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Temperature and Acidified Solvent Effect on Total Anthocyanins and RP-HPLC Phenolic Acids Determination in Selected Spices

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Abstract

Total anthocyanins of spices (*Syzygium aromaticum* L., *Coriadrum sativum* L., *Cuminum cyminum* L., *Zingiber officinale* Rosc., *Elettaria cardamomum*, *Curcuma longa*, *Rhus coriaria* L., *Cinnamomum zeylanicum* Blume, *Foeniculum vulgare* Mill and *Laurus nobilis* L.) were determined using acidified (1% HCl) solvents (methanol, ethanol and acetone) at three temperatures (20˚C, 40˚C and 60˚C). Also phenolic acids were separated and identified by RP-HPLC. Results showed that sumac and cinnamon had the highest levels of anthocyanins, while for the acetone the cinnamon indicated the highest amount of anthocyanins when methanol and ethanol were used as extracting solvents at 20˚C. At 40˚C using ethanol, sumac showed the highest level of anthocyanins whereas acetone solvent yielded the highest anthocyanin contents for cinnamon. At 60˚C, cinnamon showed the highest level of anthocyanins when methanol and acetone were the solvents, while sumac had the highest anthocyanins level using ethanol as solvent. HPLC results showed ten phenolic acids found in those spices and varied in their concentrations. Gallic acid had the highest level (1642.3 mg/100g) (cloves). Gentisic acid had the lowest level (1.2 mg/100g) in ginger. Also sumac showed the highest level of chlorogenic acid (1528.7 mg/100g). Some acids were not found in some spices, for instance, benzoic acid was not found in coriander, cumin, ginger, green cardamom, cinnamon and sweet laurel.

Keywords

Spices, Anthocyanins, Phenolic Acids, HPLC and Acidified Solvents

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1. Introduction

Anthocyanins and phenolic acids are phytochemicals and bioactive substances of plants that have been associated in the protection of human health against chronic degenerative diseases. According to Lako *et al.* [1] one class of phytochemicals is the phenolic compounds, which are a secondary metabolites in plants derived from phenylalanine and tyrosine. Phenolics are categorized into several classes such as simple phenols, phenylpropanoids, benzoic acid derivatives, flavonoids, tannins, lignan and lignin.

All types of phenolics support plants with essential characteristics such as the color, flavor and antioxidation features [2]. One significant type of phenolic-flavonoids is the anthocyanins, which determine the color of vegetables, fruits and spices. They are glycosidically bound anthocyanidins and water soluble pigments that are responsible for darkening colors [2]. Exploring the existence of anthocyanins induces the presence of phenolic compounds; this paper is interested in measuring the concentration of anthocyanins and to identify the levels of phenolic acids.

The antioxidant property in many plants is related to the presence of phenolic compounds. Nutritionally, these compounds are responsible for increasing the shelf life of foods as well as slowing the lipid, protein and enzymatic oxidation. In addition, phenolics reduce the rancidity development, which prevent off-flavor of foods [3]. The chemical composition and the microbiological status for these spices were studied [4]. The antioxidant activity and the phenolic compounds were also reported by Ereifej *et al.* [4].

Antioxidants are specific additives that inhibit oxidation through reacting with free radicals, hence forming inactive products. Antioxidants are any substance that prevents the rancidity or other flavor decline. The types of antioxidants are the natural and synthetic antioxidants; both of these additives include phenolic compounds. This paper concentrates on studying the behavior of phenolic acids in terms of measuring the natural phenolic acids and anthocyanins levels in those spices [5].

The metrics that reflect the anthocyanins, antioxidant activity and hence the role of phenolic acids in plants are the temperature, food composition, food structure and the levels of oxygen. The temperature and the levels of oxygen are dependent factors related to the composition and the structure of food. The purpose of this research is to report on the influence of temperature and the acidified extractants on the levels of anthocyanins in some spices used in food preparation and also to quantify and identify the phenolic acids in those spices.

2. Materials and Methods

2.1. Spices Samples

About 500 g of the investigated spices (Cloves, Coriander, Cumin, Ginger, Green Cardamom, Turmeric, Sumac, Cinnamon, Sweet Cumin, (Fennel) and Sweet Laurel, (Sweet Bay leaf)) [4] were purchased from a local market, in Irbid city in Jordan. All the spices were purchased twice with two months time interval. Their scientific and local names were obtained from different references. All the purchased spices were pulverized to a fine powder using a laboratory mill to pass a 0.5 mm sieve and kept at 4˚C in Ziploc plastic bags until time of analysis.

2.2. Spices Extract Preparation

Acidified (1% HCl v/v) acetone, ethanol and methanol were used for anthocyanins extraction and determination. One gram of each spice was weighed out (two replicates), the extraction was carried out with continuous stirring for one hour at 60˚C. The extractant was filtrated using filter paper into a 50 ml volumetric flask, the volume was made up to the mark with the same solvent, then kept in the dark at refrigerated temperature until the time of analysis.

