phenolic fraction.

Nevertheless, this is the first time that native Chilean corn accessions have been characterized with respect to their phenolic acids profiles in both free and cell-wall-linked fractions.

Calculated total phenolic acids were in the range of 147.1 to 309.0 mg/100 g DW (Table 4), which is similar to that obtained for the TPC (free and bound) (Table 3), indicating that corn phenolic compounds are mostly constituted by phenolic acids in the evaluated corn accessions.

In Vitro α-Amylase, α-Glucosidase, and ACE-I Inhibitory Activities. Strategies to counteract metabolic alterations related to hyperglycemia and subsequent type 2 diabetes include the inhibition of key enzymes such as carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase) and the angiotensin I-converting enzyme (ACE-I) relevant for hyperglycemia and hypertension, respectively.[45,46] Major drawbacks of the currently used therapeutic inhibitors are their well-known side effects such as abdominal distention, flatulence, and possibly diarrhea in the case of α-glucosidase inhibitors such as the drug acarbose.[47] Therefore, the consumption of natural inhibitors derived from food or its components would constitute an important dietary strategy for managing hyperglycemia and associated complications such as hypertension with minimal side effects.

The in vitro functionality of corn samples associated with their antihyperglycemic potential was assessed by measuring their ability to inhibit the porcine pancreatic α-amylase and the yeast α-glucosidase. The potential to inhibit the hypertension-related rabbit lung ACE-I was also screened in all free and bound phenolic extracts.

No α-amylase and ACE-I inhibitory activities were found in extracts from evaluated corn accessions. In addition, no α-glucosidase inhibitory activity was shown by all bound phenolic extracts. However, a significant inhibition against the α-glucosidase enzyme was shown by all free phenolic fractions, and this response followed a dose-dependent trend. This likely indicates that corn-linked-free fractions have potential anti-hyperglycemia activity (Table 5).

α-Glucosidase inhibitory activities ranged from 10.8 to 72.5% at the highest sample dose (25 mg). Samples 11 and 31 (Curagua and Polulo accessions, respectively) showed the highest α-glucosidase inhibitory activities (71.7 and 72.5%, respectively), followed by sample 20 (Diente de Caballo accession, 58.4%) and samples 5 and 2 (both Camelia accessions with 53.9 and 53.3% of inhibition, respectively). No correlation was found between this functional in vitro property and the TPC or a specific phenolic compound. The antioxidant capacity did not correlate to the α-glucosidase inhibitory activity either. However, the highest inhibitions were mainly related to yellow-colored accessions (Polulo accession) in contrast to darker kernels such as sample 32 (Pisankalla accession), which showed the lowest value (10.8%).

Similar findings were reported by Lee et al.,[48] who evaluated 18 corn strains and found that "yellow" samples overall had the highest yeast α-glucosidase inhibition (∼50%), followed by "white" samples (∼30%), and finally "black" samples (20−25%). Moreover, Kwon et al.[49] did not find a correlation between the DPPH scavenging antioxidant capacity and the inhibitory activity against the α-glucosidase, and the highest inhibition was associated with a yellow-pigmented corn variety (48%). No α-amylase and ACE-I inhibitory activities were also found by Ranilla et al.[50] when evaluating several thermally treated aqueous corn extracts; however, all extracts inhibited the α-glucosidase enzyme, and inhibitory ranges varied from 11 to 51% at 5 mg of sample weight. Slightly lower inhibitory activity ranges were obtained in the current work (5.8−37.2%) at the same sample dose.

Some studies have stated that inhibition of α-glucosidase would be associated with the methoxy group of certain hydroxycinnamic derivatives such as ferulic acid or due to the

Table 5. α-Glucosidase Inhibitory Activity (%) Detected in Extracts from the Free Fraction at Three Sample Doses[a]

