

Effect of Heat Treatment on Phenolic and Flavonoid Compounds and Antioxidant Activities of Some Egyptian Sweet and Chilli Pepper

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Abstract

Raw and heat-treated (boiled) sweet and hot chilli pepper (*Capsicum* spp.) were evaluated for their contents of phenolic and flavonoid compounds and free radical-scavenging activity by DPPH• assay. Boiling was performed under the Egyptian household conditions. Total phenolic and total flavonoid contents were determined. Twenty six phenolic compounds and eleven flavonoid compounds were fractionated by HPLC. DPPH• assay demonstrated that both fresh and heat-treated pepper samples had a high antioxidant activity which correlated with their high contents of total phenolic and flavonoid compounds. Finally, the resulted data showed that total phenolic and total flavonoid contents were increased as the result of boiling treatment as well as the antioxidant activity.

Keywords: Pepper; *Capsicum*; Boiling; Antioxidant activity; Phenolic compounds; Flavonoid compounds

Introduction

Free radicals may be harmful, leading to inflammation, tissue damage and development of diseases. Free radicals are involved on the pathogenesis of at least 100 different diseases, including cancer, atherosclerosis, rheumatoid arthritis, inflammatory and cataracts [1]. Humans use several lines of defense against free radicals, including endogenous enzymes and proteins as well as dietary antioxidants. The latter are commonly found in fruits and vegetables and therefore their consumption has been associated with protection against several non-communicable diseases [1-3]. Peppers are rich in free radical scavengers, including chlorophylls, carotenoids, phenolics, ascorbic acid (AA), capsaicinoids and tocopherols [4]; however, the free radical scavenging activity of peppers has been mainly attributed to their contents of polyphenols and ascorbic acid [5].

Peppers (*Capsicum* spp.) belong to the *Solanaceae* and provide several essential nutrients. Hot peppers contain phenolics (flavonoids) [6], carotenoids [7], vitamin C, vitamin E [8] and alkaloids [9], which play important roles in human health. In other studies, antioxidant activities in peppers were measured by radical-scavenging activity [10,11], inhibition of lipid peroxidation [12], and metal-chelating activity [13]. Flavonoids have been shown to act as antioxidants, and they possess anti-inflammatory [14], anti-allergic [15], anti-viral [16], and anti-bacterial activities [17].

The aim of this study was to evaluate total phenolic and total flavonoid contents of both ethanol and aqueous pepper extracts for both fresh and heat-treated sweet and hot chilli pepper samples at two ripening stages (green and red), and also determine of the antioxidant activity by DPPH• assay.

Materials and Methods

Chemicals

All the utilized chemical materials (solvents, mineral salts, etc.) were purchased from El Gomhoryia, El Allamyia, El Nasr and Middle East Pharmaceutical Chemical companies, Egypt and the solvents were purified before using. Chemicals, solvents and all standard materials which were used for fractionation and identification by HPLC, purchased from Sigma/Aldrich Chemical Company, USA.

Collection of plant materials and treatment

Fruits of hot chilli pepper (*Capsicum frutescens* var. *sina*) at immature stage (green color) and mature stage (red color) and others

of sweet pepper (*Capsicum annuum* var. *goduion*), green and red, were collected from Vegetable Breeding Research Dept., Horticultural Research Institute, Agricultural Research Center in September until December 2012 season and were identified at the same institute.

Heat treatment

Each of the four samples was washed by using of tap water, seeds were removed and divided into two groups, the first group included the fresh peppers and the other included the pepper samples which were boiled at 100°C in a covered pan (containing water in an approximate ratio of 1:1, w/v fruit to water), for 15 min. After boiling, the boiled samples were drained using a wire mesh strainer. The weight of the boiled tissues and the volume of the boiling water were determined. The boiling water was directly used for all analysis.

Preparation of extracts

Ten g and 20 g from both fresh and boiled peppers extracted with 100 ml ethanol 80% (1:10) and distilled water (1:5), respectively. These extracts were left up to 24 h at room temperature, placed in ultrasonic (BANDELIN SONOREX SUPER RK 514H) for 30 min and filtered through a Whatman paper No. 1. The resultant extracts were used to determine the total phenol and flavonoid content and antioxidant activity.

Determination of total phenolic content

Total phenolic content of each sample was determined using a Folin Ciocalteu assay according to the method of Singleton and Rossi [18] with slight modification. The reaction mixture contained 1 ml of extract and 0.5 ml of the Folin–Ciocalteu reagent, 1 ml sodium carbonate 7.5% and 7.5 ml of distilled water were added, respectively. After 45 min of reaction at ambient temperature, the absorbance at 765

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nm was measured using a UV-visible spectrophotometer (Beckman). A blue color indicated the presence of phenols. A calibration curve was calculated by using of gallic acid standard (0.1 mg/ml). The total phenolic content of samples was determined in triplicates and the results were expressed as dry weight basis (DW) as mg gallic acid equivalents (GAE), per g of each sample.