2.3. Total Anthocyanins Determination

Anthocyanins content in extract was determined according to the procedures described by Rabino and Mancinelli [6] and Randy [7]. All extracts were kept at dark for two hours, then the absorbance for all extracts was measured at 530 and 657 nm using a spectrophotometer (CE CELL, model 1020). The absorbance was corrected, then used to compute the anthocyanin content which was expressed as milligram of Cyanidin-3-lucoside equivalent per gram of sample weigh (dry eight basis). The concentration of anthocyanin was calculated as follows:

$$A = (A_{530} - 0.25A_{657}) \text{ corrected absorbance}$$

(Absorbance/29600) × 449.2 × dilution factor)/sample weight (gm)

where 29600 = molar extinction coefficient, 449.2 = molecular weight of cyaniding-3-glucoside. Dilution factor = final volume/initial volume.

2.4. Effect of Extraction Solvent and Temperature

One gram of each spice was weighed out (in duplicate) and transferred to a 250 ml beaker. The spice was soaked with 50 ml of acidified methanol or ethanol or acetone as the extractant. Extractions were carried out at 20, 40 and 60˚C for one hour under stirring. The extracted material was filtered using a Whatman filter paper into 50 ml volumetric flask. The volume was completed to the mark using the same solvent. The filtrate was kept at dark at refrigerated temperature until the time of analysis.

2.5. Phenolic Acids Identification by RP-HPLC

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) was used for separation and quantification of phenolic acids. An RP-HPLC system was used, which consists D-7000 interface, L-7455 Diode array detector, L-7200 autosampler, L-7150 pump, L-7612 solvent degasser all Merch Hitachi-Germany. Tow mobile phases were used (mobile phase A consists 10% acetonitrile and 0.1% trichloroacetic acid, mobile phase B consists 50% acetonitrile and 0.1% trichloroacetic acid), using the column of Thermo Electronic Corporation Hyper Sil Gold; 150 × 4.6 mm with 5 micron of particle size. Ten phenolic acids as standards were used for phenolic acids separation and quantification [2]. The standard phenolic acids were; Gallic, protocatechuic, catechin, gentisic, chlorogenic, vanillic, syringic, caffeic, epicatechin and benzoic acid. The following elution programme was conducted during 0 - 50 min with a flow rate of 1.5 mL/min from phase B, 51 - 65 min with a flow rate of 1.5 mL/min from phase B and during 66 - 85 min with a flow rate of 1 mL/min of 100% from phase A. Data analyses were carried out using the Merck Hitachi software.

2.6. Statistical Analysis

The collected data were statistically analyzed using analysis of variance (ANOVA), using the general linear model procedure of SAS institute [8]. Data for each test were analyzed as a completely randomized design (CRD). Differences among treatment means were analyzed using the least significant differences (LSD) at $P \leq 0.05$.

3. Results and Discussion

3.1. Total Anthocyanin

Data on total anthocyanin in spices extracted using three acidified solvents and extracted at different temperatures are shown in **Table 1**. Data showed that the extracting solvents (acidified methanol, ethanol and acetone) used at 60˚C affected the concentrations of anthocyanins. The results showed that the levels of anthocyanins varied significantly among the spices. Anthocyanins content ranged between 7.1 mg/100g (cumin) and 231.5 mg of cyanidin-3-glucoside/100g (cinnamon), when the acidified methanol was used as extractant. Sumac, sweet laurel and cloves found to have the highest anthocyanins levels (231.2, 101.5 and 45.6 mg of cyanidin-3-glucoside/100g, respectively). On the other hand, sweet cumin, coriander and ginger had the lowest content of anthocyanins (8.8, 11.5 and 18.2 mg of cyanidin-3-glucoside/100g, respectively).

Sumac had the highest anthocyanins concentrations using ethanol as extractant at 60˚C (213.3 mg/100g), followed by cinnamon (79.7 mg/100g) and turmeric (34.2 mg/100g). The lowest levels of anthocyanins showed by sweet cumin, cumin and green cardamom (1.1, 6.8 and 7.4 mg/100g of dry weight, respectively).

Cinnamon, turmeric, sumac and sweet laurel had the highest content of anthocyanins (145.5, 58.2, 54.4 and 24.6 mg/100g, respectively) compared with cumin, sweet cumin, coriander and cloves (2.4, 3.1, 3.2 and 9.0 mg/100g respectively) which had the lowest content of anthocyanins using acidified acetone. From data presented in **Table 1**, we conclude that acidified methanol was the best extractant at 60˚C for anthocyanins. Significant differences in the total anthocyanins concentrations using acidified methanol, ethanol and acetone at 40˚C, except for sweet cumin as shown in **Table 1**. Sumac, cinnamon, turmeric and cloves showed the highest anthocyanins content using acidified methanol as the extractant, values were, 231.3, 204.4, 83.6 and 54.1 mg of cyanidin-3-glucoside/100g, respectively. When ethanol was used as extractant, the highest anthocyanins concentrations

Table 1. Effect of different extractant and temperatures on anthocyanin levels in spices[a].

