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ARTICLE

Content of Insoluble Bound Phenolics in Millets and Their Contribution to Antioxidant Capacity

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Soluble and insoluble-bound phenolic extracts of several varieties of millet (kodo, finger, foxtail, proso, pearl, and little millets) whole grains were evaluated for their phenolic contents and antioxidative efficacy using trolox equivalent antioxidant capacity (TEAC), reducing power (RP), and β-carotene−linoleate model system as well as ferrous chelating activity. In addition, ferulic and *p*-coumaric acids were present in soluble and bound phenolic fractions of millets, and their contents were determined using high-performance liquid chromatography (HPLC) and HPLC−mass spectrometry (MS). Kodo millet had the highest total phenolic content, whereas proso millet possessed the least. All millet varieties showed high antioxidant activities, although the order of their efficacy was assay dependent. HPLC analysis of millet phenolic extracts demonstrated that the bound fractions contained more ferulic and *p*-coumaric acids compared to their soluble counterparts. The results of this study showed that soluble as well as bound fractions of millet grains are rich sources of phenolic compounds with antioxidant, metal chelating, and reducing power. The potential of whole millets as natural sources of antioxidants depends on the variety used. The importance of the insoluble bound fraction of millet as a source of ferulic acid and *p*-coumaric acid was established, and their contribution to the total phenolic content must be taken into account in the assessment of the antioxidant activity of millets.

KEYWORDS: β-Carotene−linoleate model; *p*-coumaric; ferrous chelating activity; ferulic; flavonoids; HPLC; proanthocyanidins; reducing power; TEAC

INTRODUCTION

Cereals are staple foods for many populations around the world. Epidemiological studies have demonstrated that regular consumption of whole grain cereals and their products can protect against the risk of cardiovascular diseases, type 2 diabetes, gastrointestinal cancers, and a range of other disorders (*1*). In addition to major macronutrients, whole grains contribute significant quantities of micronutrients and non-nutrient phytochemicals, the latter including phenolic compounds, in the human diet. In the past two decades there has been a renewed interest in polyphenols as "life span essentials" due to their role in maintaining body functions and health throughout the adult and latter phases of life. This is to address concerns about oxidative stress caused by imbalance between antioxidant defense mechanisms and increased production of free radicals, which is considered to be a leading cause in the development of chronic degenerative diseases. Therefore, consumption of whole grains is recommended to achieve optimal health. Canada's food guide recommends six to seven and eight daily servings of grain products, particularly whole grains, for females and males, respectively.

The potency of phenolic compounds to act as antioxidants arises from their ability to donate hydrogen atoms via hydroxyl groups on benzene rings to electron-deficient free radicals and in turn form a resonance-stabilized and less reactive phenoxyl radical. Plant phenolics may also act as reducing agents, singlet oxygen quenchers, and metal chelators (*2*). Whole grain cereals are a significant source of phenolic compounds, especially phenolic acids such as ferulic, *p*-coumaric, vanillic, caffeic, syringic, and sinapic acids and to a lesser extent flavonoids (*2*). Phenolic compounds in grains exist as free, soluble conjugates and insoluble bound forms (*3*). Phenolics are not equally distributed in the grain, and a high proportion is found in the outer layers, namely, the aleurone layer, testa, and pericarp, which form the main components in the bran fraction. Studies have shown that cereal brans mainly consist of ferulic acid and its oxidatively coupled products, the diferulic acids. Although insoluble-bound phenolics are not readily available for absorption, they can be released under the low pH conditions of the gastrointestinal tract (*4*) and upon colonic fermentation (*5*). Upon release, they can exert a localized effect on the gut lumen or could be absorbed into the bloodstream. Therefore, determination of bound phenolics and their antioxidant activity is of paramount importance to the understanding of the health benefits of grains.

Millets are important crops in semiarid and tropical regions of the world due to their resistance to pests and diseases, short growing season, and productivity under heat and drought conditions when major cereals cannot be relied upon to provide sustainable yields. Of the total millet produced in the world about 90% is utilized in the developing countries, and about two-thirds of millets produced are consumed as food (*6*). Major phenolic acids reported in millets are in general ferulic and *p*-coumaric

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acids among others (*7*). However, more is known about finger millet varieties than other millet grains. Phenolic compounds of finger millets are concentrated in the testa, and the content of phenolic compounds differs according to millet variety (*8*). Flavonoids so far reported in millets are flavones. Finger millets are reported to contain proanthocyanidins, also known as condensed tannins (*9*).

In addition to antioxidant properties, polyphenols of millets, particularly finger millet, possess other health benefits such as antimicrobial, anti-inflammatory, antiviral, anticancer, antiplatelet aggregation, and cataractogenesis inhibitory activities (*10*). Hegde et al. (*11*) reported that whole grain meals of kodo and finger millets protect against hyperglycemic and alloxan-induced oxidative stress in Wistar rats. Millets are underutilized in many developed countries. There is an immense potential to process millet grains into value-added foods and beverages in developing countries. Furthermore, millets, as they do not contain gluten, are good for coeliacs.

Although several studies have reported the phenolic compounds of principal cereals and their antioxidant properties, there remains an apparent gap in the early literature for such information on different millet varieties. Limited published data indicate that millets are good sources of phenolic compounds among different cereal grains. Millets, although named as a whole group due to the small size of the seeds, belong to a range of different species of the family Gramineae. Due to the fact that millets are consumed in several forms, they can contribute different nutrients and bioactive compounds to the diet, including phenolic compounds. Therefore, it is necessary to assess the phenolic compounds of millets to explore and possibly promote their use as functional food ingredients. The objectives of present study were (a) to investigate the potential antioxidant activity of phenolic compounds in whole grain millet varieties of kodo, finger, foxtail, proso, little, and pearl; (b) to determine the contribution of the insoluble bound fraction of phenolic compounds to the antioxidant activity; and (c) to determine the content of major phenolic acids (ferulic and *p*-coumaric) present in the grain samples.

MATERIALS

Seven millet grain samples, namely, foxtail (*Setaria italica*), proso (*Panicum miliacium*), two finger millet (*Elusine coracana*) varieties (Ravi and local), kodo (*Paspalum scrobiculatum*), little millet (*Panicum sumatrense*), and pearl millet (*Pennisetum glaucum*), were used in this study. All grain samples, with the exception of pearl millet, were obtained from the Field Crop Research and Development Center, Mahailluppallama, Sri Lanka. Pearl millet (dark green cultivar), grown in India, was obtained through Dr. Uttam Chavan, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India.

Folin−Ciocalteu's reagent, ferulic acid, *p*-coumaric acid, vanillin, catechin, aluminum chloride, sodium nitrite, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), sodium chloride, trolox, potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, ferrous chloride, ascorbic acid, ethylenediaminetetraacetic acid trisodium salt (Na_3EDTA), mono- and dibasic potassium phosphates, Tween 40 (polyoxyethylene sorbitan monopalmitate), β-carotene, linoleic acid, butylated hydroxyanisole (BHA), and 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4,4-disulfonic acid sodium salt (Ferrozine) were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Diethyl ether, ethyl acetate, hexane, acetone, methanol, chloroform, acetonitile, formic acid, hydrochloric acid, sodium hydroxide, and sodium carbonate were purchased from Fisher Scientific Ltd. (Ottawa, ON, Canada).

