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Full Length Research Paper

Comparison of total phenolic content and composition of individual phenolic acids in testae and testa-removed kernels of 15 Valencia-type peanut (*Arachis hypogaea* L.) genotypes

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A successful peanut breeding to obtain genotypes with greater phenolic content requires information on type and content of phenolic compounds in parental peanut genotypes. The aim of this study was to investigate the total phenolic contents and phenolic acid profiles of 15 Valencia-type peanut genotypes both in peanut testae and testa-removed kernels (cotyledons and embryonic axes). Total phenolic content and phenolic acid profiles were analyzed using Folin-Ciocalteu method and high-performance liquid chromatography (HPLC), respectively. The total phenolic contents of testae and testa-removed kernels varied from 2.47 ± 0.96 to 84.53 ± 5.57 and 0.07 ± 0.01 to 0.12 ± 0.01 mg gallic acid equivalent (GAE)/g dry weight, respectively. Testae of KK4 and ICG14710 genotypes had the greatest and least total phenolic content, respectively. The peanut testae with pink color (NM044, NM071, ICG15042 and KK4) had significantly greater phenolic content than those with gray (ICG397 and ICG14710) and yellow (NM001) colors. The present study demonstrates that *p*-coumaric and vanillic acids were the two predominant phenolic acids in the testae of nearly all peanut genotypes tested, except for KK4 genotype whose predominant phenolic acid in its testa was *p*-hydroxybenzoic acid. All the testa-removed kernels tested contained significantly greater amount of *p*-coumaric acid than other identified phenolic acids. These results would be useful for peanut breeding to obtain peanut genotypes with greater phenolic acid and other favorable traits.

Key words: Valencia peanuts, peanut testae, phenolic acids, *p*-coumaric acid, vanillic acid.

INTRODUCTION

Overproduction of reactive oxygen species (ROS), including the production of hydrogen peroxide, superoxide anions and hydroxyl radicals, can lead to DNA damage, lipid peroxidation and protein oxidation in cells of the human body (Klaunig et al., 2010). Oxidative stress is a key factor in the development of cancer, cardiovascular diseases and neurodegenerative diseases, which are the leading causes of death in the United States (Murphy et al., 2012). Consumption of plant-based foods rich in phenolic compounds has been recommended for the prevention of oxidative stress (Pellegrini et al., 2003; Poljsak, 2011).

Phenolic compounds are secondary metabolites distributed throughout plants from the roots to the seeds

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Abbreviations: HPLC, High-performance liquid chromatography; **GAE,** gallic acid equivalent.

(Antolovich et al., 2000). The structures of phenolic compounds range from simple structures with one aromatic ring to highly complex structures such as tannins and lignins (Sumbul et al., 2011). Phenolic compounds play a role as antioxidants through their redox properties, making them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators. Antioxidants are a group of substances that significantly delay or prevent the oxidation of oxidizable substrates, thereby, decreasing oxidative stress-induced damage. Phenolic compounds present in foods are considered to have an important role in promoting human health through preventing diseases associated with oxidative stress (Dykes and Rooney, 2007). In addition to their health benefits, phenolic compounds are also needed to replace synthetic antioxidants for the maintenance of nutritional quality and shelf-life of foods (Sang et al., 2002). The commercial development of plants as sources of antioxidants (mostly phenolic compounds) to benefit human health and food preservation is of current interest (Luthria et al., 2006; Ramamoorthy and Bono, 2007; Hajaji et al., 2010; Win et al., 2011).

Peanut (*Arachis hypogaea* L.) is a potential source of natural phenolic compounds and one of the most popular foods consumed worldwide. Information on type and content of phenolic compounds in peanut genotypes is required for successful peanut breeding to obtain genotypes with greater phenolic content. Peanut kernels and testae (seed coats) have been reported to be rich in phenolic content with potent antioxidant activity (Nepote et al., 2005; Yu et al., 2005; Wang et al., 2007; Cheng et al., 2009; Chukwumah et al., 2009). There are four major types of peanuts cultivated worldwide, Runner, Virginia, Spanish and Valencia market-types. Chukwumah et al. (2009) demonstrated that most Valencia-type peanut varieties had significantly greater total phenolic content than the other major types. However, there is no comparative data on type and content of phenolic acids in testae and kernels without testa (testa-removed kernels) of peanuts. Thus, the objectives of our study were to determine total phenolic content and composition of individual phenolic acids in both testae and testa-removed kernels of 15 Valencia-type peanut genotypes. This information will be useful for peanut breeding to obtain peanut genotypes with greater phenolic content.

