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ARTICLE

Isoflavones, Flavan-3-ols, Phenolic Acids, Total Phenolic Profiles, and Antioxidant Capacities of Soy Milk As Affected by Ultrahigh-Temperature and Traditional Processing Methods

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The objectives of this work were to assess antioxidant activities and phenolic compounds of soy milk as affected by traditional and ultrahigh-temperature (UHT) processing. Three soybean varieties were processed into raw soy milk and then cooked soy milk by indirect and direct UHT methods (both at 143 °C for 60 s) and traditional cooking (stove cooking and steam injection) methods (both at 100 °C for 20 min). Total phenolic content (TPC), total flavonoid content (TFC), phenolic acids, isoflavones, flavan-3-ols, and anthocyanins were quantified. DPPH free radical scavenging activity, ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) were analyzed. As compared to the raw soy milk, all thermal processing significantly ($p < 0.05$) reduced TPC values and significantly ($p < 0.05$) increased TFC values for all soybean varieties. All processing methods significantly ($p < 0.05$) increased DPPH and FRAP values in the soy milk processed from yellow soybean varieties Proto and IA 2032. UHT processing increased their ORAC values, but traditional and steam processing reduced their ORAC values. However, in the case of the soy milk from black soybean, all processing reduced ORAC values as compared to the raw soy milk. None of processing affected total phenolic acids, chlorogenic, and *trans*-cinnamic acid, as well as (+)-catechin. However, all processing significantly ($p < 0.05$) affected contents of total isoflavones and individual isoflavones. Thermal processing caused significant ($p < 0.05$) increases in 7-*O*-β-glucosides and acetylglucosides, but caused significant ($p < 0.05$) decreases in malonylglucosides and aglycones. Indirect UHT processing transformed more isoflavones from malonylglucosides into 7-*O*-β-glucosides than the direct UHT did.

KEYWORDS: Soy milk; processing; UHT; total phenolics; isoflavones; phenolic acids; antioxidants; DPPH; FRAP; ORAC; HPLC

INTRODUCTION

Soybeans and soy foods contain significant amounts of health-promoting components. However, they also contain undesirable components such as beany flavor and trypsin inhibitors that affect their consumption and utilization. Tofu and soy milk are the most popular soy foods consumed in China, Korea, Japan, Thailand, and Singapore. Soy milk is the water extract of soybean, which provides high-quality proteins and essential fatty acids while containing no cholesterol, gluten, or lactose. Therefore, soy milk is an excellent dietary protein source for common consumers, vegetarians, and people with lactose intolerance and milk allergy. Despite the beneficial attributes of soy milk, its consumption in the Western world has been limited due to its unacceptable beany flavor (*1*, *2*) and flatulence factor. Therefore, maximizing the retention of desired components such as essential amino acids and antioxidants and the removal or reduction of unwanted components will improve the quality and utilization of soy milk.

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In general, thermal treatment is required for soy milk preparation during and/or after grinding to destroy pathogenic microorganisms, inhibit the undesired enzymes, and degrade antinutritional factors. Traditionally, soy milk manufacturing procedures include heating freshly prepared soy milk to boiling in an open pot for 20−30 min. This partly destroys the antinutritional factors and improves flavor. However, traditional cooking methods take a long time and often result in the partial loss of food quality and are difficult to automate. Ultrahigh-temperature (UHT) processing is relatively new for processing soy milk in the modern soy milk industry. UHT can heat liquid foods in a wide range of the combinations of temperature and time to retain high food quality characteristics. The UHT system, because of its capability to heat quickly to a high temperature (up to 150 °C) in a short period of time (≤1 min), has been widely used in conjunction with the aseptic packaging technology to extend the shelf life of liquid foods. UHT in conjunction with a rapid cooling system also is used widely for preserving the bioactive ingredients of heat-sensitive beverages, health foods, and nutraceuticals. Although there are potential advantages using the UHT system, the effects of indirect or direct UHT processing on the

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compositions and structures of phenolic phytochemicals (phenolic acids, isoflavones, and anthocyanins) of soy milk and their antioxidant capacities have not been reported.

Thermal processing generally involved two major processing variables (temperature and time). The UHT processing conditions for soy milk have been optimized for the elimination of beany flavor and trypsin inhibitors (*3*, *4*). The UHT processing conditions of 143 °C for 60 s have been reported to reduce trypsin inhibitor activities to 10−20% of that in the raw soy milk (*3*, *4*) for maximizing protein nutritive value. The specific objectives of this study are to investigate the effects of these selected UHT processing conditions on the retention of desired phenolic phytochemicals such as phenolic acids, isoflavones, and their antioxidant capacity and to compare their phenolic profiles and antioxidant capacities with those of soy milk cooked by traditional stove cooking and steam injection methods.

MATERIALS AND METHODS

Chemicals and Reagents. Sixteen phenolic acids and three aldehydes, HPLC-grade trifluoroacetic acid (TFA), 2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$), fluorescein disodium (FL), Folin−Ciocalteu reagent, sodium carbonate, 6-hydroxyflavone (HFL), 2,4,6-tri (2-pyridyl)-*s*-triazine (TPTZ), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Nine isoflavone standards, including daidzin, genistin, glycitin, daidzein, genistein, glycitein, acetyldaidzin, acetylgenistin, and malonylgenistin, were purchased from LC Laboratories (Woburn, MA). A mixture of six unimolar anthocyanin standards (3-*O*-*β*-glucosides of delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin) was purchased from Polyphenols Laboratories (Sandnes, Norway). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). 2,4,4′-Trihydroxybenzoin (THB, one of the internal standards for isoflavone quantification) was synthesized and purified in our laboratory. HPLC-grade solvents (methanol and acetonitrile, B&J Brand), analytical grade acetic acid, and other analytical grade solvents using for extraction were purchased from VWR International (West Chester, PA). Deionized water (18 MΩ·cm) was prepared using a Nanopure ultrapure water system (Barnstead International, Dubuque, IA). Poly(vinylidene difluroide) (PVDF) syringe filters with a pore size of 0.2 *μ*m were purchased from National Scientific Co. (Duluth, GA).

Soybean Materials. The dry mature soybeans (harvested in 2005) of one yellow soybean variety [*Glycine max* (L.) Merr. cv. Proto] and one black soybean variety [*Glycine max* (L.) Merr. cv C-1] were provided by Sinner Brothers & Bresnahan (Casselton, ND). A lipoxygenase-null yellow soybean variety (harvested in 2005) (*Glycine max* (L.) Merr. cv. IA2032) was obtained from Stonebridge Ltd. (Cedar Falls, IA). Broken seeds, damaged seeds, and foreign materials were removed from the samples. Phenolic quantification and antioxidant activities were expressed on a dry weight basis.

Preparation of Raw Soy Milk. For each batch of soy milk, 2 kg of soybeans was soaked overnight in 20 L of tap water at room temperature (solid/liquid ratio 1:10, w/v). The hydrated beans were drained, rinsed, and ground with tap water [the ratio of water to dry bean was 9:1 (w/w)]. In the traditional stove cooking treatment, the soaked soybeans were ground for 3 min at high speed using a Hamilton Beach blender (model 585-1, Peabody, MA). The soy slurry was filtered through a muslin cloth to separate the okara from the soy milk. For the traditional direct steam injection and direct/indirect UHT treatments, the soaked soybeans were ground using an automated soy milk grinder/extractor (Chang-Seng Mech. Co., Taoyuan City, Taiwan), which was equipped with a centrifugal 120 mesh screen to separate raw soy milk automatically from the residues. Approximately 100 mL of raw soy milk from each batch processing was sampled in duplicate after grinding and filtration. The raw soy milk was freeze-dried and stored for further analyses.

Traditional Thermal Processing of Soy Milk. Two types of traditional processing methods were used.

(A) Traditional Stove Cooking. The raw soy milk (1 L) in a small pot was heated within a larger pot, which contained boiling water on a stove, which was set at the highest heat level, to approximately 90 °C, and then the small soy milk pot was switched to the hot stove surface to heat to a boiling temperature of 100 °C and held at this temperature with stirring to prevent foaming and overflowing for 20 min. Soy milk (approximately 100 mL) was sampled in duplicate at 20 min after boiling. Immediately after sampling, the soy milk in a small beaker was cooled in an ice bath. The soy milk was freeze-dried and stored for further analyses.

(B) Traditional Direct Steam Injection. The raw soy milk coming out of the continuous grinder was immediately injected with live food-grade steam at about 45 psi to boiling and held at 100 °C for 20 min. The Bryan electric steam boiler (model BE 165Q4T6, Bryan Steam Corp.) was used to generate the steam. The steam injection apparatus consisted of two stainless steel cross-tubes with 12 nozzles located at the bottom side of each tube. Therefore, there were 24 nozzles to ensure homogeneous heating of soy milk during steam injection. Approximately 2 L of soy milk was placed in a stainless steel pot, and the steam injector was immersed in the soy milk. It took approximately 15 s for the steam to heat the raw soy milk to boiling, which time was referred to as the 0 min boiling. The soy milk (approximately 100 mL) from each batch processing was sampled in duplicate at 20 min after boiling. Immediately after sampling, the soy milk in a small beaker was cooled in an ice bath. The soy milk was freeze-dried and stored for further analyses.

