Effect of Roasting on Phenolics Content and Antioxidant Activity of Proso Millet

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***Abstract*—The influence of roasting on the antioxidant activity and phenolics content of two varieties of proso millet (*Panicum miliaceum* L.) was studied. Changes of phenolic and flavonoid contents and the antioxidant properties during the roasting of soaked proso millet seeds at 180 ℃ were measured by the DPPH, ABTS, FRAP and ORAC assays. These contents and properties increased markedly after roasting for 10 min. The positive correlation coefficients between the total content of phenolics, total content of flavonoids and antioxidant activity were high; the highest was between total content of phenolics and FRAP (r 0.995). Nine individual phenolic compounds were identified in roasted proso millet. The predominant phenolic acids were p-coumaric acid and trans-ferulic acid in the free fraction and vanilic acid, p-coumaric acid and trans-ferulic acid in the bound fraction. This study showed that roasting increased the content of phenolic compounds and the antioxidant activity of proso millet.**

***Index Terms*—roasting, Proso millet, phenolics, antioxidant activity**

I. Introduction

Proso millet (*Panicum miliaceum* L.) ranks sixth in cereal grain production worldwide. It is an important minor cereal and a valuable component of the human diet, particularly in arid and semi-arid regions. *P. miliaceum* is resistant to salt, alkali, cold and drought and can be cultivated in various types of soil even under poor growing conditions [1]. Proso millet grain is rich in starch, protein, dietary fiber and many trace elements. It has been reported that the protein content is ~13.6% (w/w) and the content of essential amino acids is higher compared to wheat, rice and maize [2].

Phenolics, which are products of secondary metabolism, provide essential functions in the reproduction and growth of plants and act as defense mechanisms against pathogens, parasites and predators as well as contributing to the color of plants [3]. Proso millet is a reserve of several phenolics, including phenolic acids and flavonoids [4], which have important biological properties related to improved gut health and reduced risk of coronary heart disease, as well as antimutagenic, anti- inflammatory, anticarcinogenic and antioxidant activities [5].

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Roasting, an important processing method, can improve the flavor, crispness, color and crunchy texture of food products [6]. Roasted proso millet is a traditional food in Inner Mongolia, China [7]. There are reports of the effect of roasting on the phenolics content and the antioxidant capacity of some other species of millet [5], [8] but similar studies on proso millet are scarce. It has been reported that the content of phenolics and the antioxidant capacity of the crude phenolic extract from proso millet were increased after roasting. For example, a significant increase in total phenolic compounds was observed in roasted finger millet and roasted pearl millet [5]. Also, Pradeep & Guha, (2011) [8]reported that the content of total phenolics and flavonoids of roasted little millet was increased significantly compared to native and other processed millets, as well as the DPPH radical scavenging activity and the iron reducing power. The objective of this work was to study the effect of roasting on the content of total phenolics, antioxidant status and phenolic profiles of two varieties of proso millet.

II. Materials and Methods

A. Materials

Samples of the Nei Mei 8 (NM8) variety of proso millet were provided by the Ordos Agricultural Science Institute, Inner Mongolia, China. Samples of the Xi Nong 10-4 (XN) variety of proso millet were provided by the Northwest A&F University, Shanxi, China. Phenolic acid standards, Folin–Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchroman -2-carboxylic acid (Trolox), 2,2-diphenyl-1 -picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethyl -benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, fluorescein, 2,2′-azobis(2- methylpropionamidine) dihydro -chloride (AAPH) and 2,4,6-tri(2-pyridyl) -striazine (TP TZ) were purchased from Sigma-Aldrich (China). Rutin was purchased from the Sinopharm Chemical Reagent Co., Ltd (China). All chemicals were of HPLC grade and triple-distilled water was used for all procedures.

B. Methods

1) Roasting

Dehulled proso millet seeds were soaked in five volumes (w/v) of water for 12 h in darkness then roasted in a toaster (Electrolux, Guangzhou, China) for 5, 10, 20

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and 30 min, cooled to room temperature, powdered and stored at –20 ℃.