MATERIALS AND METHODS

15 Valencia-type peanut kernels of *A. hypogaea* L. of the 2010 crop (October 2010 to February 2011) were obtained from the Field Crop Research Station of Khon Kaen University (KKU), Khon Kaen Province, Thailand. The kernels were deposited in plastic bags and stored at 4°C until used. Testae were removed from kernels by carefully prying off pieces with a razor blade. The pure standards of *m*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid were purchased from Fluka (Buchs, Switzerland) and Acros Organics (Geel, Belgium), respectively. Additional phenolic acid pure standards necessary to complete this study were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA).

Crude methanolic extraction

Testae and testa-removed kernels (cotyledons and embryonic axes) were weighed and ground with a pestle and mortar. Forty milliliters of methanol was added to a beaker containing 1 g of ground testae or 10 g of ground testa-removed kernels. The mixture was stirred for 2 h at room temperature. The extracted suspension was then centrifuged at 1,700 x*g* for 10 min at room temperature, and filtered through filter paper (Whatman grade No. 4). The filtrate was evaporated to 2 ml using a rotary evaporator. The concentrated extracts were stored at -20°C until used for determination of total phenolic content.

Determination of total phenolic content

Total phenolic content of crude methanolic extracts was determined spectrophotometrically using the Folin-Ciocalteu method as described by Torres et al. (1987). This method is based on the reduction of Folin-Ciocalteu reagent (phosphomolybdic and phosphotungstic acids) by phenolic groups of phenolic compounds. Three milliliters of water was added to a tube containing 0.05 ml of varied concentrations of crude extract (100, 50, 25, 12.5 and 6.25% v/v). The 0.25 ml of Folin-Ciocalteu reagent was added followed by addition of 0.75 ml of 20% sodium carbonate solution. Total volume was adjusted to 5 ml by adding distilled water. The mixture was incubated at 50°C for 2 h and then absorbance at 765 nm was recorded. The intensity of light absorption at this wavelength is proportional to the concentration of phenols. The tests were carried out in triplicate. Total phenolic content was calculated from a calibration standard curve of gallic acid and the results were given as mg gallic acid equivalent (GAE) per gram dry weight.

Sample preparation for high-performance liquid chromatography (HPLC) analysis of phenolic acid compositions

Phenolic extraction was carried out using acidic hydrolysis method (Krygier et al., 1982) with some modifications. Forty milliliters of 70% methanol was added to a beaker containing 10 g of testa-removed ground kernels or 1 g of ground testae. The mixture was stirred for 2 h at room temperature. The extracted suspension was filtered through Whatman grade no.1 filter paper. The filtrate was added with 30 ml of 2 N NaOH and stirred continuously for 12 h at room temperature. The mixture was centrifuged at 1,700 x*g* for 10 min and then filtered through Whatman grade no.1 filter paper. The supernatant was repeatedly extracted three times with 30 ml of diethyl ether, in which the aqueous phase was collected and the diethyl ether phase was discarded. The aqueous phase was adjusted to pH 1.5 by 10 N HCl. The aqueous phase was filtered through Whatman grade no.1 filter paper and extracted with 30 ml of diethyl ether for three times using separating funnel. The portion of diethyl ether was collected. The diethyl ether phase was added with sodium sulphate (Na_2SO_4) anhydrous and then filtered through Whatman grade no.1 filter paper. The filtrate was evaporated to 2 ml using a rotary evaporator and finally evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved with 2 ml of 50% methanol solution and then filtered through 0.2 µm syringe filter. The filtrate was analyzed for phenolic acid compositions using HPLC.