METHODS

Sample Preparation. Whole millet grains, cleaned using seed cleaners to remove soil and other particles, were ground using a coffee bean grinder (model CBG5 series, Black & Decker, Canada Inc., Brockville, ON, Canada) to obtain a fine powder that passed through mesh 16 (sieve opening 1 mm, Tylor test sieve, Mentor, OH). All samples were defatted by blending with hexane (1:5 w/v, 5 min three times) in a Waring blender (model 33BL73, Waring Products Division Dynamics Co. of America, New Hartford, CT) at ambient temperature. Defatted samples were vacuum packed in polythene pouches and stored at −20 °C until used within 1 week for extraction of phenolics.

Extraction of Soluble Phenolic Compounds. An ultrasonic-assisted extraction procedure was used for soluble phenolic compounds. Defatted meal (5 g) was mixed with 100 mL of 70% (v/v) acetone and then placed in an ultrasonic bath (300 Ultrasonik, Whittemore Enterprises, Inc., Rancho Cucamonga, CA) and sonicated at the maximum power for 25 min under refluxing conditions. After centrifugation of the resulting slurry for 5 min at 4000*g* (IEC Centra MP4, International Equipment Co., Needham Heights, MA), the supernatant was collected and extraction was repeated two more times. Combined supernatants were evaporated in vacuo at 40 °C (Buchi, Flawil, Switzerland) and lyophilized for 72 h at −46 °C and 34×10^{-3} mbar (Freezone, model 77530, Labconco Co., Kansas City, MO). Residues of whole grain samples were air-dried for 12 h and stored at −20 °C until used to extract bound phenolic compounds within a week. During all stages, extracts were protected from light by covering them with aluminum foil. Lyophilized crude phenolic extracts were stored at −20 °C until used for further analysis.

Extraction of Free and Esterified Phenolic Compounds. Free phenolic acids and those liberated from soluble esters were extracted from the lyophilized crude phenolic extract (*12*). An aqueous suspension of extract (250 mg in 10 mL) was adjusted to pH 2 with 6 M HCl, and free phenolics were extracted five times into diethyl ether and ethyl acetate (1:1, v/v). The free phenolic extract was evaporated to dryness under vacuum at room temperature. The water phase was neutralized to pH 7 with 2 M NaOH and then lyophilized. The resulting residue was dissolved in 10 mL of 2 M NaOH and hydrolyzed for 4 h at room temperature under a nitrogen atmosphere. After acidification to pH 2 with 6 M HCl, phenolic acids released from soluble esters were extracted from the hydrolysates five times with diethyl ether (1:1, v/v) and evaporated to dryness under vacuum.

Extraction of Bound Phenolic Compounds. The residue of the whole grain sample obtained after extraction of soluble phenolics was hydrolyzed with 2 M NaOH at room temperature for 4 h with stirring under nitrogen. The resulting slurry was acidified to pH 2 with 6 M HCl and extracted five times with hexane to remove fatty acids, which are released during alkaline hydrolysis. Bound phenolic compounds were extracted five times with diethyl ether and ethyl acetate (1:1, v/v) and subsequently desolventized to dryness at room temperature in a rotary evaporator. Phenolic compounds were reconstituted in 5 mL of HPLC grade methanol and stored at −20 °C until used.

Determination of Total Phenolic Content (TPC). The TPC of each extract was determined using the method described by Singleton and Rossi (*13*) with slight modifications. Briefly, the crude extracts of soluble phenolic compounds were dissolved in methanol to obtain a concentration of 2.5 mg/mL. Folin− Ciocalteu's reagent (0.5 mL) was added to centrifuge tubes containing 0.5 mL of extract, and the contents were mixed thoroughly by vortexing. The reaction was neutralized by adding 1 mL of saturated sodium carbonate to each tube, followed by the addition of distilled water (8 mL) and thorough mixing. Tubes were allowed to stand at room temperature in the dark for 35 min followed by centrifugation for 10 min at 4000*g*. The absorbance of the resulting blue color supernatant was measured at 725 nm (model HP 8452A diode array spectrophotometer, Agilent Technologies, Palo Alto, CA) using appropriate blanks for background subtraction. The content of total phenolics in each extract was determined using a standard curve prepared for ferulic acid and expressed as micromoles of ferulic acid equivalents (FAE) per gram of defatted meal.

Determination of Total Flavonoid Content (TFC). The TFC was determined using a colorimetric method described by Kim et al. (*14*) with slight modifications. One milliliter of aliquot of the extract, dissolved in methanol (2.5 mg/mL), was mixed with 4 mL of distilled water in a 50 mL centrifuge tube; 0.3 mL of 5% $NaNO_2$ was then added to the tube, which was allowed to react for 5 min. Subsequently, 0.3 mL of 10% $AlCl_3$ was added to the reaction mixture and allowed to stand for 1 min. Finally, 2 mL of 1 M NaOH and 2.4 mL of distilled water were added and mixed

immediately. Centrifuge tubes were kept in the dark at room temperature for 15 min followed by centrifugation for 5 min at 4000*g*. The absorbance was read at 510 nm against a blank prepared in a similar manner by replacing the extract with distilled water. The TFC calculated from a standard curve for catechin was expressed as micromoles of catechin equivalents (CE) per gram of defatted meal.

Determination of Proanthocyanidin Content (PC). The PC of crude phenolic extracts of millets was determined colorimetrically as described by Price et al. (*15*). To 1 mL of a methanolic solution of the extract was added 5 mL of 0.5% vanillin−HCl reagent [0.5% vanillin (w/v) in 4% concentrated HCl in methanol] followed by incubation for 20 min at room temperature. A separate blank for each sample was read with 4% HCl in methanol. The absorbance was read at 500 nm, and the content of proanthocyanidins was expressed as micromoles of CE per gram of defatted meal.

Trolox Equivalent Antioxidant Capacity (TEAC). The total antioxidant capacity of the millet extracts was determined according to the method explained by van den Berg et al. (*16*) and modified in our laboratory (*12*, *17*). The TEAC assay is based on the scavenging of the long-lived 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical anion ($ABTS^{\bullet-}$). An $ABTS^{\bullet-}$ solution was prepared by mixing 2.5 mM AAPH with 2.0 mM ABTS in 100 mM saline phosphate buffer (pH 7.4, 0.15 M NaCl) (PBS). The solution was heated for 16 min at 60 °C, protected from light by covering in a tin foil, and stored at room temperature. The $ABTS^{\bullet-}$ solution was filtered using medium-porosity filter papers (Fisher Scientific Co., Pittsburgh, PA) before mixing with the extracts. A blank was used for each measurement to account for the decrease in the absorbance of the radical solution itself with time. Millet extracts were dissolved in PBS at a concentration of 1 mg/mL and further diluted to fit within the range of values in the standard curve (6.25−50 μM prepared using trolox). The total antioxidant capacity was measured by mixing 40 μL of the sample with 1960 μL of the $ABTS^{\bullet-}$ solution. Absorbance of the reaction mixture was measured at 734 nm immediately at the point of mixing (t_0) and after 6 min (t_6). The decrease in absorbance at 734 nm after 6 min of addition of trolox and extract was calculated using the following equation: $\Delta A_{\text{trolox}} = (A_{t0,\text{trolox}} - A_{t6,\text{trolox}}) - (A_{t0,\text{blank}} - A_{t6,\text{blank}})$, where ΔA is the reduction of absorbance and A the absorbance at a given time. TEAC values were expressed as micromoles of trolox equivalents (TE) per gram of defatted meal.