Spices	Temperature 60˚C			Temperature 40˚C			Temperature 20˚C		
	Methanol	Ethanol	Acetone	Methanol	Ethanol	Acetone	Methanol	Ethanol	Acetone
Cloves	45.6^{d}	21.7^{d}	9.0^{e}	54.1^{d}	28.1^{c}	8.1^{f}	45.4^{e}	34.3^{c}	16.2^{d}
Coriander	11.5^{g}	14.1^{e}	3.2^{f}	9.5^{f}	4.0^{g}	2.7^{f}	19.4^{f}	17.2^{f}	3.0^{f}
Cumin	7.1^{h}	6.8^{fg}	2.4^{f}	12.6^{f}	8.0^{f}	2.9^{f}	8.7^{g}	7.0^{e}	3.0^{f}
Ginger	18.2^{f}	8.4^{ef}	14.8^{d}	12.3^{f}	0.1^{i}	26.7^{e}	19.9^{f}	5.6^{ef}	18.7^{d}
Green Cardamom	40.9^{e}	7.4^{f}	10.4^{de}	10.4^{f}	4.2^{g}	5.6^{f}	19.2^{f}	4.6^{ef}	7.9^{e}
Turmeric	71.8^{c}	34.2^{c}	58.2^{b}	83.6^{c}	20.0^{d}	56.5^{d}	80.8^{c}	19.5^{d}	53.8^{f}
Sumac	231.2^{a}	213.3^{a}	54.4^{b}	231.3^{a}	227.0^{a}	82.9^{c}	266.9^{a}	235.8^{b}	49.2^{c}
Cinnamon	231.5^{a}	79.7^{b}	145.5^{a}	204.4^{b}	52.0^{b}	128.0^{a}	138.0^{b}	263.1^{a}	296.5^{a}
Sweet Cumin	8.8^{gh}	1.1^{g}	3.1^{f}	3.0^{g}	2.0^{h}	1.7^{f}	4.1^{h}	0.5^{g}	4.5^{ef}
Sweet Laurel	101.5^{b}	20.4^{d}	24.6^{c}	31.0^{e}	15.9^{e}	93.9^{b}	60.0^{d}	32.7^{c}	55.3^{b}
LSD at P ≤ 0.05	4.1	5.9	5.0	5.1	1.8	10.4	4.0	2.5	4.5

[a]Means are average of two replicates and expressed as mg of cyanidin-3-glucoside/100g of dry weight. *Means with different letters in the same column are significantly different at P ≤ 0.05.

were found in sumac (227 mg/100g), cinnamon (52 mg/100g) and cloves (28.1 mg/100g). But when acidified acetone was employed as extractant, cinnamon presented the highest anthocyanins concentration (128 mg/100g), sweet laurel and sumac, values were, 93.9 mg/100g and 82.9 mg/100g respectively.

At ambient temperature and methanol as extractant. Anthocyanin contents for sumac, cinnamon and sweet laurel were the highest, values were (266.9, 138, 60 mg/100g, respectively) as shown in **Table 1**. Sumac, cloves and sweet laurel found to have the highest anthocyanins levels (263.1, 235.8, 34.3 and 32.7 mg/100g respectively) when ethanol was used.

When acetone was the extractant; cinnamon, sweet laurel turmeric had anthocyanin levels 296.5, 55.3 and 53.8 mg/100gm. **Table 1** illustrate that acidified methanolic extract for all the spices showed the greatest anthocyanin levels and was superior to ethanol and acetone.

Prata and Oleviera [9] reported a value of (20.3 mg/100g) anthocyanin in fresh coffee using methanol as extractant, our anthocyanin values are higher than those reported previously. Also Wang and Ballington [10] reported higher range of anthocyanins in deerberry samples using acetone as extractant. The anthocyanin contents ranged from 371.4 to 630.6 mg/100g.

The effect of temperatures on anthocyanins levels when applying acidified methanol, anthocyanins varied significantly as the temperature elevated. At the ambient temperature, sumac, cinnamon and turmeric had the highest anthocyanins concentration (266.9, 138 and 80 mg/100g). As the temperature increased to 40˚C, the anthocyanins concentration for the sumac, cinnamon and turmeric were 231.3, 204.4 and 83.6 mg/100g respectively. When the temperature elevated to 60˚C, cinnamon and sumac showed the same value of anthocyanins content (231.5 and 231.2 mg/100g respectively), on the other hand; sweet laurel and turmeric presented 101.5 and 71.8 mg/100g respectively. Most of the spices showed higher content of anthocyanin at 60˚C when compared with lower temperatures used.

Data on the concentrations of anthocyanins using acidified ethanol at different temperatures 20˚C, 40˚C and 60˚C are shown in **Table 1**. Cloves, cumin and sweet cumin did not vary significantly, whereas, anthocyanin content in other spices showed significant differences. At 20˚C; cinnamon, sumac and cloves had the highest concentrations (263.1, 235.8 and 34.4 mg/100g respectively). At 40˚C, sumac had the highest concentrations (227 mg/100g), followed by cinnamon (52 mg/100g) then cloves (28.1 mg/100g). Despite the increase of temperature to 60˚C, the highest concentrations were shown by sumac, cinnamon and turmeric (213.3, 79.7 and 34.2 mg/100g respectively). The highest concentration of anthocyanin was achieved at ambient temperature when ethanol was used. The anthocyanins concentrations showed significant differences among different temperatures,

cumin and sweet cumin showed almost the same content of anthocyanin at 20˚C, 40˚C and 60˚C when acetone was used. The highest concentration of anthocyanin in cinnamon, sweet laurel, turmeric and sumac at 20˚C were 296.5, 55.3, 53.8 and 49.2 mg/100g respectively.

