Variability in antioxidant activity and phenolic profile in different parts of sunflower (*Helianthus annuus* L.) genotypes

Asaad Aghaei[1], Reza Darvishzadeh[2,3*], Ali Asghar Hatamnia[4], Farhad Ghavami[5]

[1]Department of Biology, Faculty of Science, Urmia University, Urmia, Iran.
[2]Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Urmia University, Urmia, Iran.
[3]Institute of Biotechnology, Urmia University, Urmia, Iran.
[4]Department of Biology, Faculty of Science, Ilam University, Ilam, Iran.
[5]Chief Science Officer (CSO) Eurofins BioDiagnostics Inc, River Falls, Wisconsin, USA.
[*]Corresponding author, E-mail: r.darvishzadeh@urmia.ac.ir, Tel: 04432779558.

Abstract
Sunflower (*Helianthus annuus* L.) is largely consumed in Iran as an oleaginous and confectionery product. In this study, the total phenolic and flavonoid contents, antioxidant activity and phenolic composition in different parts of the seeds from six sunflower genotypes were investigated. The antioxidant activity of extract was evaluated by different assays. The total phenolic and flavonoid contents in kernel were 6.8 and 4.1 times higher than those in shell. Antioxidant activity of kernel extract was significantly higher than shell. It can be attributed to higher total phenolic and flavonoid content. In kernel extracts, a positive correlation coefficient was observed between total phenolic content and FRAP (r = 0.636) and Nitric oxide radical inhibition (r= 0. 721) assays. The amount of identified phenolic compounds varied in different extracts and ranged from 0.29 µg/g (Vanillic acid, shell of S5 genotype) to 433.6 µg/g (Syringic acid, kernel of S2 genotype). Therefore, the result indicated that kernel sunflower seeds can be used as potent natural antioxidants in diet.

Key words: Antioxidant activity, Kernel, Phenolic composition, Shell, Sunflower.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most ancient crop species in North America. It belongs to the family Compositae (Asteraceae) and the genus *Helianthus*. Nowadays, two main types of sunflower are grown: oil seed and non-oilseed or confectionery types (Gonzalez-Perez and Vereijken, 2007; Salunkhe *et al.*, 1992). Confectionery sunflower produces large seeds with low oil and high protein content and is used for baking and as snack (Lu and Hoeft, 2009). Confectionery kernels are roasted and salted (or not salted), and marketed as snacks. Confectionery type is one of the most popular and important crops in Iran which is cultivated in all parts of the country, especially in northwest regions.

Reactive oxygen species (ROS) that are produced in living organisms generate oxidative stress by oxidizing the bio-molecules in our body, such as nucleic acids, proteins and lipids (Harborne and Williams, 2000; Heim *et al.*, 2002; Shahidi *et al.*, 1992). Oxidative stress leads to different diseases including cardiovascular disease, cancer, aging, and cataracts. The antioxidants are important substances because of their inhibition activity against excessive free radical accumulation in cells. Since antioxidants are effective elements in preventing oxidative stress, widespread attention has been focused on the importance of natural antioxidants and their utilization in food (Kourie, 1998; Temple, 2000). Among antioxidant materials, phenolic compounds as bioactive substances extensively present in plants are important in human diet (Hatamnia *et al.*, 2014; Kornsteiner *et al.*, 2006).

Butylated-hydroxyl anisole (BHA) and butylated-hydroxyl toluene (BHT) have been used as synthetic

antioxidants since the beginning of this century. Nowadays, using these compounds is not recommended as they may increase carcinogenesis effects. Therefore, special attention is paid to find safe sources such as herbal antioxidants as a replacement (Hatamnia *et al.*, 2014; Hatamnia *et al.*, 2016a; Mahdavi and Salunkhe, 1995).

Among dietary fruits and vegetables containing natural antioxidants, sunflower seed is an important source for high level of antioxidants (Halvorsen *et al.*, 2002; Velioglu *et al.*, 1998). However, the amount of phenolic compounds in different parts of the seed is different from small percentage (0.7- 5.4% of total phenolics) in shell to higher percentage in kernel (Pedrosa *et al.*, 2000).

Due to the high consumption of sunflower seeds as sources for oil production and confectionery use, the aims of this study were: (1) to estimate and compare the total phenolic and flavonoid contents as well as antioxidant activity in different parts of seed (kernel and shell), (2) to estimate phenolic composition by HPLC (high performance liquid chromatography), and (3) to determine the correlation between total phenolic content, flavonoid content and different assays of antioxidant activity in six sunflower genotypes.