HPLC analysis of phenolic and aromatic compounds

Phenolic and aromatic compounds were detected by HPLC according to the method of Goupy et al. [19] as follows: the extracts were centrifuged at 10000 rpm (in ICE Micro-MB Centrifuge/NARP 64606 instrument) for 10 min and the supernatant was filtrated through a 0.2 µm Millipore membrane filter, then 1-3 ml were collected in a vial for injection into HPLC Agilent (Series 1200) equipped with auto sampler injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quaternary HP pump (Series 1100). The column [Agilent 5HC-C18 (2) 250 × 4.6 mm] temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. Phenolic acid standards from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area of the tested samples were calibrated against standard solutions of different phenolic and aromatic compounds concentration by the data analysis of HEWLETT Packed (HP) software.

Determination of total flavonoid content

Total flavonoid content was measured by $AlCl_3$ colorimetric assay according to the method of Tacouri et al. [20]. Briefly, 500 µl of extract and 2 ml of distilled water, 150 µl of 5% sodium nitrate were added. After 5 min, 150 µl of 10% $AlCl_3$ was added. A total of 2000 µl of sodium hydroxide (1 M) were added after 1 min and followed by 1200 µl of distilled water. The mixture was incubated for 30 min. The absorbance was measured at 510 nm against a prepared blank. A yellow color indicated the presence of flavonoids. A calibration curve was calculated using quercetin standard (0.1 mg/ml). Total flavonoid content of samples was determined in triplicates and the results were expressed on dry weight basis (DW) as mg quercetin equivalents (QE), per g of each sample.

HPLC analysis of flavonoid compounds

Flavonoid fractions were also identified by HPLC according to the method of Mattila et al. [21] as follows: the extracts were centrifuged at 10000 rpm (in ICE Micro-MB Centrifuge/NARP 64606 instrument) for 10 min and the supernatant was filtrated through a 0.2 µm Millipore membrane filter, then 1-3 ml were collected in a vial for injection into the previous HPLC Agilent (Series 1200) and HP software were used. The ultraviolet (UV) detector was set at 330 nm and the other conditions were set as that previously used in the fractionation of phenolic compounds.

DPPH[•]-scavenging activity assay

Free radical scavenging was determined using the free radical generator DPPH[•] (2, 2- diphenyl-1- picrylhydrazyl) assay based on slight modifications [22]. One ml of each extract was added to 1 ml of 0.002% methanol solution of DPPH[•]. The mixture was thoroughly mixed using BioCote/Stuart vortex instrument and kept in the dark for 30 min. The absorbance, using a spectrophotometer, was measured at 517 nm against a blank of methanol without DPPH[•].

The inhibition percent was calculated as:

$$[A \text{ control} - A \text{ extract}/A \text{ control}] \times 100$$

Statistical analysis

All results were expressed as means ± standard deviation. Statistical Analysis System (SAS) 9.1 software package was used to analysis of data and significant differences between mean values were determined by least significant difference (LSD) test at $P > 0.05$.

Results and Discussion

Polyphenols contents and antioxidant activities

Total phenolic contents (TPC) of ethanolic extracts ranged from 19.21 to 28.43 and 21.81 to 37.64 mg GAE/g DW in fresh and boiled peppers, respectively. Boiling treatment increased the total phenolic contents. These contents were increased also with maturation and chilli peppers had a high content than sweet peppers.

The present results are in agreement with Howard et al. [23] who found that total soluble phenolics ranged from 2656 to 5788 mg/kg FW and total phenols were increased with maturation. In contrast, Ghasemzad et al. [24] reported that phenolic content was decreased with maturity and following ripening. Hassimoto et al. [25] also showed that phenolic contents in green and red sweet peppers were 119 and 131 mg/100 g FW, respectively. Lin and Tang [26] observed that total phenols for green, yellow and red pepper were 206.0, 191.2 and 180.3 mg GAE/100 g FW, respectively. Isabelle et al. [27] reported that total phenolic content for *Capsicum annum* var. grossum, green and red, was 0.59 and 1.70 mg GAE/g FW, respectively, and its content in *Capsicum annum* var. longum, red and green chilli, was 2.77 and 1.07 mg GAE/g FW, respectively. Serrano et al. [28] found that total phenols were 142 mg/100 g FW in sweet pepper. Park et al. [29] reported that TPC ranged from 80.53 in green pepper to 122.82 mg GAE/g in red pepper, with significantly higher values found in the red and orange bell pepper, and significantly lower TPC values found in the green bell pepper. Zhuang et al. [30] also reported that the phenolic contents ranged from 1078.26 µg GAE/g FW for Longline Pepper Green (LPG) to 4992.40 µg GAE/g FW for Fructus Capsici Red (FCR). FCR showed significantly higher phenolic contents than others ($p < 0.05$). Phenolics were accumulated along the developmental stages of pepper fruit, so the fully colored Long-Point Pepper Red (LPPR) and Point Pepper Red (PPR) showed significantly higher phenolic contents than those of green fruits ($p < 0.05$). Tundis et al. [31] showed that in immature stage the content of phenols varied from 781.4 mg chlorogenic acid equivalent/100g FW in Fiesta cultivar to 1207.5 mg chlorogenic acid equivalent/100 g FW in Cayenne Golden cultivar. In mature stage the phenols content ranged from 648.6 mg chlorogenic acid equivalent/100g FW for Acuminatum cultivar to 679.6 mg/100 g FW chlorogenic acid equivalent for Orange Thai cultivar. Aliakbarlu et al. [32] found that TPC in red pepper was 34 mg GAE/g DW. Chen and Kang [33] observed that TPC of red peppers in pericarp, placenta and stalk were ranged from 43.3 to 91.9 mg GAE/g DW. Finally, Nascimento et al. [34] revealed that total phenolic contents of *Capsicum frutescens* whole fruits were 4.9 and 110.6 mg GAE/g DW in hexane and acetonitrile extracts, respectively. On the other hand, Ornelas-Paz et al. [4] found that boiling caused a sequential increase in total phenolic content in all pungent peppers from 1745.9 to 2549.7 µg GAE/g FW. Increases of phenolic content in pungent peppers by cooking ranged from minimal to large (7.4–137%). In this regard, Turkmen et al. [35] found that several cooking methods (including boiling) caused increases (2–26%) in the phenolic content of peppers. Boiling also increased three fold the antioxidant capacity of peppers [36]. Increments of phenolic content of vegetables by cooking have been attributed to dehydration of food matrix and an improved