At 40˚C, cinnamon, sweet laurel and sumac had concentrations levels 128, 82.9 and 56.5 mg/100g respectively. But at 60˚C, cinnamon, turmeric and sumac anthocyanins values were 145.5, 58.2 and 54.4 mg/100g respectively.

It was reported that methanol was the most effective solvent for anthocyanins extraction and the best solvent for catechin extraction, whereas a better yield for procyanidins was obtained with 70 % acetone [11].

Our anthocyanin values are in agreement with values reported previously on fresh blueberry fruits despite the different extracting method [8]. Also our anthocyanin values are comparable with values reported on common fruits and vegetables such as strawberry, red grapes, red cabbage and elderberry [12].

Anthocyanins in dried strawberry, dried peach and dried apple using an extractant mixed of acetone, methanol, water and acetic acid were studied [13]. Dried strawberry had the highest content of anthocyanin (53.39 mg/100g) whereas dried peaches had 5.09 mg/100g and dried apple had 3.81 mg/100g. Anthocyanin content in the current investigation of spices (**Table 1**) showed higher levels of anthocyanin when compared to these dried fruits.

Anthocyanin levels in these spices compare very will with values reported on eggplant, red cabbage and red onion [14].

3.2. Phenolic Acids Identification Using RP-HPLC

Data on phenolic acid separation and quantification by HPLC are illustrated in **Figures 2-6**. **Figure 1** presents the HPLC separation of standard phenolic acids used as internal standard for identification and quantification of phenolic acids in spices extract. The concentration of these phenolic acids is shown in **Table 2**. Data showed that gallic, protocatechuic and gentisic acid were the dominating phenolic acids in cloves (**Figure 2**); cloves had a range of total phenolic acids between 2.7 mg/100g (benzoic acid) and 1642.3 mg/100g (gallic acid) (**Table 2**). Vanillic and caffeic acids were the dominating phenolic acid in coriander having a concentration of 237.0 and 38.6 mg/100g respectively, whereas, syringic and benzoic acids were not detected in coriander. Results also showed that gallic, vanillic and chlorogenic acids were the dominant phenolic acids in cumin, but gentisic, syringic, epicatechin and benzoic acid were not detected in cumin, (**Figure 3**). Ginger found to have vanillic, chlorogenic and catechin as the dominant phenolic acids; their levels were 20.4, 12.5 and 8.0 mg/100g, respectively. On the other hand, caffeic and benzoic acids were not detected (**Figure 3**).

Figure 4 showed that protocatechuic, vanillic, syringic, caffeic, epicatechin and benzoic acids were not present in green cardamom, whereas, gallic and chlorogenic acids were the dominant phenolic acids. Chlorogenic, caffeic acid and epicatechin were not detected in turmeric (**Figure 4**); but, catechin was the predominant phenolic acid (35.8 mg/100g) in turmeric (**Table 2**). Protocatechuic and benzoic acid showed the lowest and the same concentrations found in turmeric (1.9 mg/100g).

Figure 5 showed the concentrations of phenolic acids in sumac which ranged from 3.3 mg/100g (gentisic acid) to 1528.7 mg/100g (chlorogenic acid), caffeic acid was not detected in sumac. Syringic, caffeic, epicatechin and benzoic acid were not detected in cinnamon (**Figure 5**); vanillic acid (**Table 2**) showed the highest concentration (187.1 mg/100g) whereas; gentisic acid was the lowest concentration in cinnamon (3.3 mg/100g).

Caffeic acid and epicatechin were not detected in sweet cumin, whereas; epicatechin and benzoic acid were not detected in sweet laurel (**Figure 6**). The main phenolic acids in cloves and sumac had been found to be gallic and chlorogenic acid. These data are in agreement with data reported by Surveswaran *et al.* [15] on some spices.

4. Conclusion

From this work we can conclude that the extracted anthocyanins found to increase as temperature increases from 20˚C to 60˚C. 1% acidified methanol was a superior extractant over acetone and ethanol. Also acidified methanol and 60˚C were excellent anthocyanin extraction combination. HPLC system found to be helpful for separation, identification and quantification of ten phenolic acids. Benzoic, epicatechin, caffeic and syringic acids were not detected in coriander, cumin, ginger and green cardamom. Gallic acid showed the highest level (1642.3 mg/100g) in cloves. Gentisic acid had the lowest level (1.2 mg/100g) in ginger; sumac had the highest level of chlorogenic acid (1528.7 mg/100g).