UHT Processing of Soy Milk. The raw soy milk coming out of the continuous grinder was immediately pumped into the Microthermics Direct/Indirect Steam Injection Processor (DIP, Microthermics, Inc., Raleigh, NC) for UHT processing. The Microthermics processor heated the soy milk in two stages. The soy milk was preheated quickly (time was controlled by adjusting the temperature/flow rate) in the first stage to 110 °C, followed by processing at two different UHT treatments: a direct UHT process and an indirect UHT process, respectively.

(A) Direct UHT Processing. The direct UHT process was carried out at 143 °C for 60 s. Briefly, steam was injected directly into the product, and soy milk was in direct contact with the heating medium (steam) at 143 °C. The heated soy milk was pumped through a holding tube in which the heat processing was continued for 60 s. The direct method included a vacuum evaporation chamber for cooling and removal of odors and the added water, which condensed from the steam injected. The product was further cooled by circulating cold tap water in a tubular heat exchanger. The soy milk at the final product outlet was approximately 25 °C and collected.

(B) Indirect UHT Processing. The indirect system was based on tubular heat exchangers whereby heat was transferred from the steam to the product across a heat exchange tube. The indirect UHT process was also carried out at 143 °C for 60 s. The heated soy milk coming out of the holding tube was cooled only by a tubular heat exchanger using cold tap water without going through the vacuum chamber. The soy milk at the final product outlet was approximately 25 °C and collected. The UHT-processed soy milk from each batch processing was sampled in duplicate, freeze-dried, and stored for further analyses.

Extraction of Total Phenolics from Soy Milk. After the traditional and UHT processing, all soy milk samples were immediately frozen and then freeze-dried. The moisture content of freeze-dried soy milk was determined by drying the sample after 24 h at 105 °C in an air oven until a constant weight was obtained (*5*). The freeze-dried soy milk flours (0.5 g in triplicate) were accurately weighed into a set of centrifuge tubes. Extraction procedures were carried out according to our previously published method (*6*). Briefly, dried soy milk was extracted twice with 5 mL of acetone/water (50:50, v/v). The two extracts were combined and stored at 4 °C in the dark for use.

Determination of Total Phenolic Content (TPC). The TPC was determined by a Folin−Ciocalteu assay (*7*) with slight modifications (*6*) using gallic acid (GA) as the standard. The absorbance was measured using an UV−visible spectrophotometer (UV 160, Shimadzu) at 765 nm against the reagent blank. The TPC was expressed as milligrams of gallic acid equivalents per gram of freeze-dried soy milk (mg of GAE/g) through the calibration curve of gallic acid.

Determination of Total Flavonoid Content (TFC). The TFC was determined using a colorimetric method described previously (*8*). The absorbance was measured at 510 nm using an UV−visible spectrophotometer (UV 160, Shimadzu). The TFC was expressed as milligrams of catechin equivalents per gram of freeze-dried soy milk (mg of CAE/g) using the calibration curve of (+)-catechin.

HPLC Analysis of Free Phenolic Acids (FPA). *(A) Extraction of FPA.* The extraction of free phenolic acids was performed by modifying the method of Luthria and Pastor-Corrales (*9*). Briefly, the freeze-dried soy milk samples (0.5 g in triplicate) were extracted twice at room temperature with a total 10 mL of methanol/water/acetic acid/BHT (85:15:0.5:0.2, v/v/v/w) by shaking extraction tubes on an orbital shaker at 300 rpm for 4 h. The extracts were concentrated at 45 °C under vacuum to remove solvents; the dry residue was dissolved in 5 mL of water and then freeze-dried. The freeze-dried extracts (10 mg) were dissolved in 1 mL of 25% methanol. The methanol solution was centrifuged and then filtered through a 0.2 μm PVDF syringe filter and analyzed for FPA composition by HPLC.

(B) HPLC Analysis of FPA. The quantitative analysis of FPA was performed by HPLC according to Xu and Chang (*10*). A Waters Associates (Milford, MA) chromatography system equipped with a model 720 system controller, a model 6000A solvent delivery system, a model 7125 loading sample injector, and a model 418 LC spectrophotometer set at 270 nm was used. A 4.6 mm × 250 mm, 5 μm, Zorbax Stablebond Analytical SB-C_{18} column (Agilent Technologies, Rising Sun, MD) was used for separation at 40 °C, which was maintained with a column heater. HPLC elution was performed according to previous description (*9*) without modification. Identification and quantification of FPA were performed according to our previous method (*10*). In addition, (+)-catechin (flavan-3-ol) in soy milk was detected during the FPA assay; therefore, (+)-catechin content in soy milk was quantified with phenolic acids together. Phenolic acid contents were expressed as micrograms of phenolic acid per gram of dry soy milk (μg/g).

HPLC Analysis of Isoflavone Content. *(A) Extraction of Isoflavones.* Isoflavones were extracted by modifying the methods of Murphy et al. (*11*) and Hou and Chang (*12*). Briefly, the freeze-dried raw and processed soy milk samples (1.0 g in triplicate) were accurately weighed into a set of 15 mL screw-top VWR centrifuge tubes. Five milliliters of acetonitrile, 4.5 mL of distilled water, 0.25 mL of internal standard THB (0.1 mg/mL), and 0.25 mL of internal standard HFL (0.1 mg/mL) were added to each tube. The tubes were capped, and the mixtures were shaken at 250 rpm at room temperature on an orbital shaker for 2 h. Then the slurry was centrifuged by an Allegra 21R Centrifuge (Beckman Coulter Ltd., Palo Alto, CA) at 5500 rpm for 20 min. The supernatant was filtered through Whatman no. 42 filter paper into a 125 mL flask and evaporated to dryness on a rotary evaporator at 34 °C. The residues in the flask were dissolved in 5 mL of 80% methanol and kept in a freezer (−20 °C) for < 12 h before analysis. An aliquot of sample solution was filtered through a 0.2 μm PTFE syringe filter prior to HPLC assay.

(B) HPLC Analysis of Isoflavones. The quantitative analysis of soy milk isoflavones was performed by HPLC according to the method of Xu and Chang (*13*). The same Waters Associates chromatography system as used for phenolic acids analysis was used for quantitative analysis of isoflavones, and a spectrophotometer set at 262 nm was used. A YMC-Pack ODS-AM-303 C_{18} reversed phase column (250 mm × 4.6 mm i.d., 5 μm particle size) was obtained from Waters and employed for chromatographic separation at 34 °C, which was maintained with a column heater. HPLC operation, peak identification, and quantification of compounds were performed according to our previous description (*13*). Isoflavone contents were expressed as micrograms of isoflavone per gram of freeze-dried soy milk (μg/g).

HPLC Analysis of Anthocyanin Content. The free phenolic acid extracts were also used for anthocyanin analysis; the analysis was performed on an HP 1090 series HPLC (Hewlett-Packard, Waldbronn, Germany) equipped with a filter photometric detector, using a YMC Pack ODS-AM column (4.6 × 250 mm, S-50 μm, 120A) according to the method in our previous publication (*14*). HPLC conditions were as follows: solvent A, 0.1% TFA/H_2O; solvent B, CH_3CN/H_2O/TFA (50:50:0.1, v/v/v); linear gradient, initial percentage of B (15%) to 60 min (40%); column temperature, 40 °C; flow rate, 0.5 mL/min. The filter detector was set at 540 nm. The identifications and peak assignments of anthocyanins were primarily based on comparison of their retention times with those of standards and a blueberry reference sample according to our previous description (*14*). Anthocyanin contents were expressed as micrograms of anthocyanin per gram of freeze-dried soy milk (μg/g).

Radical DPPH Scavenging (DPPH) Activity. The DPPH activity of soy milk was evaluated according to our previous study (*6*). The DPPH free radical scavenging activity of soy milk was expressed as micromoles of Trolox equivalent per gram of freeze-dried soy milk (μmol of TE/g) using the calibration curve of Trolox.

Ferric Reducing Antioxidant Power (FRAP) Assay. The FRAP assay was performed as previously described by Benzie and Strain (*15*). The soy milk sample solution analyzed was first properly diluted with deionized water to fit within the linearity range. The FRAP value was expressed as millimoles of Fe^{2+} equivalents per 100 g of freeze-dried soy milk (mmol of FE/100 g) using the calibration curve of Fe^{2+}.

Oxygen Radical Absorbing Capacity (ORAC) Assay. Hydrophilic ORAC assays were carried out on a BMG Fluostar Optima Microplate Reader (BMG Labtech GmbH, Offenburg, Germany), which was equipped with two autoinjectors, an incubator, and wavelength-adjustable fluorescence filters. The procedures were based on the previous paper by Prior et al. (*16*) with slight modifications (*6*). The ORAC value was expressed as micromoles of Trolox equivalents per gram of freeze-dried soy milk (μmol of TE/g) using the calibration curve of Trolox.

Statistical Analysis. All processes were performed in triplicate. Further chemical analyses and antioxidant activity evaluations were performed on the basis of triplicate processed samples, and the data were expressed as mean ± standard deviation. Statistical analysis was performed using 2005 SAS (version 9.1, SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was conducted. Duncan's multiple-range tests were used to determine the significant differences between group means ($p = 0.05$). Pearson's correlation test was conducted to determine the correlation coefficients between variables.