	sample dose		
no.	5 mg	12.5 mg	25 mg
1	15.1 ± 3.6	27.0 ± 2.5	39.6 ± 2.6
2	20.2 ± 3.3	35.0 ± 2.7	53.3 ± 3.4
3	11.1 ± 3.1	21.8 ± 1.8	31.4 ± 2.6
4	9.8 ± 3.5	22.1 ± 2.1	32.5 ± 2.3
5	19.9 ± 2.2	38.5 ± 3.6	53.9 ± 2.6
6	15.1 ± 2.1	26.4 ± 3.6	48.7 ± 2.2
7	8.8 ± 4.2	19.0 ± 2.5	31.4 ± 2.4
8	7.9 ± 2.7	16.8 ± 1.8	27.1 ± 2.2
9	11.9 ± 3.6	22.9 ± 3.0	36.2 ± 2.4
10	8.1 ± 1.9	14.5 ± 1.4	20.3 ± 2.1
11	37.2 ± 1.0	59.0 ± 2.6	71.7 ± 1.8
12	ND	25.7 ± 5.3	39.4 ± 4.3
13	ND	22.6 ± 2.9	27.7 ± 4.6
14	5.8 ± 0.7	12.5 ± 1.4	25.6 ± 3.2
15	12.9 ± 1.6	21.0 ± 3.8	26.2 ± 2.2
16	ND	29.4 ± 6.2	34.6 ± 3.6
17	9.1 ± 1.9	15.3 ± 1.6	19.0 ± 1.2
18	18.7 ± 1.5	32.3 ± 1.8	43.0 ± 2.3
19	8.3 ± 1.9	17.7 ± 1.5	26.0 ± 2.8
20	21.7 ± 1.7	40.9 ± 3.1	58.4 ± 3.4
21	10.8 ± 2.9	22.4 ± 2.8	33.8 ± 4.6
22	8.2 ± 2.7	17.7 ± 1.6	25.3 ± 1.3
23	11.9 ± 1.9	18.0 ± 3.3	44.5 ± 3.6
24	9.9 ± 2.3	16.6 ± 4.4	32.5 ± 5.5
25	12.0 ± 7.4	16.8 ± 1.4	27.8 ± 2.5
26	15.8 ± 1.6	27.1 ± 2.0	37.7 ± 3.1
27	15.8 ± 3.2	24.4 ± 2.7	48.9 ± 4.1
28	18.2 ± 3.1	33.6 ± 2.4	48.2 ± 2.9
29	17.5 ± 4.4	33.5 ± 3.1	49.7 ± 4.5
30	12.6 ± 3.7	27.2 ± 7.2	37.8 ± 5.1
31	33.5 ± 2.6	58.8 ± 1.2	72.5 ± 2.1
32	ND	ND	10.8 ± 1.0
33	12.4 ± 5.0	21.5 ± 1.8	32.7 ± 2.2
mean	13.2	25.6	37.8
min	ND	ND	10.8
max	33.5	59.0	72.5
accessions	*	*	*
race	*	*	*

[a]Mean ± SD; ND = not detected. *Significant at $p < 0.001$.

presence of hydroxy or methoxy groups at the 4-position in the case of *trans*-cinnamic acid.[51] Both ferulic and *p*-coumaric acids were found in all free phenolic fractions of evaluated corn accessions together with hydroxybenzoic derivatives such as vanillin and vanillic and protocatechuic acids, but no correlation was found between any of these detected phenolic acids and the inhibition against α-glucosidase. This suggests that other undetected conjugated forms of soluble phenolic compounds may likely be involved. Certain flavones, flavanones, and flavonol glycosides related to yellow-colored grains have been shown to possess α-glucosidase inhibitory activity in cereal grains.[52,53] In addition, nonphenolic compounds with α-glucosidase inhibitory activity could have been extracted under the experimental conditions of the current work. Further chemical screening is being developed by our laboratory to elucidate the other soluble phenolic or nonphenolic compounds likely present in free fractions of selected corn accessions.

Principal Component Analysis (PCA). PCA was conducted to confirm any relationships among analyzed variables from evaluated corn samples. After the statistical analysis of all data, the PCA model retained five principal components (PC), which explained 73.1% of the total variability. The score and loading plots of the first two principal components are shown in Figure 3.

No defined groups were observed, and relationships among variables were more related to the accession type than to the race (Figure 3a). PC1 revealed an inverse relationship among the TPC, DPPH, and ABTS antioxidant capacity results from the free fractions and the color parameters (L^*) and (b^*), thus indicating that darker corn accessions would be associated with higher TPC and antioxidant capacity in free fractions. Dark samples such as sample 18 (Negrito Chileno accession) and sample 24 (Capio Chileno accession) among others, were located to the left of the score plot exhibiting the highest TPC and antioxidant capacities among free phenolic extracts. Similar PCA results were reported by Szydłowska-Czerniak et al.[54] for measured color parameters and antioxidant capacities when several rapeseed cultivars were evaluated. PC2 arranged samples according to their high phenolic acid contents (top to bottom), revealing a strong relationship between the content of ferulic and *p*-coumaric acids and the antioxidant capacities in bound fractions (samples at the top of score plot). Sample 32 (Pisankalla accession) exhibited the highest ferulic and *p*-coumaric acid contents. These results are in agreement with those obtained with Pearson correlations. PC3 (data not shown) arranged samples according to their high α-glucosidase inhibitory activities, which corresponded to sample 11 (Curagua accession) and sample 31 (Polulo accession). A clearer relationship between the inhibition of α-glucosidase and yellow colored-samples was observed when PCA was run excluding sample 11. However, this functional property did not show a correlation with other variables such as TPC or the antioxidant capacity.