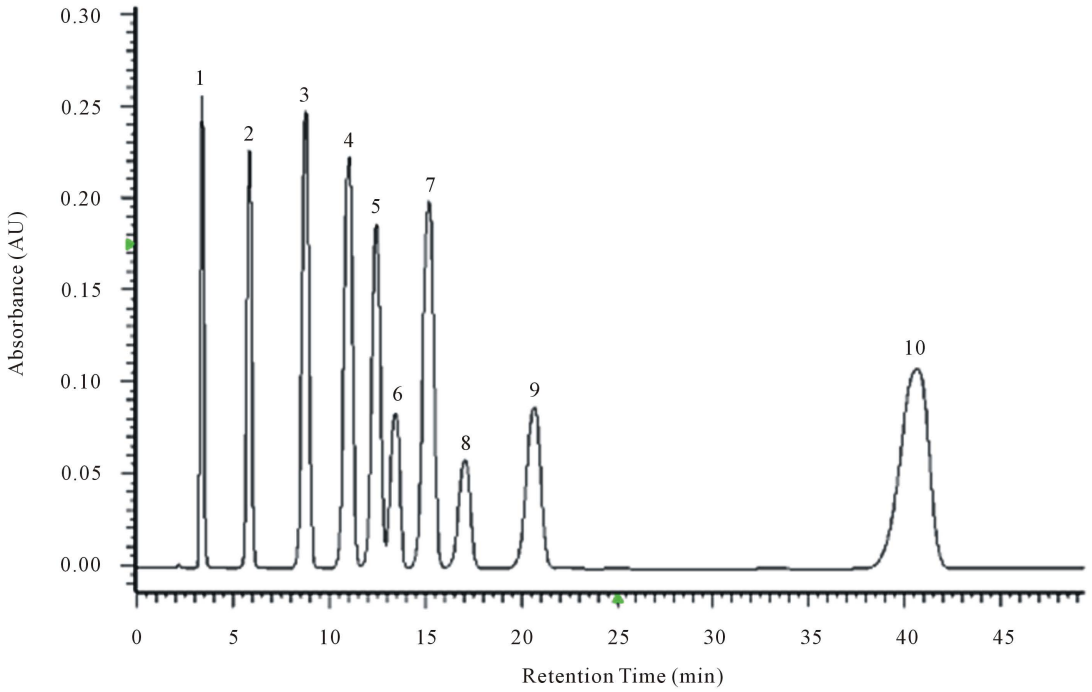

Figure 1. High-performance liquid chromatography profiles for the standard phenolic acids; 1: Gallic acid; 2: Protocatechuic acid; 3: Catechin; 4: Gentisic acid; 5: Chlorogenic acid; 6: Vanillic acid; 7: Syringic acid; 8: Caffeic acid; 9: Epicatechin; 10: Benzoic acid.

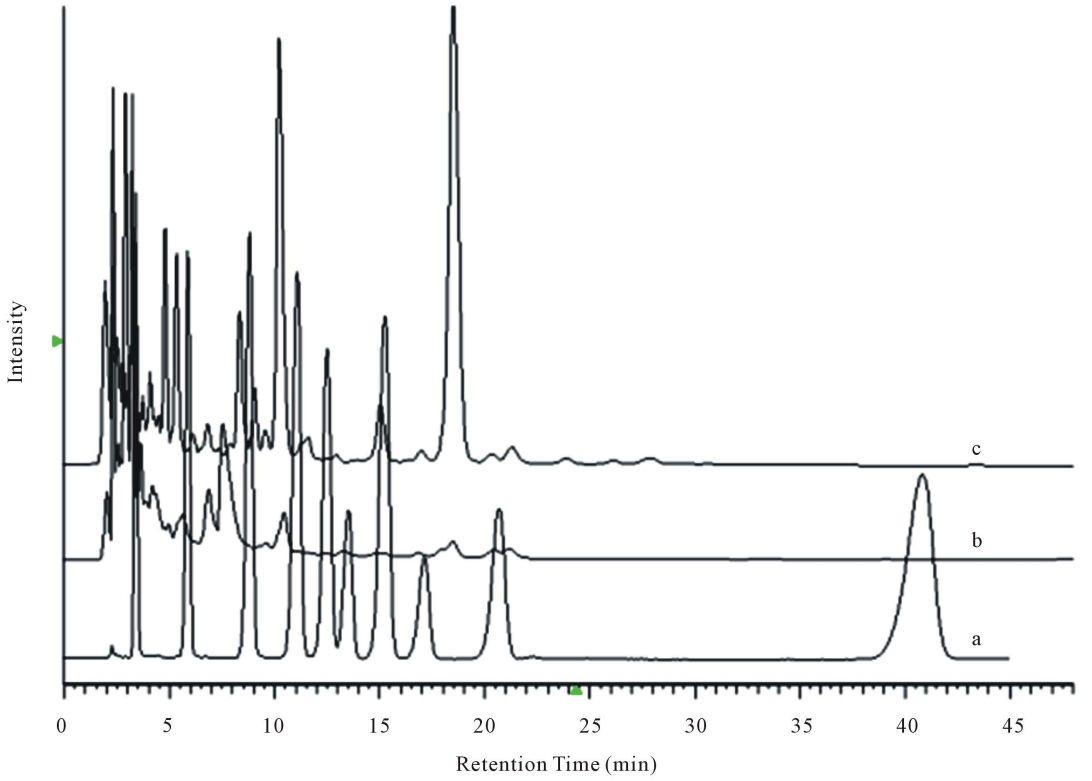

Figure 2. RP-HPLC profiles for the content of phenolic acids for; a: standard phenolic acids; b: cloves; and c: coriander.