Reducing Power (RP). The reducing power of soluble and bound phenolic extracts of millets was determined according to the method of Oyaiza (*18*). The extracts (1 mL) were mixed with 2.5 mL of a phosphate buffer solution (0.2M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%, w/v) in a centrifuge tube. The mixture was incubated at 50 °C for 20 min, and 2.5 mL of 10% TCA was added followed by centrifugation at 1750*g* for 10 min. The supernatant (1 mL) was transferred into a tube containing 2.5 mL of deionized water and 0.5 mL of 0.1% (w/v) $FeCl_3$, and the absorbance was read using a spectrophotometer at 700 nm. The standard curve was prepared using ascorbic acid. Increased absorbance of the reaction mixture indicated increased reducing power. The results were expressed as micromoles of ascorbic acid equivalents (AAE) per gram of defatted meal.

***β*-Carotene−Linoleate Model System.** The antioxidant activity of extracts was evaluated in a β-carotene−linoleate model system as explained by Jayaprakasha et al. (*19*) with some modifications. Briefly, 0.5 mL of β-carotene (1 mg/mL) dissolved in chloroform was pipetted into a 50 mL round-bottom flask. After chloroform was removed under vacuum, using a rotary evaporator at room temperature, 20 mg of linoleic acid, 200 mg of Tween 40 emulsifier, and 50 mL of aerated distilled water were added to the flask with vigorous agitation to form an emulsion. Emulsion was freshly prepared for each experiment. Absorbance measurement was carried out using a microplate reader equipped with a built-in incubator (FLUOstar OPTIMA, BMG LABTECH GmbH, Offenburg, Germany). Extracts in methanol (20 μL) were manually pipetted into sample wells of a Costar flat-bottom 96-well assay plate (Corning Inc., Corning, NY), and the injector pump was programmed to inject β-carotene−linoleic acid emulsion (200 μL) in each of the wells with automatic mixing. The microplate was incubated at 45 °C, and absorbance was read at 450 nm. Gain adjustment was done to improve the sensitivity of measurements before the start. The microplate reader was programmed to perform additional shaking of the contents in wells before each reading was taken. Readings of samples were recorded immediately at zero time and every 10 min up to 120 min. An equal amount of methanol was used for the control. Blank samples devoid of β-carotene were prepared for background subtraction. Butylated hydroxyanisole (BHA) and ferulic acid (200 ppm) in methanol were used as reference standards. The antioxidant activity coefficient (AAC) after 120 min of incubation was calculated using the following equation: $\text{AAC} = (A_{a(120)} - A_{c(120)})/(A_{c(0)} - A_{c(120)})$, where $A_{a(120)}$ and $A_{c(120)}$ are the absorbance values measured at 120 min for the sample and the control, respectively, and $A_{c(0)}$ is the absorbance value of the control at 0 min. The results were expressed as AAC per gram of defatted meal.

Ferrous Ion Chelating Activity. The ability of millet phenolic extracts to chelate ferrous ions was measured according to the method described by Dinis et al. (*20*). Different concentrations (0.5−4 mg/mL) of soluble phenolic extracts were used to measure chelating activity of ferrous ions. Briefly, 0.4 mL of extracts in distilled water was added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by adding 5 mM Ferrozine (0.2 mL), and the total volume was adjusted to 4 mL with distilled water. The mixture was vigorously shaken and left at room temperature for 10 min. The absorbance of the reaction mixture was measured at 562 nm. For the control distilled water was used instead of the extract. Appropriate blanks were prepared with 0.4 mL of the sample and 3.6 mL of distilled water for background subtraction. Different concentrations (0.05−2 mM) of Na_3EDTA were used to prepare the standard curve. The inhibition percentage of Ferrozine−ferrous ion complex formation was calculated by the following equation: metal chelating effect (%) = [1 − (absorbance of the sample − absorbance of the control)] × 100. The results were expressed as micromoles of EDTA equivalents per gram of defatted meal.

Determination of Ferulic and *p*-Coumaric Acid Contents. *HPLC Analysis*. Ferulic and *p*-coumaric acid contents of free, esterifed, and insoluble bound phenolic fractions of millet grains were determined by HPLC analysis. The RP-HPLC analysis was carried out using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a G1311A quaternary pump, a G1379A degasser, a G1329A ALS automatic sampler, a G1330B ALS Therm, a G1316A Colcom column compartment, a G1315B diode array detector (DAD), and a system controller linked to a Chem Station Data handling system (Agilent Technologies). Separations were conducted with a Supelcosil LC-18 column (4.6 × 250 mm, 5 μm; Merck). The mobile phase consisted of 1% formic acid (eluent A) and methanol/acetonitrile/formic acid (94:5:1; v/v/v) (eluent B). Gradient elution was used as follows: 0 min, 20% B; 10 min, 30% B; 15 min, 40% B; 18 min, 45% B; 20 min, 50% B; 30 min, 70% B; and 40 min, 85% B. The flow rate was adjusted to 0.5 mL/min, and the detection of compounds was performed at 254, 280, and 320 nm. All samples were filtered through a 0.45 μm PTFE membrane syringe filter (Whatman Inc., Florham Park, NJ) before injection. Ferulic and *p*-coumaric acids were identified by comparing their relative retention times and UV and ESI-MS spectra with authentic compounds. An external standard method with ferulic and *p*-coumaric acids was used for quantification purposes.

HPLC-ESI-MS Analysis. HPLC-MS analysis was performed under the HPLC analytical conditions explained above using an Agilent 1100 series capillary liquid chromatography−mass selective detector (LC-MSD) ion trap system in electrospray ionization (ESI) negative ion mode. Complete system control and data evaluation were achieved with Agilent LC-MSD Trap software (Agilent Technologies). The mass spectrometer was operated in a scan range from m/z 100 to 700 using a drying gas (N_2) temperature of 350°, a drying gas flow of 10 L/min, and a nebulizer gas (N_2) pressure of 60 psi.

Statistical Analysis. All experiments were carried out in triplicates unless otherwise stated, and data were reported as mean ± standard deviation. The significance of differences between soluble and bound extracts of millets was determined using Student's t test at $p \leq 0.05$. The differences of mean values among millet varieties were determined using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Differences (HSD) multiple-rank test at $p \leq 0.05$ significance level. Correlation analysis was performed between phenolic contents and antioxidant activity of soluble and bound extracts using Pearson and Spearman's correlations, respectively. All statistical analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The fat contents of millet grains generally range from 1.5 to 5 g/100 g of edible portion at 12% moisture level (*6*). Thus, ground

Table 1. Yield and Total Phenolic (TPC) and Flavonoid (TFC) Contents of Soluble and Bound Phenolic Extracts of Whole Millets