This study provides for the first time important insights about the health-relevant functionality of 33 Chilean native corn accessions (from 14 races) related to their phenolic profiles, antioxidant capacity and in vitro potential to inhibit carbohydrate-hydrolyzing and intestinal absorption enzymes (α-amylase and α-glucosidase) and ACE-I relevant for hyperglycemia and hypertension management, respectively. A high variability was observed in all studied variables among evaluated samples independently of the race and accession. TPC and antioxidant capacity measured by the DPPH and ABTS methods were significantly higher in cell wall-linked fractions than in free forms. Around 88% of TPC were found in the bound form. Major phenolic compounds detected by HPLC were vanillin, vanillic, ferulic, and *p*-coumaric acids in free fractions, whereas only ferulic and *p*-coumaric acids were detected in all bound fractions. Pisankalla accession was remarkable due to its highest ferulic (269.5 mg/100g DW) and *p*-coumaric acids contents (37.9 mg/100g DW). No α-amylase and angiotensin I-converting enzyme (ACE-I) inhibitory activities were found. However, all free fractions significantly inhibited the α-glucosidase enzyme indicating potential antihyperglycemia activity. Correlations among studied variables were better revealed by PCA than by Pearson correlations. PCA showed that free fractions from darker kernels with lower color (L^*) and (b^*) parameter values had higher TPC and antioxidant capacity, whereas yellow-colored kernels were generally associated with high α-glucosidase inhibitory activities (Polulo accession). On the basis of results

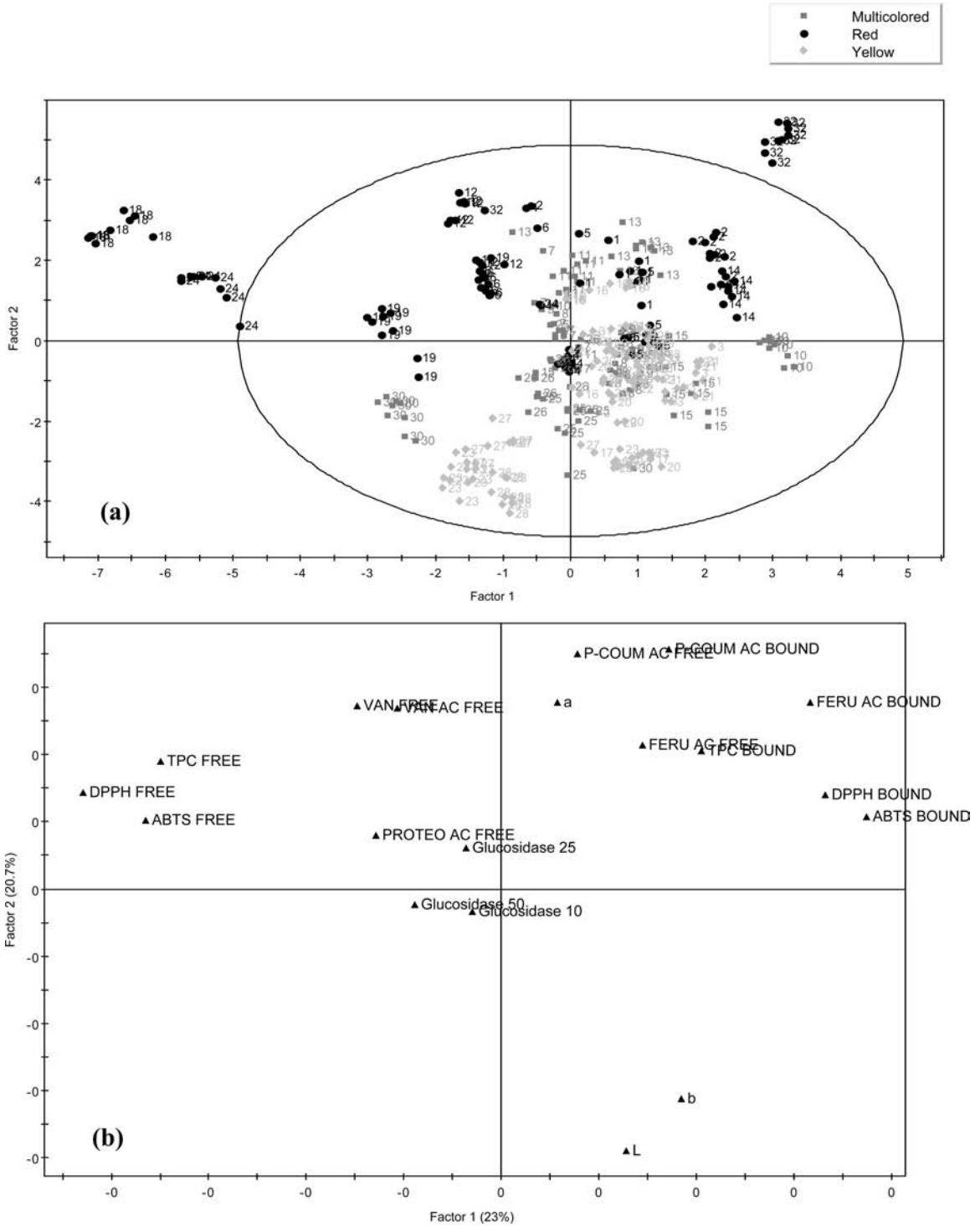

Figure 3. Score plot (a) and loading plot (b) for principal component analysis (PCA) for the first two factors.

from the current work, Chilean native corn accessions may be important natural sources of phenolic antioxidants with the potential of controlling early stages of postprandial hyperglycemia. Also, this research provides the biochemical basis for further in vivo studies and a rationale for functional food and ingredient design.

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Notes

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