2) Sample preparation

The powder was mixed with five volumes (w/v) of hexane for 5 min three timesin an ultrasonic bath (Ningbo Scientz Biotechnology Co. Ltd, China) with ice water to remove lipids and lipid-soluble components. The defatted samples were dried at 45 ℃ in a vacuum oven (Boxun Inc., Shanghai, China) before placing into polythene pouches, sealed and stored at –20 ℃.

3) Extraction of soluble phenolic compounds

Soluble phenolics were extracted as described by Chandrasekara (2012) [9] but with minor modification. An ultrasound-assisted extraction procedure was used to extract phenolic compounds from the defatted powder. A sample (2 g) of defatted extract was mixed with 20 mL of 80% (v/v) acetone and then placed into an ultrasonic bath (Scientz Inc., Ningbo, China) with ice water, sonicated at maximum power for 30 min and blended by vortex mixing at intervals of 2 min (IKA, German). After centrifugation of the resulting slurry at 6000 *g* (Sigma, Germany) for 10 min, the supernatant was collected and the extraction was repeated twice (i.e., three times in all). Supernatants were combined, evaporated *in vacuo* at 45 ℃ (Buchi, Flawil, Switzerland) and then freeze-dried (CHRIST, German). The crude phenolic extracts were dissolved in 2 mL of 70% (v/v) methanol and stored at –80 ℃. Residues were dried in a vacuum oven (Boxun Inc., Shanghai, China) and then stored at –20 ℃.

4) Extraction of bound phenolic compounds

Soluble phenolic compounds were extracted as described by Adom *et al.* (2002) [10] but with minor modification. The residues were digested with 2 M sodium hydroxide under nitrogen gas at room temperature for 1 h with shaking. The mixture was neutralized by the addition of an appropriate amount of hydrochloric acid. The solution was extracted five times with 10 mL ethyl acetate and then evaporated to dryness. The phenolic compounds were dissolved in 2 mL of 70% (v/v) methanol and stored at –80 ℃.

5) Determination of Total Phenolics Content (TPC)

TPC of the millet extracts was determined by spectrophotometry as described by Singleton & Rossi (1965) [11] but with modification. A mixture of 50 μL of extract and 1.5 mL of distilled water was treated with 125 μL of Folin–Ciocalteu's reagent (1:2 dilution with water) and 375 μL of 7% (w/v) sodium carbonate, mixed thoroughly and made to 2.5 mL with distilled water . A blue color developed and the absorbance at 760 nm was measured after 90 min. A calibration curve was prepared using a standard solution of gallic acid and the results were expressed (in mg) as gallic acid equivalents (GAE)/100 g defatted sample (dry wt basis).

6) Determination of Total Flavonoids Content (TFC)

TFC was determined by a colorimetric method as described by Bao *et al.* (2005) [12] but with modification. Samples (0.25 mL) of appropriately diluted extracts were placed into 15 mL polypropylene conical tubes containing 0.75 mL of distilled water and mixed with 100 μL of 5% (w/v) $NaNO_2$. After 6 min, 100 μL of 10% (w/v) $Al_2(NO_3)_3$ was added and after a further 5 min, 1 mL of 1 M NaOH was added to the mixture. The reaction solution was mixed well, made to 2.5 mL with distilled water and the absorbance at 510 nm was measured after 15 min. The total flavonoid content was calculated using a rutin standard curve and expressed (in mg) as rutin equivalents (RE)/100 g defatted sample (dry wt basis).

7) Determination of the DPPH value

The DPPH assay was done as described by Brand-Williams *et al.* (1995) [13] and Thaipong et al. (2006) [14] but with minor modification. Samples (200 μL) of appropriately diluted extracts were allowed to react with 3.8 mL of DPPH solution for 30 min in darkness before the absorbance at 517 nm was measured. The results were calculated using a standard Trolox curve and expressed (in μmol) as Trolox equivalents (TE)/g defatted sample (dry wt basis).

