THE CONTENT OF ANTIOXIDANTS IN SUNFLOWER
SEED AND KERNEL

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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• radical scavenging activity, the content of phenolic components and tocopherols (α-, β-, γ-, and δ-) 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 µg/g and from 256.62 to 267.49 µg/g in sunflower kernels where α-tocopherol was the dominant isomer in all samples. The α-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• scavenging. Accordingly, sunflower kernels had a higher DPPH• 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-
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: α (5,7,8-trimethyltocol), β (5,8-dimethyltocol), γ (7,8-dimethyltocol), and δ (8-methyltocol), 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 α-tocopherol, which accounts for more than 90% of the total tocopherols. β- and γ-tocopherols can be present in sunflower seeds usually in amounts below 2% of the total tocopherols.
(Demurin, 1993). α-Tocopherol has the highest vitamin properties in biological systems and the lowest antioxidant properties in oils and foods containing them. Conversely, γ-tocopherol is the most powerful antioxidant in vitro but its in vivo activity is low. β- and δ-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 µm), 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 µl) 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 IC50 value that represents the amount of flour (in mg) providing 50% inhibition of 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH•) (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 contribute 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).

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

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>Seed</th>
<th>Kernel</th>
<th>LSD0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid</td>
<td>Es Petunia</td>
<td>Allium</td>
<td>Albarte</td>
</tr>
<tr>
<td></td>
<td>9.68c</td>
<td>12.17b</td>
<td>9.53a</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.079a</td>
<td>0.092a</td>
<td>0.085a</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.060a</td>
<td>0.056a</td>
<td>0.053a</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>0.128ab</td>
<td>0.084c</td>
<td>0.141a</td>
</tr>
<tr>
<td>Myricetin</td>
<td>0.053bc</td>
<td>0.062b</td>
<td>0.042c</td>
</tr>
<tr>
<td>Rutin</td>
<td>0.023c</td>
<td>n.d. d</td>
<td>0.028bc</td>
</tr>
<tr>
<td>Total phenolics</td>
<td>15.00d</td>
<td>18.24b</td>
<td>14.68a</td>
</tr>
</tbody>
</table>

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

Table 1: Phenolic compounds (mg/g d.m.) in sunflower seeds and kernels
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 a 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 α-tocopherol. These antioxidants in combination have a synergistic effect (Marinova et al., 2008).

All sunflower samples were examined for their α-, β-, γ-, and δ-tocopherols (Table 2 and 3). The sum of α-tocopherol, γ+β-tocopherol and δ-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 µg/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 Velasco et al. (2004). The total tocopherols content in sunflower kernels was higher and ranged from 256.62 to 267.49 µg/g. α-Tocopherol was the dominant isomer in all samples. The highest α-tocopherol content was estimated in kernels of the hybrid Allium (262.74 µg/g d.m.) and the lowest in seeds of the hybrid Es Petunia (196.70 µg/g d.m.). The content of α-tocopherol averaged 98% of the total tocopherols in all analysed samples. γ+β-Tocopherols presented in sunflower samples in amounts of 1.28 to 1.61% of the total tocopherols. The level of δ-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 (µg/g d.m.) in sunflower seeds and kernels

<table>
<thead>
<tr>
<th>Tocopherols</th>
<th>Seed</th>
<th>Kernel</th>
<th>LSD0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Es Petunia</td>
<td>Allium</td>
<td>Albarte</td>
</tr>
<tr>
<td>(\alpha)-tocopherol</td>
<td>196.70f</td>
<td>207.06e</td>
<td>215.83d</td>
</tr>
<tr>
<td>(\gamma+\beta)-tocopherol</td>
<td>2.99d</td>
<td>3.42ab</td>
<td>3.08cd</td>
</tr>
<tr>
<td>(\delta)-tocopherol</td>
<td>0.98d</td>
<td>0.91a</td>
<td>1.14b</td>
</tr>
<tr>
<td>Total tocopherol</td>
<td>200.67f</td>
<td>211.39e</td>
<td>220.05d</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Tocopherols</th>
<th>Seed</th>
<th>Kernel</th>
<th>LSD0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha)-tocopherol</td>
<td>98.02a</td>
<td>97.70a</td>
<td>98.08a</td>
</tr>
<tr>
<td>(\gamma+\beta)-tocopherol</td>
<td>1.49ab</td>
<td>1.61a</td>
<td>1.41bc</td>
</tr>
<tr>
<td>(\delta)-tocopherol</td>
<td>0.49ab</td>
<td>0.43ab</td>
<td>0.52a</td>
</tr>
</tbody>
</table>

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 \((r=0.85, r=0.79, \text{respectively})\). All these nutrients with antioxidant properties influenced the capacity of DPPH• scavenging. The DPPH• scavenging activity of sun-
flower seeds and kernels are shown in Figure 1. All sunflower samples had a very strong DPPH• scavenging activity. The IC₅₀ 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•. According to (Žilić et al., 2009a; Žilić et al., 2009b) IC₅₀ 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 IC₅₀ value than sunflower seeds (P<0.05). Same as the total phenolic content, there was no significant differences (P<0.05) between the IC₅₀ value in seeds, as well as, in kernels of hybrids Es Petunia and Albatre.

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 α-tocopherol content, like that of total tocopherols, was observed in the investigated genotypes. α-Tocopherol was the dominant isomer in all samples. The content of α-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• scavenging. Accordingly, sunflower kernels had a higher DPPH• 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.

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