extractability of phenolics from the food [23,37,38]. Cooking is able to inactivate the polyphenol oxidase enzyme during heating, leading to the inhibition of polyphenolics degradation [39]. These studies are agreed the results in Table 1. In contrast, Ornelas-Paz et al. [40] reported that decreases in the antioxidant activity of cruciferous vegetables during aqua thermal processing were attributed to the losses of water-soluble antioxidants i.e. ascorbic acid and polyphenols [41]. Interestingly, boiling significantly increased the antiradical activity of non-pungent peppers

Total flavonoid contents ranged from 10.28 to 15.52 and 11.63 to 17.62 mg QE/g DW in fresh and boiled peppers, respectively. Similar to total phenolic contents, total flavonoids increased by boiling treatments and with maturation from green to red color. Data are approved by Materska and Perucka [5] who monitored that flavonoids ranged from 16.7 to 40.7 mg/g DW. Lin and Tang [26] also observed that total flavonoids for green, yellow and red pepper were 7.8, 4.1 and 10.4 mg QE/100 g FW, respectively. Ghasemzhad et al. [24] reported that the changes of flavonoids such as quercetin and catechin were depended on the pepper cultivars. The Zorro cultivar showed the highest concentration of quercetin and catechin at both ripening stages. Finally, Tundis et al. [31] showed that the flavonoids content followed the same trend as for the phenols content, decreasing with maturity. In immature stage Fiesta type showed high flavonoids content (115.7 mg QE/100 g FW). Other cultivars showed a content ranged from 93.1 to 110.2 mg QE/100 g FW. As a whole, in mature stage flavonoids content ranged from 34.9 mg QE/100 g FW for Fiesta type to 61.5 mg QE/100 g FW for Orange Thai cultivar. In contrast Perucka and Materska [42] found low flavonoid contents ranged from 81.2 to 91.0 mg QE/100 g DW.

A method commonly used to verify the antioxidant ability consists of measuring the ability of extracts or pure molecules to scavenge DPPH•. Antioxidants are able to reduce the DPPH• from purple color to yellow color upon receiving an electron or a hydrogen radical [43]. The radical scavenging effects and antioxidant activity are also demonstrated in Table 2. All of the samples were able to reduce the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) to the yellow-

colored diphenylpicrylhydrazine, with inhibition values ranged from 81.10 to 91.84% and 92.20 to 93.45% in fresh and boiled peppers, respectively. In contrast, free radical scavenging activity was increased in the immature peppers (green color) than mature (red) and in chilli peppers than sweet.

Total phenolic contents of aqueous extracts ranged from 11.09 to 22.13, 11.20 to 26.14 and 56.40 to 75.40 mg GAE/g DW in fresh, boiled and cooking water, respectively. Similar to ethanolic extracts, boiling caused increment of TPC because of cooking is able to inactivate the polyphenol oxidase enzyme during heating, leading to the inhibition of polyphenolics degradation. TPC of boiled peppers were lower than cooking water due to reach the phenolic compounds from tissues into water during boiling treatment. As the same of ethanolic extracts, in aqueous extracts TPC increased with maturation.

Total flavonoid contents of aqueous extracts ranged from 1.13 to 1.51, 2.70 to 5.00 and 3.04 to 15.26 mg QE/g DW in fresh, boiled and boiling water, respectively. Boiling also caused increment of total flavonoids and boiled peppers had lower contents than cooking water as the same reason of TPC, except boiled sweet peppers.

Inhibition values of the free radical scavenging activity of aqueous pepper extracts on DPPH• ranged from 83.09 to 94.54% in fresh peppers. These values were increased in boiling water than boiled peppers due to increment of total phenolic and flavonoid contents in these extracts.