8) Determination of the ABTS value

The ABTS assay was performed as described by Arnao *et al.* (1995) [15] and Thaipong et al. (2006) [14] but with minor modification. Samples (150 μL) of appropriately diluted extracts were allowed to react with 2850 μL of the DPP^+ solution for 30 min in darkness before the absorbance at 734 nm was measured. The results were calculated using the standard Trolox curve and expressed (in μmol) as Trolox equivalents (TE)/g defatted sample (dry wt basis).

9) Determination of the FRAP value

The ABTS assay was performed as described by Benzie and Strain (1996, 1999) [16], [17] but with minor modification. Samples (150 μL) of appropriately diluted extracts were allowed to react with 2850 mL of the FRAP solution for 30 min in darkness before the absorbance at 593 nm was measured. The results were calculated using the standard Trolox curve and expressed (in μmol) as Trolox equivalents (TE)/g defatted sample (dry wt basis).

10) Determination of the ORAC value

The ORAC procedure used 96-well plates [18] and an automated plate reader (2104 Multilabel Reader, PerkinElmer, MA). Samples (20 μL) of appropriately diluted extracts, blank or Trolox standard or 200 μL of fluoroscein working solution were placed into the appropriate wells and incubated for 10 min. A portion (200 μL) of the fluoroscein working solution was added into each well and the plate was incubated at 37 ℃ for at least 20 min. Plates were read 35 times immediately after adding 20 μL of AAPH every 5 min. Fluorescence conditions were as follows: excitation at 485 nm and emission at 520 nm. The results were calculated using a standard Trolox curve and expressed (in μmol) as Trolox equivalents (TE)/g defatted sample (dry wt basis).

11) Fractionation of phenolic compounds

The composition of free and bound phenolic fractions of roasted millet grain was determined by Ultra Performance Liquid Chromatography (UPLC). Reverse Phase (RP)-UPLC analysis was performed with a Waters Acquity UPLC system (Waters Corporation, Milford, MA) equipped with a Waters Acquity photodiode array (PDA) detector (Waters Corporation, Milford, MA). Separation of phenols was achieved with an ACQUITY

UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm; Waters Corporation, Milford, MA). The mobile phase consisted of methanol (solvent A) and 1% (v/v) formic acid (solvent B). The column was equilibrated with 5% solvent A and the gradient elution protocol was: 30% solvent A for 15 min; 100% solvent A for 20 min. The flow rate was adjusted to 0.3 mL/min and the detection of compounds was achieved by measuring the absorbance at 200–500 nm. The quantification of compounds was performed at the largest absorption wavelength. All samples were filtered through a 0.45 μm pore size PTFE membrane syringe filter (Jinteng Inc., Tianjin, China) before injection.

12) Statistical analysis

All experiments were performed in triplicate and data were reported as mean ± SD except for the UPLC analysis. Statistical significance and correlation analysis were calculated using SPSS version 17.0. The figures were drawn with Origin 8.6 software.

III. Results and Discussion

A. Total Content of Phenolics and Flavonoids

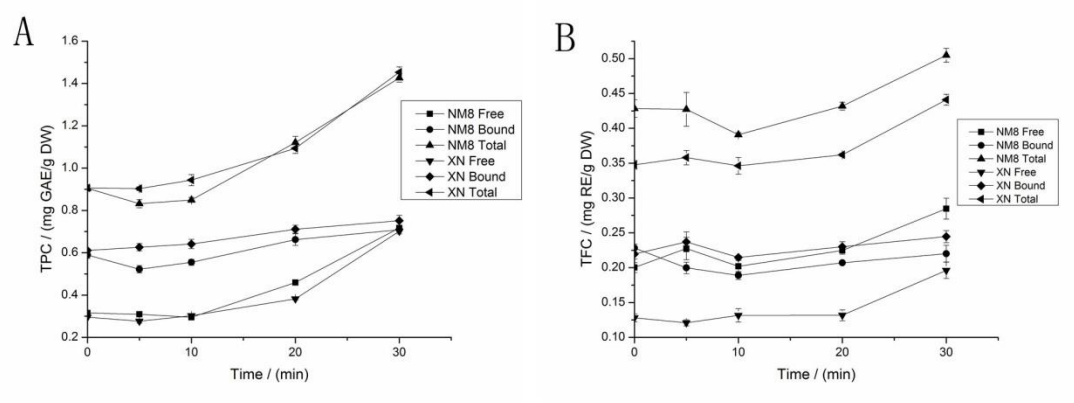

Figure 1. Total phenolics (A) and flavonoids (B) content of two proso millet varieties.

