

## THE CONTENT OF ANTIOXIDANTS IN SUNFLOWER SEED AND KERNEL

---

Žilić, S.<sup>\*1\*</sup>, Maksimović Dragišić, J.<sup>2</sup>, Maksimović, V.<sup>2</sup>, Maksimović, M.<sup>3</sup>,  
Basić, Z.<sup>3</sup>, Crevar, M.<sup>1</sup>, Stanković, G.<sup>1</sup>

---

<sup>1</sup>Maize Research Institute, Zemun Polje, Slobodana Bajića 1,  
Belgrade-Zemun, Serbia

<sup>2</sup>Institute for Multidisciplinary Research, Kneza Višeslava 1, Belgrade, Serbia

<sup>3</sup>Military Medical Academy, Institute of Hygiene, Crnotravska 17,  
Belgrade, Serbia

Received: September 5, 2009

Accepted: May 28, 2010

### SUMMARY

The primary objective of this research was to determine differences among investigated sunflower genotypes and whether the analysed hybrids could be sources of phenols and tocopherols important for storage stability of sunflower seeds and their derived products. DPPH<sup>•</sup> radical scavenging activity, the content of phenolic components and tocopherols ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -) in seeds and kernels of three sunflower hybrids were analysed. In the present study, six different phenolic compounds were separated by the HPLC analysis. Chlorogenic acid was the most abundant phenol. The chlorogenic acid content strongly correlated with total phenols ( $r=0.93$ ). Other marked phenolics were caffeic acid, ferulic acid, rosmarinic acid, myricetin and rutin. The total tocopherols were significantly higher ( $P<0.05$ ) in kernels than in seeds of all sunflower hybrids. Concentrations in sunflower seeds ranged from 200.67 to 220.05  $\mu\text{g/g}$  and from 256.62 to 267.49  $\mu\text{g/g}$  in sunflower kernels where  $\alpha$ -tocopherol was the dominant isomer in all samples. The  $\alpha$ -tocopherol content was 98% of averaged of the total tocopherols in all analysed samples. All these nutrients with antioxidant properties influenced the capacity of DPPH<sup>•</sup> scavenging. Accordingly, sunflower kernels had a higher DPPH<sup>•</sup> scavenging activity, and a higher nutritive value than sunflower seeds.

**Key words: sunflower, phenolics components, tocopherols, total antioxidants**

### INTRODUCTION

The frequently cited cause of seed deterioration is lipid peroxidation. Also, lipid oxidation occurring in food products is one of the major concerns in food technology. It is responsible for rancid odours and flavours of the products, with a conse-

---

\* Corresponding author: Phone: +381 37 56 704; Fax: +381 37 54 994;  
e-mail: szilic@mrizp.rs

quent decrease in nutritional quality and safety caused by the formation of secondary, potentially toxic compounds.

Cells contain a complex system of antioxidant defences to protect against the harmful consequences of activated oxygen species. Generally, the antioxidant activity is the greatest at lower concentrations and decreases or may become prooxidant at higher concentrations of its components. Also, antioxidants serve as food additives inhibiting the oxidation process, especially in edible fats. The phenolics components and tocopherols are the most important antioxidants for storage stability, as well as, nutritional quality of food made from sunflower seeds.

Numerous scientific articles refer to several natural phenols delaying the *in vitro* oxidation of simple or complex lipid matrices (Fukumoto and Mazza, 2000). Nevertheless, at the moment, the industrial use of natural phenolics, pure or in the extract form, is still hardly put into practice. Besides having the same safety and technological requirements as synthetic compounds, a natural antioxidant additive should also satisfy the following requirements:

- a) to be originated from an abundant and inexpensive vegetable matrix;
- b) to be produced by an economic and compatible technology;
- c) to be properly effective (De Leonardis *et al.*, 2005).