Phenolic compounds fractionation

Data of HPLC of phenolic and aromatic compounds of fresh peppers are presented in Table 3. They show that pyrogallol, 3-hydroxy tyrosol, chlorogenic, catechol, oleuropein, E-vanillic and benzoic acids were presented at higher levels than other phenolic acids. Also, ethanolic extracts had high contents of phenolic compounds compared with aqueous extracts. In contrast, ellagic acid was not found in all extracts, except aqueous SGC, GGS and ethanolic GRS extracts. The data are approved by Zhuang et al. [30] who reported that the phenolic profiles in nine fresh peppers showed that the content of gallic acid in CPG (Creasing pepper green) was the highest. The content of

Component	Samples	SGC	SRC	GGS	GRS
Total phenolic contents (mg GAE/g DW)	Fresh	19.21 ± 0.41 ^f	28.43 ± 0.31 ^b	20.52 ± 0.02 ^{ef}	24.48 ± 2.14 ^c
	Boiled	28.68 ± 0.88 ^b	37.64 ± 0.29 ^a	21.81 ± 0.20 ^{de}	24.61 ± 1.92 ^c
Total flavonoid contents (mg QE/g DW)	Fresh	13.62 ± 0.71 ^c	15.55 ± 0.21 ^a	10.28 ± 0.15 ^e	15.52 ± 0.37 ^a
	Boiled	14.42 ± 0.09 ^b	17.62 ± 0.18 ^a	11.63 ± 0.45 ^d	15.71 ± 0.26 ^a
DPPH•-scavenging activities (%)	Fresh	91.84 ± 0.18 ^c	81.10 ± 0.85 ^h	89.52 ± 0.06 ^d	82.55 ± 0.07 ^g
	Boiled	93.45 ± 0.50 ^b	84.80 ± 0.42 ^f	95.50 ± 0.08 ^a	92.20 ± 0.14 ^e

Each value represents the mean ± Standard Deviation; the mean values with different letters within a specific row indicate significant differences (P<0.05). SGC=Sina green chilli, SRC=Sina red chilli, GGS=Godiun green sweet, GRS=Godiun red sweet.

Table 1: Total phenolic and total flavonoid contents and antioxidant activities of ethanolic extracts.

Component	Samples	SGC	SRC	GGS	GRS
Total phenolic contents (mg GAE/g DW)	Fresh	11.09 ± 0.96 ^h	13.96 ± 3.07 ^g	13.78 ± 5.27 ^g	22.13 ± 0.05 ^{de}
	Boiled	11.20 ± 0.25 ^b	16.12 ± 0.05 ^f	20.92 ± 0.65 ^e	26.14 ± 0.78 ^d
	B.W.	68.81 ± 1.08 ^b	75.40 ± 6.14 ^a	56.40 ± 1.57 ^c	72.36 ± 1.26 ^{ab}
Total flavonoid contents (mg QE/g DW)	Fresh	1.13 ± 0.02 ^h	1.34 ± 0.09 ^b	1.48 ± 0.10 ^h	1.51 ± 0.04 ^h
	Boiled	2.70 ± 0.03 ^g	4.10 ± 0.01 ^{de}	3.59 ± 0.10 ^{efg}	5.00 ± 0.19 ^c
	B.W.	11.40 ± 0.32 ^b	15.26 ± 1.93 ^a	3.04 ± 0.16 ^{fg}	4.60 ± 0.04 ^{de}
DPPH•-scavenging activities (%)	Fresh	91.00 ± 0.71 ^d	92.05 ± 0.21 ^c	83.09 ± 0.05 ^f	94.54 ± 0.30 ^a
	Boiled	86.20 ± 0.28 ^e	74.68 ± 0.92 ⁱ	78.30 ± 0.57 ^g	77.10 ± 0.57 ^h
	B.W.	93.00 ± 0.28 ^b	94.45 ± 0.21 ^a	92.71 ± 0.05 ^{bc}	94.43 ± 0.40 ^a

SGC=Sina green chilli, SRC=Sina red chilli, GGS=Godiun green sweet, GRS=Godiun red sweet, B.W.=Boiling water resulted from boiling tea.

Table 2: Total phenolic, total flavonoid contents and antioxidant activities of aqueous extracts.

3,4-dihydroxybenzoic acid in FCR was higher than that in other peppers. The contents of catechin showed no significant difference ($p>0.05$) in all peppers, except FCR. LPPR had significantly higher vanillin content than other peppers. The content of benzoic acid in LPPR was 176.76 $\mu\text{g/g}$ FW, and was significantly higher than that in others peppers ($p<0.05$). The red peppers had significantly higher salicylic acid contents than the green peppers ($p<0.05$). The content of luteolin in the peppers was similar to that of catechin. FCR had the highest content, showing 0.84 $\mu\text{g/g}$ FW, and the other peppers had no significant difference ($p>0.05$).

HPLC of phenolic and aromatic compounds of boiled pepper extracts are presented in Table 4. The data mentioned that some extracts decreased in their contents of phenolics, some not contained and others increased in their contents. So that boiling treatment caused loss of the contents of phenolic acid fractions in some extracts. Finally, HPLC of phenolic and aromatic compounds of boiling water are also observed in Table 4. The data monitored that the phenolic fractions were decreased in all boiling water.