		TPC[a]		TFC[b]	
millet type	yield[c]	soluble	bound	soluble	bound
kodo	28 ± 0.5	32.39 ± 0.93 a	81.64 ± 0.15 a	33.71 ± 0.73 a	4.53 ± 0.14 a
finger (local)	43 ± 0.1	31.39 ± 1.22 a	3.20 ± 0.19 d	7.86 ± 0.33 b	0.36 ± 0.05 c
finger (Ravi)	42 ± 0.4	21.16 ± 0.31 b	3.83 ± 0.18 d	7.01 ± 0.06 b	1.05 ± 0.06 b
foxtail	49 ± 0.8	10.79 ± 0.82 c	11.59 ± 0.23 b	1.26 ± 0.03 c	0.47 ± 0.09 c
little	62 ± 0.4	12.67 ± 0.33 c	9.64 ± 0.28 c	1.59 ± 0.17 c	0.40 ± 0.06 c
pearl	59 ± 0.1	8.63 ± 0.38 d	9.14 ± 0.17 c	1.67 ± 0.01 c	0.28 ± 0.10 c
proso	32 ± 0.1	7.19 ± 0.12 d	2.21 ± 0.01 e	1.18 ± 0.07 c	0.44 ± 0.05 c

[a] Expressed as μmol of ferulic acid equiv/g of defatted meal. Values in each column having the same letter are not significantly different ($p > 0.05$). [b] Expressed as μmol of catechin equiv/g of defatted meal. Values in each column having the same letter are not significantly different ($p > 0.05$). [c] Yield of soluble phenolic extract expressed as mg/g of defatted meal .

millet grains were defatted to remove lipids and lipid-soluble components that may affect phenolic compounds and antioxidant activity assays. Preliminary studies showed that ultrasonic-assisted extraction with 70% acetone under reflux conditions affords high phenolic yield and antioxidant activity. **Table 1** presents yields of crude extracts after lyophilization. To the best of our knowledge this study is the first to report on the total phenolic content and antioxidant capacity of insoluble bound phenolic extracts of kodo, proso, foxtail, little, and pearl millets as well as the content of soluble and insoluble bound flavonoids in the extracts of millet grains.

TPC. The TPC of soluble and bound fractions of whole grain millets were determined using Folin−Ciocalteu's assay. In this method, under alkaline conditions, phenolic groups are deprotonated, leading to the formation of phenolate ions, which reduce the phosphotungstic−phosphomolybdic complex in the Folin−Ciocalteu reagent to a blue color. The soluble phenolic fraction includes both free and soluble conjugates, which are responsible for the in vitro antioxidant capacity of the extracts.

Total phenolic contents of soluble and insoluble bound fractions of different millet varieties ranged from 7.19 ± 0.12 to 32.39 ± 1.22 μmol of FAE/g of defatted meal and from 2.21 ± 0.01 to 81.64 ± 0.15 μmol of FAE/g of defatted meal, respectively (**Table 1**). Similar to other cereals (*21*−*27*), an influence of genotype on the content of phenolic compounds was observed in this study. Kodo millet showed the highest soluble phenolic content followed by finger (local), finger (Ravi), little, foxtail, pearl, and proso millets. In general, the phenolic contents of millets obtained in the present study were higher than those noted in other cereals such as barley and wheat. According to Madhujith and Shahidi (*24*) the TPC of different barley cultivars ranged from 0.35 to 6.13 μmol of FAE/g of defatted meal. Beta et al. (*26*) reported that the TPC of mostly hull-less barley cultivars ranged from 13.8 to 20.3 μmol of FAE/g of barley. Phenolic contents of whole grains of soft and hard wheats were 3.96 and 6.65 μmol of FAE/g of defatted meal, respectively (*27*). In the present study, millets with dark brown pigmented testa and pericarp (kodo and two finger millet varieties) possessed a higher phenolic content of soluble phenolic fractions than those with white or yellow testa and pericarp (pearl, proso, foxtail, and little millets), in agreement with the findings of others (*28*).

The comparison between soluble and insoluble bound phenolic contents showed an inconsistency in their trend among different millet types. Kodo millet exhibited 2.5 times higher phenolic content in bound phenolic extract compared to its soluble phenolic counterpart. A higher phenolic content for bound phenolic extracts of whole grains of corn, wheat, oats, rice, and barley than their soluble counterparts was documented in the literature (*17*, *22*, *29*). However, foxtail and pearl millets had similar ($p < 0.05$) soluble and bound phenolic contents. On the other hand, the TPCs of bound extracts of finger (local), finger (Ravi), little, and proso millets were lower ($p < 0.05$) than those of their corresponding soluble phenolic counterparts. In agreement with this finding, some studies have reported a lower phenolic content for bound phenolic extracts for whole grains of finger millet and buckwheat than their soluble counterparts (*8*, *30*). The variety of cereals may account for differences in the results obtained as well as possible variations in the extraction conditions employed in the present study. Extraction of phenolic compounds may vary due to their chemical nature, extraction method employed, sample particle size, ratio of sample to solvent, extraction time, pH, and temperature. Ultrasonic-assisted extraction employed in the present study is a simple alternative to conventional extraction methods. Passage of ultrasonic waves produces acoustic cavitations in the solvent, and the mechanical effect exerted by ultrasonic waves allows greater penetration of the solvent into the sample matrix and increases the contact surface area between the solid and liquid phases. This facilitates the diffusing of the solute from the solid phase to the solvent. Furthermore, in addition to phenolics other compounds such as simple carbohydrates and/or amino acids may be present in the crude extracts and could interfere with determinations of TPC by the Folin−Ciocalteu assay, leading to discrepancies of the results obtained in the present work (*21*).

TFC. Flavonoids are the most studied group of phenolic compounds and are known to possess antioxidant, anticancer, antiallergic, anti-inflammatory, antineuro-inflammatory, and gastroprotective properties. TFC was quantified using the chelating power of flavonoids with aluminum(III). Flavonoids form a pink-colored complex with aluminum(III) through the 4-keto and neighboring hydroxyl groups or through adjacent hydroxyl groups in the B ring.

Among soluble extracts of different millet varieties studied, kodo millet had the highest TFC followed by finger (local), finger (Ravi), pearl, little, foxtail, and proso millets as shown in **Table 1**. The TFC of soluble extracts ranged from 1.18 ± 0.07 to 33.71 ± 0.73 μmol of CE/g of defatted meal. The soluble extracts of little, foxtail, pearl, and proso millets did not show any significant ($p > 0.05$) difference among varieties and, in general, were 4−7- and 20−29-fold lower than that of two finger millet varieties and kodo millet, respectively. As noted in TPC, varieties with dark pigmented testa and pericarp showed a higher TFC than those with white or yellow pigmented testa. Recently, Shen et al. (*31*) also reported that white rice had a lower mean flavonoid content compared to red rice and black rice. The TFC varied significantly ($p < 0.05$) between soluble and bound fractions for all millet varieties tested in this study. In general, all soluble extracts had higher TFC than their corresponding bound extracts. The bound phenolic extract of kodo had the highest (4.53 ± 0.14 μmol of CE/g of defatted meal) TFC, whereas pearl millet showed the lowest (0.28 ± 0.10 μmol of CE/g of defatted meal). In contrast to the results obtained in this study, Adom and Liu (*34*) reported that soluble extracts of corn, wheat, oat, and rice contained a lesser TFC than their bound counterparts. The TFCs reported in the soluble extracts of oat, rice, corn, and wheat were 0.45, 0.33, 0.16, and 0.09 μmol of CE/g of grain, respectively, whereas the TFCs for corresponding bound extracts were 0.71, 0.6, 1.52, and 1.15 μmol of CE/g of grain, respectively (*22*). Adom et al. (*32*), using different varieties of wheat, further showed that bound TFC was higher than soluble TFC. In general, values of TFC reported in the present study for different millet varieties were higher than those reported for other cereals, namely, corn, wheat, oat, and rice (*22*, *32*). This study reported for the first time the