RESULTS AND DISCUSSION

Effects of Traditional and UHT Processing on Total Phenolics of Soy Milk. Total phenolic content (TPC) and total flavonoid content (TFC) of the soy milk extracts are presented in **Table 1**. Significant differences ($p < 0.05$) in TPC and TFC values were found among most soy milk processed by traditional and UHT methods. TPC and TFC in soy milk had not been reported previously. In the present study, it was found that about 15−18% of TPC was reduced in traditional stove-cooked soy milk, about 8.5−11% of TPC was reduced in steam injection processed soy milk, about 15.5−42.5% of TPC was reduced in direct UHT processed soy milk, and about 7.5−16% of TPC was reduced in indirect UHT processed soy milk from three soybean varieties, as compared to the respective raw soy milk. However, traditional stove cooking increased by 20−23% TFC, steam injection cooking increased by 28−65% TFC, direct UHT processing increased by 48−90% TFC, and indirect UHT processing increased by 74−113% TFC. For each individual variety, direct UHT processing lost more TPC than the other processing treatments, whereas indirect UHT increased TFC more than the other processing treatments. These results indicated that processing caused complex changes on chemical compositions. Thermal processing might cause the degradation of polyphenols and release of bound phenolic compositions. Previously, we found that the increased TFC values of thermally processed yellow soybeans might be related to the release of free phenolic substances from polymerized structural substances (such as lignin) in cell walls upon thermal processing (*10*). As there are no data available on TPC and TFC values of soy milk, it is impossible to compare current data with the literature. However, a comparison can be done with their original materials—soybeans—to determine the changes when soybeans are processed into raw soy milk. We found that there were no big differences in TPC values in terms of per unit dry matter between yellow soybeans (2.45 mg of GAE/g for Proto, 2.54 mg of GAE/g for IA2032) as reported in our recent paper (*13*) and soy milk (2.34 mg of GAE/g for Proto, 2.79 mg of GAE/g for IA2032) made from them as shown in **Table 1**. These results indicated that most phenolic substances in the yellow soybeans could be transferred to soy milk products. However, when black soybeans were processed into soy milk, tremendous

Table 1. Effect of UHT and Traditional Processing on Phenolic Contents of Soy Milk[a]

soybean material	soy milk	processing conditions	TPC (mg of GAE/g)	loss in TPC[b] (%)	TFC (mg of CAE/g)	increase in TFC[b] (%)
Proto (yellow soybean)	raw		2.34 ± 0.21a		0.13 ± 0.00c	
	stove cooked	100 °C, 20 min	1.99 ± 0.12c	14.9	0.16 ± 0.00b	23.1
	steam injection	100 °C, 20 min	2.14 ± 0.04b	8.5	0.17 ± 0.01a	30.8
Proto (yellow soybean)	raw UHT		2.39 ± 0.15a		0.10 ± 0.01b	
	direct UHT	143 °C, 60 s	2.02 ± 0.07c	15.5	0.19 ± 0.02a	90
	indirect UHT	143 °C, 60 s	2.21 ± 0.08b	7.5	0.21 ± 0.02a	110
black soybean	raw		2.67 ± 0.07a		0.25 ± 0.01b	
	stove cooked	100 °C, 20 min	2.22 ± 0.09c	16.8	0.30 ± 0.01a	20
	steam injection	100 °C, 20 min	2.40 ± 0.05b	10.1	0.32 ± 0.01a	28
black soybean	raw UHT		3.20 ± 0.14a		0.24 ± 0.01c	
	direct UHT	143 °C, 60 s	2.51 ± 0.19c	21.6	0.36 ± 0.01b	48
	indirect UHT	143 °C, 60 s	2.85 ± 0.14b	10.9	0.51 ± 0.02a	112.5
IA 2032 (yellow soybean)	raw		2.79 ± 0.05a		0.20 ± 0.02c	
	stove cooked	100 °C, 20 min	2.30 ± 0.13c	17.6	0.24 ± 0.00b	20
	steam injection	100 °C, 20 min	2.49 ± 0.08b	10.7	0.33 ± 0.03a	65
IA 2032 (yellow soybean	raw UHT		2.99 ± 0.06a		0.19 ± 0.00c	
	direct UHT	143 °C, 60 s	1.72 ± 0.04c	42.5	0.30 ± 0.01b	57.9
	indirect UHT	143 °C, 60 s	2.52 ± 0.09b	15.7	0.33 ± 0.01a	73.7

[a] Data are expressed as mean ± standard deviation ($n = 3$) on dry weight basis. Values marked by the same letter within each group in each column are not significantly different ($p < 0.05$). [b] The values of raw soy milk were considered to be 100%.

Table 2. Effect of UHT and Traditional Processing on Antioxidant Capacity of Soy Milk[a]

soybean material	soy milk	processing conditions	DPPH (μmol of TE/g)	FRAP (mmol of FE/100 g)	ORAC (μmol of TE/g)
Proto (yellow soybean)	raw		0.35 ± 0.04c	0.64 ± 0.03c	84.62 ± 6.14a
	stove cooked	100 °C, 20 min	0.68 ± 0.00b	0.95 ± 0.04b	66.94 ± 5.85c
	steam injection	100 °C, 20 min	0.87 ± 0.05a	1.11 ± 0.04a	73.23 ± 5.12b
Proto (yellow soybean)	raw UHT		0.35 ± 0.06c	0.67 ± 0.02c	69.28 ± 1.78b
	direct UHT	143 °C, 60 s	0.48 ± 0.04b	0.95 ± 0.02b	83.76 ± 2.30a
	indirect UHT	143 °C, 60 s	0.94 ± 0.05a	1.19 ± 0.03a	83.25 ± 4.52a
black soybean	raw		0.99 ± 0.03b	1.12 ± 0.03b	55.09 ± 2.42a
	stove cooked	100 °C, 20 min	0.86 ± 0.01c	1.11 ± 0.05b	42.76 ± 2.48c
	steam injection	100 °C, 20 min	1.09 ± 0.07a	1.56 ± 0.03a	49.27 ± 1.97b
black soybean	raw UHT		1.19 ± 0.03c	1.49 ± 0.02b	58.63 ± 0.65a
	direct UHT	143 °C, 60 s	1.73 ± 0.04b	1.56 ± 0.06b	50.34 ± 3.21b
	indirect UHT	143 °C, 60 s	2.51 ± 0.02a	1.82 ± 0.02a	53.25 ± 2.37b
IA 2032 (yellow soybean)	raw		1.10 ± 0.07b	1.16 ± 0.04b	76.03 ± 3.71a
	stove cooked	100 °C, 20 min	1.22 ± 0.04a	1.28 ± 0.07a	59.14 ± 4.32b
	steam injection	100 °C, 20 min	1.25 ± 0.06a	1.33 ± 0.02a	62.98 ± 1.18b
IA 2032 (yellow soybean)	raw UHT		0.90 ± 0.04c	1.02 ± 0.01c	60.08 ± 4.67b
	direct UHT	143 °C, 60 s	1.11 ± 0.07b	1.23 ± 0.04b	92.15 ± 3.38a
	indirect UHT	143 °C, 60 s	1.43 ± 0.05a	1.41 ± 0.02a	87.21 ± 3.60a

[a] Data are expressed as mean ± standard deviation ($n = 3$) on dry weight basis. Values marked by the same letter within each group in each column are not significantly different ($p < 0.05$).

reduction in TPC values occurred when compared to the raw material black soybeans (8.75 mg of GAE/g) as reported in our recent paper (*13*); the reduction could be partly attributed to the leaching of water-soluble anthocyanins in the seed coats during soaking. In addition, partial losses might be due to the separation of soy milk from the soy residue (okara), which contained black seed coat fragments.

Effect of Traditional and UHT Processing on Antioxidant Capacities of Soy Milk. Antioxidant activities of the raw and processed soy milk, including DPPH, FRAP, and ORAC, are presented in **Table 2**. Significant differences ($p < 0.05$) in DPPH, FRAP, and ORAC values were found among most treatments. In the case of yellow soybean varieties (Proto and IA 2032), as compared to the raw soy milk, both traditional and UHT processes caused significant ($p < 0.05$) increases in DPPH and FRAP, whereas traditional processes caused significant ($p < 0.05$) decreases in ORAC and UHT processes caused significant ($p < 0.05$) increases in ORAC. In the case of black soybean, as compared to the raw soy milk, both traditional and UHT processes caused significant ($p < 0.05$) decreases in ORAC,