Flavonoid fractionation

HPLC of flavonoid compounds of both ethanolic and aqueous extracts of fresh peppers are observed in Table 5. Data show that naringin, rutin, hisperdin, rosmarinic and quercetrin were presented at higher levels than other flavonoids. Also, ethanol extracts had high contents of flavonoid profiles compared with aqueous extracts.

HPLC of flavonoid compounds of both extracts of boiled pepper samples are revealed in Table 6. Data show that naringin was increased in all extracts, except ethanol SGC and aqueous SRC extracts. Rutin was increased in all extracts, except ethanol SGC and aqueous SRC extracts. Quercetrin was increased in all extracts, except ethanol red pepper extracts. Quercetin, naringenin and keampferol were increased in all extracts, except ethanol SGC and aqueous SRC extracts. Hisperdin was increased in all extracts, except ethanol chilli pepper extracts. Apeginin was decreased only in ethanol and aqueous SGC and aqueous SRC extract. Finally, 7-hydroxy flavone was decreased only in ethanol SGC, aqueous SRC and GRS extracts. In contrast, hisperdin was decreased in all extracts, except aqueous SGC and GGS extracts. Also, rosmarinic was decreased in all extracts, except aqueous SGC, ethanol and aqueous GGS extracts.

HPLC of flavonoid compounds of boiling water resulted from boiling treatment are also shown in Table 6 and investigated that these water had the lowest contents of flavonoid fractions.

Conclusion

This study demonstrated that Egyptian sweet and chilli peppers, at two ripening stages (green and red), are good sources of phenolic and flavonoid compounds. Both fresh and heat-treated peppers exhibited a good free radical scavenging activity on the DPPH• and also they contained high contents of total phenolic and total flavonoid compounds in both ethanol and aqueous extracts.

Phenolic fractions	SGC		SRC		GGS		GRS	
	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous
Gallic	0.61	0.24	0.87	0.31	0.38	0.11	0.71	0.69
Pyrogallol	11.66	11.87	65.33	43.55	13.98	6.22	12.16	35.99
4-amino benzoic	0.32	0.38	0.73	0.21	0.23	0.09	0.33	0.84
3-hydroxy tyrosol	9.51	5.58	18.21	7.87	3.01	1.01	9.25	4.01
Protocatechoic	0.47	0.37	1.07	0.68	0.37	0.12	0.82	0.39
Catechin	0.70	0.26	0.72	0.49	0.30	0.14	2.69	0.98
Chlorogenic	13.96	21.51	29.28	49.28	12.24	9.86	30.91	51.13
Catechol	21.75	5.12	8.98	6.07	9.05	2.25	13.40	8.09
Epicatechin	1.03	1.71	6.88	5.81	1.60	0.29	2.06	1.43
Caffeine	0.11	0.16	4.08	0.38	0.15	0.08	0.17	0.50
P-hydroxy benzoic	5.87	1.95	11.45	4.79	1.70	0.49	6.74	4.35
Caffeic	1.60	0.22	5.39	0.36	0.16	0.10	0.59	0.59
Vanillic	5.10	0.47	3.60	7.24	0.63	0.67	4.61	2.31
Ferulic	1.59	0.12	7.28	1.82	0.93	0.07	ND	0.83
Isoferulic	1.43	0.07	1.15	0.39	0.32	0.01	0.58	0.45
Reverstrol	1.56	0.04	0.75	0.29	0.13	0.03	0.69	0.10
Oleuropein	17.53	0.24	12.72	2.18	1.49	0.46	3.89	5.68
Ellagic	ND	0.48	ND	ND	ND	0.14	0.44	ND
E-vanillic	90.49	1.46	44.57	8.01	24.61	1.09	13.51	6.02
α -coumaric	1.92	0.05	ND	0.08	0.07	0.01	0.04	0.06
Benzoic	65.52	2.98	94.82	8.11	32.32	3.49	43.30	8.27
3,4,5-methoxy cinnamic	0.22	0.02	0.20	0.08	0.08	0.02	0.18	0.19
Coumarin	0.84	0.10	1.00	0.37	0.32	0.18	0.34	0.13
Salicylic	11.87	0.40	10.79	0.86	2.70	0.07	0.60	0.57
P-coumaric	1.98	0.06	1.51	0.24	0.12	0.04	0.11	0.28
Cinnamic	7.86	0.46	1.53	1.00	1.22	0.37	0.99	0.79

SGC=Sina green chilli, SRC=Sina red chilli, GGS=Godiuon green sweet, GRS=Godiuon red sweet.

Table 3: HPLC of phenolic and aromatic compounds of both ethanolic and aqueous extracts of fresh peppers.