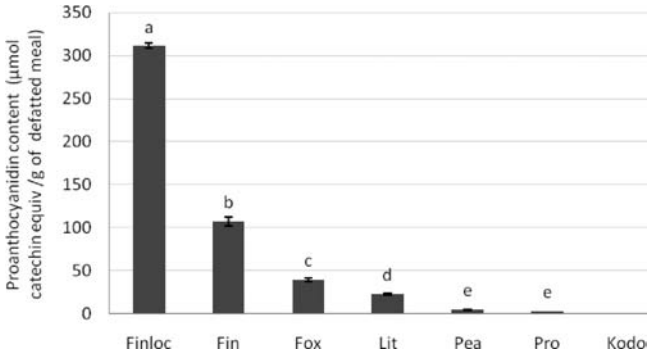

Figure 1. Proanthocyanidin content of whole millet varieties: Finloc, finger (local); Fin, finger (Ravi); Fox, foxtail; Lit, little; Pea, pearl; Pro, proso; Kodo, kodo millets. Bars with different letters are significantly different ($p < 0.05$).

Table 2. Trolox Equivalent Antioxidant Activity (TEAC) and Reducing Power (RP) of Soluble and Bound Phenolic Extracts of Whole Millets

	TEAC[a]		RP[b]	
millet type	soluble	bound	soluble	bound
kodo	41.68 ± 0.24 a	86.13 ± 2.60 a	18.79 ± 0.34 a	29.33 ± 2.60 a
finger (local)	12.37 ± 0.08 b	6.77 ± 0.90 d	26.75 ± 0.36 b	7.10 ± 0.90 b
foxtail	11.14 ± 0.68 b	40.61 ± 4.66 b	5.02 ± 0.01 c	8.25 ± 4.66 c
proso	6.73 ± 0.73 c	11.14 ± 0.55 d	3.64 ± 0.10 d	2.96 ± 0.55 d
finger (Ravi)	6.29 ± 0.33 c	5.03 ± 0.73 d	17.06 ± 0.35 e	6.30 ± 1.35 e
pearl	4.15 ± 0.24 d	6.77 ± 1.37 d	6.69 ± 0.17 f	11.29 ± 1.37 f
little	3.70 ± 0.73 d	18.34 ± 0.17 c	3.93 ± 0.07 d	4.17 ± 0.17 g

[a] Expressed as μmol of Trolox equiv/g of defatted meal. Values in each column having the same letter are not significantly different ($p > 0.05$). [b] Expressed as μmol of ascorbic acid equiv/g of defatted meal. Values in each column having the same letter are not significantly different ($p > 0.05$).

TFC of soluble and insoluble bound phenolic extracts of millets as determined using a colorimetric method. Limited information is so far available on the quantification and characterization of millet flavonoids, and their identification is being further pursued.

Proanthocyanidin Content (PC). Proanthocyanidins are oligomeric or polymeric flavonoids consisting of flavan-3-ol units. They are biologically active and, when present in sufficient quantities, may lower the nutritional value and biological availability of proteins and minerals (*33*). Several in vivo assays have demonstrated their anti-inflammatory, antiviral, antibacterial, and antioxidant properties. **Figure 1** shows the PC expressed as micromoles of CE per gram of defatted meal. Among the millet varieties used in this study, finger (local) millet had the highest PC (311.28 ± 3.0 μmol of CE/g of defatted meal) followed by finger (Ravi), foxtail, little, pearl, and proso millets. Low amounts of PC were detected in proso and pearl millets. Proanthocyanidins were not detected in kodo millet. The values reported for millets were higher than those for barley. Tannin contents reported for barley showed a range of 0.21−0.20 μmol of CE/g of barley (*26*). The vanillin test used in this study is quite specific for flavanols (monomers and polymers) and dihydrochalcones with a single bond at the 2- and 3-positions and free meta-oriented hydroxyl groups on the B ring (*2*). Condensed tannins are generally more potent antioxidants than their corresponding monomers. These have so far been reported only in finger millet varieties. Nevertheless, in the present study all millet varieties, except kodo, had a positive reaction with vanillin reagent and, in addition to the two finger millet varieties foxtail and little millets, also showed considerable quantities of proanthocyanidins of 39 and 23 μmol of CE/g of defatted meal, respectively (**Figure 1**). The solvent system (70% acetone) used in this study could enhance the extraction of proanthocyanidins. Chavan et al. (*33*) reported that PC of 70% acetone extract of beach pea was 11 times higher than that of 70% methanol extract. However, previous studies have documented that a number of compounds other than condensed tannins such as eriodictyol and luteoforol may give a positive response in the vanillin reaction. Therefore, it is required to confirm the availability of proanthocyanidins using specific tests such as the Clorox bleach test (*34*).

TEAC. The present analysis shows that antioxidant activities of millet were different among varieties and between soluble and bound fractions of the same grain. The TEAC assay is widely used to assess the antioxidant capacity of different biological matrices. The ability of antioxidant compounds to reduce the ABTS radical anion to its nonradical form is compared with that of trolox, which is a water-soluble analogue of α-tocopherol. In this study, the TEAC test is performed in an aqueous buffer; thus, only water-soluble compounds are measured. In the modified TEAC assay (*16*) that was used in the present work, ABTS radical anions with a characteristic blue-green color were pregenerated by heating ABTS with the thermolabile azo compound AAPH before addition of the extracts. As shown in previous studies (*16*), some compounds show a biphasic reaction pattern that includes fast and slow reactions in the TEAC assay. Therefore, TEAC values depend on the time point used to read the absorbance (*16*). In the present study TEAC at 6 min was chosen as it includes more of the slow reaction as many antioxidants also demonstrate a slow reaction as well with ABTS radical anion (*24*, *27*).

Table 2 shows the TEAC of soluble and bound phenolics of millets. The TEAC value ranged from 3.70 ± 0.73 to 41.48 ± 0.24 μmol of TE/g of defatted meal for soluble phenolics. The soluble extract of kodo millet showed 4−10 times higher TEAC than the other six millet varieties tested in the present study. Except for two finger millet varieties, bound extracts of all other varieties examined showed higher ($p < 0.05$) TEAC values than their soluble counterparts. Bound phenolic extracts of kodo millet (86.13 ± 2.6 μmol of TE/g of defatted meal) showed the highest TEAC, which was 17-fold higher than that of finger (Ravi) millet (5.03 ± 0.73 μmol of TE/g of defatted meal), which had the lowest.