Fig. 1 shows TPC and TFC of proso millets during roasting. TPC of the free and bound fractions increased significantly ($p < 0.05$) with time. TPC of the free and bound fractions (except XN bound phenolics) decreased slightly during the first 5 min. From 5~10 min, TPC of the free and bound fractions (except XN free phenolics) increased slightly. After 10 min, TPC of the free and total fractions increased rapidly and TPC of the bound fraction increased slightly. After roasting for 30 min, TPC of the free, bound and total fractions of NM8 increased 2.3, 1.2 and 1.6-fold, respectively and the corresponding values for XN were 2.4, 1.2 and 1.6-fold, respectively. The proportion of the free fraction increased from 34.9% to 50.3% for NM8 and from 32.6% to 48.3% for XN, indicating the content of the free fraction increased faster compared to the bound fraction.

Similar results are reported for cashew nut, little millet, finger millet, pearl millet and other grains [19], [5], [8], [20]. The decrease of TPC of the free fraction by 5 min might be caused by the high temperature, which induces oxidization and degradation of free phenolics [21], [22]. The increase of free phenolics after roasting for 30 min could have occurred because the phenolics accumulated in the vacuoles during thermal processing might be released due to the breakdown of cellular constituents and membranes [23] and be easier to extract with 80% (v/v) acetone. During thermal treatment, reaction between reducing sugars and amino acids (the Maillard reaction) can occur during roasting and lead to the formation of a variety of byproducts, intermediates and brown pigments (melanoidins), which might contribute to the TPC value [19]; a significant increase in phenolic compounds reflected an increase in tannin content [5]. The increase of bound phenolics after roasting might be caused by the breakdown of cells during roasting, which could release some of the bound phenolics that could not be extracted at first.

There are reports that roasting leads to a decrease of free phenolics in faba beans and guava seeds [24], [25]. A different identity and content of free and bound phenolics in different samples, a different effect on free and bound phenolics during roasting, different extraction methods as well as different temperature and time during roasting might all lead to different results [25].

There was a similar increase in the flavonoids content after roasting the two proso millet varieties. TFC of both free and total fractions (but not the bound fraction) was increased significantly ($p < 0.05$). In contrast to TPC, the lowest content of TFC occurred after roasting for 10 min. After 30 min, the TFC of free, bound and total fractions of the NM8 variety increased 1.4, 0.96 and 1.2-fold, respectively, and the corresponding values for the XN variety were 1.5-, 1.1- and 1.3-fold, respectively. A similar increase in the flavonoids content during roasting was reported for little millet [8].

B. Antioxidant Properties

Fig. 2 shows the antioxidant properties, including DPPH, ABTS, FRAP and ORAC, of two proso millet varieties during roasting. The antioxidant properties of the free fractions increased significantly ($p < 0.05$), whereas the change of the bound fractions depended on the variety of millet and the antioxidant assays used. Changes of the four antioxidant properties were similar compared to TPC.

DPPH radical scavenging activity of the free fractions decreased at first and then increased significantly,

whereas the activity of the bound fraction increased slightly during roasting. Before 10 min of roasting, the DPPH value of the free, bound and total fractions declined slightly or was unchanged; after 10 min, the value of the free and total fractions increased significantly whereas a decline was observed at 20 min for the bound fraction. The change of DPPH was similar compared to TPC, indicating the increase of DPPH was related directly to TPC, which was increased significantly during roasting [8]. After roasting for 30 min, the free fraction contributed 53.3% and 44.6% of the total DPPH value of the NM8 and XN varieties, respectively. The corresponding values for TPC were 50.3% and 48.3%, respectively, indicating the DPPH radical scavenging activity of the free and the bound fractions were similar.