Sunflower seeds are rich in phenols (1 - 4% of d.m.), above all, in chlorogenic acid, but the phenols are present only in traces in cold-pressed sunflower seed oils (Leung *et al.*, 1981). The principal phenolic constituents of sunflower seeds are chlorogenic acid (CGA), smaller quantities of caffeic acid (CA), cinnamic, coumaric, ferulic, sinapic and hydroxy-cinnamic and finally traces of vanillic, syringic and hydroxy-benzoic acids (Pedrosa *et al.*, 2000). Chlorogenic acid causes a significant reduction in the availability and digestibility of the sunflower proteins, but from another point of view, chlorogenic acid is an important component of the hydroxy-cinnamates, compounds that are ubiquitous in plants and have interesting biological activities, including antioxidant properties (Clifford, 2000). Chlorogenic acid delayed the oxidation of sunflower seed oil by 11% and 41% at 30°C and 110°C, respectively, in comparison to the control. Caffeic acid, produced by alkaline hydrolysis of the chlorogenic acid methanol extract, showed the same antioxidant effectiveness as the original extract at low temperatures, but it was significantly more effective at 110°C (118% compared to the control) (De Leonardis *et al.*, 2003).

Tocopherols (vitamin E) are the most powerful fat-soluble antioxidants. They exist in four homologous isomers:  $\alpha$  (5,7,8-trimethyltolcol),  $\beta$  (5,8-dimethyltolcol),  $\gamma$  (7,8-dimethyltolcol), and  $\delta$  (8-methyltolcol), which differs in number or a position of methyl groups in the molecules. The various tocopherols differ in their biological activities and their ability to protect fats and oils from oxidative rancidity (Hashim *et al.*, 1993). Standard sunflower seeds mainly contain  $\alpha$ -tocopherol, which accounts for more than 90% of the total tocopherols.  $\beta$ - and  $\gamma$ -tocopherols can be present in sunflower seeds usually in amounts below 2% of the total tocopherols

(Demurin, 1993).  $\alpha$ -Tocopherol has the highest vitamin properties in biological systems and the lowest antioxidant properties in oils and foods containing them. Conversely,  $\gamma$ -tocopherol is the most powerful antioxidant *in vitro* but its *in vivo* activity is low.  $\beta$ - and  $\delta$ -tocopherols exhibit intermediate properties (Pongracz *et al.*, 1995).

The primary objective of this research was to determine differences among investigated sunflower genotypes and whether the analysed hybrids could be sources of phenols and tocopherols important for storage stability of sunflower seeds and their derived products. A more detailed knowledge of the variability of antioxidants accumulation among ZP sunflower genotypes could facilitate ongoing efforts to improve the sunflower seed composition.

## MATERIAL AND METHODS

### Plant material

The three European condition adapted oil sunflower (*Helianthus annuus* L.) hybrids selected for this investigation were Es Petunija, Allium and Albatre, all developed at the cooperation of Maize Research Institute, Zemun Polje, (MRIZP), Serbia with the French Agri Business Cooperative *Euralis*. All hybrids are of a high yielding potential and characterised by high tolerance to lodging and *Phomopsis* spp. Seeds were collected at full maturity stage from plants grown in a field-trial at the MRIZP, in the 2009 growing season.

The wholemeal flour (particle size < 500  $\mu\text{m}$ ), obtained by grounding sunflower seeds and kernels on a Cyclotec 1093 lab mill (FOSS Tecator, Sweden), was used in the analyses.

### DPPH radical scavenging activity

For the DPPH test the sunflower seed extract was prepared by dissolving 0.15 g of flour in 10 ml of 70% (v/v) acetone. After continuous shaking for 30 min at room temperature, the solution was centrifuged for 20 min at 20,000 g. An aliquot of extract (50  $\mu\text{l}$ ) was mixed with the ethanol DPPH solution (0.5 mM, 0.25 ml) and the acetate buffer (100 mM, pH 5.5, 0.5 ml). After standing for 30 min in the dark, the absorbance was measured at 517 nm against a blank containing absolute ethanol instead of a sample aliquot. The results are expressed as an  $\text{IC}_{50}$  value that represents the amount of flour (in mg) providing 50% inhibition of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH $^{\bullet}$ ) (Kolečkár *et al.*, 2007).

### The total phenolics content

Total phenolics were determined by the method of Singleton and Rossi (1965), using the same extract as for the DPPH test. Briefly, 0.1 ml extract was mixed with the 0.25 ml Folin reagent, 1.25 ml 20% sodium carbonate, and 0.4 ml deionised water. After standing for 40 min at room temperature, the absorbance was meas-

ured at 725 nm. The total phenolics content was calculated as a chlorogenic acid equivalents (eq.) from the calibration curve of chlorogenic acid standard solutions and expressed as mg of chlorogenic acid per g of dry matter (d.m.).