MATERIALS AND METHODS

Plant materials

Seeds of two oily sunflower genotypes and four confectionery sunflower genotypes from different regions were kindly provided by The Seed and Plant Improvement Institute (SPII), Karaj, and Department of Plant Breeding and Biotechnology, Urmia University, Urmia, respectively (Table 1). Seeds were planted in round pots (30 cm in diameter, 26 cm deep) filled with a mixture of one part sand and one part top soil to about 1 cm from the top of the pots. Each genotype was planted in 15 pots, each pot representing a replication. Pots were arranged in a completely randomized design with a spacing of 60 × 40 cm outside the greenhouse in an open-air area under natural environmental conditions. Irrigation was done using a drip irrigation system. The pots were watered on a daily basis until the plants reached maturity. Water-soluble fertilizer (20-20-20: N-P-K) was applied twice a week until flowering. The grains were harvested at physiological maturity stage.

Preparation of methanolic extracts

Seed parts, shell and kernel, were separated and air dried in shade and then ground to a fine powder. The fine powder (2g) was extracted with pure methanol (50 ml) in a soxhlet apparatus at 60°C for 30 min (Wijeratne *et al.*, 2006). The supernatant was filtered through filter paper and kept at 4°C.

Determination of total phenolic content

Total phenolic content was determined for the extracts using the Folin-Ciocalteu colorimetric method (Singleton *et al.*, 1999; Tsantili *et al.*, 2010), with a few modifications. Briefly, 0.2 ml of extract was added to distilled water (2.6 ml) plus 0.2 ml of Folin- Ciocalteu Reagent (FCR) and mixed thoroughly. The reaction was neutralized with 2 ml of 7% sodium carbonate. The reaction mixture was incubated for 90 min at the room temperature and then its absorbance was read at 750 nm using spectrophotometer (Biowave, WPA S2100, UK). A mixture of distilled water and reagents was used as a blank. All the tests were carried out in triplicate. The total phenolic content was expressed as chlorogenic acid (mg) equivalents (CAEs) per 1 g of sample.

Total flavonoid content assay

The total flavonoid concentration was determined using Lenucci *et al.* (2006) method with some modifications. Briefly, 0.1 ml of extract was diluted with distilled water (0.9 ml) up to 1 ml. Then 0.05 ml of sodium nitrite solution (5%) was added to the mixture and remained for 5 min at room temperature and subsequently the mixture was supplemented with 10% aluminum chloride solution (0.1 ml). The mixture was allowed to react

Table 1. Details of sunflower genotypes used in the present experiment.

Genotype	Cultivar	Origin	Type
Iloflor (S1)	Single cross hybrid	France	Oily
Azargol (S2)	Single cross hybrid	Iran	Oily
Bane-Ghalami (S3)	Local landrace	Iran	Confectionary
Urmia-Pesteii (S4)	Local landrace	Iran	Confectionary
Hamedan-Ghalami (S5)	Local landrace	Iran	Confectionary
Saghez-Ghalami (S6)	Local landrace	Iran	Confectionary

for 6 min and the final composition was made by adding 0.5 ml of 1M sodium hydroxide and 1ml of distilled water. The mixture absorbance was immediately measured at 510 nm and the flavonoid content was expressed as mg of catechin equivalents (CEs) per g of sample. All tests were run in triplicate.

DPPH radical scavenging activity assay

DPPH radical scavenging activity was evaluated as described by Wu *et al.* (2003) with a slight modification. An aliquot of 100 µl of extracts was mixed with 1.5 ml of methanolic solution containing 0.1 mmol of DPPH (2, 2- diphenyl- 1- picrylhydrazyl). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min (until stable absorbance values were obtained). The reaction of the DPPH radical was estimated by measuring the absorption at 515 nm. Samples absorbance was measured at 515 nm and DPPH radical scavenging activity (RSA) was measured by using the following equation:

$$RAS\% = (A_0 - A_1) / A_0 \times 100$$

Where A_0 is the absorbance of control and A_1 is the absorbance of the sample. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of the scavenging effect percentage against the corresponding extract concentration. BHA was used as the reference compound.

The ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed as described by Benzie, Iris and Nilsson (Benzie and Strain, 1996; Iris *et al.*, 1999; Nilsson *et al.*, 2005). The FRAP reagent was prepared by mixing 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM HCl, 20 mM $Fe_2(SO_4)_3.7H_2O$ and acetate buffer (0.3 M, pH= 3.6) at the proportion of 1:1:10. Acetate buffer was contained 3.1 g sodium acetate trihydrate and 16 ml acetic acid in 1L distilled water. The mixture was incubated for 30 min at room temperature.