The TEAC values for different barley cultivars ranged from 3.74 to 6.82 μmol of TE/g of defatted meal (*24*), and those for whole grains of wheat were 4.24 and 4.99 μmol of TE/g of defatted meal for soft and hard wheat, respectively (*27*). According to Yu et al. (*35*) the TEAC of hard winter wheat varieties ranged from 1.08 to 1.91 μmol of TE/g of grain. In general, the results of the present study had higher TEAC values for both soluble and bound extracts than those reported for barley and wheat, suggesting their potential as important sources of natural antioxidants. The TEAC of soluble extracts of whole grain millets was associated with TPC ($r^2 = 0.696$; $p < 0.01$) and TFC ($r^2 = 0.965$; $p < 0.01$). Furthermore, the TEAC of bound phenolic extracts positively and significantly correlated with their corresponding TPC ($r^2 = 0.705$; $p < 0.01$). Many prior studies have reported a significant correlation between phenolic content and the TEAC of the extracts of cereals (*27*). Although soluble extracts of kodo and finger (local) millets showed similar TPC, finger (local) millet had a 3.4 times lower TEAC value than that of kodo millet. This demonstrates that the contents of phenolics alone may not sufficiently explain the observed antioxidant activity of plant phenolic extracts, which are mixtures of different compounds with variable activities in test systems employed in the determination of antioxidant activity of samples. However, the ABTS radical anion is a synthetic organic radical, which is not relevant in biological and food systems. Thus, interpretation of TEAC of millet phenolic extracts in relation to such systems could

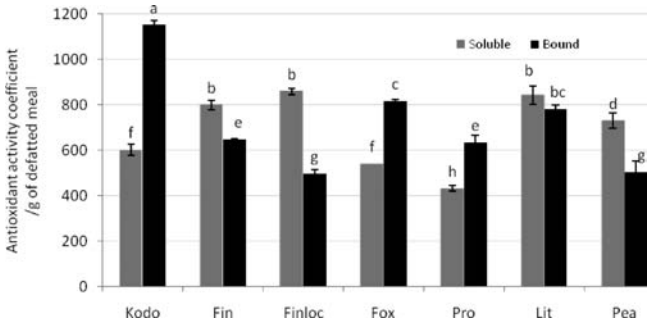

Figure 2. Antioxidant activity of soluble and bound phenolic extracts of whole millets in β-carotene−linoleate model system: Kodo, kodo; Fin, finger (Ravi); Finloc, finger (local); Fox, foxtail; Pro, proso; Lit, little; Pea, pearl millets. Bars with different letters are significantly different ($p < 0.05$). The letter "a" represents the highest value.

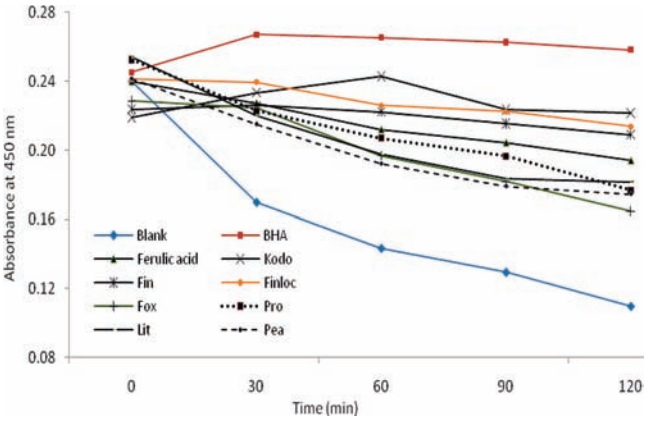

Figure 3. Absorbance of retained β-carotene at the presence of soluble phenolic extracts of whole millets and reference standards in the β-carotene−linoleate model system: Kodo, kodo; Fin, finger (Ravi); Finloc, finger (local); Fox, foxtail; Pro, proso; Lit, little; Pea, pearl millets.

be difficult. Therefore, determination of the inhibitory activity of millet phenolics on peroxyl and hydroxyl radicals and other reactive oxygen species is underway and will be communicated separately.

RP. Compounds with RP are capable of donating electrons and thus reducing the oxidized intermediates of peroxidation by acting as antioxidants. Reductants in the extracts reduce the ferric/ferricyanide complex to the ferrous form. Millet extracts tested in the present study exhibited a considerable RP, thereby acting as effective reductones. Reducing power of soluble phenolic extracts of millets ranged from 3.64 ± 0.1 to 26.75 ± 0.36 μmol of AAE/g of defatted meal (**Table 2**). Soluble phenolics of finger (local) millet had the highest RP, whereas proso millet showed the lowest.

Reducing power of bound phenolics ranged from 2.96 ± 0.55 to 29.33 ± 2.6 μmol of AAE/g of defatted meal. Bound phenolics of the two finger millet varieties and proso millet exhibited a lower RP than their soluble counterparts. Reducing power of bound phenolic extracts showed a significant ($p < 0.05$) difference from that of soluble phenolic extracts except for little millet. In the present study, bound extracts of kodo, foxtail, and pearl millets showed generally 1.6 times higher RP than their soluble counterparts. In agreement with other assays used in this study the RP of dark brown pigmented varieties was quite higher than those of other varieties investigated. Reducing power of soluble phenolic extracts positively associated with TPC ($r^2 = 0.93$; $p < 0.01$), TFC ($r^2 = 0.551$; $p < 0.01$), and PC ($r^2 = 0.773$; $p < 0.01$), whereas only TPC ($r^2 = 0.669$; $p < 0.01$) of the bound extracts positively correlated with their corresponding RP. These results suggest that phenolic compounds of millet extracts serve as a viable source of electron donors.

***β*-Carotene−Linoleate Model System.** In the β-carotene−linoleate aqueous emulsion system, heat-induced oxidation results in the formation of free radicals, which are formed by abstracting a hydrogen atom from the active bis-allylic methylene group of linoleic acid in C-11 between two double bonds. Free radicals attack β-carotene, causing the molecule to lose its conjugation, resulting in the loss of the characteristic yellow-orange color of the molecule. Phenolic compounds protect the β-carotene from bleaching by reacting with linoleate free radical. Thus, in this system the antioxidant potential of millet extracts is rendered in an aqueous medium, which is more relevant to foods and biological systems.

Antioxidant activity coefficients of soluble and bound phenolic extracts of millets are presented in **Figure 2**. Soluble extracts of the two finger millet varieties and little millet showed high AAC followed by pearl, kodo, foxtail, and proso millets. Bound extracts of kodo showed the highest AAC among the others. The antioxidative efficacy of bound extracts was in the order kodo > foxtail > little > finger (Ravi) > proso > pearl > finger (local). Except little millet, all other varieties tested demonstrated a significant ($p < 0.05$) difference in β-carotene bleaching, between soluble and bound phenolic extracts.

Figure 3 shows the change of corrected absorbance of β-carotene retained with time in the presence of soluble extracts of phenolic compounds and reference standards, ferulic acid, and a synthetic antioxidant, BHA. BHA showed greater antioxidant activity than ferulic acid and all soluble phenolic extracts. This was also observed for all bound extracts (data not shown). Synthetic antioxidants have been reported to contain high antioxidant activity in emulsion systems. Kodo and two finger millet varieties studied exhibited a higher antioxidant activity than ferulic acid, whereas foxtail, proso, little, and pearl millet soluble extracts had a lower activity at 200 ppm.

Both soluble and bound phenolic extracts showed high potential antioxidant activity against linoleate and any other free radicals formed within the system. Rao and Muralikrishna (*8*) reported higher AAC in soluble extracts than the corresponding bound extracts in finger millet varieties as noted in the present study. The observed activity of millet extracts can be attributed to their phenolic content and the profile of phenolic compounds present therein. The antioxidant activity coefficient of bound phenolic extracts was positively and significantly associated with TPC ($r^2 = 0.73$; $p < 0.01$) and TFC ($r^2 = 0.616$; $p < 0.01$). However, the AAC of soluble phenolic extracts of millet showed a significant correlation only with PC ($r^2 = 0.562$; $p < 0.01$), whereas TPC and TFC were insignificant ($p > 0.05$). On the basis of the results shown here it can be speculated that besides the content, the composition of phenolics and other factors may also play a role in the antioxidant activity of millet phenolics in this system.