Phenolic fractions	SGC			SRC			GGS			GRS		
	Ethanolic	Aqueous	B.W.	Ethanolic	Aqueous	B.W.	Ethanolic	Aqueous	B.W.	Ethanolic	Aqueous	B.W.
Gallic	0.29	0.49	0.20	0.34	0.22	0.05	0.38	1.08	0.01	0.31	1.45	0.14
Pyrogallol	17.25	28.57	1.72	29.16	21.28	1.02	15.74	43.49	1.11	36.82	39.82	2.71
4-amino benzoic	0.70	1.38	0.11	0.15	0.22	0.01	0.18	0.61	0.01	0.51	0.98	0.21
3-hydroxy tyrosol	4.97	7.79	ND	5.18	4.84	0.18	1.80	3.42	ND	8.13	2.09	0.01
Protocatechoic	0.73	5.99	0.28	0.46	1.44	0.06	0.23	2.38	0.04	1.49	1.46	0.10
Catechin	0.59	0.67	0.25	0.37	0.59	0.06	0.18	1.40	0.06	0.42	0.68	0.17
Chlorogenic	7.82	1.70	0.44	17.22	3.03	0.59	11.43	5.29	0.10	21.77	6.12	0.71
Catechol	2.43	10.29	ND	3.64	3.48	0.34	1.75	6.77	0.04	2.41	3.75	NF
Epicatechin	1.23	0.81	0.03	1.79	0.97	0.04	1.35	0.74	0.06	2.42	0.62	0.20
Caffeine	0.33	0.70	0.07	2.27	0.35	0.06	0.12	0.15	0.02	0.19	0.36	0.07
P-hydroxy benzoic	2.06	1.67	0.14	10.56	4.20	0.16	2.38	3.07	0.31	5.55	3.97	0.51
Caffeic	0.10	1.19	0.17	0.65	1.00	0.06	0.23	0.07	0.02	0.49	0.15	0.10
Vanillic	0.32	1.64	0.02	0.92	0.40	0.01	0.14	0.34	0.004	1.04	0.89	0.01
Ferulic	3.30	0.51	0.07	0.68	1.27	0.07	0.05	0.06	0.01	0.18	0.27	0.10
Isoferulic	0.12	0.27	0.02	0.26	0.22	0.04	0.04	0.15	0.01	0.46	0.30	0.02
Reverstrol	0.25	0.74	0.02	0.12	0.19	0.03	0.04	0.07	0.01	0.10	0.32	0.02
Oleuropein	0.59	ND	1.83	5.01	ND	0.97	0.34	ND	ND	2.19	11.70	0.29
Ellagic	ND	7.54	ND	ND	ND	0.17	ND	7.76	ND	ND	ND	0.10
E-vanillic	10.36	8.61	0.86	39.48	14.06	1.27	1.58	ND	0.02	3.91	3.92	0.11
α -coumaric	0.02	ND	0.01	0.14	0.03	0.01	0.01	ND	0.02	0.06	0.04	0.01
Benzoic	57.01	17.94	0.36	55.51	4.31	ND	14.41	12.49	0.12	32.03	7.46	NF
3,4,5-M. cinnamic	0.02	ND	0.01	0.14	0.04	0.01	0.03	0.01	0.01	0.09	0.13	0.01
Coumarin	0.05	0.16	0.04	0.52	0.09	0.02	0.04	ND	0.01	0.13	0.04	0.01
Salicylic	0.11	0.84	ND	1.20	0.63	0.10	0.12	0.05	0.02	0.58	0.93	0.15
P-coumaric	0.04	0.04	0.03	0.15	0.06	0.01	0.01	0.02	ND	0.11	0.03	0.01
Cinnamic	0.40	0.78	0.12	1.32	0.30	0.03	0.15	0.22	ND	0.51	0.60	0.03

SGC=Sina green chilli, SRC=Sina red chilli, GGS=Godioun green sweet, GRS=Godioun red sweet, B.W.=Boiling Water, ND=Not Detected.

Table 4: HPLC of phenolic and aromatic compounds of both ethanol and aqueous extracts of boiled peppers and boiling water.

Flavonoid fractions	SGC		SRC		GGS		GRS	
	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous
Naringin	12.71	1.67	2.01	3.53	1.43	0.15	2.44	0.56
Rutin	22.21	0.79	10.78	2.19	3.13	0.36	2.82	5.94
Hesperdin	151.69	9.88	46.09	15.57	32.63	4.95	21.97	11.10
Rosmarinic	11.95	0.40	2.23	2.55	1.77	0.63	2.35	0.98
Quercetrin	36.21	1.73	51.61	5.07	17.37	1.50	25.09	3.43
Quercitin	1.58	0.13	0.99	0.41	0.03	0.04	0.08	0.03
Narenginin	0.47	0.05	0.13	0.29	0.03	0.02	0.05	0.03
Keampferol	0.51	0.27	1.49	1.08	0.12	0.04	0.21	0.14
Hispertin	4.99	0.18	1.88	0.52	0.11	0.05	0.18	0.04
Apegenin	0.51	0.09	0.34	0.57	0.04	0.03	0.05	0.04
7-hydroxy flavone	1.43	0.19	0.62	1.35	0.07	0.06	0.13	0.19

Table 5: HPLC of flavonoid compounds of both ethanolic and aqueous extracts of fresh peppers.