In brief, the extracts (50 µl) were added to the diluted FRAP reagent in methanol (1 ml FRAP reagent mixed with 2 ml methanol) and absorbance at 593 nm was recorded after 30 min at room temperature against a blank (all the FRAP reagents without the extract). Results were calculated according to the following equation that was obtained with ascorbic acid from a calibration curve and then expressed as ascorbic acid equivalents AEAC. All the tests were carried out in triplicate.

Nitric oxide radical inhibition assay

Nitric oxide was determined by using Griess Illosvoy reaction according to the method of Garrat (1964). The reaction mixture including sodium-nitroprocid (10 mmol/L), phosphate saline buffer (0.5 ml) and 100 µl extracts were incubated for 150 min at the room temperature. After incubation, 1ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was added to 0.50 ml of the reaction mixture. Then 1ml 0.1% naphthyl ethylenediamine dihydrochloride was added to the reaction mixture and remained for 30 min at the room temperature. A pink colored chromophore was formed in diffused light. Finally, 2 ml of distilled water was added to all samples. The absorbance of the solution was recorded at 540 nm against the corresponding blank solutions.

Nitric oxide radical scavenging % = A blank – A sample / A blank × 100

Extraction and hydrolysis for HPLC

For HPLC analysis, 0.5 g of dried and powdered plant material was extracted with 50% methanol/ water for 2 h at room temperature. The plant extract was hydrolyzed with 1.2 M HCl by refluxing in a water bath for 1 h. All samples were filtered through a 0.45 µm pore size syringe- driven filter before injection (Hertog *et al.*, 1992).

Chromatographic separation of phenolic compounds by HPLC

A 20 µl aliquot of sample solution was separated using HPLC (high performance liquid chromatography) system (Knuer, Germany) equipped with UV-Vis detector and a eurospher 100-5 C-18 column (25cm × 4.6cm; 5µm). The mobile phase contained solvents A (purified water with 2% acetic acid, pH= 3.2) and B (methanol). Solvent gradient was used as the following: 0- 5 min, 5% B; 5- 15 min, 10% B; 15- 26 min, a linear gradient of 10 -100% B. Total run time of analysis was 26 min. The flow rate was 0.8 ml/min, and the temperature was set at 25°C. Phenolic compounds were detected at the wavelength of 280 nm and identified by comparing their relative retention times and UV spectra with solutions of each pure commercial compound.

Statistical analysis

All the assays were carried out in triplicate. The results are expressed as mean values and standard error (SE) of the mean. Data analysis were performed using SPSS software version 18 and the means were compared using Tukey's multiple comparison test at $p < 0.05$ following analysis of variance (ANOVA). Correlation between various parameters was also investigated.

Table 2. Total phenolic and flavonoid contents of different parts of sunflower seeds. Results are mean of three replicates with standard errors (Means ± S.E, n=3). In each column different letters indicate significant differences $p < 0.05$.

Genotype	Phenolic contents (mg CAEs/g)		Flavonoid contents (mg CEs/g)	
	Kernel	Shell	Kernel	Shell
S1	^{cd}17.59 ± 0.16	^{a}5.38 ±0.03	^{bc}4.88 ± 0.03	^{b}1.61 ± 0.15
S2	^{a}24.29 ± 0.16	^{d}1.76 ± 0.11	^{a}7.21 ± 0.09	^{d}0.89 ± 0.03
S3	^{b}20.12 ± 0.33	^{b}4.15 ± 0.07	^{d}3.25 ± 0.35	^{a}2.29 ± 0.27
S4	^{bc}18.72 ± 0.39	^{c}2.26 ± 0.09	^{b}5.69 ± 0.49	^{b}1.58 ± 0.18
S5	^{b}19.47 ± 0.32	^{d}1.88 ± 0.02	^{a}7.13 ± 0.11	^{c}1.14 ± 0.16
S6	^{d}16.77 ± 0.35	^{d}1.55 ± 0.05	^{cd}3.90 ± 0.38	^{e}0.29 ± 0.02
Mean	19.49 ± 0.27	2.83 ± 0.06	5.34 ± 0.24	1.30 ± 0.08

RESULTS AND DISCUSSION

Total phenolic and flavonoid content

Total phenolic content in the shell and kernel of different sunflower genotypes are shown in Table 2. There were significant differences between genotypes for total phenolic content (TPC). The shell's TPC ranged from 1.55 (for S6 genotype) to 5.38 (for S1 genotype) mg CAEs/g DW and in kernel of seed it was from 16.77 (in S6 genotype) to 24.29 (S2 genotype) mg CAEs/g DW. The significant differences in total phenolic content were found between different parts of seed, so that total phenolic content in kernel were 6.8 times higher than that of shell. Furthermore, Table 2 shows flavonoid content in different genotypes of sunflower shells and kernels.