HPLC analysis of phenolic acid compositions

The identification of individual phenolic acids was carried out using

HPLC-Shimadzu Scientific Instruments, based on matching spectrum and retention times of standards. The HPLC system consisted of Waters In-Line Degasser AF, SIL-20A Autosampler and SPD-M20A Photo Diode Array Detector. The column used was a Waters reverse-phase C18 column (3.9 mm i.d. x 150 mm, 5 µm particle diameter). The temperature of the column was 25°C and the flow rate of mobile phase was 1.0 ml/min. The compounds were eluted with a gradient elution of mobile phase A (100% acetonitrile) and B (1% acetic acid in deionized water), where A increased from 3 to 8% in 5 min, to 10% in 25 min and was maintained at 10% for 20 min, then returned to initial condition (3%) in 10 min and remained for 15 min before next injection. Elutes were detected by photodiode array (PDA) detector at the ultraviolet wavelength of 280 nm. Concentrations of individual phenolic acids in samples were calculated by comparing their peak areas with those of standard phenolic acids run under the same elution conditions. Internal phenolic acid standards were used to ensure accuracy of the phenolic acid identification.

Statistical analysis

Data are expressed as means ± standard deviation (SD) from three replicate experiments. Statistical analyses were carried out using statistical program of SPSS version 11.5 for windows (SPSS Corporation, Chicago, IL). Significant differences among peanut genotypes were analyzed by using one-way (analysis of variance) ANOVA with Duncan's post hoc test. The criterion for statistical significance was set at $P<0.05$.

RESULTS AND DISCUSSION

The results given in Table 1 indicate that total phenolic contents of all peanut testae were much greater than those of testa-removed peanut kernels. Total phenolic content in the testae varied from 2.47 ± 0.96 to 84.53 ± 5.57 mg GAE/g dry weight. Previous research indicated that the color of peanut testa has a strong correlation with total phenolic content of peanut kernels (Chukwumah et al., 2009). In this study, peanut testae with pink color (NM044, NM071, ICG15042 and KK4) had significantly greater phenolic content than those with gray (ICG397 and ICG14710) and yellow (NM001) colors. Peanut testae with red color showed a wide range of variation with respect to total phenolic content (16.33 ± 9.14 to 61.44 ± 4.43 mg GAE/g dry weight), but all of them had lesser phenolic content than those with pink color. Shem-Tov et al. (In press) reported that Israeli peanut testae with pink color had greater total phenolic content than most of peanut testae with red, purple or white color.

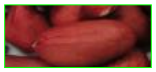
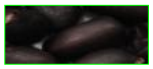
Among all testa extracts in this study, testa methanolic extract of KK4 had the greatest total phenolic content (84.53 ± 5.57 mg GAE/g dry weight), whereas, testa methanolic extract of ICG14710 had the least total phenolic content (2.47 ± 0.96 mg GAE/g dry weight). Yu et al. (2005) reported that one gram of peanut testae from local grocery store (North Carolina, USA) contained 90.1 ± 4.90 mg total phenolics when methanol was used as the extraction solvent. Wang et al. (2007) demonstrated that total phenolic content of defatted peanut testae from local herbal pharmacy (Beijing, China) was 97.0 ± 3.0 mg GAE/g dry weight when extracted with 50% ethanol. These results were comparable to total phenolic contents of KK4 and ICG15042 peanut testae tested in this study; however, peanut genotypes, extraction solvents and extraction procedures had significant effect on total extractable phenolic compounds (Yu et al., 2005; Chukwumah et al., 2009). Testa content of the 15 peanut genotypes varied from 2.09 ± 0.04 to 3.52 ± 0.07% (w/w) with an average of 2.62 ± 0.19% of whole kernel, which was comparable to the testa content of 2.4% reported by Shem-Tov et al. (In press). However, the observed testa content was less than 3.3 and 3.4% reported by Yu et al. (2005) and Cheng et al. (2009), respectively. The differences in growing conditions and peanut cultivars may also contribute to the differences.

In contrast to peanut testae, testa-removed kernels (cotyledons and embryonic axes) had a lesser total phenolics with a range of 0.07 to 0.12 mg GAE/g dry weight (Table 1). There was less variation among the tested genotypes. This observation is in agreement with the result of a previous study which also indicated no significant variation in total phenolic content of peanut seed cotyledons among the tested peanut lines (Shem-Tov et al., In press). In addition, the color of peanut testae was not correlated with total phenolic content of the testa-removed kernels, which is consistent with the results of Shem-Tov et al. (In press).