### **Phenolics components**

Quantification of phenolics was done by HPLC. Samples (40% ethanolic extract, with the exception of a browning sample extracted in the presence of 5 mM ascorbate in 40% ethanol) were injected in a Waters HPLC system consisting of 1525 binary pumps, thermostat and 717+ autosampler connected to a Waters 2996 diode array detector (Waters, Milford, USA). The separation of phenolics was performed on a Symmetry C-18 RP column 125 × 4 mm with 5 μm particle size (Waters) with an appropriate guard column. Two mobile phases, A 0.1% phosphoric acid and B acetonitrile (J.T. Baker, Deventer, the Netherlands), were used at a flow of 1 ml/min with the following gradient profile: 20 min from 10-22% B, 20 min with a linear rise to 40% B, 5 min reverse to 10% B, and additional 5 min equilibration time. Identified peaks were confirmed and quantified by data acquisition and spectral evaluation using Waters Empower 2 chromatographic software (Waters).

### **Tocopherols content**

The tocopherol content (μg/g d.w.) was determined by the HPLC method described by Branković (2006). Ten μm of samples were injected into a column (Nova Pak C 18, 150 mm × 3.9 mm diameter, 4 μm, Waters, UK). The mobile phase consisted of acetonitrile and methanol in a ratio of 97 to 3 (v/v) at a flow rate of 1.3 ml/min and 40°C. The fluorescence detector was set at 295 nm.

### **Statistical analyses**

All chemical analyses were performed in three replicates and the results were statistically analysed. Significant statistical differences of observed chemical parameters means of sunflower were determined by the Fisher's least significant differences (LSD) test, after the analysis of variance (ANOVA) for trials set up according to the RCB design.

## **RESULTS AND DISCUSSION**

Sunflower (*Helianthus annuus* L.) is one of larger sources of vegetable oil and protein of good nutritional quality. However, the presence of phenolic compounds in the seeds contributes to dark colour of formulated foods and prevents the use of sunflower defatted meal. On the other hand, phenols have interesting biological activities, including antioxidant properties.

In our study the total phenolic content varied over the investigated ZP sunflower genotypes (Table 1). However, the significant differences ( $P < 0.05$ ) between the total

phenolic content in seeds, as well as, in kernels of hybrids Es Petunia and Albatre, were not detected. According to our results the total phenolic content, expressed in chlorogenic acid eq. (mg/g d.w.) was significantly higher ( $P < 0.05$ ) in kernels than in seeds of all sunflower hybrids. The content of total phenolic was lower by 10% in seeds of all sunflower hybrids. The highest content of total phenolics was recorded in seeds and kernels of the hybrid Allium (18.24 and 20.13 eq. chlorogenic acid mg/g d.m., respectively), while the lowest was detected in seeds of hybrids Albatre and Es Petunia (14.68 and 15.00 eq. chlorogenic acid mg/g d.m., respectively). According to (De Leonardis *et al.*, 2005), the content of total phenols in sunflower seeds was similar in 60% ethanol and 60% acetone extracts *i.e.*, 1.11 and 1.15 eq. chlorogenic acid mg/ml, respectively. The levels of phenolic compounds were expressed as mg of ferulic acid per g of d.m. to that reported by (Velioglu *et al.*, 1998) for sunflower seeds (16 mg/g) and sunflower hulls (94 mg/g). Sunflower meal represents a protein source of a great interest as a human food. However, the preparation of sunflower protein isolates for food products is prevented by the presence of undesirable phenolic compounds in the seed, such as chlorogenic, caffeic and quinic acids (Sodini and Canella, 1977). These acids bind to polar groups of the proteins at alkaline pH values usually employed for protein extraction, giving dark-green or yellow products, strongly reducing the both their digestibility and functionality, as well as, the available lysine content (González-Pérez *et al.*, 2002). During processing, the cells in sunflower seeds may be ruptured, releasing polyphenoloxidase, which catalyses the oxidation of polyphenols to o-quinones. The o-quinones are highly reactive and may bind covalently with thiol or amino groups of proteins. Polyphenolic compounds may also bind noncovalently with protein via hydrogen-bonding, ionic, and hydrophobic interactions (Saeed and Cheryan, 1989).