The flavonoid content ranged from 0.29 (for S6 genotype) to 2.29 (for S3 genotype) mg CEs/g DW in shell of seed and from 3.25 (S3 genotype) to 7.21 (S2 genotype) mg CEs/g DW in kernel. The flavonoid content in kernel was 4.1-fold higher than that of shell. The average TPC value of kernels in this study (19.49 mg/g) was higher than TPC amount reported by Hamed *et al.* (2012). They reported the average TPC content as 10.88 mg CAEs/ g in the kernels of Egyptian and 8.86 mg CAEs/g in Chinese sunflower seeds. The TPC observed in our study was almost two times greater than that reported by Pedrosa *et al.* (2000). Considerable differences in total phenolic and flavonoid content between genotypes may be due to the different regional conditions of plant growth and the genetic factors (Akbari *et al.*, 2012; Hatamnia *et al.*, 2014; Hatamnia *et al.*, 2016b).

DPPH radical scavenging activity assay

Reducing power of sunflower extracts as neutralizing DPPH radicals was investigated. Neutralization of the DPPH radicals could be seen and measured by changing the samples color from purple to yellow because of the electron donation. The stable $DPPH^{\bullet}$ is neutralized by accepting a hydrogen atom from the hydroxyl group of phenolic compound resulting in the reduced form, DPPH-H (Huang *et al.*, 2005). The antioxidant capacity of sunflower extracts was also investigated by EC_{50} index, the concentration at which antioxidant compounds scavenge fifty percent of radicals. The higher value of EC_{50} indicates a lower antiradical activity and vice versa. The results of DPPH radical quenching and EC_{50} values were compared to BHT activity as a synthetic antioxidant (Table 3). Radical scavenging activities of samples ranged from 22.83% to 94.36%. The kernel of S2 genotype exhibited a strong antioxidant activity with 94.36% DPPH scavenging and EC_{50} value of 0.065 mg/ml. Moreover, other studies previously reported that antioxidant activities are affected by phenolic compound type and structure (Thiago Inacio *et al.*, 2008). A positive correlation was observed between the TPC and DPPH radical scavenging in kernel and shell extracts ($r= 0.483$ and $r= 0.904$, respectively). There was a negative and strong correlation between DPPH and EC_{50} as two parallel assays that confirmed the results of each other (Table 4). As it was previously mentioned, the genetic factor and condition of plant environment impressed the phenolic compounds synthesis and target site of phenolics accumulation and consequently, antioxidant potential of specific parts of plants (Hatamnia *et al.*, 2014; Kalt, 2005).

Table 3. Antioxidant activity of different parts of sunflower seeds investigated by FRAP, nitric oxide radical inhibition and DPPH radical scavenging assays. Results are mean of three replicates with standard errors (Means ± S.E, n=3). In each column different letters indicate significant differences (P ≤0.05).