Separation and identification of individual phenolic acids in the base hydrolyzed extracts of testae and testa-removed kernels indicated that not all the 10 free phenolic acids prepared for HPLC analyses were present in the extracts (Figure 1). The six phenolic acids present in the base hydrolyzed testa extracts were identified as *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, ferulic and sinapinic acids (Table 2). Vanillic acid and *p*-coumaric acid were the predominant phenolic acids in the base hydrolyzed testa extracts of almost all peanut genotypes tested, except for KK4 genotype whose predominant phenolic acid was *p*-hydroxybenzoic acid. A large amount of ferulic acid was present in testa extracts of several peanut genotypes, especially KS2. Ferulic, *p*-hydroxybenzoic and *p*-coumaric acids were previously reported in peanut testae (Yu et al., 2005; Win et al., 2011). The type and content of individual phenolic acids in peanut extracts was significantly affected by the extraction procedures (Yu et al., 2005). The six compounds were also identified in the testa-removed kernel extracts, where *p*-coumaric acid was the predominant phenolic acid in all extracts tested (Figure 2 and Table 3). *p*-Coumaric acid has antioxidant activity in addition to tyrosinase inhibitory activity (An et al., 2010) and histone deacetylase inhibitory activity (Waldecker et al., 2008). In addition to their antioxidant activity, both vanillic and ferulic acids possess anti-inflammatory activity (Cheng et al., 2008; Kim et al., 2011).

The NM genotypes (NM001-NM077) had different testa colors, but exhibited a similar phenolic acid composition

Table 1. Color of testa, testa content, and total phenolic content of testae and testa-removed kernels extracted with 100% methanol of 15 Valencia peanut genotypes.

Peanut genotype	Color of testa		Testa content (% of whole kernel; w/w)	Total phenolic (mg GAE/g dry weight)	
				Testa	Testa-removed kernel
NM001		Yellow	2.09 ± 0.04^{a}	22.11 ± 0.83^{b}	0.09 ± 0.01^{bcd}
NM018		Red	2.17 ± 0.00^{ab}	61.44 ± 4.43^{def}	0.12 ± 0.00^{fg}
NM025		Dark purple	2.87 ± 0.07^{cd}	52.72 ± 0.71^{d}	0.09 ± 0.01^{bcd}
NM039		Dark purple	2.78 ± 0.00^{bcd}	60.03 ± 7.18^{de}	0.07 ± 0.01^{a}
NM044		Pink	2.42 ± 0.06^{abc}	72.81 ± 4.57^{fgh}	0.07 ± 0.00^{a}
NM071		Pink	2.79 ± 0.15^{cd}	70.39 ± 6.81^{efg}	0.12 ± 0.02^{g}
NM077		Dark purple	2.76 ± 0.10^{bcd}	49.63 ± 3.48^{cd}	0.12 ± 0.01^{fg}
2019(K vemena)		Red	2.48 ± 0.03^{abc}	23.56 ± 0.31^{b}	0.11 ± 0.01^{efg}
ICG397		Gray	2.60 ± 0.16^{abcd}	2.59 ± 1.07^{a}	0.12 ± 0.01^{fg}
ICG8517		Red	2.40 ± 0.18^{abc}	39.59 ± 3.08^{c}	0.10 ± 0.01^{cde}
ICG14710		Gray	2.12 ± 0.06^{a}	2.47 ± 0.96^{a}	0.10 ± 0.01^{cde}
ICG15042		Pink	3.52 ± 0.07^{e}	84.46 ± 8.10^{h}	0.07 ± 0.01^{a}
KS1		Red	2.58 ± 0.19^{abcd}	16.33 ± 9.14^{b}	0.08 ± 0.01^{abc}
KS2		Vericolor	3.12 ± 0.31^{de}	82.00 ± 7.20^{gh}	0.09 ± 0.01^{bcd}
KK4		Pink	2.57 ± 0.09^{abcd}	84.53 ± 5.57^{h}	0.09 ± 0.01^{bcd}

Values are means ± standard deviation of three replicates. Different superscript letters within the same column indicate significant differences ($P<0.05$). GAE = gallic acid equivalent.

Table 2. Phenolic acid compositions of different base hydrolyzed extracts of peanut testae analyzed by HPLC. Elutes were detected by the SPD-M20A PDA detector. The detection wavelength was 280 nm.