Table 1: Phenolic compounds (mg/g d.m.) in sunflower seeds and kernels

Phenolic compounds	Seed			Kernel			LSD <sub>0.05</sub>
	Es Petunia	Allium	Albatre	Es Petunia	Allium	Albatre	
Chlorogenic acid	9.68 <sup>c</sup>	12.17 <sup>b</sup>	9.53 <sup>c</sup>	12.63 <sup>b</sup>	18.68 <sup>a</sup>	12.80 <sup>b</sup>	0.956
Caffeic acid	0.079 <sup>a</sup>	0.092 <sup>a</sup>	0.085 <sup>a</sup>	0.049 <sup>b</sup>	0.046 <sup>b</sup>	0.050 <sup>b</sup>	0.015
Ferulic acid	0.060 <sup>a</sup>	0.056 <sup>a</sup>	0.053 <sup>a</sup>	0.065 <sup>a</sup>	0.060 <sup>a</sup>	0.055 <sup>a</sup>	0.015
Rosmarinic acid	0.128 <sup>ab</sup>	0.084 <sup>c</sup>	0.141 <sup>a</sup>	0.138 <sup>a</sup>	0.096 <sup>c</sup>	0.123 <sup>b</sup>	0.015
Myricetin	0.053 <sup>bc</sup>	0.062 <sup>b</sup>	0.042 <sup>c</sup>	0.056 <sup>b</sup>	0.079 <sup>a</sup>	0.051 <sup>bc</sup>	0.015
Rutin	0.023 <sup>c</sup>	n.d. <sup>d</sup>	n.d. <sup>d</sup>	0.028 <sup>bc</sup>	0.057 <sup>a</sup>	0.042 <sup>b</sup>	0.015
Total phenolics*	15.00 <sup>d</sup>	18.24 <sup>b</sup>	14.68 <sup>e</sup>	16.67 <sup>c</sup>	20.13 <sup>a</sup>	16.28 <sup>c</sup>	1.121

<sup>a-f</sup> Means followed by the same letter within the same row are not significantly different ( $P < 0.05$ ); Means of total phenols marked with \* are expressed in mg chlorogenic acid/g d.m.; n.d.- not detected

In all samples, six different phenolic compounds were separated by the HPLC analysis (Table 1). Chlorogenic acid was the most abundant phenol, *i.e.*, from 9.53 to 12.17 mg/g d.m. in seeds and from 12.63 to 18.68 mg/g d.m. in kernels of analysed sunflower hybrids. Other marked phenolics were the caffeic acid, ferulic acid, rosmarinic acid, myricetin and rutin, which were, however, all present in quantities

of less than 0.15 mg/g d.m. The results of (De Leonardis *et al.*, 2005) showed that phenolic spectrum of sunflower seeds included seven components (chlorogenic acid, protocatechuic, caffeic acid *o*-cinnamic acids, ferulic acid and syringic acid). Only one compound, with an elution time of 25.4 min, was not identified. These authors also reported that the chlorogenic acid was the most abundant phenol (79.4% of total phenols). Caffeic acid was equal to 4.1%, protocatechuic acid 5.2%, *o*-cinnamic acids 5.8, ferulic acid 1.4 and syringic acid 0.6% of total phenols. In our study, the chlorogenic acid content strongly correlated with total phenols ( $r=0.93$ ,  $P<0.05$ ). Same as total phenols, the chlorogenic acid content was significantly higher ( $P<0.05$ ) in kernels than in seeds of all sunflower hybrids. In seeds of sunflower hybrids Es Petunia, Allium and Albatre the content chlorogenic acid was lower by 23.4, 34.9 and 25.4%, respectively. The highest content of chlorogenic acid was detected in kernels of the hybrid Allium (18.64 mg/g d.m.). Chlorogenic acid is a natural phenol formed by one molecule of caffeic acid and one of quinic acid (De Leonardis *et al.*, 2006). In contrast to chlorogenic acid, the caffeic acid content was significantly higher ( $P<0.05$ ) in seeds than in kernels of all sunflower hybrids. Caffeic acid is stronger antioxidant than chlorogenic acid. The antioxidative activity of polyphenols is generally ascribed to their hydroxyl groups (Chen and Ho, 1997). Also, the presence of a second hydroxyl group in the ortho or para position is known to increase the antioxidative activity. Chen and Ho (1997) reported that the rosmarinic acid, which has four hydroxyl groups, showed the strongest DPPH scavenging potency of all phenols. In our study, rosmarinic acid negatively correlated with chlorogenic acid ( $r=-0.56$ ). The lowest content of rosmarinic acid was in seeds and kernels of the hybrid Allium (0.84 and 0.96 mg/g d.m., respectively). However, the myricetin content strongly correlated with chlorogenic acid ( $r=0.90$ ,  $P<0.05$ ). Myricetin is an important dietary flavonoid and is a stronger antioxidant than  $\alpha$ -tocopherol. These antioxidants in combination have a synergistic effect (Marinova *et al.*, 2008).