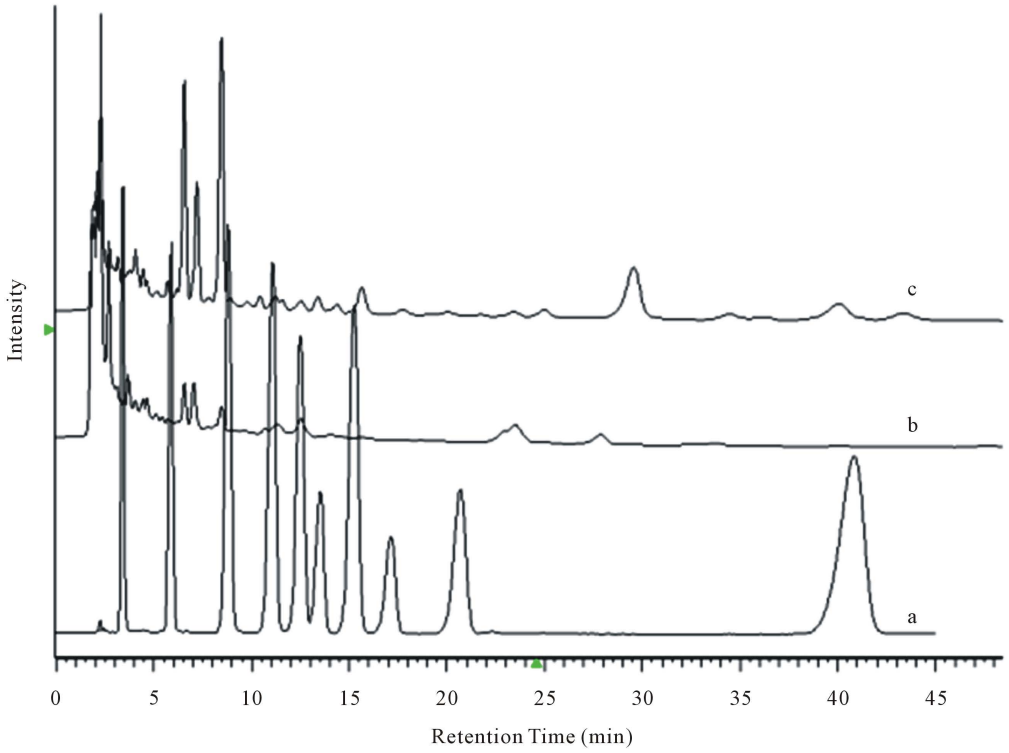

Figure 3. RP-HPLC profiles for the content of phenolic acids for; a: standard phenolic acids; b: cumin; and c: ginger.

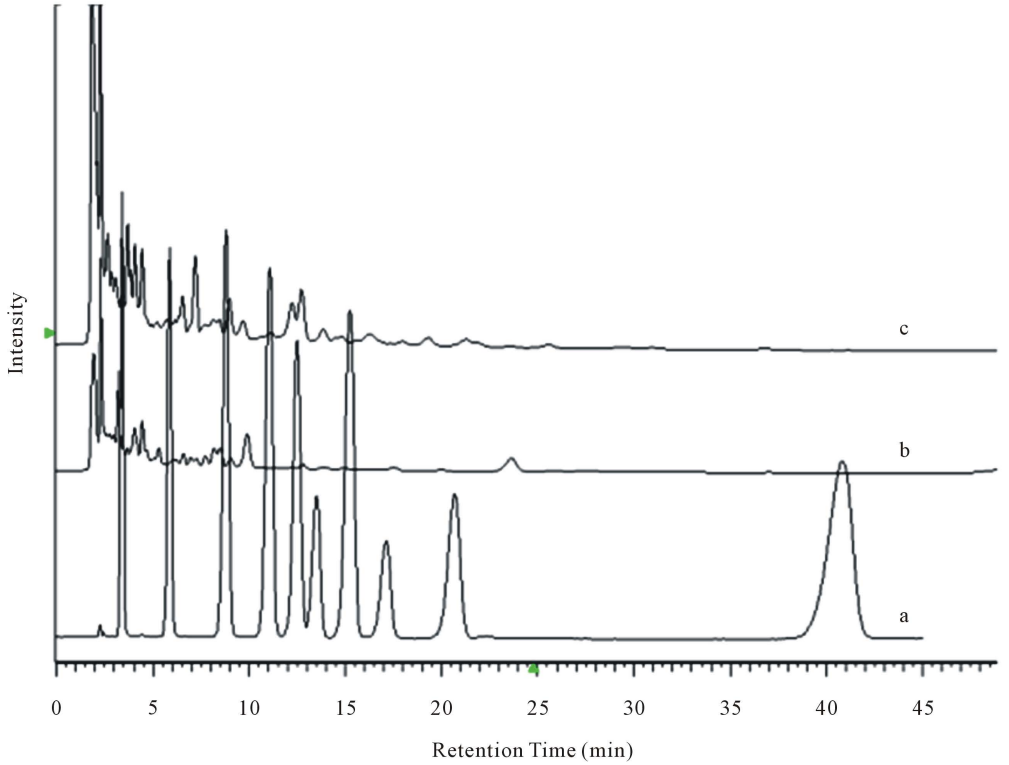

Figure 4. RP-HPLC for the content of phenolic acids for; a: standard phenolic acids; b: green cardamom; and c: turmeric.