Peanut testae	Phenolic acid (µg/g dry weight)					
	p-Coumaric acid	Vanillic acid	Ferulic acid	*p*-Hydroxybenzoic acid	Sinapinic acid	Syringic acid
NM001	36.35 ± 0.29^{g}	67.07 ± 0.00^{m}	11.93 ± 0.08^{d}	11.52 ± 0.00^{c}	0.36 ± 0.00^{c}	3.95 ± 0.00^{e}
NM018	20.93 ± 0.10^{d}	39.90 ± 0.35^{i}	13.53 ± 0.13^{e}	29.40 ± 0.11^{h}	0.73 ± 0.46^{g}	8.10 ± 0.51^{i}
NM025	48.22 ± 0.03^{i}	50.71 ± 0.02^{k}	14.46 ± 0.05^{e}	26.54 ± 0.02^{f}	0.66 ± 0.03^{f}	7.28 ± 0.32^{f}
NM039	36.86 ± 0.04^{h}	5.31 ± 0.03^{a}	13.56 ± 0.08^{e}	11.80 ± 0.10^{c}	0.58± 0.04^{e}	6.40 ± 0.40^{d}
NM044	33.25 ± 0.00^{f}	9.55 ± 0.02^{c}	20.48 ± 0.00^{f}	28.43 ± 0.17^{g}	1.07 ± 0.01^{h}	11.82 ± 0.10^{k}
NM071	72.74 ± 0.04^{l}	13.76 ± 0.15^{d}	26.64 ± 0.15^{g}	34.44 ± 0.37^{j}	2.50 ± 0.02^{k}	27.70 ± 0.22^{l}
NM077	62.33 ± 0.11^{j}	64.26 ± 0.01^{l}	21.41 ± 0.17^{f}	33.12 ± 0.04^{i}	1.33 ± 0.03^{i}	14.73 ± 0.29^{b}
2019 (K vemena)	12.97 ± 0.02^{c}	46.29 ± 0.04^{j}	6.25 ± 0.10^{b}	20.81 ± 0.27^{d}	0.35 ± 0.03^{c}	3.90 ± 0.38^{h}
ICG397	105.47 ± 0.13^{m}	91.16 ± 0.14^{n}	10.83 ± 0.15^{d}	6.76 ± 0.07^{a}	0.26 ± 0.01^{b}	2.83 ± 0.01^{e}
ICG8517	112.40 ± 0.73^{n}	21.72 ± 0.12^{g}	29.28 ± 1.83^{h}	54.06 ± 0.39^{m}	1.70 ± 0.02^{j}	18.86 ± 0.18^{j}
ICG14710	24.88 ± 0.11^{e}	117.29 ± 0.03^{o}	6.47 ± 0.04^{b}	9.19 ± 0.14^{b}	0.15 ± 0.01^{a}	1.64 ± 0.21^{i}
ICG15042	33.34 ± 0.40^{f}	8.14 ± 0.12^{b}	8.69 ± 0.41^{c}	23.68 ± 0.10^{e}	0.43 ± 0.01^{d}	4.80 ± 0.06^{c}
KS1	6.15 ± 0.06^{a}	30.13 ± 0.19^{h}	3.35 ± 0.04^{a}	23.56 ± 0.00^{e}	0.24 ± 0.01^{b}	2.67 ± 0.13^{a}
KS2	68.96 ± 0.19^{k}	18.38 ± 0.37^{f}	47.56 ± 0.73^{i}	35.77 ± 0.01^{k}	0.63 ± 0.02^{f}	6.69 ± 0.18^{b}
KK4	6.93 ± 0.03^{b}	18.03 ± 0.02^{e}	2.91 ± 0.08^{a}	52.11 ± 0.41^{l}	0.23 ± 0.02^{b}	2.55 ± 0.19^{g}

Values are means ± standard deviation of three replicates. Different superscript letters within the same column indicate significant differences (P<0.05).

Table 3. Phenolic acid compositions of different base hydrolyzed extracts of testa-removed peanut kernels analyzed by HPLC. Elutes were detected by the SPD-M20A PDA detector. The detection wavelength was 280 nm.