Genotype	DPPH		FRAP (mg/ml)	Nitric oxide scavenging (%)
	EC_{50} (mg/ml)	Radical scavenging (%)		
Kernel				
S1	^{cd}0.072 ± 0.012	^{a}93.44 ± 0.29	^{cd}1.28 ± 0.05	^{b}79.29 ± 0.19
S2	^{d}0.065 ± 0.001	^{a}94.36 ± 0.26	^{a}1.49 ± 0.04	^{a}89.00 ± 0.20
S3	^{bcd}0.092 ± 0.014	^{b}87.53 ± 0.38	^{d}1.25 ± 0.01	^{c}70.96 ± 0.30
S4	^{a}0.126 ± 0.015	^{d}62.46 ± 0.25	^{c}1.28 ± 0.02	^{d}56.19 ± 0.11
S5	^{abc}0.099 ± 0.015	^{b}87.52 ± 0.31	^{b}1.35 ± 0. 02	^{c}71.56 ± 0.30
S6	^{ab}0.110 ± 0.005	^{c}72.17 ± 0.16	^{b}1.34 ± 0.01	^{d}44.71 ± 0.25
Mean	0.094 ± 0.010	82.91 ± 0.27	1.33 ± 0.04	68.62 ± 0.21
Shell				
S1	^{c}0.15 ± 0.02	^{a}47.70 ± 0.59	^{a}0.72 ± 0.017	^{a}31.42 ± 0.1
S2	^{b}0.45 ± 0.12	^{c}31.14 ± 0.80	^{c}0.28 ± 0.014	^{c}23.64 ± 1.01
S3	^{bc}0.39 ± 0.08	^{b}39.30 ± 0.69	^{d}0.21 ± 0.011	^{b}29.54 ± 0.74
S4	^{a}0.77 ± 0.11	^{d}24.01 ± 0.60	^{b}0.44 ± 0.006	^{d}19.70 ± 0.05
S5	^{a}0.74 ± 0.13	^{d}22.83 ± 1.31	^{c}0.28 ± 0.001	^{d}16.01 ± 0.11
S6	^{ab}0.57 ± 0.01	^{c}28.58 ± 0.62	^{e}0.11 ± 0.007	^{e}9.38 ± 0.03
Mean	0.51 ± 0.07	32.30 ± 0.37	0.34 ± 0.003	21.61 ± 0.51
BHA	0.052 ± 0.001	96.49 ± 0.11	0.67 ± 0.0085	-

Table 4. Correlation between Total phenolic content (mg CAEs/g), flavonoid content (mg CEs/g), nitric oxide scavenging (%), FRAP (mg AEAC/g extract), DPPH radical Scavenging (%) and EC_{50} assays in different parts of sunflower seeds.

Character	Total phenolic contents	Flavonoid contents	FRAP assay	Nitric oxide scavenging	DPPH radical scavenging	EC_{50} Assay
Kernel						
Total phenolic contents	1	0.549*	0.636**	0.721**	0.483*	-0.504*
Flavonoid contents		1	0.560*	0.480*	0.222	-0.213
FRAP assay			1	0.814**	0.477*	-0.381
Nitric oxide scavenging				1	0.864**	-0.792**
DPPH radical scavenging					1	-0.855**
EC_{50} assay						1
Shell						
Phenolic contents	1	0.699**	0.685**	0.846**	0.904**	- 0.758**
Flavonoid contents		1	0.367	0.786**	0.452	- 0.291
FRAP assay			1	0.614**	0.541*	- 0.445
Nitric assay				1	0.791**	- 0.691**
DPPH assay					1	- 0.907**
EC_{50} assay						1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 probability level (2-tailed).

Table 5. The amounts of individual phenolic compounds (µg/g DW) detected by HPLC. Results are mean of three replicates with standard errors (Means ± S.E, n=3). DW: dry weight. (1) Ascorbic acid; (2) Gallic acid; (3 Rutin; (4) Caffeic acid; (5) P-hydroxybenzoic acid; (6) Vanillic acid; (7) P-coumaric acid; (8) Syringic acid; (9) Ferulic acid; (10) Sinapic acid. r.t: retention time; ND: not detected.

	1	2	3	4	5	6	7	8	9	10
r.t (min)	4.03	6.20	6.66	7.06	9.33	18.10	20.35	21.25	23.18	23.66
Kernel										
S1	31.5±0.54	15.0±0.43	N.D.	N.D.	20.4±0.83	N.D.	N.D.	69.5±3.7	42.1±6.3	29.9±0.88
S2	N.D.	N.D.	N.D.	88.9 ± 1.3	N.D.	N.D.	N.D.	433.6±12.7	118.2±8.9	20.6±0.55
S3	N.D.	N.D.	17.1 ± 53.2	18.9 ± 1.3	24.6±2.4	0.42±0.01	N.D.	14.0±0.51	46.0±4.1	28.5±1.8
S4	N.D.	18.3±1.3	N.D.	17.1 ± 0.94	24.9±0.76	4.32±0.02	N.D.	N.D.	N.D.	112.2±10.6
S5	N.D.	19.0±0.79	N.D.	27.8 ± 0.33	23.6±0.89	2.11±0.01	N.D.	59.4±7.5	N.D.	189.9±13.9
S6	243.1±7.9	53.2±2.4	N.D.	N.D.	N.D.	0.30±0.01	N.D.	N.D.	N.D.	107.3±3.8
Shell										
S1	3.97±0.06	N.D.	25.4±4.2	N.D.	24.9±3.6	N.D.	N.D.	16.7±0.55	78.4±7.5	22.9±1.2
S2	243.1±24.6	N.D.	N.D.	18.4±2.2	22.1±3.1	N.D.	N.D.	N.D.	N.D.	32.2±3.5
S3	5.22±0.54	31.1±2.4	30.1 ± 18.3	12.9±3.2	31.2±12.3	0.66±0.03	10.9±0.2	105.1±5.8	N.D.	105.1±6.3
S4	166.2±7.2	15.2±0.92	N.D.	28.2±5.2	N.D.	N.D.	8.9±0.8	N.D.	50.3±7.4	N.D.
S5	N.D.	15.9±2.4	N.D.	N.D.	N.D.	0.29±0.01	3.41±0.1	21.6±3.4	49.8±4.4	116.1±10.3
S6	N.D.	15.0±2.5	0.37 ± 0.02	N.D.	30.6±11.2	0.53±0.04	ND	52.3±10.5	45.7±6.3	46.4±8.3