Table 3. Effect of UHT and Traditional Processing on Free Phenolic Acids of Soy Milk[a]

			individual benzoic acid derivates (μg/g)						
soybean material	soy milk	processing conditions	GA	PA	TBA	PCD	HBA	VA	subtotal, benzoics
Proto (yellow soybean)	raw		7.88 ± 0.01b	nd[b]	nd	nd	9.74 ± 0.09a	nd	17.63 ± 0.08b
	stove cooked	100 °C, 20 min	72.28 ± 4.46a	5.37 ± 0.47a	10.56 ± 1.97a	nd	1.62 ± 1.03b	nd	89.82 ± 3.99a
	steam injection	100 °C, 20 min	9.69 ± 0.03b	1.57 ± 0.46b	3.40 ± 0.43b	nd	1.93 ± 0.13b	nd	16.61 ± 0.73b
Proto (yellow soybean)	raw UHT		20.66 ± 1.99c	nd	nd	nd	7.77 ± 0.80a	nd	28.44 ± 2.79c
	direct UHT	143 °C, 60 s	58.51 ± 3.66b	3.82 ± 0.05a	9.43 ± 0.36b	nd	1.09 ± 0.10b	nd	72.85 ± 3.36b
	indirect UHT	143 °C, 60 s	48.02 ± 0.73a	7.60 ± 1.31a	29.27 ± 0.23a	nd	1.37 ± 0.69b	nd	86.26 ± 1.49a
black soybean	raw		85.92 ± 2.62a	nd	nd	28.78 ± 2.73a	5.96 ± 0.67a	2.88 ± 0.27b	123.54 ± 6.29a
	stove cooked	100 °C, 20 min	69.34 ± 1.89b	5.58 ± 1.23a	19.51 ± 0.13a	nd	3.48 ± 0.47b	4.53 ± 0.41a	102.45 ± 2.93b
	steam injection	100 °C, 20 min	36.69 ± 1.63c	4.35 ± 0.73a	18.27 ± 0.57a	2.48 ± 0.10b	0.08 ± 0.06c	3.77 ± 0.05ab	65.63 ± 3.02c
black soybean	raw UHT		7.95 ± 1.00c	12.33 ± 1.64a	3.71 ± 0.11c	22.49 ± 2.78a	5.00 ± 1.93a	2.03 ± 0.10b	53.51 ± 3.69b
	direct UHT	143 °C, 60 s	74.03 ± 6.38a	7.87 ± 0.05b	50.25 ± 3.73b	23.08 ± 1.18a	3.86 ± 0.19a	5.72 ± 0.09ab	164.82 ± 11.23a
	indirect UHT	143 °C, 60 s	46.31 ± 1.31b	10.12 ± 0.19ab	69.98 ± 0.03a	24.31 ± 0.82a	1.82 ± 0.93a	1.34 ± 0.07c	153.87 ± 2.09a
IA 2032 (yellow soybean)	raw		21.69 ± 4.61c	7.98 ± 0.55a	6.54 ± 0.61c	nd	nd	2.27 ± 0.23a	38.48 ± 6.00b
	stove cooked	100 °C, 20 min	67.44 ± 6.22a	2.03 ± 0.67b	11.22 ± 0.77b	6.84 ± 0.68b	1.88 ± 0.31a	1.23 ± 0.11b	90.65 ± 8.15a
	steam injection	100 °C, 20 min	39.02 ± 0.75b	2.04 ± 0.07b	14.52 ± 1.02a	13.32 ± 0.14a	1.24 ± 0.11a	2.27 ± 0.19a	72.41 ± 1.26a
IA 2032 (yellow soybean)	raw UHT		62.79 ± 1.12a	34.18 ± 7.77a	15.77 ± 2.14b	nd	nd	2.39 ± 0.11b	115.13 ± 4.39a
	direct UHT	143 °C, 60 s	57.42 ± 1.10b	3.65 ± 0.05b	18.81 ± 0.15b	17.41 ± 0.12a	2.54 ± 0.01a	8.06 ± 0.49a	107.89 ± 0.94a
	indirect UHT	143 °C, 60 s	35.05 ± 1.88c	4.79 ± 0.46b	27.18 ± 1.73a	11.18 ± 0.12b	1.88 ± 0.38a	8.37 ± 0.42a	88.47 ± 0.77b

			individual cinnamic acid derivates								
soybean material	soy milk	processing conditions	CFA	CLA	PCA+SD	MCA+FA	SPA	OCA	TCA	subtotal, cinnamics	total phenolic acids
Proto (yellow soybean)	raw		nd	1109.2 ± 26.9a	17.02 ± 0.33a	nd	nd	nd	187.2 ± 3.9a	1313.4 ± 31.2a	1330.9 ± 31.2a
	stove cooked	100 °C, 20 min	nd	996.1 ± 78.3ab	13.39 ± 1.26b	0.47 ± 0.04a	16.67 ± 1.29a	1.21 ± 0.14a	133.8 ± 13.2b	1161.6 ± 93.9a	1251.4 ± 97.9a
	steam injection	100 °C, 20 min	nd	930.7 ± 45.5b	12.66 ± 0.00b	0.14 ± 0.09b	17.56 ± 1.20a	1.63 ± 0.04a	165.6 ± 4.3a	1128.3 ± 51.1a	1144.9 ± 51.9a
Proto (yellow soybean)	raw UHT		nd	861.8 ± 19.9b	14.06 ± 0.29b	nd	nd	nd	142.5 ± 4.9a	1018.4 ± 15.2b	1046.8 ± 12.4b
	direct UHT	143 °C, 60 s	nd	810.2 ± 15.4b	12.49 ± 0.21c	0.22 ± 0.19b	18.61 ± 0.04b	1.95 ± 0.20b	122.6 ± 3.1b	966.0 ± 17.9b	1038.9 ± 21.3b
	indirect UHT	143 °C, 60 s	nd	1054.9 ± 26.6a	17.72 ± 0.30a	0.88 ± 0.04a	23.65 ± 1.15a	3.32 ± 0.24a	124.6 ± 4.0b	1225.0 ± 31.3a	1311.3 ± 32.8a
black soybean	raw		nd	1002.9 ± 99.7a	17.71 ± 2.02a	0.74 ± 0.03b	3.31 ± 0.07b	1.54 ± 0.38a	98.4 ± 10.5a	1124.6 ± 112.7a	1248.1 ± 118.9a
	stove cooked	100 °C, 20 min	45.03 ± 1.01	1105.4 ± 153.7a	16.96 ± 0.89a	4.12 ± 0.79a	23.01 ± 1.53a	1.31 ± 0.05a	81.9 ± 0.0a	1277.7 ± 155.8a	1380.2 ± 158.8a
	steam injection	100 °C, 20 min	nd	1032.1 ± 61.1a	11.72 ± 0.61b	3.69 ± 0.34a	21.18 ± 1.35a	0.94 ± 0.12a	78.6 ± 5.4a	1148.2 ± 68.9a	1213.8 ± 71.9a
black soybean	raw UHT		43.29 ± 0.57a	915.1 ± 132.5ab	17.09 ± 1.83a	1.33 ± 0.21a	20.26 ± 1.86b	4.49 ± 1.24a	64.7 ± 5.5ab	1066.2 ± 142.6a	1119.8 ± 146.3b
	direct UHT	143 °C, 60 s	43.82 ± 0.49a	768.9 ± 48.3b	16.24 ± 0.02a	0.89 ± 0.03b	23.45 ± 0.19b	3.10 ± 0.03a	73.6 ± 5.5a	930.1 ± 54.5a	1094.9 ± 65.7b
	indirect UHT	143 °C, 60 s	nd	1134.9 ± 110.0a	15.32 ± 1.41a	0.15 ± 0.04c	31.82 ± 3.62a	3.46 ± 0.24a	53.5 ± 5.7b	1239.2 ± 120.5a	1393.0 ± 118.4a

Table 3. Continued

soybean material	soy milk	processing conditions	individual cinnamic acid derivates								
			CFA	CLA	PCA+SD	MCA+FA	SPA	OCA	TCA	subtotal, cinnamics	total phenolic acids
IA 2032 (yellow soybean)	raw		20.56 ± 0.47a	905.5 ± 56.3a	8.05 ± 0.65a	0.01 ± 0.01b	28.70 ± 2.07a	2.39 ± 0.25a	177.0 ± 8.5a	1142.3 ± 67.3a	1180.7 ± 73.3a
	stove cooked	100 °C, 20 min	10.98 ± 0.02c	774.9 ± 8.0b	5.50 ± 0.20b	0.53 ± 0.11a	30.13 ± 4.44a	1.62 ± 0.11a	142.6 ± 11.3b	966.3 ± 24.2b	1056.9 ± 32.3a
	steam injection	100 °C, 20 min	13.09 ± 0.12b	763.5 ± 5.3b	6.23 ± 0.02b	0.76 ± 0.06a	25.98 ± 1.07a	2.43 ± 0.57a	181.1 ± 0.2a	993.1 ± 5.6b	1065.5 ± 4.4a
IA 2032 (yellow soybean)	raw UHT		22.33 ± 0.06	907.2 ± 90.1a	8.67 ± 0.19a	0.53 ± 0.19a	23.86 ± 5.73a	2.06 ± 0.02a	170.3 ± 10.3a	1134.9 ± 74.0a	1250.1 ± 78.4a
	direct UHT	143 °C, 60 s	nd	801.2 ± 25.3a	5.77 ± 0.39c	0.45 ± 0.04a	25.19 ± 0.23a	1.86 ± 0.45a	164.8 ± 2.9a	999.3 ± 29.3a	1107.2 ± 30.2a
	indirect UHT	143 °C, 60 s	nd	869.6 ± 40.1a	7.25 ± 0.45b	0.29 ± 0.01a	30.21 ± 0.23a	1.88 ± 0.35a	116.6 ± 5.7b	1025.8 ± 45.8a	1114.3 ± 44.9a

[a] Data are expressed as mean ± standard deviation ($n = 3$) on dry weight basis. Values marked by the same letter within each group in each column are not significantly different ($p < 0.05$). Phenolic acids: GA, gallic acid; PA, protocatechuic acid; TBA, 2,3,4-trihydroxybenzoic acid; PCD, protocatechualdehyde; HBA, *p*-hydroxybenzoic acid; VA, vanillic acid; CFA, caffeic acid; CLA, chlorogenic acid; PCA+SD, *p*-coumaric acid + syringaldehyde; MCA+FA, *m*-coumaric acid + ferullic acid; SPA, sinapic acid; OCA, *o*-coumaric acid; TCA, *trans*-cinnamic acid. [b] nd, not detectable.

whereas traditional steam injection and indirect UHT caused significant ($p < 0.05$) increases in DPPH and FRAP. Interestingly, in the case of Proto and black varieties, traditional steam injection cooked soy milk exhibited significantly ($p < 0.05$) higher antioxidant activities (DPPH, FRAP, ORAC) than traditional stove-cooked soy milk, whereas in the case of the IA 2032 variety, traditional steam injection and stove-cooked soy milk exhibited similar antioxidant activities (DPPH, FRAP, ORAC). For all three soybean varieties, indirect UHT processed soy milk exhibited significantly ($p < 0.05$) higher antioxidant activities in DPPH and FRAP values than direct UHT-processed soy milk, whereas there were no significant differences in ORAC values between indirect and direct UHT processing.