Testa-removed kernel	Phenolic acid (µg/g dry weight)					
	p-Coumaric acid	Vanillic acid	Ferulic acid	*p*-Hydroxybenzoic acid	Sinapinic acid	Syringic acid
NM001	170.04 ± 0.07^{i}	2.25 ± 0.01^{a}	7.57 ± 0.01^{c}	1.38 ± 0.00^{c}	0.28 ± 0.00^{a}	0.23 ± 0.00^{d}
NM018	195.87 ± 0.03^{k}	2.84 ± 0.00^{c}	8.76 ± 0.01^{e}	2.35 ± 0.00^{g}	0.65 ± 0.00^{d}	0.58 ± 0.12^{g}
NM025	169.99 ± 0.00^{i}	2.24 ± 0.00^{a}	7.56 ± 0.00^{c}	1.38 ± 0.00^{c}	0.28 ± 0.00^{a}	0.04 ± 0.01^{a}
NM039	238.72 ± 0.02^{l}	6.66 ± 0.01^{i}	14.99 ± 0.04^{m}	2.37 ± 0.04^{g}	1.59 ± 0.05^{i}	0.12 ± 0.00^{b}
NM044	155.27 ± 0.03^{e}	2.13 ± 0.04^{a}	9.77 ± 0.04^{f}	1.36 ± 0.02^{c}	0.91 ± 0.06^{g}	0.17 ± 0.00^{c}
NM071	163.23 ± 0.03^{f}	2.24 ± 0.13^{a}	11.00 ± 0.02^{j}	1.83 ± 0.04^{e}	0.54 ± 0.03^{c}	0.59 ± 0.01^{g}
NM077	166.81 ± 0.00^{i}	5.29 ± 0.00^{g}	10.41 ± 0.00^{h}	1.46 ± 0.01^{d}	2.30 ± 0.03^{k}	0.41 ± 0.01^{ef}
2019 (K vemena)	238.94 ± 0.05^{m}	6.20 ± 0.05^{h}	19.91 ± 0.02^{n}	3.09 ± 0.00^{i}	0.50 ± 0.03^{bc}	0.87 ± 0.00^{i}
ICG397	179.73 ± 0.02^{j}	2.99 ± 0.00^{d}	8.66 ± 0.01^{d}	0.95 ± 0.01^{a}	0.51 ± 0.01^{bc}	0.61 ± 0.01^{g}
ICG8517	145.98 ± 0.01^{c}	2.53 ± 0.01^{b}	6.75 ± 0.01^{b}	1.91 ± 0.02^{f}	1.02 ± 0.00^{h}	0.45 ± 0.00^{f}
ICG14710	164.74 ± 0.01^{g}	3.18 ± 0.01^{f}	11.55 ± 0.02^{k}	0.98 ± 0.00^{a}	0.85 ± 0.01^{f}	0.44 ± 0.02^{f}
ICG15042	166.24 ± 0.03^{h}	3.51 ± 0.15^{e}	5.51 ± 0.04^{a}	2.70 ± 0.07^{h}	0.48 ± 0.01^{b}	1.08 ± 0.05^{j}
KS1	138.03 ± 0.00^{b}	3.10 ± 0.05^{d}	11.97 ± 0.01^{l}	1.34 ± 0.00^{c}	0.72 ± 0.01^{e}	0.74 ± 0.00^{h}
KS2	154.05 ± 0.06^{d}	3.08 ± 0.10^{d}	10.76 ± 0.00^{i}	1.93 ± 0.03^{f}	4.42 ± 0.01^{l}	1.07 ± 0.02^{j}
KK4	132.80 ± 0.02^{a}	2.18 ± 0.06^{a}	10.29 ± 0.01^{g}	1.28 ± 0.00^{b}	1.17 ± 0.00^{i}	0.40 ± 0.00^{e}

Values are means ± standard deviation of three replicates. Different superscript letters within the same column indicate significant differences (P<0.05).