Flavonoid fractions	SGC			SRC			GGS			GRS		
	Ethanolic	Aqueous	B.W.	Ethanolic	Aqueous	B.W.	Ethanolic	Aqueous	B.W.	Ethanolic	Aqueous	B.W.
Naringin	1.62	0.52	0.06	2.40	0.46	0.11	0.91	0.340	0.010	3.40	0.32	0.02
Rutin	0.93	1.34	0.07	5.73	2.24	0.71	0.69	0.700	0.010	1.05	2.98	0.02
Hesperdin	7.08	7.98	0.06	6.13	5.62	0.05	7.88	8.230	0.002	15.92	7.82	0.04
Rosmarinic	2.26	4.65	0.5	1.88	1.01	0.31	0.86	0.150	ND	3.09	0.40	0.06
Quercetrin	40.99	9.74	ND	36.26	2.36	0.17	9.73	7.910	0.002	21.02	4.41	0.03
Quercitin	0.01	0.13	0.01	1.06	0.13	0.10	0.08	0.010	0.006	0.07	0.13	0.01
Narenginin	0.01	0.01	0.01	0.41	0.05	0.05	0.14	0.004	0.001	0.17	0.01	0.01
Keampferol	0.06	0.06	0.03	0.67	0.06	ND	0.13	0.010	0.012	0.09	0.03	ND
Hispertin	0.12	0.15	0.04	1.21	0.14	0.10	0.09	0.030	0.001	0.18	0.02	0.01
Apegenin	0.04	0.07	0.01	0.38	0.02	0.01	0.16	0.010	0.006	0.07	0.03	0.01
7-hydroxy flavone	0.45	0.04	0.02	1.62	0.04	0.02	0.08	0.140	0.020	0.26	0.05	0.02

Table 6: HPLC of flavonoid compounds of ethanolic and aqueous extracts of boiled peppers and boiling water.