All sunflower samples were examined for their  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols (Table 2 and 3). The sum of  $\alpha$ -tocopherol,  $\gamma$ + $\beta$ -tocopherol and  $\delta$ -tocopherol was significantly higher ( $P<0.05$ ) in kernels than in seeds of all sunflower hybrids. Concentrations in sunflower seeds ranged from 200.67 to 220.05  $\mu\text{g/g}$ . This range was lower than that of 669.1 mg/kg reported for seeds of commercial hybrids cultivated in Spain by Velasco *et al.* (2002) and slightly lower than that of 328 mg/kg found in seeds of wild *Helianthus* spp. by Velasko *et al.* (2004). The total tocopherols content in sunflower kernels was higher and ranged from 256.62 to 267.49  $\mu\text{g/g}$ .  $\alpha$ -Tocopherol was the dominant isomer in all samples. The highest  $\alpha$ -tocopherol content was estimated in kernels of the hybrid Allium (262.74  $\mu\text{g/g}$  d.m.) and the lowest in seeds of the hybrid Es Petunia (196.70  $\mu\text{g/g}$  d.m.). The content of  $\alpha$ -tocopherol averaged 98% of the total tocopherols in all analysed samples.  $\gamma$ + $\beta$ -Tocopherols presented in sunflower samples in amounts of 1.28 to 1.61% of the total tocopherols. The level of  $\delta$ -tocopherol was not significant in all sunflower samples and

ranged from 0.42 to 0.52% of the total tocopherols. In seeds of commercial hybrids cultivated in Spain the total tocopherol content made up of 92.4%  $\alpha$ -tocopherol, 5.6%  $\beta$ -tocopherol and 2.0%  $\gamma$ -tocopherol (Velasco *et al.*, 2002). The average tocopherol profile of the wild *Helianthus* spp. germplasm evaluated by Velasco *et al.* (2004) consisted of 99.0%  $\alpha$ -tocopherol, 0.7%  $\beta$ -tocopherol and 0.3%  $\gamma$ -tocopherol. New sunflower lines containing  $\gamma$ -tocopherol as a major natural antioxidant instead of the  $\alpha$ -tocopherol characteristic of standard sunflower were developed (Demurin *et al.*, 1996). Standard sunflower oil with a higher content of  $\alpha$ - and  $\beta$ -tocopherols has good properties for low temperature food applications (salad dressings, emulsions, *etc.*) (Garcés *et al.*, 2009). At moderate temperature,  $\alpha$ -tocopherol was a better antioxidant than  $\gamma$ -tocopherol at low concentrations, but the opposite was found at high tocopherol concentrations. At high temperature,  $\gamma$ -tocopherol was a better inhibitor of polymerisation reactions than  $\alpha$ -tocopherol (Marmesat *et al.*, 2008). Processes for food preparation involving high temperature, *i.e.* baking and frying, require fats and oils of high thermal stability, to delay undesirable modifications. In triacylglycerol (TAG) model systems and in different edible oils, it has been reported that the loss of tocopherols at frying temperatures depended on the degree of unsaturation of the lipid substrate, with  $\alpha$ -tocopherol being the least stable among the four natural tocopherols (Barrera-Arellano *et al.*, 1999). In this respect, the development of genetically modified oils and tocopherol components has significantly increased the availability of oils of high thermal stability.