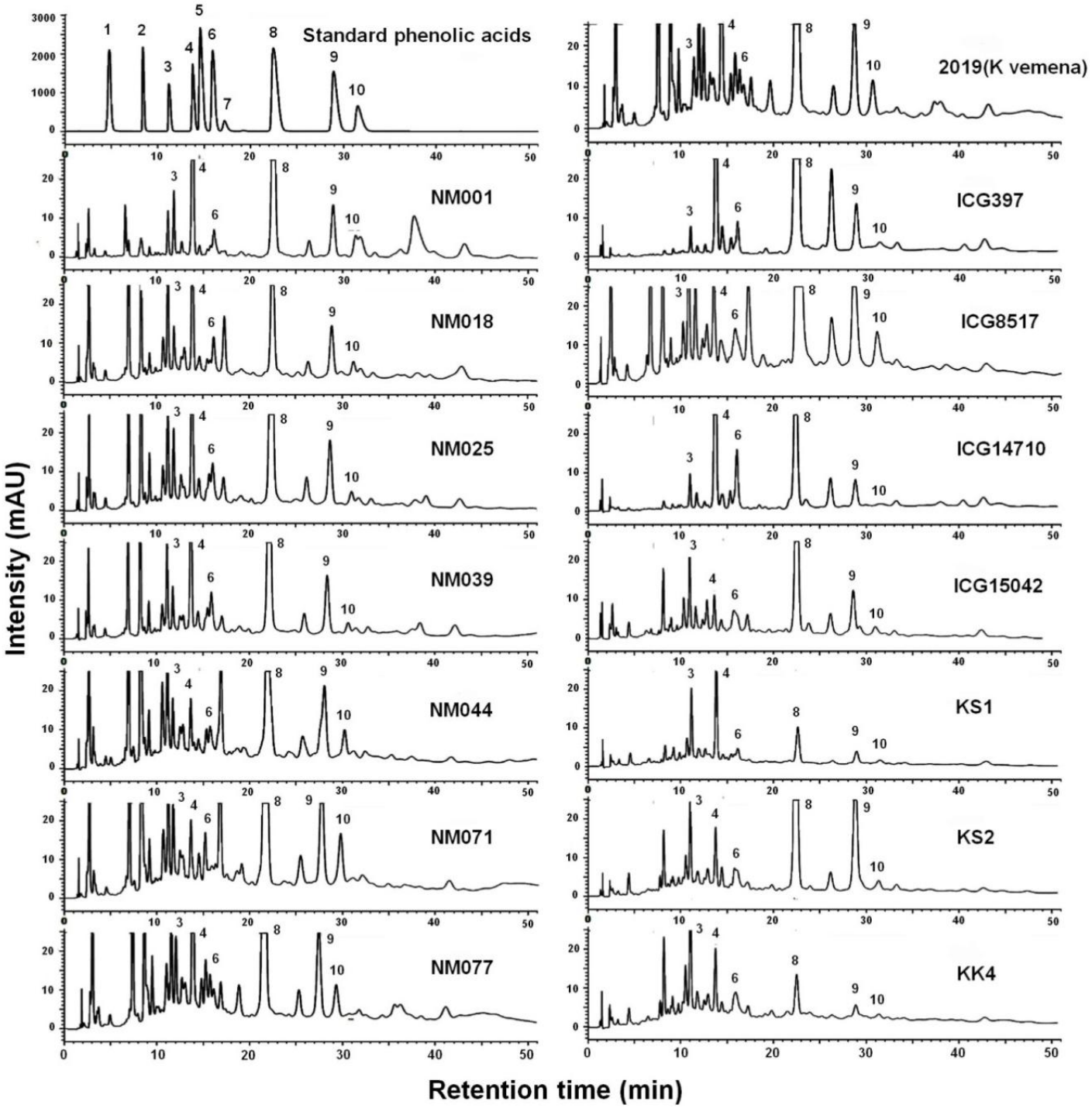


Figure 1. HPLC chromatograms of phenolic acid standards and base hydrolyzed extracts of testae of 15 peanut genotypes: 1, gallic acid; 2, protocatechuic acid; 3, *p*-hydroxybenzoic acid; 4, vanillic acid; 5, caffeic acid; 6, syringic acid; 7, *m*-hydroxybenzaldehyde; 8, *p*-coumaric acid; 9, ferulic acid and 10, sinapinic acid.

profile of free phenolic acids in their testa extracts (Figure 1). Phenolic acid composition of testa-removed kernel extracts were similar; however, some distinct unidentified peaks were observed (Figure 2). The base hydrolysis was used in the extraction procedure in order to quantitatively determine total phenolic acids, both free and bound, as phenolic acids can occur in multiple conjugated forms with sugars, acids and other phenolic compounds.