To the best of our knowledge, only three studies (*17*−*19*) have previously focused on antioxidant activities of raw or processed soy milk. However, all of those antioxidant research works were based on fermented soy milk cultured with kefir, lactic acid bacteria, and bifidobacteria. Although their findings have demonstrated that fermented soy milk possessed significantly higher antioxidant properties than unfermented soy milk, the antioxidant properties of the raw unfermented soy milk or heat-processed unfermented soy milk have not been systematically investigated. It is well-known that natural antioxidants contained in foods may be significantly reduced during processing. Nevertheless, it was recently demonstrated that thermal treatments can induce the formation of compounds with new antioxidant properties (*20*). In the present study, we found that thermal processing including traditional and UHT processes increased antioxidant activities of soy milk made from yellow soybean varieties in terms of free radical scavenging activities and reducing antioxidant power. In addition, UHT processing also increased oxygen radical absorbance capacity of both yellow soybean varieties. Similar positive heat effects were found in pasteurization of tea extracts (*21*), which caused an increase in the antioxidant activity of tea. In addition, previous research on fruits and vegetables indicated that processing increased antioxidant potential due to improvement of antioxidant properties of naturally occurring compounds or formation of novel compounds such as Maillard reaction products having antioxidant activity (*22*, *23*). The Maillard reaction might have occurred as we observed the darkening of soy milk after heating (color data not shown; will be published in another paper). The increases of antioxidant activities of processed soy milk may be attributed to, in part, the formation of new compounds with new antioxidant properties or transformation between originally existing compounds, which transformed some compounds from lower antioxidant properties to higher antioxidant status.

However, soy milk made from a black soybean variety did not demonstrate similar trends as compared to the two yellow soybean varieties in antioxidant activities. The differences may be attributable, in part, to the differences of chemical composition between black and yellow soybeans. Previously, we have found unique pigments (anthocyanins) to exist in the seed coats of black soybeans, but yellow soybeans contained no anthocyanins in their seed coats (*13*, *14*), whereas the anthocyanins in the black soybeans could easily be degraded upon thermal processing (*10*). Meanwhile, we found that DPPH and FRAP of soy milk always exhibited similar trends, whereas ORAC of soy milk exhibited trends similar to the former two antioxidant assays in UHT processes but different trends from the former two antioxidant assays in the traditionally processed soy milk. These differences may be attributed to the differences of antioxidant mechanisms: the ORAC reaction involves hydrogen atom transfer mechanism, whereas DPPH and FRAP involve the same mechanism of single electron transfer (*24*).

Besides natural phenolic compounds, soybean protein-derived peptides have also been reported to exhibit radical scavenging activities. Chen et al. (*25*) reported that a total of 22 peptides that derived from proteolytic digests of a soybean major storage protein, β-conglycinin (7S), exhibited some DPPH radical scavenging activities. In our current study, soy milk obtained by traditional and UHT procesing demonstrated higher antioxidant power in radical scavenging activities and reduced antioxidant power compared with the raw soy milk, suggesting that the increased activities may be attributable, in part, to the active peptides (with antioxidant activities) released from soybean storage protein during soy milk processing.

The changes in the overall antioxidant properties of processed soy milk can be attributed to the synergistic actions or counteractions of several types of oxidative reaction, transformation, formation, or breakdown of antioxidant compositions. To better understand the role and fate of natural and process-induced antioxidants on food stability and human health, further research as described below was performed to investigate the molecular mechanisms responsible for the losses or formation of antioxidants and the interactions between natural and heat-induced antioxidants and their effects on the overall antioxidant properties of processed soy milk.

Effect of Traditional and UHT Processing on Phenolic Acid Compositions. The free phenolic acid (FPA) contents of raw and processed soy milk are presented in **Table 3**. Two phenolic acids of the benzoic type (gallic and *p*-hydroxybenzoic acid) and three phenolic acids of the cinnamic type (chlorogenic, *p*-coumaric, and *trans*-cinnamic acid) were detected in both raw and processed soy milk from Proto. Two phenolic acids of the benzoic type (gallic and vanillic acid) and six phenolic acids of the cinnamic type (chlorogenic, *p*-coumaric, *m*-coumaric, sinapic, *o*-coumaric, and *trans*-cinnamic acid) were detected in both raw and processed soy milk from IA 2032 and black soybean. Among these detected compounds, gallic, chlorogenic, and *trans*-cinnamic acids are the predominant phenolic acids in the raw and processed soy milk. Significant differences ($p < 0.05$) in FPA contents were found among most treatments of soy milk from all soybean varieties.

In the case of yellow soybean Proto (**Table 3**), as compared to the raw soy milk, indirect UHT processes caused significant ($p < 0.05$) increases in free gallic, protocatechuic, 2,3,4-trihydroxybenzoic, chlorogenic, *p*-coumaric, sinapic acid, subtotal benzoic acids, subtotal cinnamic acids, and total phenolic acids, but caused significant ($p < 0.05$) decreases in *p*-hydroxybenzoic acid and *trans*-cinnamic acid. Direct UHT processes caused significant ($p < 0.05$) increases in free gallic, protocatechuic, 2,3,4-trihydroxybenzoic, sinapic acid, and subtotal benzoic acids, but caused significant ($p < 0.05$) decreases in *p*-hydroxybenzoic acid and *trans*-cinnamic acid and caused insignificant ($p > 0.05$) changes in chlorogenic acid, subtotal cinnamic acids, and total phenolic acids. The traditional stove cooking caused significant ($p < 0.05$) increases in free gallic, protocatechuic, 2,3,4-trihydroxybenzoic, sinapic acid, and subtotal benzoic acids, but caused significant ($p < 0.05$) decreases in *p*-hydroxybenzoic, chlorogenic, *p*-coumaric, *trans*-cinnamic acid and caused insignificant ($p > 0.05$) changes in subtotal cinnamic acids and total phenolic acids. The traditional steam injection cooking caused significant ($p < 0.05$) increases in free protocatechuic, 2,3,4-trihydroxybenzoic, and sinapic acid, but caused significant ($p < 0.05$) decreases in *p*-hydroxybenzoic, chlorogenic, *p*-coumaric, and *trans*-cinnamic acid and caused insignificant ($p > 0.05$) changes in subtotal benzoic acid, subtotal cinnamic acids, and total phenolic acids.

In the case of black soybean (**Table 3**), as compared to the raw soy milk, both indirect and direct UHT processes caused significant ($p < 0.05$) increases in free gallic, protocatechuic, 2,3,4-trihydroxybenzoic acid, and subtotal benzoic acids, while caused insignificant ($p > 0.05$) changes in protocatechualdehyde, *p*-hydroxybenzoic acid, *p*-coumaric acid, *o*-coumaric acid, and subtotal cinnamic acids. Traditional cooking (including indirect stove cooking and direct steam injection cooking) caused significant ($p < 0.05$) increases in free protocatechuic, 2,3,4-trihydroxybenzoic, vanillic, *m*-coumaric, and sinapic acid, but caused significant ($p < 0.05$) decreases in gallic, *p*-hydroxybenzoic acid, and subtotal benzoic acids and caused insignificant ($p > 0.05$) changes in chlorogenic, *o*-coumaric, *trans*-cinnamic acid, subtotal cinnamic acids, and total phenolic acids.

In the case of yellow soybean IA 2032 (**Table 3**), as compared to the raw soy milk, both indirect and direct UHT processes caused significant ($p < 0.05$) increases in free protocatechualdehyde and vanillic acid, but caused significant ($p < 0.05$) decreases in gallic acid and protocatechuic acid and insignificant ($p > 0.05$) changes in chlorogenic, *m*-coumaric, sinapic, *o*-coumaric acid, subtotal cinnamic acids, and total phenolic acids. Traditional cooking (including indirect stove cooking and direct steam injection cooking) caused significant ($p < 0.05$) increases in free gallic, 2,3,4-trihydroxybenzoic, *p*-hydroxybenzoic, protocatechualdehyde, and subtotal benzoic acid, but caused significant ($p < 0.05$) decreases in protocatechuic, caffeic, chlorogenic, *p*-coumaric acid, and subtotal cinnamic acids andcaused insignificant ($p > 0.05$) changes in sinapic, *o*-coumaric acid, and total phenolic acids.

Among the phenolic phytochemicals in soy foods, free and conjugated phenolic acids are far less studied than isoflavones. Phenolic acid content in soy milk had not been reported previously. In the present study, we found that thermal processing caused complex variations in phenolic acid profiles of soy milk. The variations depended on processing methods and soybean materials. However, despite significant changes in individual phenolic acids caused by processing, there were no significant differences in total phenolic acid contents between raw soy milk and traditionally processed soy milk from yellow soybean Proto and black soybean and no significant differences between raw soy milk and UHT processed soy milk from yellow soybean IA 2032 and black soybean. Furthermore, there were no significant differences in total phenolic acids between direct and indirect UHT processed soy milk.