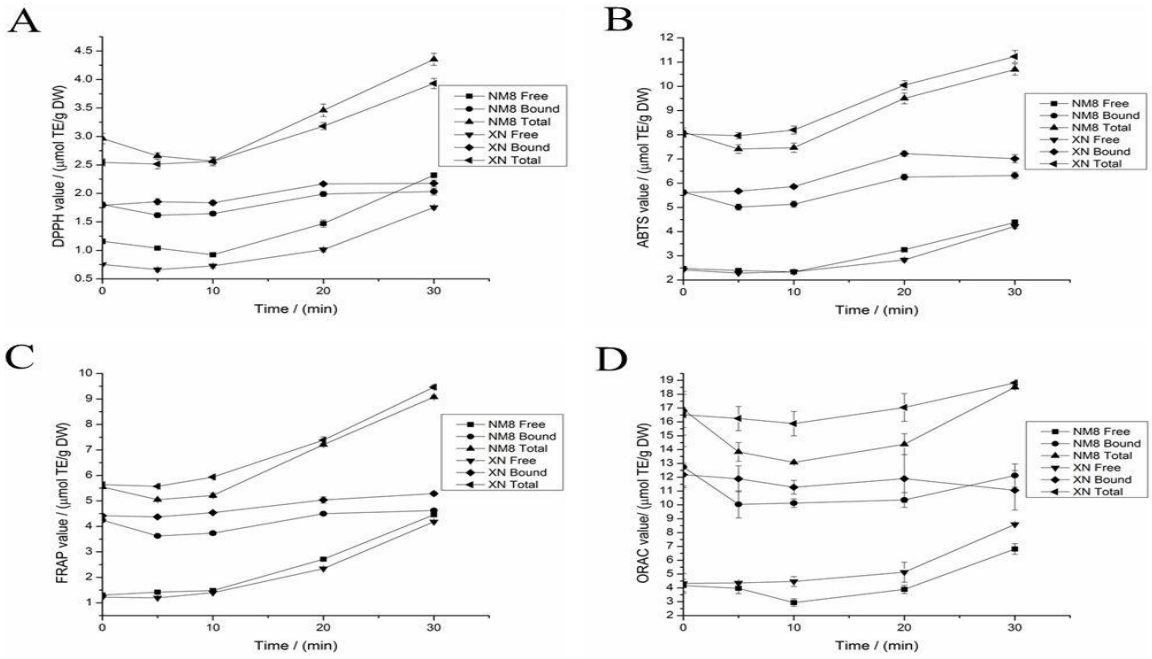

Figure 2. Antioxidant properties (DPPH (A), ABTS (B), FRAP (C) and ORAC (D)) of two proso millet varieties during roasting.

The change of the ABTS radical scavenging activity was similar compared to TPC for the free and the bound fraction. After roasting for 30 min, the ABTS value of the free and bound fractions of the NM8 variety increased 1.8- and 1.1-fold, respectively, and contributed 40.9% and 59.1%, respectively, to the total ABTS value of the NM8 variety. For the XN variety, ABTS values of the free and bound fractions increased 1.7- and 1.2-fold, respectively, and contributed 37.6% and 62.4%, respectively, to the total ABTS value. The contribution from the free fraction to the total ABTS value was less than the percentage of free phenolics in the total TPC. The different identities of the free and bound phenolics in samples could have different ABTS radical scavenging activity, leading to this result.

The tendency for changes of the FRAP radical scavenging activity of the free and bound fractions was similar compared to TPC. After roasting for 30 min, the FRAP value of the free and bound fractions of NM8 was increased 3.4- and 1.1-fold, respectively, compared to before roasting, contributing 49.1% and 50.9% to the total FRAP value of NM8. The corresponding FRAP value of the free and bound fractions increased 3.4- and 1.2-fold, respectively, contributing 44.2% and 55.8% to the total FRAP value for XN. These results indicate that the FRAP radical scavenging activity of the free and the bound fractions were similar.