The ferric reducing antioxidant power (FRAP) assay

FRAP assay is a quick, simple, sensitive and inexpensive method that measures the antioxidant potential of extracts. This method is based on the reduction of ferric ion Fe^{3+} to ferrous Fe^{2+} form that reflects the antioxidant capacity of extracts (Prior and Cao, 1999; Santas *et al.*, 2008). The antioxidant potential was expressed as AEAC (ascorbic acid equivalent antioxidant capacity) and compared to the standard commercial antioxidant (BHA).

The significant differences were observed among genotypes and different parts of seed for FRAP assay (Table 3). The extracts of kernel (the mean value for kernels = 1.33 mg/ml) were two times more active than BHA, while activity of shell extracts were lower than BHA (Table 3). The kernel of S2 and the shell of S6 genotypes displayed the highest (1.493 mg/ml) and the lowest (0.11 mg/ml) antioxidant power for ferric ions reduction, respectively. A positive correlation between FRAP assay and phenolic content was observed in kernel (r= 0.636) and shell extracts (r= 0.685), which are consistent with Szydłowska-Czerniak *et al.* (2011) reports. It has been suggested that the FRAP assay was one of the major trials to confirm antioxidant activity (Farhoosh *et al.*, 2011; Hatamnia *et al.*, 2016a). Comparison between FRAP assay and commercial antioxidant (BHA) revealed the considerable antioxidant potential of kernel. Since, the consumption of sunflower seed product is almost high, therefore, the sunflower seeds can be used as natural antioxidants in human diet.

Nitric oxide radical inhibition assay

The nitrite oxide radical scavenging of kernel and shell extracts in each genotype of sunflower is shown in Table 3. The results obtained from nitrite oxide radical scavenging assay indicated that, there were significant differences between different parts of seed and genotypes (Table 3). The nitrite oxide radical scavenging ranged from 9.38% (for S6 genotype) to 31.42% (S1 genotype) in shell extracts and from 44.71% (in S6 genotype) to 89.00% (S2 genotype) in kernel extracts. A positive correlation was observed between flavonoid

content and nitrite oxide radical scavenging power of shell extracts (r= 0.786) but not in kernel (Table 4). The nitrite oxide radical scavenging activity of sunflower extracts can be attributed to phenolic content, so that a positive correlation was observed between nitric oxide radical scavenging activity and phenolic content in kernel and shell extracts (r= 0.721 and r= 0.846, respectively). The high correlation between TPC and antioxidant activity in sunflower seeds have also been reported in other studies (Karamac *et al.*, 2005; Szydłowska-Czerniak *et al.*, 2011).

Determination of phenolic compounds by HPLC analysis

Some members of phenolic compounds (ascorbic acid, gallic acid, rutin, caffeic acid, p-hydroxybenzoic acid, vanillic acid, p-coumaric acid, syringic acid, ferulic acid and sinapic acid) were assessed and determined in all fractions of sunflower seeds (Table 5). Identification of phenolic compounds was on the basis of comparing their retention times and UV-Vis spectra properties to those of reference standards. In kernel extract, the largest and smallest amounts of detected phenolic compounds were Syringic acid (433.6 µg/g, S2 genotype) and vanillic acid (0.30 µg/g, S6 genotype) respectively. In shell extract, vanillic acid with 0.29 µg/g (S5 genotype) and ascorbic acid with 243.1 µg/g (S2 genotype) were observed in the lowest and the highest amount of identified phenolic compounds, respectively (Table 5). Sinapic acid was found in the kernel of all investigated genotypes, while p- coumaric acid was not. In addition, all individual phenolics with the exception of ferulic acid were determined in the shell of S3 genotype (Table 5). Considering not only the number of phenolic compounds but also their contents, the results indicated significant differences between different parts of fruits as well as between different genotypes. Similar to the results mentioned for total phenol and flavonoid content, the significant difference in phenolic composition can be probably due to different factors such as genetic, climate and environmental conditions (Barreira *et al.*, 2008; Hatamnia *et al.*, 2014; Hatamnia *et al.*, 2016b). The results showed that different genotypes contain various types of phenolic compounds which lead to different antioxidant potentials.