Table 2: Tocopherol content ( $\mu\text{g/g}$  d.m.) in sunflower seeds and kernels

Tocopherols	Seed			Kernel			LSD <sub>0.05</sub>
	Es Petunia	Allium	Albarte	Es Petunia	Allium	Albarte	
$\alpha$ -tocopherol	196.70 <sup>f</sup>	207.06 <sup>e</sup>	215.83 <sup>d</sup>	254.94 <sup>b</sup>	262.74 <sup>a</sup>	252.07 <sup>c</sup>	0.384
$\gamma$ + $\beta$ -tocopherol	2.99 <sup>d</sup>	3.42 <sup>ab</sup>	3.08 <sup>cd</sup>	3.47 <sup>ab</sup>	3.60 <sup>a</sup>	3.30 <sup>bc</sup>	0.230
$\delta$ - tocopherol	0.98 <sup>d</sup>	0.91 <sup>e</sup>	1.14 <sup>c</sup>	1.21 <sup>b</sup>	1.15 <sup>c</sup>	1.25 <sup>a</sup>	0.015
Total tocopherol	200.67 <sup>f</sup>	211.93 <sup>e</sup>	220.05 <sup>d</sup>	259.62 <sup>b</sup>	267.49 <sup>a</sup>	256.62 <sup>c</sup>	1.161

<sup>a-f</sup> Means followed by the same letter within the same row are not significantly different ( $P < 0.05$ )

Table 3: Tocopherol content (% of total tocopherols) in sunflower seeds and kernels

Tocopherols	Seed			Kernel			LSD <sub>0.05</sub>
	Es Petunia	Allium	Albarte	Es Petunia	Allium	Albarte	
$\alpha$ -tocopherol	98.02 <sup>a</sup>	97.70 <sup>a</sup>	98.08 <sup>a</sup>	98.19 <sup>a</sup>	98.22 <sup>a</sup>	98.22 <sup>a</sup>	0.630
$\gamma$ + $\beta$ -tocopherol	1.49 <sup>ab</sup>	1.61 <sup>a</sup>	1.41 <sup>bc</sup>	1.33 <sup>bc</sup>	1.34 <sup>bc</sup>	1.28 <sup>c</sup>	0.163
$\delta$ - tocopherol	0.49 <sup>ab</sup>	0.43 <sup>ab</sup>	0.52 <sup>a</sup>	0.46 <sup>ab</sup>	0.42 <sup>b</sup>	0.48 <sup>ab</sup>	0.094

<sup>a-c</sup> Means followed by the same letter within the same row are not significantly different ( $P < 0.05$ )

Phenolic compounds and tocopherols in seeds and kernels of sunflower deserve much more attention because the total phenolic content, as well as, the total tocopherols content strongly correlate with the total antioxidant activity ( $r=0.85$   $r=0.79$ , respectively). All these nutrients with antioxidant properties influenced the capacity of DPPH<sup>\*</sup> scavenging. The DPPH<sup>\*</sup> scavenging activity of sun-

flower seeds and kernels are shown in Figure 1. All sunflower samples had a very strong DPPH<sup>\*</sup> scavenging activity. The IC<sub>50</sub> values ranged from 0.99 to 1.29 mg d.w. and 0.86 to 0.95 mg d.w. in sunflower seeds and kernels, respectively. To compare, under our experimental conditions, 8.8 μg of ascorbic acid was able to scavenge 50% of DPPH<sup>\*</sup>. According to (Žilić *et al.*, 2009a; Žilić *et al.*, 2009b) IC<sub>50</sub> value ranged from 6.50 to 8.28 mg in soybean, 10.18 to 11.78 mg in wheat and from 3.27 to 5.91 mg in maize. According to our results, sunflower kernels had a significantly higher IC<sub>50</sub> value than sunflower seeds ( $P < 0.05$ ). Same as the total phenolic content, there was no significant differences ( $P < 0.05$ ) between the IC<sub>50</sub> value in seeds, as well as, in kernels of hybrids Es Petunia and Albatre.