The tendency for changes of the ORAC radical scavenging activity of the free and bound fractions was similar compared to TPC. After roasting for 30 min, the ORAC value of the free and bound fractions of NM8 increased 1.6- and 0.95-fold, respectively, compared to before roasting, contributing 36.8% and 63.2% to the total ORAC value of NM8. The ORAC value of the free and bound fractions increased to 2.0- and 0.95-fold, respectively, contributing 44.2% and 55.8% to the total ORAC value for XN. The contribution to the total ABTS value from the free fraction of NM8 was less than the percentage of free phenolics in total TPC, but similar compared to XN. A possible reason for this result is the difference between the varieties of proso millet as well as the relatively greater standard deviation compared to other antioxidant assays.

There have been studies showing that the antioxidant properties of plant extracts were enhanced during roasting. Şahin *et al.* (2009) [26] found that the antioxidant properties of carob powder extracts increased continuously during roasting for 60 min. A similar phenomenon was reported for soybean [27]. The increased antioxidant property after roasting for 30 min might be because the breakdown of cellular constituents and membranes during heating allows more phenolics to be extracted. The Maillard reaction occurs during heating and allows the formation of a variety of byproducts,

intermediates and brown pigments (melanoidins), which might contribute to antioxidant properties [26], [19].

C. Correlation Analysi

Table I shows the Pearson's correlation coefficient between total phenolics content, total flavonoids content and antioxidant activity. There was a strong correlation (r >0.9, p <0.01) between TPC and antioxidant activity (but not ORAC). TFC was poorly correlated to antioxidant activity (but not DPPH). The highest correlation coefficient was between TPC and FRAP (r = 0.995, p <0.01). This finding is similar to the results of other published work [28], [26], [16]. The correlation coefficient between ORAC and the other three assays was low, which might be because two methods were used to measure total antioxidant activity. The ORAC assay is hydrogen atom transfer-based, whereas the other three assays are electron transfer-based. Unlike the other assays, the ORAC assay takes into account the kinetic action of antioxidants, which might explain the discrepancy between the results obtained with the ORAC assay compared to the other procedures [28].

TABLE I. PEARSON'S CORRELATION COEFFICIENT BETWEEN TOTAL PHENOLICS CONTENT, TOTAL FLAVONOIDS CONTENT AND ANTIOXIDANT ACTIVITY

	TPC	TFC	DPPH	ABTS	FRAP	ORAC
TPC	1					
TFC	0.638*	1				
DPPH	0.951**	0.812**	1			
ABTS	0.967**	0.515	0.911**	1		
FRAP	0.995**	0.584	0.937**	0.986**	1	
ORAC	0.730*	0.279	0.635*	0.739*	0.729*	1

* $p < 0.05$; ** $p < 0.01$. The other values were not statistically significant ($p > 0.05$).