Effect of Traditional and UHT Processing on Isoflavone Compositions. Isoflavones are the most studied of the soybean phytochemicals. Isoflavone profiles in raw and processed soy milk have been investigated in several earlier reports (*26*−*31*). However, no systematic analyses were performed to compare yellow with black soybeans as processed by traditional and UHT methods in one study. The isoflavone contents of the raw and processed soy milk are presented in **Table 4**. The total isoflavones in the raw and processed soy milk made from yellow soybean and black soybean varieties were 1700−2200 and 1000−1200 μg/g, respectively. Most isoflavones existed as glucosides. The highest content was 6″-*O*-malonyl-β-glucosides, followed by 7-*O*-β-glucosides, whereas 6″-*O*-acetyl-β-glucosides and aglycones occurred in only very small amounts.

There was a significant impact on the retention and distribution of isoflavones as a result of different processing methods. As shown in **Table 4** and **Figure 1**, malonylglucosides in soy milk decreased dramatically with an increase in 7-*O*-β-glucosides and acetylglucosides after traditional and UHT processing. Significant differences ($p < 0.05$) in isoflavone contents were found among most processing treatments for all three soybean varieties. As compared to the raw soy milk, all thermal processing

Table 4. Effect of UHT and Traditional Processing on Isoflavones (Micrograms per Gram) of Soy Milk [a]

			7-*O*-β-glucosides			malonylglucosides			acetylglucosides		
soybean material	soy milk	processing conditions	Din	Gin	Gly	MDin	MGin	MGly	ADin	AGin	AGly
Proto (yellow soybean)	raw		120.7 ± 5.8c	189.1 ± 12.8c	68.13 ± 4.62b	927.3 ± 24.1a	1629.9 ± 60.3a	137.9 ± 11.5a	4.69 ± 0.49b	18.81 ± 1.47c	34.26 ± 1.26b
	stove cooked	100 °C, 20 min	357.1 ± 9.9a	625.8 ± 15.4a	108.89 ± 7.71a	667.1 ± 37.3c	1186.9 ± 17.4c	99.81 ± 1.2c	27.02 ± 2.73a	57.16 ± 2.81a	45.02 ± 6.46a
	steam injection	100 °C, 20 min	282.7 ± 11.9b	548.0 ± 33.1b	97.47 ± 5.62a	763.4 ± 44.9b	1323.1 ± 69.7b	106.09 ± 1.8b	3.40 ± 0.17b	37.95 ± 2.46b	10.12 ± 0.24c
Proto (yellow soybean)	raw UHT		100.2 ± 1.2c	229.9 ± 3.4b	39.59 ± 2.81b	861.7 ± 20.0a	1291.7 ± 65.6a	108.38 ± 3.1a	3.29 ± 0.25c	47.12 ± 1.36c	34.00 ± 1.80c
	direct UHT	143 °C, 60 s	298.1 ± 18.6b	529.4 ± 35.9a	79.57 ± 5.40a	692.9 ± 59.7b	1275.0 ± 119.8a	99.85 ± 21.7ab	43.63 ± 8.12b	218.91 ± 7.73b	58.35 ± 0.76a
	indirect UHT	143 °C, 60 s	357.6 ± 9.9a	569.7 ± 11.2a	88.51 ± 0.05a	659.3 ± 40.5b	1116.8 ± 71.3a	89.57 ± 0.7b	62.95 ± 1.09a	280.15 ± 1.62a	47.06 ± 1.99b
black soybean	raw		92.6 ± 4.7c	151.7 ± 9.5c	62.82 ± 1.55c	586.8 ± 32.0a	921.5 ± 27.3a	204.62 ± 9.4a	7.10 ± 0.29a	47.34 ± 1.90a	24.93 ± 1.24a
	stove cooked	100 °C, 20 min	133.6 ± 0.0b	204.0 ± 7.1b	88.55 ± 0.10b	486.2 ± 5.2b	769.3 ± 4.8b	138.13 ± 2.7b	2.01 ± 0.18c	10.79 ± 2.58b	16.49 ± 0.59b
	steam injection	100 °C, 20 min	177.5 ± 12.1a	301.1 ± 3.7a	118.04 ± 8.94a	495.6 ± 25.2b	776.0 ± 34.1b	200.89 ± 16.4a	4.61 ± 0.78b	9.34 ± 1.20b	27.23 ± 0.49a
black soybean	raw UHT		70.0 ± 1.1c	111.9 ± 5.8c	56.31 ± 2.51c	415.0 ± 11.7a	546.4 ± 19.9ab	170.68 ± 0.4a	0.76 ± 0.28c	98.68 ± 1.53b	16.35 ± 0.48c
	direct UHT	143 °C, 60 s	145.8 ± 10.8b	241.2 ± 17.3b	80.84 ± 3.79b	357.3 ± 23.9b	600.2 ± 43.5a	141.29 ± 11.3b	19.72 ± 0.32b	99.20 ± 2.72b	20.98 ± 0.45b
	indirect UHT	143 °C, 60 s	228.9 ± 0.5a	366.3 ± 7.2a	116.86 ± 0.62a	274.3 ± 16.2c	471.4 ± 16.4b	133.47 ± 1.4c	49.61 ± 2.21a	204.37 ± 8.88a	35.34 ± 0.86a
IA 2032 (yellow soybean)	raw		281.4 ± 21.9c	397.9 ± 10.8c	49.74 ± 0.87c	1342.1 ± 46.5a	1403.4 ± 19.8a	107.93 ± 32.6a	7.81 ± 0.23b	49.58 ± 1.46b	61.50 ± 4.01a
	stove cooked	100 °C, 20 min	512.4 ± 35.7a	701.6 ± 15.7a	70.99 ± 2.91a	970.8 ± 50.5c	1281.5 ± 55.1c	66.96 ± 5.2b	46.85 ± 1.41a	59.33 ± 5.78a	50.59 ± 1.77b
	steam injection	100 °C, 20 min	484.8 ± 27.6b	636.8 ± 15.1b	61.60 ± 0.97b	1204.5 ± 78.2b	1362.7 ± 132.7b	68.55 ± 0.9b	5.84 ± 0.56b	42.36 ± 2.34b	54.48 ± 4.03ab
IA 2032 (yellow soybean)	raw UHT		153.8 ± 13.9c	431.9 ± 20.1b	29.94 ± 0.21b	804.9 ± 26.7a	1401.9 ± 32.4a	49.27 ± 3.9b	4.02 ± 0.18c	42.45 ± 1.12c	25.27 ± 1.26b
	direct UHT	143 °C, 60 s	373.4 ± 23.2b	523.3 ± 21.6b	44.55 ± 0.48a	701.5 ± 32.2b	1344.4 ± 7.4b	66.02 ± 2.9a	6.68 ± 1.04b	222.24 ± 6.00b	93.70 ± 4.02a
	indirect UHT	143 °C, 60 s	548.3 ± 44.8a	807.9 ± 66.9a	50.22 ± 6.66a	670.8 ± 41.7b	994.4 ± 35.4c	42.40 ± 3.7b	30.16 ± 0.56a	462.31 ± 7.39a	103.10 ± 9.80a

			aglycones			total individuals			
soybean material	soy milk	processing conditions	Dein	Gein	Glein	T-Dein	T-Gein	T-Glein	total isoflavones
Proto (yellow soybean)	raw		15.69 ± 3.69a	36.69 ± 1.23a	nd[b]	562.2 ± 7.4a	973.1 ± 33.9b	137.3 ± 5.0ab	1672.6 ± 27.2b
	stove cooked	100 °C, 20 min	11.37 ± 1.61b	22.48 ± 5.75b	nd	573.7 ± 26.0a	1059.8 ± 6.9a	149.0 ± 8.8a	1782.5 ± 41.1a
	steam injection	100 °C, 20 min	9.66 ± 0.15b	20.96 ± 0.80b	nd	570.4 ± 29.7a	1074.7 ± 59.2a	130.1 ± 2.7b	1775.3 ± 86.2a
Proto (yellow soybean)	raw UHT		91.84 ± 4.17a	164.43 ± 8.00a	nd	590.9 ± 13.4a	1008.2 ± 40.8a	103.0 ± 0.9b	1702.1 ± 55.1b
	direct UHT	143 °C, 60 s	14.86 ± 1.60b	38.41 ± 3.50b	nd	571.7 ± 47.6a	1158.6 ± 92.8a	137.9 ± 6.8a	1868.1 ± 147.2a
	indirect UHT	143 °C, 60 s	14.65 ± 0.51b	35.45 ± 1.26b	nd	601.5 ± 17.2a	1133.2 ± 43.9a	131.8 ± 0.7a	1866.5 ± 27.4a
black soybean	raw		32.33 ± 2.15a	65.44 ± 5.11a	nd	389.7 ± 11.3a	667.5 ± 12.4a	163.9 ± 4.8b	1221.2 ± 18.9a
	stove cooked	100 °C, 20 min	15.14 ± 0.72b	31.45 ± 0.45c	nd	342.6 ± 1.9b	661.8 ± 14.9a	139.9 ± 1.0c	1144.4 ± 15.1b
	steam injection	100 °C, 20 min	18.10 ± 0.00b	43.23 ± 3.38b	nd	379.8 ± 19.7a	641.2 ± 22.8a	198.6 ± 3.3a	1219.6 ± 45.8a
black soybean	raw UHT		90.71 ± 7.46a	181.01 ± 10.99a	nd	343.9 ± 2.1a	591.9 ± 16.9ab	136.7 ± 1.6b	1072.6 ± 17.3ab
	direct UHT	143 °C, 60 s	14.51 ± 0.75b	36.32 ± 1.62b	nd	295.3 ± 17.3b	556.4 ± 30.7b	147.3 ± 6.8b	999.1 ± 48.4b
	indirect UHT	143 °C, 60 s	13.40 ± 1.32b	30.47 ± 0.89b	nd	319.5 ± 10.4ab	621.6 ± 17.2a	166.5 ± 1.6a	1107.6 ± 8.4a