References

1. Yahia EM, Ornelas-Paz J De J (2010) Chemistry, stability and biological actions of carotenoids. In LA De la Rosa, Alvarez-Parrilla E, González-Aguilar GA (eds.), *Fruit and Vegetable Phytochemicals: Chemistry, Nutritional Value and Stability*, Iowa: Blackwell Publishing: 177-222.
2. Davey MW, Van Montagu M, Inzé D, Sanmartin M, Kanellis A, et al. (2000) Plant L-ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. *J Sci Food Agric* 80: 825-860.
3. Harrison FE, May JM (2009) Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2. *Free Radic Biol Med* 46: 719-730.
4. Ornelas-Paz J, Martínez-Burrola MJ, Ruiz-Cruz S, Santana-Rodríguez V, Ibarra-Junquera V, et al. (2010) Effect of cooking on the capsaicinoids and phenolics contents of Mexican peppers. *Food Chem* 119: 1619-1625.
5. Materska M, Perucka I (2005) Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annum* L.). *J Agric Food Chem* 53: 1750-1756.
6. Bae H, Jayaprakasha GK, Jifon J, Patil BS (2012) Variation of antioxidant activity and the levels of bioactive compounds in lipophilic and hydrophilic extracts from hot pepper (*Capsicum* spp.) cultivars. *Food Chem* 134: 1912-1918.
7. Ha SH, Kim JB, Park JS, Lee SW, Cho KJ (2007) A comparison of the carotenoid accumulation in *Capsicum* varieties that show different ripening colours: deletion of the capsanthin-capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. *J Exp Bot* 58: 3135-3144.
8. García-Closas R, Berenguer A, José Tormo M, José Sánchez M, Quirós JR, et al. (2004) Dietary sources of vitamin C, vitamin E and specific carotenoids in Spain. *Br J Nutr* 91: 1005-1011.
9. Srinivas Ch, Sai Pavan Kumar ChN, China Raju B, Jayathirtha Rao V, Naidu VG, et al. (2009) First stereoselective total synthesis and anticancer activity of new amide alkaloids of roots of pepper. *Bioorg Med Chem Lett* 19: 5915-5918.
10. Conforti F, Statti AG, Menichini F (2007) Chemical and biological variability of hot pepper fruits (*Capsicum annum* var. *acuminatum* L.) in relation to maturity stage. *Food Chem* 102: 1096-1104.
11. Oboh G, Ademiluyi AO, Faloye YM (2011) Effect of combination on the antioxidant and inhibitory properties of tropical pepper varieties against α -amylase and α -glucosidase activities in vitro. *J Med Food* 14: 1152-1158.
12. Menichini F, Tundis R, Bonesi M, Loizzo RM, Conforti F, et al. (2009) The influence of fruit ripening on the phytochemical content and biological activity of *Capsicum chinense* Jacq. CV Habanero. *Food Chem* 114: 553-560.
13. Ciz M, Cizova H, Denev P, Kratchanova M, Slavov A, et al. (2010) Different methods for control and comparison of the antioxidant properties of vegetables. *Food Cont* 21: 518-523.
14. Loke WM, Proudfoot JM, Stewart S, McKinley AJ, Needs PW, et al. (2008) Metabolic transformation has a profound effect on anti-inflammatory activity of flavonoids such as quercetin: Lack of association between antioxidant and lipoxygenase inhibitory activity. *Biochem Pharmacol* 75: 1045-1053.
15. Seelinger G, Merfort I, Wölfl U, Schempp CM (2008) Anti-carcinogenic effects of the flavonoid luteolin. *Molecules* 13: 2628-2651.
16. Liu RH (2003) Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr* 78: 517S-520S.
17. Hong H, Landauer MR, Foriska MA, Ledney GD (2006) Antibacterial activity of the soy isoflavone genistein. *J Basic Microbiol* 46: 329-335.
18. Singleton VL, Rossi JAJ (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Viticult* 16: 144-158.
19. Goupy P, Hugues M, Biovin P, Amiot MJ (1999) Antioxidant composition and activity of barely (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. *J Sci Food Agric* 79: 1625-1634.
20. Tacouri DD, Ramful-Baboolall D, Puchooa D (2013) In vitro bioactivity and phytochemical screening of selected spices used in Mauritian foods. *Asian Pacific J Tropical Disease* 3: 253-261.
21. Mattila P, Astola J, Kumpulainen J (2000) Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. *J Agric Food Chem* 48: 5834-5841.
22. Blois MS (1958) Antioxidant determinations by the use of a stable free radical. *Nature* 181: 1199-1200.
23. Howard LR, Talcott ST, Brenes CH, Villalon B (2000) Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *J Agric Food Chem* 48: 1713-1720.
24. Ghasemnezhad M, Sherafati M, Payvast GA (2011) Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annum*) fruits at two different harvest times. *J Fun Foods* 3: 44-49.
25. Hassimotto NM, Genovese MI, Lajolo FM (2005) Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *J Agric Food Chem* 53: 2928-2935.
26. Lin YJ, Tang YC (2007) Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem* 101: 140-147.
27. Isabelle M, Lee LB, Lim TM, Koh W, Huang D, et al. (2010) Antioxidant activity and profiles of common vegetables in Singapore. *Food Chem* 120: 993-1003.
28. Serrano M, Zapata JP, Castillo S, Guillén F, Martínez-Romero D, et al. (2010) Antioxidant and nutritive constituents during non-pungent pepper development and ripening are enhanced by nitrophenolate treatments. *Food Chem* 118: 497-503.
29. Park J, Jeon G, Kim J, Park E (2012) Antioxidant activity and antiproliferative action of methanol extracts of 4 different colored bell peppers (*Capsicum annum* L.). *Food Sci Biotechnol* 21: 543-550.
30. Zhuang Y, Chen L, Sun L, Cao J (2012) Bioactive characteristics and antioxidant activities of nine peppers. *J Functional Foods* 4: 331-338.
31. Tundis R, Menichini F, Bonesi M, Conforti F, Statti G, et al. (2013) Antioxidant and hypoglycaemic activities and their relationship to phytochemicals in *Capsicum annum* cultivars during fruit development. *Food Sci Technol* 53: 370-377.
32. Aliakbarlu J, Mohammad S, Khalili S (2014) A study on antioxidant potency and antibacterial activity of water extracts of some spices widely consumed in Iranian diet. *J Food Biochem* 38: 159-166.
33. Chen L, Kang Y (2014) In vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of red pepper (*Capsicum annum* L.) including pericarp, placenta and stalk. *J Food Biochem* 38: 300-306.
34. Nascimento LAP, Nascimento CEST, Ramos SMN, Silva RG, Gomes EGJ, et al. (2014) Quantification, antioxidant and antimicrobial activity of phenolics isolated from different extracts of *Capsicum frutescens* (pimenta malagueta). *Molecules* 19: 5434-5447.
35. Turkmen N, Sari F, Velioglu YS (2005) The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chem* 93: 713-718.
36. Shobana S, Naidu KA (2000) Antioxidant activity of selected Indian spices. *Prostaglandins Leukot Essent Fatty Acids* 62: 107-110.
37. Huffman VL, Schadle ER, Villalon B, Burns EE (1978) Volatile components and pungency in fresh and processed jalapeno peppers. *J Food Sci* 43: 1809-1811.
38. Schweiggert U, Schieber A, Carle R (2006) Effects of blanching and storage on capsaicinoid stability and peroxidase activity of hot chili peppers (*Capsicum frutescens* L.). *Innovative Food Sci Emerging Technol* 7: 217-224.
39. Chuah MA, Lee Y, Yamaguchi T, Takamura H, Yin L, et al. (2008) Effect of cooking on the antioxidant properties of coloured peppers. *Food Chem* 111: 20-28.
40. Ornelas-Paz J, Cira-Chávez AL, Gardea-Béjar AA, Guevara-Arauz CJ, Sepúlveda RD, et al. (2013) Effect of heat treatment on the content of some bioactive compounds and free radical-scavenging activity in pungent and non-pungent peppers. *Food Res Intl* 50: 519-525.
41. Sikora E, Cieslik E, Leszczynska T, Filipiak-Florkiewicz F, Pisulewski PM (2008) The antioxidant activity of selected cruciferous vegetables subjected to aquathermal processing. *Food Chem* 107: 55-59.
42. Perucka I, Materska M (2003) Antioxidant activity and content of capsaicinoids isolated from paprika fruits. *Polish J Food Nutri Sci* 12: 15-18.
43. Bamoniri A, Ebrahimabadi AH, Mazoochi A, Behpour M, Kashi FJ, et al. (2010) Antioxidant and antimicrobial activity evaluation and essential oil analysis of *Semenovia*. *Food Chem* 122: 553-558.