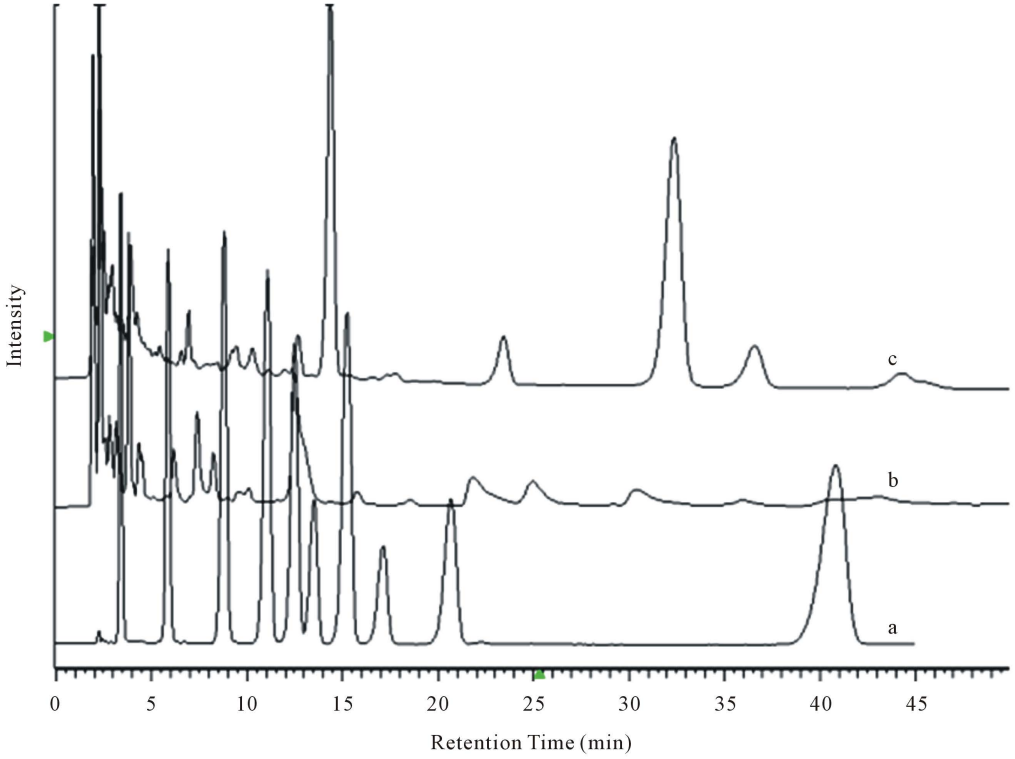

Figure 5. RP-HPLC for the content of phenolic acids for; a: standard phenolic acids; b: sumac; and c: cinnamon.

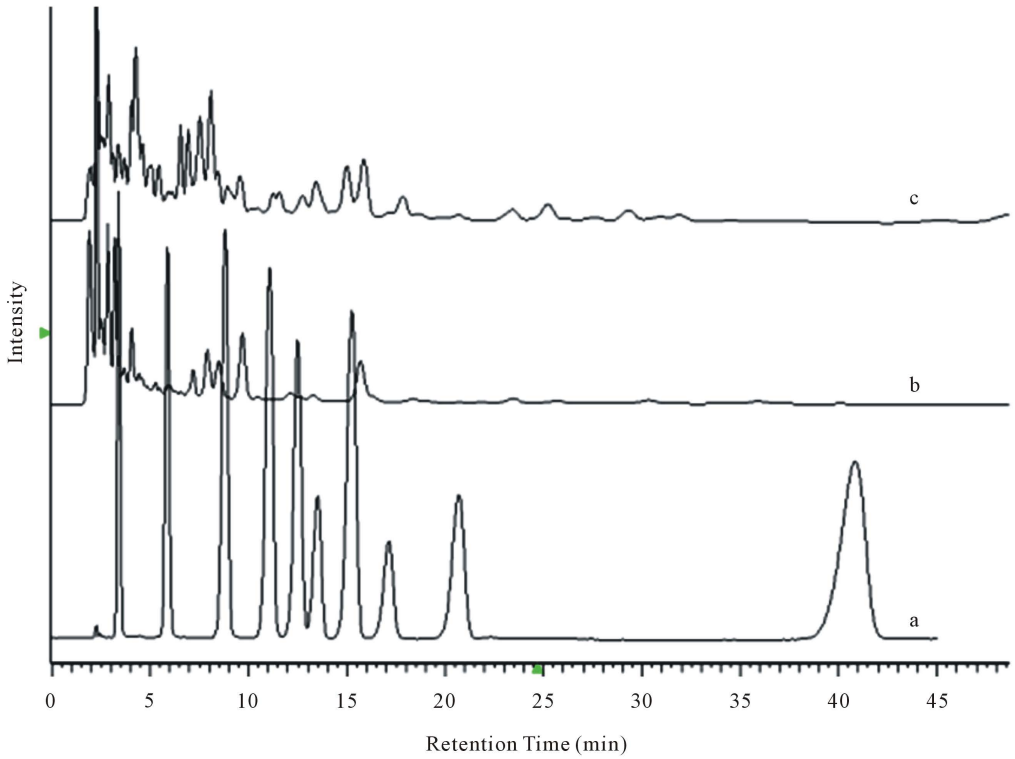

Figure 6. RP-HPLC profiles for the content of phenolic acids for: a: standard phenolic acids; b: sweet cumin; and c: sweet laurel.

Table 2. Phenolic acids identified in spices by RP-HPLC (mg/100g).