Table 4. Continued

soybean material	soy milk	processing conditions	aglycones			total individuals			
			Dein	Gein	Glein	T-Dein	T-Gein	T-Glein	total isoflavones
IA 2032 (yellow soybean)	raw		145.43 ± 2.00a	327.05 ± 9.31a	nd	901.9 ± 8.3a	1194.5 ± 6.6a	125.3 ± 4.1a	2221.6 ± 23.4a
	stove cooked	100 °C, 20 min	34.74 ± 3.30b	80.46 ± 6.63b	nd	863.6 ± 43.4b	1224.5 ± 38.6a	110.6 ± 1.3b	2198.7 ± 82.8a
	steam injection	100 °C, 20 min	17.19 ± 0.73b	36.74 ± 0.73c	nd	926.5 ± 56.6a	1225.5 ± 75.2a	94.9 ± 3.4c	2246.9 ± 133.5a
IA 2032 (yellow soybean)	raw UHT		154.65 ± 13.46a	317.23 ± 1.74a	nd	658.0 ± 18.5ab	1385.7 ± 27.1a	60.2 ± 1.5b	2103.8 ± 47.1a
	direct UHT	143 °C, 60 s	20.16 ± 0.68b	52.04 ± 1.22b	nd	606.7 ± 25.4b	1206.5 ± 15.8b	118.3 ± 1.3a	1931.5 ± 37.1b
	indirect UHT	143 °C, 60 s	24.64 ± 1.39b	59.39 ± 8.87b	nd	715.6 ± 49.7a	1345.9 ± 71.4a	114.8 ± 9.6a	2176.3 ± 129.2a

[a] Data are expressed as mean ± standard deviation ($n = 3$) on dry weight basis. Values marked by the same letter within each group in each column are not significantly different ($p < 0.05$). Din, daidzin; Gin, genistin; Gly, glycitin; MDin, malonyldaidzin; MGin, malonylgenistin; MGly, malonylglycitin, ADin, acetydaidzin; AGin, acetylgenistin; AGly, acetylglycitin; Dein, daidzein; Gein, genistein; Glein, glycitein. T-Dein, subtotal daidzein; T-Gein, subtotal genistein; T-Glein, subtotal glycitein. [b] nd, not detectable.

significantly ($p < 0.05$) increased the content of 7-*O*-β-glucosides (daidzin, glycitin, genistin) and significantly ($p < 0.05$) decreased the content of malonylglucosides (malonyldaidzin, malonylglycitin, malonylgenistin) and aglycones (daidzein and genistein) in soy milk made from both yellow and black soybean varieties; traditional processes (including traditional indirect stove cooking and steam direct injection) had no significant effects on contents of acetylglucosides (acetyledaidzin, acetylglycitin, acetylgenistin), but both direct and indirect UHT processing methods significantly ($p < 0.05$) increased contents of acetylglucosides in soy milk made from both yellow and black soybean varieties.

In terms of total individual contents (moles of each form of isoflavone multiplied by the respective molecular weight of their aglycone form), indirect UHT increased contents of total individuals of daidzein, glycitein, and genistein groups in soy milk made from yellow soybean IA 2032 and black soybean as compared to the respective raw soy milk; direct UHT did not affect the contents of total individuals of daidzein and genistein groups in soy milk made from all three soybean varieties. Traditional stove cooking significantly ($p < 0.05$) reduced the contents of total individuals of daidzein, genistein, and glycitein groups in soy milk made from yellow soybean IA 2032 and black soybean. In terms of total isoflavone contents (sum of total individuals of aglycones), as compared to the raw soy milk, indirect UHT significantly ($p < 0.05$) increased the total isoflavone contents in soy milk made from yellow soybean Proto and black soybean, but indirect UHT did not increase the total isoflavone contents in soy milk made from yellow soybean IA 2032. Both traditional stove cooking and steam injection cooking significantly ($p < 0.05$) increased total isoflavone content in soy milk made from yellow soybean Proto, but traditional stove cooking significantly reduced total isoflavone content in soy milk made from black soybean, whereas neither traditional stove cooking not steam injection cooking significantly ($p < 0.05$) affected total isoflavone content in soy milk made from IA 2032.

As compared to the direct UHT, indirect UHT processing yielded significantly ($p < 0.05$) higher 7-*O*-β-glucosides and higher acetylglucosides in soy milk made from all three soybean varieties (**Table 4**). Meanwhile, indirect UHT processed soy milk exhibited lower malonylglucosides than direct UHT processed soy milk. As compared to the steam injection processes, traditional stove cooking yielded significantly ($p < 0.05$) higher 7-*O*-β-glucosides and acetylglucosides in soy milk made from yellow soybean varieties (Proto and IA 2032); meanwhile, traditional stove-cooked soy milk exhibited significantly ($p < 0.05$) lower malonylglucosides. These results demonstrate that indirect UHT and traditional stove cooking transformed more malonylglucosides into 7-*O*-β-glucosides and acetylglucosides than direct UHT and steaming injection processing did, respectively. These results were different from the findings of Probhakaran and Perera (*31*), who found direct and indirect UHT (143 °C, 10 s) methods produced similar effects on the transformation of isoflavones. The discrepancy may be due to the fact that our processing conditions of 143 °C for 60 s were much more severe than their 10 s process.

The findings of the present study are consistent with the results that the isoflavones contained in soybeans and soy milk are mainly 6″-*O*-malonyl-β-glucosides, which are partly transformed to 7-*O*-β-glucosides, 6″-*O*-acetyl-β-glucosides upon thermal processing (*10*, *26*, *28*, *32*). All thermal processing methods caused significant decreases in aglycones. This is consistent with the findings of Huang et al. (*30*) that aglycones (daidzein and glycitein) decreased rapidly during the early stage of heating.

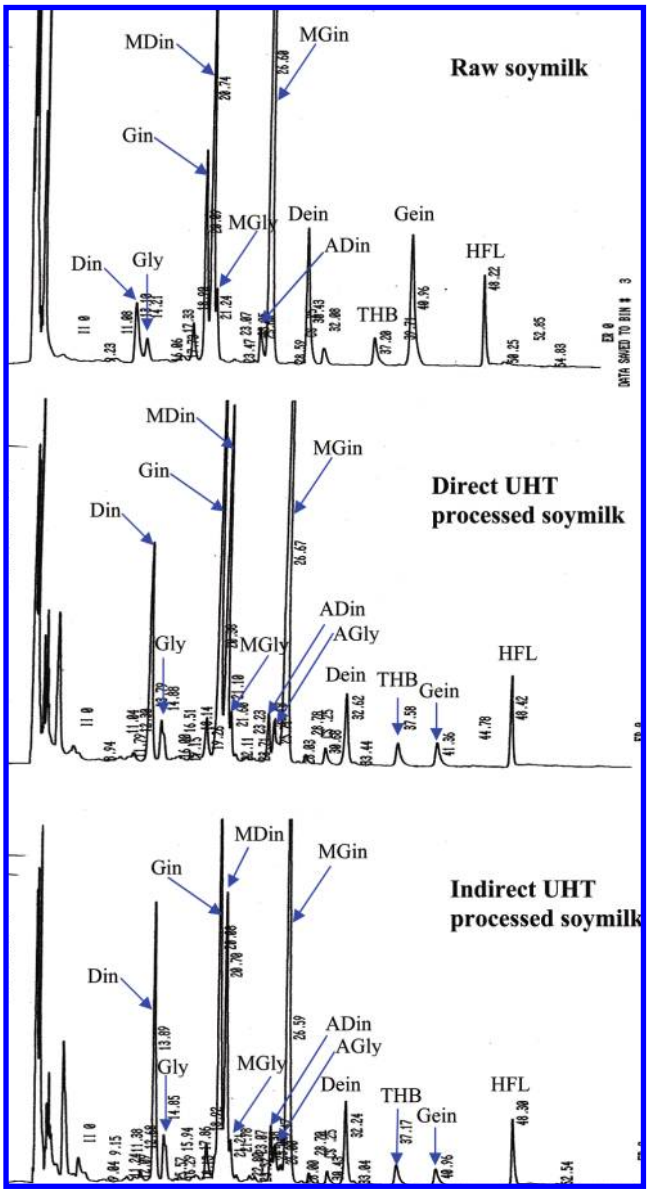

Figure 1. Effects of UHT processing on isoflavone profiles of soy milk. Din, daidzin; Gin, genistin; Gly, glycitin; MDin, malonyldaidzin; MGin, malonylgenistin; MGly, malonylglycitin; ADin, acetyldaidzin; AGly, acetylglycitin; Dein, daidzein; Gein, genistein; THB, internal standard 1; HFL, internal standard 2.