Ferrous Ion Chelating Activity. Ferrous ion is a key transition metal ion responsible for initiation of peroxidation in foods and biological systems. In the body ferrous ions contribute to the generation of hydroxyl radicals via Fenton's reaction and lead to the destruction of biomolecules, thus causing disease conditions and aging. Chelating agents reduce the concentration of metal ions available for catalyzing peroxidation and thus are known to serve as effective secondary antioxidants. In this assay ferrous ions form a complex with Ferrozine, and the intensity of the purple color of the complex decreases in the presence of chelating agents.

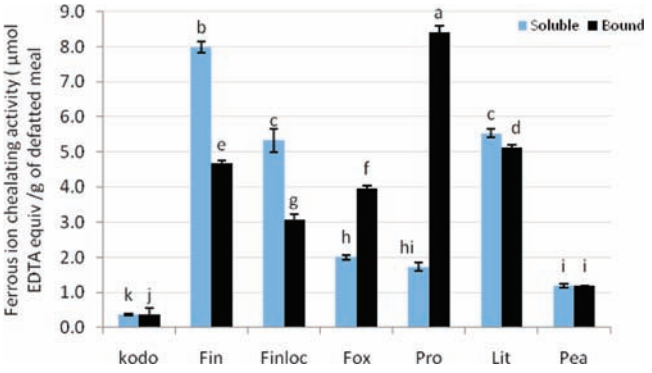

Figure 4. Ferrous ion chelating activity (μmol of EDTA equiv/g of defatted meal) of soluble and bound phenolic extracts of whole millet varieties: Kodo, kodo; Fin, finger (Ravi); Finloc, finger (local); Fox, foxtail; Pro, proso; Lit, little; Pea, pearl millets. Bars with different letters are significantly different ($p < 0.05$). The letter "a" represents the highest value.

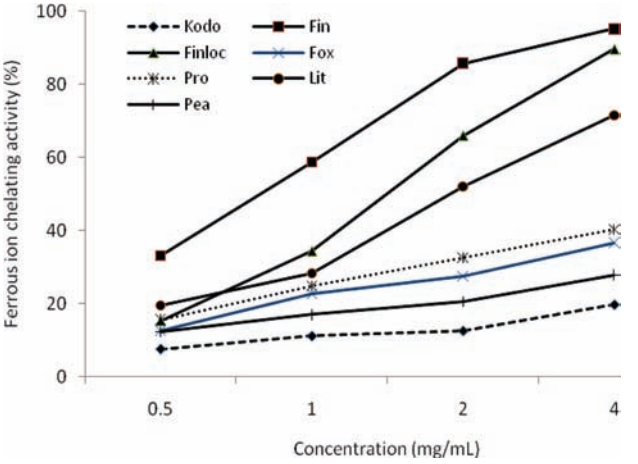

Figure 5. Ferrous ion chelating activity (%) of soluble phenolic extracts of whole millet varieties at different concentrations: Kodo, kodo; Fin, finger (Ravi); Finloc, finger (local); Fox, foxtail; Pro, proso; Lit, little; Pea, pearl millets.

Figure 4 shows the ferrous ion chelating activity of soluble and bound phenolic extracts of millets. The ferrous ion chelating activity of soluble phenolic extracts ranged from 0.37 ± 0.03 to 7.99 ± 0.16 μmol of EDTA equiv/g of defatted meal. Soluble extracts of kodo millet had the lowest ferrous ion chelating activity, whereas finger (Ravi) millet showed the highest. The results of this study indicate that phenolic extracts of millets may serve as a potential source of chelating agents inhibiting radical-mediated chain reactions. Millet varieties, except kodo millet, used in this study exhibited ferrous ion chelating activity superior to that of a number of other cereals. The ferrous ion chelating activity of soluble extracts of barley varieties ranged from 1.1 to 2.1 μmol of EDTA equiv/g of defatted meal (*24*), whereas soluble extracts of soft and hard wheat whole grains were 2.4 and 2.5 μmol of EDTA equiv/g of defatted meal, respectively (*27*). The chelating capacities of hard winter varieties ranged from 6.5 to 18.8 μmol of EDTA equiv/g of extract (*35*). In this study, the ferrous ion chelating activity of soluble millet extracts decreased in the order finger (Ravi) > little > finger (local) > foxtail > proso > pearl > kodo (**Figure 4**). Interestingly, the ferrous ion chelating activity of soluble extracts of millets did not show a significant ($p > 0.05$) correlation with either TPC or TFC, but demonstrated a significant positive correlation with PC ($r^2 = 0.551$; $p < 0.01$). Proanthocyanidins form stable complexes with metal ions acting as an effective ferrous ion chelator. Thus, soluble extracts of two finger millet varieties, which were rich sources of PC, showed a higher ferrous ion chelating activity than that of the kodo millet, despite the fact that it has a high TPC, similar to that of finger millets. Consistent with the present findings, lack of correlation between phenolic contents and ferrous ion chelating activity was reported in several studies (*7*, *20*). These results further suggest that factors other than phenolic content affect the ferrous chelating activity of millet extracts. As shown in **Figure 5**, soluble phenolic extracts of millets had a dose dependent ferrous ion chelating activity. For all tested concentrations of soluble extracts, finger (Ravi) millet exhibited a higher ferrous ion chelating activity than others, whereas kodo millet showed the lowest.

In this study, the ferrous ion chelating activity of bound phenolic extracts ranged from 0.85 ± 0.19 to 8.42 ± 0.18 μmol of EDTA equiv/g of defatted meal. Proso millet, which contained the lowest TPC, showed the highest activity among bound phenolic extracts. As suggested by other authors, this could be due to the presence of nonphenolic antioxidant compounds such as phytic acid (*4*). Phytic acid is found in various concentrations in cereals, legumes, nuts, and oilseeds and serves as a potential antioxidant by virtue of forming iron−phytate chelate. Extraction conditions employed for bound fractions in the present study may release phytates, thus contributing to high ferrous chelation activity. Except for pearl millet all other varieties exhibited significant differences in ferrous ion chelating activity between soluble and bound extracts. Bound extracts showed a negative correlation with TPC ($r^2 = -0.53$; $p < 0.05$) and an insignificant negative correlation with TFC ($r^2 = -0.09$; $p > 0.05$).

Ferulic and *p*-Coumaric Acid Contents. In general, ferulic and *p*-coumaric acids are reported as the major hydroxycinnamic acids in cereals (*24*, *27*, *36*, *37*). **Table 3** presents the contents of ferulic and *p*-coumaric acids of free, esterified, and insoluble bound phenolic fractions of different millet varieties. Pearl millet had the highest free ferulic acid content (22.37 ± 1.16 μg/g of defatted meal), which was at least 3 times higher ($p < 0.05$) than that of other millet varieties tested. The esterified ferulic acid content ranged from 8.47 ± 0.44 to 196.18 ± 5.77 μg/g of defatted meal. Kodo millet had the highest total soluble ferulic acid content with a high contribution from esterified fraction. Total soluble ferulic acid contents of all millet varieties used in this study were lower ($p < 0.05$) than those of their bound counterparts.