CONCLUSIONS

The present study revealed a positive correlation between total phenolic content and antioxidant activity in sunflower seeds. Moreover, the levels of total phenolic and flavonoid contents, as well as antioxidant activity changed depending on different parts of seeds and genotypes. The overall results indicated that, the S2 genotype possessed the highest total phenolic content and antioxidant activity among all genotypes. The differences between the genotypes emphasize on the influence of genetic factors on phenolic compounds and their compositions. Nowadays, there is a considerable interest in natural dietary antioxidants since most additive oxidative factors generate various diseases. Since sunflower has a significant contribution to human dietary and it contains considerable amount of phenolic compounds with high antioxidant potential, this crop may be exploited as an important source of natural antioxidants.

REFERENCES

Akbari V., Jamei R., Heidari R., and Jahanban Sfahlan, A. (2012). Antioxidant activity of different parts of Walnut (*Juglans regia* L.) fruit as a function of genotype. *Food Chemistry*, 135: 2404–2410.

Barreira J. C. M., Ferreira I. C. F. R., Oliveira M. B. P. P., and Pereira, J. A. (2008). Antioxidant activity and bioactive compounds of ten Portuguese regional and commercial almond cultivars. *Food and Chemical Toxicology*, 46: 2230-2235.

Benzie I. F., and Strain J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. *Analytical Biochemistry*, 239: 70-76.

Farhoosh R., Tavassoli- Kafrani M. H., and Sharif A. (2011). Antioxidant activity of the fractions separated from the unsaponifiable matter of bene hull oil. *Food Chemistry, 126*, 583-589.

Garrat D. C. (1964). *The quantitative analysis of drugs*. (3th ed.). Tokyo: Chapman and Hall.

Gonzalez-Perez S., and Vereijken J. M. (2007). Sunflower proteins: Overview of their physicochemical, structural and functional properties. *Journal of the Science of Food and Agriculture*, 87: 2173-2191.

Halvorsen B. L., Holte K., Myhrstad M. C., Barikmo I., Hvattum E., Remberg S. F., Wold A. B., Haffner K., Baugerod H., Andersen L. F., Moskaug J. O., Jacobs D. R., and Blomhoff R. (2002). A systematic screening of total antioxidants in dietary plants. *The Journal of Nutrition*, 132: 461-471.

Hamed S. F., Wagdy S. M., and Megahed M. G. (2012). Chemical Characteristics and Antioxidant Capacity of Egyptian and Chinese Sunflower Seeds: A Case Study. *Life Science Journal*, 9: 320-328.

Harborne J. B., and Williams C. A. (2000). Advances in flavonoids research since 1992. *Phytochemistry*, 55: 481-504.

Hatamnia A. A., Abbaspour N., and Darvishzadeh R. (2014). Antioxidant activity and phenolic profile of different parts of Bene (*Pistacia atlantica subsp. kurdica*) fruits. *Food Chemistry*, 145: 306-311.

Hatamnia A. A., Rostamzad A., Malekzadeh P., Darvishzadeh R., Abbaspour N., Hosseini M., Nourollahi Kh., and Sheikh Akbari Mehr R. (2016a). Antioxidant activity of different parts of *Pistacia khinjuk* Stocks fruit and its correlation to phenolic composition. Natural Product Research, DOI: 10.1080/14786419.2015.1060593.

Hatamnia A. A., Rostamzad A., Hosseini M., Abbaspour N., Darvishzadeh R., Malekzadeh P., and Mohammad Aminzadeh, B. (2016b). Antioxidant capacity and phenolic composition of leaves from ten Bene (*Pistacia atlantica subsp. kurdica*) genotypes. *Natural Product Research*, 30: 600-604.

Heim K. E., Tagliaferro A. R., and Bobilya D. J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry*, 13: 572-584.