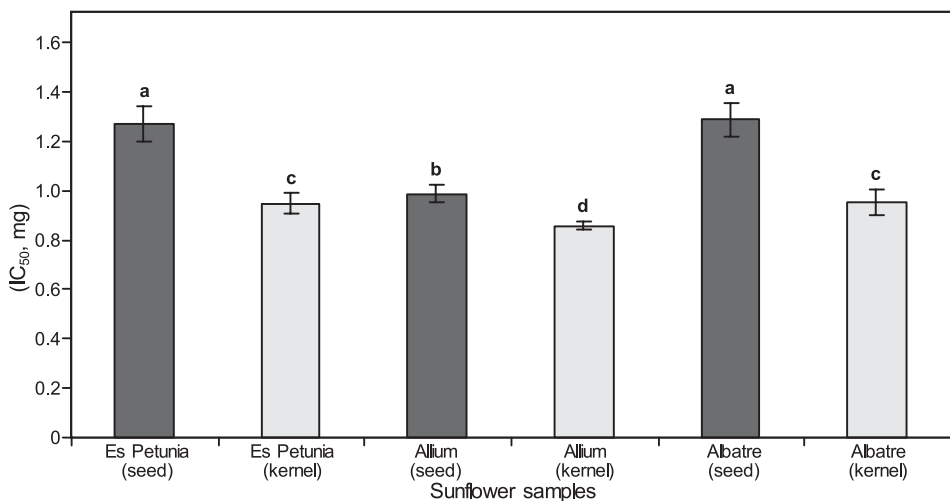


Figure 1: DPPH radical-scavenging activity in sunflower seeds and kernels. Bars with different letters are statistically significantly different ( $P < 0.05$ ).

## CONCLUSION

The total phenolics content varies over the investigated ZP sunflower genotypes. Chlorogenic acid was the most abundant phenol. Other remarkable phenols were caffeic acid, ferulic acid, rosmarinic acid, myricetin and rutin. The high variability of  $\alpha$ -tocopherol content, like that of total tocopherols, was observed in the investigated genotypes.  $\alpha$ -Tocopherol was the dominant isomer in all samples. The content of  $\alpha$ -tocopherol averaged 98% of the total tocopherols. The content of phenolics and tocopherols was more abundant in sunflower kernels than in sunflower seeds. All these nutrients with antioxidant properties influenced the capacity of DPPH<sup>\*</sup> scavenging. Accordingly, sunflower kernels had a higher DPPH<sup>\*</sup> scavenging activity, and a higher nutritive value than sunflower seeds.

Analysed ZP sunflower hybrids, especially Allium, may be considered as a potential source of natural antioxidants. According to our results, oil from standard



ZP sunflower hybrids has an antioxidant activity adequate for low temperature food applications (salad dressings, emulsions, *etc.*). The results suggest that the improvement of the sunflower antioxidant characteristics could be reached through the breeding activity.

The primary objective of this research was to verify, in the laboratory, the concrete possibility to produce a phenolic antioxidant from sunflower seeds that could be utilised in edible oils and fats. Simultaneously, dephenolisation of sunflower seeds could improve the composition of seeds and their derived products for further uses.

### ACKNOWLEDGEMENTS

*This study was supported by the Ministry of Science and Technological Development of the Republic of Serbia, Project OI 143020. Also, the author would like to thank her colleague Dr Vesna Hadži-Tašković Šukalović for all suggestions and help.*