Little millet had the highest total soluble *p*-coumaric acid content among all millet varieties tested. Total soluble *p*-coumaric acid content ranged from 1.22 to 109.62 μg/g of defatted meal. Except for pearl millet, bound phenolic fractions of all other millet varieties showed higher ($p < 0.05$) *p*-coumaric acid contents than their soluble counterparts. Proso millet contained the highest level of *p*-coumaric acid among the bound phenolic extracts, whereas pearl millet had the lowest (**Table 3**). In addition, bound phenolic extracts of little, foxtail, and kodo millets contained 44, 40, and 32 times higher *p*-coumaric acid levels, respectively, than pearl millet.

HPLC analysis revealed that the content of each phenolic acid in the bound fraction was higher than that in the soluble fraction of all millet varieties examined in this study. In the soluble fractions of millets ferulic acid was the most abundant hydroxycinnamate except for little millet, and the ratios of ferulic acid to *p*-coumaric acid content of kodo, finger (Ravi), finger (local), foxtail, proso, little, and pearl millets were 11.6, 4.0, 15.8, 1.32, 2.0, 0.92, and 3.92 μg/g of defatted meal, respectively. However, in the insoluble bound fraction, ferulic acid was dominant only in kodo, finger (Ravi), finger (local), and pearl millets, and the ratios

Table 3. Content (Micrograms per Gram of Defatted Meal) of Ferulic and *p*-Coumaric Acids in Free, Esterified, and Insoluble Bound Phenolic Extracts of Whole Millets

millet type	free[a]	esterified[a]	soluble[a,b]	bound[a]
		Ferulic Acid		
kodo	5.79 ± 0.07 bc	196.18 ± 5.77 a	201.97 ± 5.70 a	1685.04 ± 1.00 a
finger (Ravi)	3.94 ± 0.05 cd	8.47 ± 0.44 e	12.40 ± 0.49 e	325.54 ± 7.47 d
finger (local)	2.61 ± 0.99 d	16.20 ± 0.10 e	18.81 ± 0.89 e	263.85 ± 2.27 e
foxtail	3.46 ± 0.31 cd	93.14 ± 0.47 b	96.60 ± 0.15 b	592.92 ± 3.33 c
proso	4.40 ± 0.48 cd	28.95 ± 3.26 d	33.35 ± 3.74 d	325.50 ± 1.34 d
little	7.50 ± 0.53 b	94.09 ± 0.80 b	101.58 ± 0.27 b	178.82 ± 7.89 f
pearl	22.37 ± 1.16 a	60.14 ± 0.26 c	82.51 ± 1.42 c	638.91 ± 2.86 b
		***p*-Coumaric Acid**		
kodo	4.84 ± 0.11 c	14.31 ± 0.57 c	19.15 ± 0.46 c	679.52 ± 6.33 d
finger (Ravi)	1.31 ± 0.08 d	1.44 ± 0.05 d	2.75 ± 0.14 d	34.81 ± 1.28 ef
finger (local)	0.89 ± 0.16 d	0.33 ± 0.01 d	1.22 ± 0.15 d	38.59 ± 0.12 e
foxtail	19.11 ± 0.01 b	54.18 ± 0.70 b	73.29 ± 0.69 b	842.61 ± 1.51 c
proso	0.48 ± 0.09 d	15.86 ± 0.88 c	16.34 ± 0.96 c	1139.06 ± 1.18 a
little	39.30 ± 1.18 a	70.31 ± 2.68 a	109.62 ± 3.87 a	917.64 ± 8.22 b
pearl	3.80 ± 0.06 c	16.96 ± 0.11 c	20.77 ± 0.18 c	20.68 ± 0.04 f

[a] Values in each column having the same letter are not significantly different ($p > 0.05$). [b] Sum of free and esterified phenolic fractions.

of ferulic acid to *p*-coumaric acid of kodo, finger (Ravi), finger (local), foxtail, proso, little, and pearl millets were 2.47, 9.35, 6.84, 0.70, 0.29, 0.19, and 30.89 μg/g of defatted meal, respectively. Thus, the findings of the present study lend further support to an earlier study by Rao and Muralikrishna (*37*), who showed that ferulic and *p*-coumaric acids were the major bound phenolics present in finger millet. In this study the bound phenolic fraction accounted for 64−96 and 50−99% of total ferulic acid and *p*-coumaric acid contents of millet grains, respectively. These findings are similar to those reported in previous studies for other cereals such as rice and wheat (*36*, *38*).

According to Zhao et al. (*39*) ferulic acid can be absorbed from rat stomach in its free form and is likely to be metabolized into conjugated ferulic acid in the liver. Moreover, excretion of ferulic acid in its sulfate and glucuronide forms in human subjects has been observed, thus suggesting its absorption and metabolism in the body (*40*). This further demonstrates the importance of including bound phenolic fraction of grains in assessing the antioxidant activity of millets. Bound phenolics in cereal grains, in general, hydroxycinnamates such as ferulic and *p*-coumaric acids, are linked via an ester bond to the arabinoxylans in the plant cell wall. Andreasen et al. (*41*) reported that gastrointestinal esterase from intestinal mucosa and microflora (both human and rat) can release ferulic and diferulic acids from cereal bran. The results of the present study therefore suggest that bound phenolic compounds of millet with high antioxidant activity may also exert their health benefits locally in the colon upon their release by colonic fermentation.

The overall effect of millet phenolic extracts as effective antioxidants appears to depend on a number of factors other than the phenolic content. In this study, when soluble and bound extracts of the same variety were compared, it was generally noted that for some varieties (kodo, finger, and pearl millets) high phenolic content in either bound or soluble fraction determines the antioxidant efficacy of the extracts in different antioxidant assays. Thus, the insoluble bound fraction of kodo millet and soluble fractions of the two finger millet varieties demonstrated stronger antioxidant efficacy when compared to their soluble and insoluble bound counterparts, respectively. However, although proso and little millets had higher soluble phenolic contents, their bound phenolic counterparts showed a higher antioxidant activity. It is noteworthy that insoluble bound fractions of proso and little millets had lower ferulic acid to *p*-coumaric acid ratios of 0.29 and 0.19, respectively, compared to those of their soluble counterparts of 2.0 and 0.92, respectively. It appears that *p*-coumaric acid is contributing more, compared to ferulic acid, to the antioxidant capacity of bound extracts of these two millet varieties, in addition to other phenolics that may be present. These results further suggest that compounds in the extract may exert their antioxidant activity individually as well synergistically. Hence, further phenolic profile analysis is in progress, which is necessary to understand the relationship between composition and antioxidant properties of extracts. Furthermore, investigations of the millet phenolics as potential natural antioxidants in several food and biphasic model systems and biological substrates are currently underway.

This study demonstrated a wide variation in the phenolic contents and antioxidant capacity in whole millet grains. Varietal differences existed in the contents of phenolics as well as antioxidant capacities between soluble and insoluble bound phenolic fractions. Significant contribution from both fractions was noted for the total antioxidant capacity of whole grains of millets as assessed by in vitro antioxidant activity assays. However, their potential activity as natural antioxidants depends on the quantity as well as the type of phenolic constituents that need to be fully identified to explain the antioxidant capacity of the extracts. A considerable amount of ferulic and *p*-coumaric acids was found in the bound form in whole grains. Thus, the contribution of bound phenolic fraction should be taken into account in the assessment of the antioxidant activity of such extracts. The knowledge generated from this study may help to exploit the use of millets, among other cereals, as a nutraceutical ingredient and to promote their use in disease risk reduction and overall health.

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