Hertog M. G. L., Hollman P. C. H., and Venema D. P. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agriculture and Food Chemistry*, 40: 1591-1598.

Huang D., Ou B., and Prior R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 53: 1841-1856.

Iris F. F., Benzie I. F., and Strain J. J. (1999). Ferric reducing Antioxidant Power assay. *Methods in Enzymology*, 299: 15-27.

Kalt W. (2005). Effects of production and processing factors on major fruit and vegetable antioxidants. *Journal of Food Science*, 70: R11- R19.

Karamac M., Kosinska A., and Pegg R.B. (2005). Comparison of radical-scavenging activities for selected phenolic acids. *Polish Journal of Food and Nutrition Sciences*, 55: 165-170.

Kornsteiner M., Wagner K.H., and Elmadfa I. (2006). Tocopherols and total phenolics in 10 different nut types. *Food Chemistry*, 98: 381-387.

Kourie J. I. (1998). Interaction of reactive oxygen species with ion transport mechanism. *American Journal of Physiology: Cell Physiology*, 275: 1-24.

Lenucci M. S., Cadinu D., Taurino M., Piro G., and Dalessandro G. (2006). Antioxidant composition in cherry and high-pigment tomato cultivars. *Journal of Agricultural and Food Chemistry*, 54: 398-415.

Lu G., and Hoeft E. (2009). Sunflower. In: C. Kole, & T.C. Hall, (Eds.), *A compendium of transgenic crop plants* (pp. 125–168). London: Wiley-Blackwell.

Mahdavi D. L., and Salunkhe D. K. M. (1995). Toxicological aspects of food antioxidant. In: D. L. Mahdavi, S. S. Deshpande, & D. K. Salunkhe (Eds.), *Food antioxidants* (pp. 267–293). New York: Marcel Dekker.

Nilsson J., Pillai D., Onning G., Persson C., Nilsson A., and Akesson, B. (2005). Comparison of the 2,2'-azinobis-3-ethylbenzotiazo-line-6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP) methods to asses the total antioxidant capacity in extracts of fruit and vegetables. *Molecular Nutrition & Food Research*, 49: 239-246.

Pedrosa M. M., Muzquiz M., Garcıa-Vallejo C., Burbano C., Cuadrado C., Ayet G., and Robredo L. M. (2000). Determination of caffeic and chlorogenic acids and their derivatives in different sunflower seeds. *Journal of the Science of Food and Agriculture*, 80: 459-464.

Prior R. L., and Cao G. (1999). In vivo total antioxidant capacity: Comparison of different analytical methods. *Free Radical Biology & Medicine*, 27: 1173-1181.

Salunkhe D. K., Chavan J. K., Adsule R. N., and Kadam S. S. (1992). *World oil seeds: Chemistry, Technology, and Utilization*. New York: Van Nostrand Reinhold.

Santas R., Carbo-Gordon M. H., and Almajano M. P. (2008). Comparison of the antioxidant activity of two Spanish onion varieties. *Food Chemistry*, 107: 1210-1216.

Shahidi F., Janitha P. K., and Wanasundara P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition*, 32: 67-103.

Singleton V. L., Orthofer R., and Lamuela-Raventos R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology*, 299: 152-178.

Szydłowska-Czerniak A., Trokowski K., and Szłyk E. (2011). Optimization of extraction conditions of antioxidants from sunflower shells (*Helianthus annuus* L.) before and after enzymatic treatment. *Industrial Crops and Products*, 33: 123-131.

Temple N.J. (2000). Antioxidants and disease: more questions than answers. *Nutrition Research*, 20: 449-459.

Thiago Inacio B. L., Roberta G. C., Nidia C. Y., and Neli K. H. (2008). Radical scavenging activity of orsellinates. *Chemical & Pharmaceutical Bulletin*, 56: 1551-1554.

Tsantili E., Shin Y., Nock J. F., and Watkins C. B. (2010). Antioxidant concentrations during chilling injury development in peaches. *Postharvest Biology and Technology*, 57: 27-34.

Velioglu Y. S., Mazza G., Gao L., and Oomah B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, 46: 4113-4117.

Wijeratne S. S. K., Abou-Zaid M. M., and Shahidi F. (2006). Antioxidant polyphenols in almond and its coproducts. *Journal of Agricultural and Food Chemistry*, 54: 312-318.

Wu H. C., Chen H. M., and Shiou C. Y. (2003). Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Research International*, 36: 949-957.