Spices	Gallic acid	Protocatechuic acid	Catechin	Gentisic acid	Chlorogenic acid	Vanillic acid	Syringic acid	Caffeic acid	Epicatechin	Benzoic acid
Cloves	1642.3	316.2	31.0	232.4	8.1	83.9	30.9	55.1	41.5	2.7
Coriander	13.3	23.3	47.2	30.3	9.3	237.0	ND	38.6	13.3	ND
Cumin	46.2	5.8	5.2	ND	6.9	14.5	ND	6.4	ND	ND
Ginger	4.4	2.2	8.0	1.2	12.5	20.4	4.2	ND	3.1	ND
Green Cardamom	6.2	ND	4.8	3.6	5.2	ND	ND	ND	ND	ND
Turmeric	2.6	1.9	35.8	5.5	ND	12.1	4.9	ND	ND	1.9
Sumac	76.1	14.7	11.6	3.3	1528.7	21.4	75.0	ND	11.2	30.8
Cinnamon	3.5	3.4	5.7	3.3	9.7	187.1	ND	ND	ND	ND
Sweet Cumin	20.8	27.6	83.4	1.7	44.1	48.3	157.7	ND	ND	1.5
Sweet Laurel	13.8	6.0	5.5	1.7	6.8	36.4	13.0	42.4	ND	ND

ND = not detected.

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References

[1] Lako, J., Trenerry, V., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S. and Premier, R. (2007) Phytochemical Flavonols, Carotenoids and the Antioxidant Properties of a Wide Selection of Fijian Fruit, Vegetables and Other Readily Available Foods. *Food Chemistry*, **102**, 777-783. http://dx.doi.org/10.1016/j.foodchem.2006.01.031

[2] Shahidi, F. and Naczk, M. (2004) Phenolics in Food and Nutraceuticals. CRC Press.

[3] Kähkönen, M., Hopia, A., Vuorela, H., Rauha, J., Pihlaja, K., Kujala, T. and Heinonen, M. (1999) Antioxidant Activity of Plants Extracts Containing Phenolic Compounds. *Journal of Agricultural and Food Chemistry*, **47**, 3954-3962. http://dx.doi.org/10.1021/jf990146l

[4] Ereifej, K.I., Feng, H., Rababah, T.M., Tashtoush, S.H., Al-U'datt, M.H., Al-Rabadi, G.J., Torley, P. and Alkasrawi, M. (2015) Microbiological Status and Nutritional Composition of Spices Used in Food Preparation. *Food and Nutrition Sciences*, **6**, 1134-1140. http://dx.doi.org/10.4236/fns.2015.612118

[5] Pokorny, J., Yanishlieva, N. and Gordon, M. (2001) Antioxidants in Food: Practical Applications. CRC Press, Chapters 1 and 3.

[6] Rabino, I. and Mancinelli, A. (1986) Light, Temperature and Anthocyanin Production. *Journal of Plant Physiology*, **81**, 922-924. http://dx.doi.org/10.1104/pp.81.3.922

[7] Randy, W. (2001) Leaf Pigment and Canopy Photosynthetic Response to Early Flower Removal in Cotton. *Crop Science*, **41**, 1522-1529. http://dx.doi.org/10.2135/cropsci2001.4151522x

[8] SAS (2004) User's Guide. Release 8.2 Edition. SAS Institute Inc., Cary.

[9] Prata, E. and Oliveira, L. (2007) Fresh Coffee Husks as Potential of Anthocyanins. *Journal of Food Science and Technology*, **40**, 1555-1560. http://dx.doi.org/10.1016/j.lwt.2006.10.003

[10] Wang, S. and Ballington, J. (2007) Free Radical Scavenging Capacity and Antioxidant Enzyme Activity in Deerberry (*Vaccinium stamineum* L.). *Journal of Food Science and Technology*, **40**, 1352-1361. http://dx.doi.org/10.1016/j.lwt.2006.09.005

[11] Suhaj, M. (2006) Spice Antioxidants Isolation and Their Antiradical Activity: A Review. *Journal of Food Composition and Analysis*, **19**, 531-537. http://dx.doi.org/10.1016/j.jfca.2004.11.005

[12] Awika, J., Rooney, L. and Waniska, R. (2004) Anthocyanins from Black Sorghum and Their Antioxidant Properties. *Food Chemistry*, **90**, 293-301. http://dx.doi.org/10.1016/j.foodchem.2004.03.058

[13] Rababah, T., Ereifej, K. and Howard, L. (2005) Effect of Ascorbic Acid and Dehydration on Concentrations of Total

Phenolics, Antioxidants Capacity, Anthocyanins and Color in Fruits. *Journal of Agricultural and Food Chemistry*, **53**, 4444-4447. http://dx.doi.org/10.1021/jf0502810

[14] Wu, X., Beecher, G., Holden, J., Haytowitz, D., Gebhardt, S. and Prior, R. (2006) Concentrations of Anthocyanins in Common Foods in the United States and Estimation of Normal Consumption. *Journal of Agricultural and Food Chemistry*, **54**, 4069-4075. http://dx.doi.org/10.1021/jf060300l

[15] Surveswaran, S., Cai, Y., Corke, H. and Sun, M. (2007) Systematic Evaluation of Natural Phenolic Antioxidants from 133 Indian Medicinal Plants. *Journal of Food Chemistry*, **102**, 938-953. http://dx.doi.org/10.1016/j.foodchem.2006.06.033