In conclusion, our results demonstrate that peanut testae contained greater total phenolic content than testa-removed kernels. The varieties of peanut genotypes

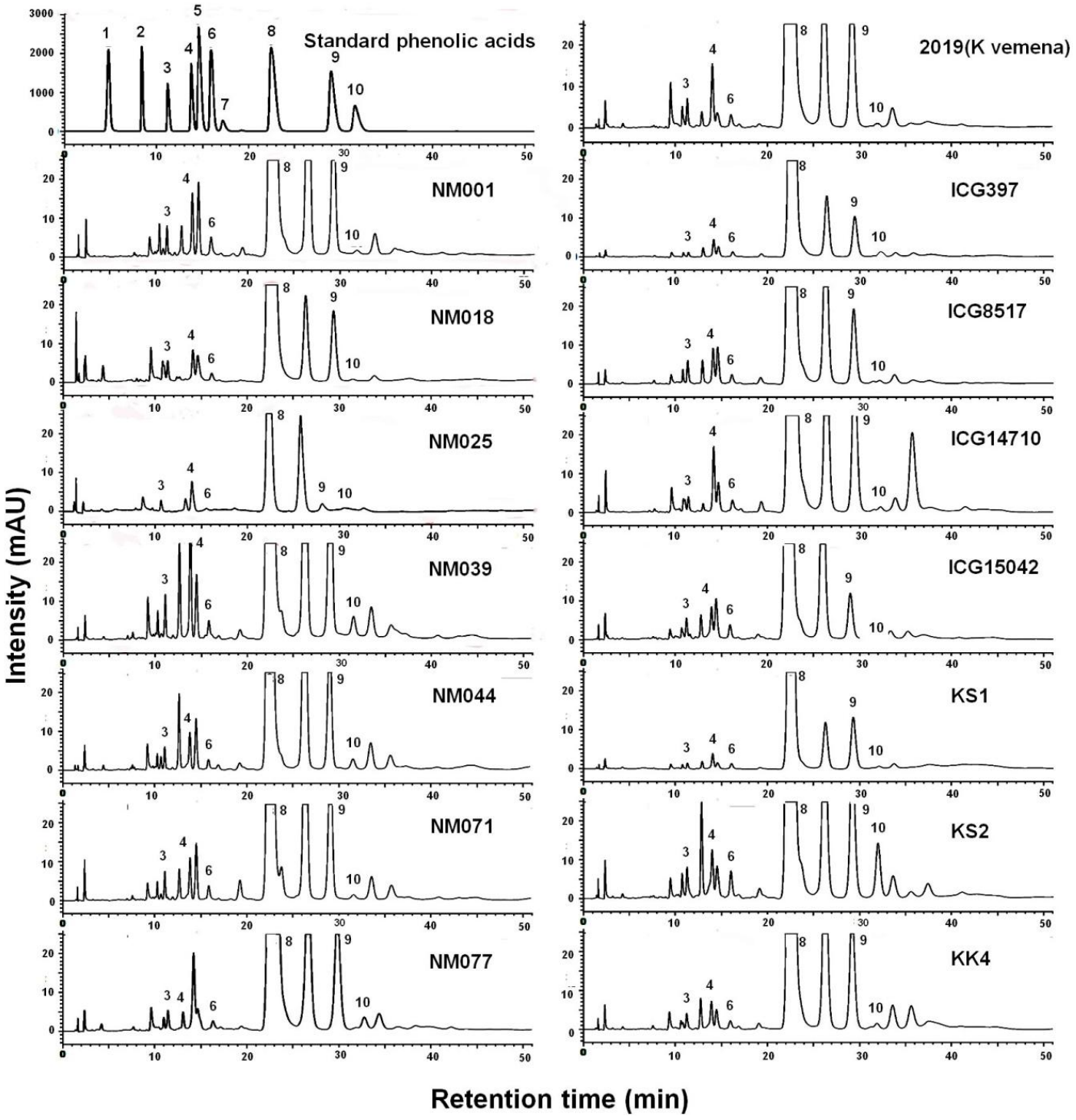


Figure 2. HPLC chromatograms of phenolic acid standards and base hydrolyzed extracts of testa-removed kernels of 15 peanut genotypes: 1, gallic acid; 2, protocatechuic acid; 3, *p*-hydroxybenzoic acid; 4, vanillic acid; 5, caffeic acid; 6, syringic acid; 7, *m*-hydroxybenzaldehyde; 8, *p*-coumaric acid; 9, ferulic acid and 10, sinapinic acid.

analyzed here can be considered as good sources of phenolic compounds, especially *p*-coumaric acid. However, more work is needed to test bioactivity of the identified phenolic acids in biological systems and to test the contribution of each individual phenolic compound to the total antioxidant activity. Furthermore, color of testa should be taken into consideration in breeding programs that aim to integrate high total phenolic content of the

testa with other favorable traits.

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