Effect of Traditional and UHT Processing on Flavan-3-ols and Anthocyanins. The flavan-3-ols and anthocyanin contents of the raw and thermally processed soy milk made from yellow and black soybean varieties are presented in **Table 5**. Anthocyanins, such as cyanindin-3-glucoside and peonidin-3-glucoside, which were found in black soybeans in our previous study (*13*), were not detectable in the raw and processed soy milk made from black soybean. As compared to the raw soy milk, there was no significant impact on the content of flavan-3-ol as a result of traditional and UHT processing. There were also no significant differences between different processing methods in terms of (+)-catechin contents in processed soy milk. About 50−60 μg/g (+)-catechin was detected in the raw and thermally processed soy milk. These results indicated that flavan-3-ol was stable in soy milk processing.

Correlations of Phenolic Compounds and Antioxidant Activities. The correlation analyses were performed by dividing soy milk into two groups, UHT processed soy milk and traditionally processed soy milk, due to their different profiles in terms of ORAC antioxidant activity, as well as phenolic acids and isoflavones. The correlation coefficients between selected predominant phenolic compositions, including TPC, TFC, gallic acid, chlorogenic acid, *trans*-cinnamic acid, total benzoic acids, total cinnamic acids, total phenolic acids, daidzin, glycitin, genistin, malonyldaidzin, malonylglycitin, malonylgenistin, subtotal daidzeins, subtotal glyciteins, subtotal genisteins, and total isoflavones, and overall antioxidant activities (DPPH, FRAP, ORAC) among the soy milk made from yellow soybean varieties (Proto and IA 2032) are summarized in **Table 6**. In the case of UHT processing (n = 12), there were significant linear correlations between TFC and DPPH (r = 0.85, p < 0.05), TFC and FRAP (r = 0.93, p < 0.0001), TFC and ORAC (r = 0.61, p < 0.05), total benzoic acids and DPPH (r = 0.82, p < 0.05), total benzoic acids and FRAP (r = 0.69, p < 0.05), daidzin and DPPH (r = 0.68, p < 0.05), daidzin and FRAP (r = 0.90, p < 0.0001), daidzin and ORAC (r = 0.79, p < 0.05), genistin and DPPH (r = 0.66, p < 0.05), genistin and FRAP (r = 0.91, p < 0.0001), genistin and ORAC (r = 0.62, p < 0.05), subtotal glyciteins and ORAC (r = 0.85, p < 0.05), subtotal genisteins and DPPH (r = 0.63, p < 0.05), subtotal genisteins and FRAP (r = 0.62, p < 0.05), total isoflavones and DPPH (r = 0.64, p < 0.05), and total isoflavones and FRAP (r = 0.69, p < 0.05). However, there were no significant linear correlations between antioxidant activities and individual phenolic acids.

In the case of traditional processing (n = 12), there were significant linear correlations between TPC and DPPH (r = 0.63, p < 0.05), TFC and DPPH (r = 0.75, p < 0.05), TFC and FRAP (r = 0.82, p < 0.05), chlorogenic acid and ORAC (r = 0.82, p < 0.05), *trans*-cinnamic acid and ORAC (r = 0.59, p < 0.05), total cinnamic acids and ORAC (r = 0.89, p < 0.05), total phenolic acids and ORAC (r = 0.78, p < 0.05), daidzin and FRAP (r = 0.86, p < 0.05), genistin and FRAP (r = 0.79, p < 0.05), malonyldaidzin and DPPH (r = 0.77, p < 0.05), malonylglycitin and ORAC (r = 0.89, p < 0.05), malonylgenistin and ORAC (r = 0.69, p < 0.05), subtotal daidzeins and DPPH (r = 0.82, p < 0.05), subtotal daidzeins and FRAP (r = 0.76, p < 0.05), subtotal genisteins and DPPH (r = 0.78, p < 0.05), subtotal genisteins and FRAP (r = 0.94, p < 0.0001), and total isoflavones and DPPH (r = 0.82, p < 0.05), as well as total isoflavones and FRAP (r = 0.84, p < 0.05). However, no significant correlations existed between the other compounds and antioxidant activities.

These correlation results indicate that different phenolic contents might have different degrees of contribution to overall antioxidant activities. β-Glucosides seemed to play a more important role than other compositions in contributing to the overall antioxidant activities of soy milk made from yellow soybean varieties. Both traditional and UHT processing transformed malonylglucosides into 7-*O*-β-glucosides and acetylglucosides and, therefore, increased the overall antioxidant activities (DPPH, FRAP) of processed soy milk products. These results indicate that daidzin, glycitin, and genistin might play an important role in the overall antioxidant activities of soy milk, whereas the other phenolic acids and isoflavones did not.

In summary, traditional thermal processing and UHT processing significantly affected the content, retention, and distribution of phenolic compounds and antioxidant activities of soy milk made from both yellow and black soybean varieties. The changes depended on the types of soybeans and processing conditions. However, traditional steam injection and indirect UHT processes caused smaller losses in total phenolic compositions and yielded higher antioxidant activities than traditional stove cooking and direct UHT processes in all three soybean varieties.

Table 5. Effect of UHT and Traditional Processing on Flavonoids (Micrograms per Gram) of Soy Milk [a]

soybean material	soy milk	processing conditions	flavan-3-ol, (+)-catechin	anthocyanins, cyanidin-3-glucoside
Proto (yellow soybean)	raw		58.60 ± 0.00a	nd[b]
	stove cooked	100 °C, 20 min	49.30 ± 2.63a	ns[c]
	steam injection	100 °C, 20 min	50.12 ± 0.42a	ns
Proto (yellow soybean)	raw UHT		55.43 ± 0.52a	ns
	direct UHT	143 °C, 60 s	47.38 ± 0.86a	ns
	indirect UHT	143 °C, 60 s	48.90 ± 2.04a	ns
black soybean	raw		50.26 ± 6.13b	nd
	stove cooked	100 °C, 20 min	64.24 ± 3.07a	nd
	steam injection	100 °C, 20 min	40.40 ± 1.51b	nd
black soybean	raw UHT		52.86 ± 1.33a	nd
	direct UHT	143 °C, 60 s	56.92 ± 1.54a	nd
	indirect UHT	143 °C, 60 s	52.51 ± 1.59a	nd
IA 2032 (yellow soybean)	raw		55.09 ± 4.31a	ns
	stove cooked	100 °C, 20 min	51.44 ± 6.08a	ns
	steam injection	100 °C, 20 min	51.72 ± 0.87a	ns
IA 2032 (yellow soybean)	raw UHT		48.19 ± 2.68a	ns
	direct UHT	143 °C, 60 s	52.65 ± 1.96a	ns
	indirect UHT	143 °C, 60 s	49.37 ± 1.82a	ns

[a] Data are expressed as mean ± standard deviation (n = 3) on dry weight basis. Values marked by the same letter within each group in each column are not significantly different ($p < 0.05$). [b] nd, not detectable. [c] ns, not sampled.

Table 6. Correlations between Antioxidant Activities and Predominant Phenolic Compounds of Soy Milk Made from Yellow Soybean Varieties

	correlation coefficient[a] (r)					
	UHT processing (n = 12)			traditional processing (n = 12)		
	DPPH	FRAP	ORAC	DPPH	FRAP	ORAC
TPC	−0.14	−0.19	−0.79*	0.63*	0.23	0.21
TFC	0.85*	0.93**	0.61*	0.75*	0.82*	−0.67*
gallic acid	0.38	0.29	−0.01	−0.16	0.36	−0.73*
chlorogenic acid	0.18	0.17	−0.14	−0.78*	−0.92**	0.82*
trans-cinnamic acid	0.19	−0.21	−0.48	0.37	−0.16	0.59*
total benzoic acids	0.82*	0.69*	0.07	0.01	0.48	−0.80*
total cinnamic acids	0.30	0.19	−0.27	−0.65*	−0.88*	0.89*
total phenolic acids	0.49	0.35	−0.22	−0.74*	−0.87*	0.78*
daidzin	0.68*	0.90**	0.79*	0.51	0.86*	−0.91**
glycitin	−0.07	0.19	0.55	−0.61*	−0.23	−0.04
genistin	0.66*	0.91**	0.62*	0.31	0.79*	−0.87*
daidzein	−0.22	−0.47	−0.94**	0.39	0.21	0.17
genistein	−0.14	−0.39	−0.93**	0.39	0.21	0.17
malonyldaidzin	−0.35	−0.61*	−0.69*	0.77*	0.47	−0.07
malonylglycitin	−0.67*	−0.56	0.28	−0.57	−0.88*	0.89*
malonylgenistin	−0.19	−0.53	−0.59*	0.02	−0.54	0.69*
subtotal daidzeins	0.50	0.57	0.00	0.82*	0.76*	−0.46
subtotal glyciteins	−0.04	0.24	0.85*	−0.89*	−0.75*	0.49
subtotal genisteins	0.63*	0.62*	−0.11	0.78*	0.94**	−0.68*
total isoflavones	0.64*	0.69*	0.04	0.82*	0.84*	−0.57

[a] *, correlation is significant at the 0.05 level (two-tailed); **, correlation is significant at the 0.0001 level (two-tailed).

ABBREVIATIONS USED

DPPH, 2-diphenyl-1-picrylhydrazyl radical; FPA, free phenolic acid; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbing capacity; TFC, total flavonoid content; TPC, total phenolic content; UHT, ultrahigh temperature.

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