### REFERENCES

- Barrera-Arellano, D., Ruiz-Méndez, M.V., Márquez-Ruiz, G. and Dobarganes, M.C., 1999. Loss of tocopherols and formation of degradation compounds in triacylglycerol model systems heated at high temperature. *J. Sci. Food Agric.* 79: 1923–1928.
- Branković, T., 2006. Izolovanje tokoferola iz biljnih ulja optimizovanim postupkom saponifikacije u cilju njihovog određivanja tečnom hromatografijom. *In: Branković, T. (Ed.), Specijalistički rad. Military Medical Academy*, pp. 33-59.
- Chen, J.H. and Ho, C.T., 1997. Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *J. Agric. Food Chem.* 45: 2374-2378.
- Clifford, M.N., 2000. Chlorogenic acids and other cinnamates: nature, occurrence, dietary burden, absorption and metabolism. *J. Sci. Food Agric.* 80: 1033–1043.
- De Leonardis, A., Albanese, T. and Macciola, V., 2006. Biodegradation in vivo and in vitro of chlorogenic acid by a sunflower-seedling (*Helianthus annuus* L.) like-polyphenoloxidase enzyme. *Eur. Food Res. Technol.* 223: 295–301.
- De Leonardis, A., Macciola, V. and Di Domenico, N., 2005. A first pilot study to produce a food antioxidant from sunflower seed shells (*Helianthus annuus* L.). *Eur. J. Lipid Sci. Technol.* 107: 220–227.
- De Leonardis, A., Macciola, V. and Di Rocco, A., 2003. Oxidative stabilization of cold-pressed sunflower oil using phenolic compounds of the same seeds. *J. Sci. Food Agric.* 83: 523–528.
- Demurin, Y., Škorić, D. and Karlović, D., 1996. Genetic variability of tocopherol composition in sunflower seeds as a basis of breeding for improved oil quality. *Plant Breeding* 115: 33-36.
- Fukumoto, L.R. and Mazza, G., 2000. Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem.* 48: 3597–3604.
- Garcés, R., Martínez-Force, E., Salas, J.J. and Venegas-Calcrón, M., 2009. Current advances in sunflower oil and its applications. *Lipid Technology* 21(4): 79-82.
- González-Pérez, S., Merck, K.B., Vereijken, J.M., van Koningsveld, G.A., Gruppen, H. and Voragen, A.G.J., 2002. Isolation and characterization of undenatured chlorogenic acid free sunflower (*Helianthus annuus*) proteins. *J. Agric. Food Chem.* 50: 1713-1719.
- Hashim, I.B., Koehler, P.E. and Eitenmiller, R.R., 1993. Tocopherols in runner and virginia peanut cultivars at various maturity stages. *J. Am. Oil Chem. Soc.* 70: 633-635.
- Kolečár, V., Jun, D., Opletal, L., Jahodár, L. and Kuča, L., 2007. Assay of radical scavenging activity of antidotes against chemical warfare agents by DPPH test using sequential injection technique. *J. Appl. Biomed.* 5: 81-84.

- Leung, J., Fenton, T.W. and Clandinin, D.R., 1981. Phenolic components of sunflower flour. *J. Food Sci.* 46: 1386-1393.
- Marinova, E., Toneva, A. and Yanishlieva, N., 2008. Synergistic antioxidant effect of  $\alpha$ -tocopherol and myricetin on the autoxidation of triacylglycerols of sunflower oil. *Food Chemistry* 106: 628-633.
- Marmesat, S., Velasco, L., Ruiz-Méndez, M.V., Fernández-Martínez, J.M. and Dobarganes, C., 2008. Thermostability of genetically modified sunflower oils differing in fatty acid and tocopherol compositions. *Eur. J. Lipid Sci. Technol.* 110: 776-782.
- Pedrosa, M.M., Muzquiz, M., Garcia-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. *J. Sci. Food Agric.* 80: 459-464.
- Pongracz, G., Weiser, H. and Matzinger, D., 1995. Tocopherole. *Antioxidanten der Natur. Fat Sci. Technol.* 97: 90-104.
- Saeed, M. and Cheryan, M., 1989. Acidic butanol removal of color-forming phenols from sunflower meal. *J. Agric. Food Chem.* 37(5): 1270-1274.
- Singleton, V.L. and Rossi, J.A., 1965. Colorimetry a total phenolics with phosphomolibdic-phosphotungstic acid reagents. *Am. J. Enol. Vitric.* 16: 144-158.
- Sodini, G. and Canella, M., 1977. Acidic butanol removal of color-forming phenols from sunflower meal. *J. Agric. Food Chem.* 25(4): 822-825.
- Velasco, L., Fernández-Martínez, J.M., García-Ruiz, R. and Domínguez, J., 2002. Genetic and environmental variation for tocopherol content and composition in sunflower commercial hybrids. *J. Agric. Sci.* 139: 425-429.
- Velasco, L., Pérez-Vich, B. and Fernández-Martínez, J.M., 2004. Evaluation of wild sunflower species for tocopherol content and composition. *Helia* 27(40): 107-112.
- Velioglu, Y.S., Mazza, G., Gao, L. and Oomah, B.D., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* 46: 4113-4117.
- Žilić, S., Hadži-Tašković Šukalović, V., Milašinović, M., Terzić, D. and Maksimović, M., 2009a. Effects of infrared radiation on protein solubility and antioxidants content in maize flour. *Czech J. Food Sci.* 27: S241-S241.
- Žilić, S., Hadži-Tašković Šukalović, V., Srebrić, M., Dodig, D., Maksimović, M. and Mladenović Drinić, S., 2009b. Chemical compositions as quality parameters of ZP soybean and wheat genotypes. *Genetika* 41(3): In press.