TABLE II. PHENOLICS COMPOSITION OF TWO PROSO MILLETS EXTRACTS DURING ROASTING

Samples	Phenolics	Time (min)				
		0	5	10	20	30
NM8 Free	*p*-Hydroxybenzaldehyde	ND	ND	ND	0.37	0.81
	Vanilic acid	ND	ND	ND	ND	1.25
	Caffeic acid	1.68	1.08	0.78	0.51	ND
	p-Coumaric acid	3.37	3.06	2.72	2.81	1.41
	trans-Ferulic acid	7.95	6.13	4.82	4.55	3.79
	Sinapic acid	0.86	0.39	0.29	ND	ND
	Total	13.86	10.66	8.62	8.24	7.27
NM8 Bound	Protocatechuic aldehyde	0.39	0.34	0.26	ND	0.26
	p-Hydroxybenzoic acid	1.43	ND	1.38	1.75	1.93
	p-Hydroxybenzaldehyde	ND	0.41	ND	0.11	0.26
	Vanilic acid	5.15	2.82	2.94	3.38	6.36
	Caffeic acid	1.54	1.44	2.18	2.44	2.98
	Syringic acid	1.94	0.72	1.71	1.86	1.39
	p-Coumaric acid	11.74	9.09	8.12	9.05	9.92
	trans-Ferulic acid	397.96	317.23	321.37	353.55	371.58
	Sinapic acid	0.92	0.88	0.64	0.91	1.06
	Total	421.07	332.93	338.60	373.06	395.75
XN Free	*p*-Hydroxybenzaldehyde	ND	ND	0.26	0.41	0.87
	Vanilic acid	2.29	ND	ND	ND	2.42
	Caffeic acid	1.69	1.46	1.29	0.70	0.16
	p-Coumaric acid	3.87	3.45	3.32	3.62	1.62
	trans-Ferulic acid	11.48	10.72	9.57	9.35	4.64
	Sinapic acid	0.73	0.76	0.65	0.50	ND
	Total	20.07	16.39	15.08	14.58	9.71
XN Bound	*p*-Hydroxybenzaldehyde	0.39	0.21	ND	0.24	0.26
	Vanilic acid	6.61	6.62	7.07	5.47	4.94
	Caffeic acid	1.02	1.72	1.65	2.13	2.42
	Syringic acid	1.75	1.87	1.15	1.39	2.13
	p-Coumaric acid	10.51	11.26	9.78	10.57	10.72
	trans-Ferulic acid	431.92	434.16	391.42	392.78	386.09
	Sinapic acid	0.75	0.83	0.68	0.73	1.14
	Total	452.96	456.66	411.75	413.32	407.69

ND, not detected; * μg/g defatted sample (dry wt basis)

D. Fractionation of Phenolics

The phenolics contents of two proso millet varieties during roasting are given in Table II. The results showed that the identity and content of phenolics varied depending on the type of millet and roasting time. Five hydroxybenzoic acids and derivatives (protocatechuic aldehyde, *p*-hydroxybenzoic acid, *p*-hydroxybenzalde -hyde, vanilic acid and syringic acid) and four hydroxycinnamic acids and derivatives (caffeic, *p*-coumaric, *trans*-ferulic and sinapic acids) were detected in the roast millet grains. Among the phenolics, *p*-coumaric and *trans*-ferulic acids were predominant in the free fraction of both varieties, whereas vanilic, *p*-coumaric and *trans*-ferulic acids were predominant in the bound fractions; *trans*-ferulic acid was the most abundant phenolic acid in both varieties, especially in the bound fractions, contributing >90% to total phenolics.

There were significant variations in the contents of phenolics after roasting. Roasting caused significant

decreases in caffeic acid, *p*-coumaric, *trans*-ferulic, sinapic and total phenolic acids but caused an increase in the *p*-hydroxybenzaldehyde and vanilic acid content of the free fraction of both varieties. Some individual phenolics and the total phenolics content in the bound fraction of NM8 first decreased and then increased during roasting. The changes of phenolics in proso millet during roasting in this study might have been due to the oxidative degradation of phenolics, including enzymatic browning or release of free acids from conjugate forms or the formation of complex structures of phenolic substances from related compounds, including proteins, tannins and anthocyanins [29].

The change of total phenolics was different from that described for TPC above, which might be due, at least in part, to the difference between the UPLC and TPC assays. Some of the phenolic compounds that cannot be determined and quantified by UPLC because of the lack of a standard can be detected by TPC as well as the formation of a variety of byproducts, intermediates and brown pigments (melanoidins) formed during the Maillard reaction [4], [19].

IV. Conclusion

Phenolics are known to be important nutraceuticals with antioxidant properties. The content of phenolics in proso millet extracts was determined by TPC and UPLC. Further, the antioxidant activity of the phenolics in roasted proso millet was determined by the DPPH, ABTS, FRAP and ORAC radical scavenging activity assays. The present study showed that roasting enhanced the antioxidant properties of proso millet significantly by increasing its content of TPC. Therefore, roasted proso millet is a potentially good source of nutraceuticals for food formulations.

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