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CHARACTERIZATION OF SUNFLOWER SEED AND KERNEL PROTEINS

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SUMMARY

Total sunflower proteins, storage proteins, and helianthinin (11S) and 2S albumin fractions and their respective subunits in seeds and kernels of three sunflower hybrids were analyzed. Protein contents were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and coupled with densitometry. The SDS-PAGE profiles of the seed and kernel proteins in the crude extracts for all genotypes showed a very similar number of protein bands (thirty two) in the electrophoretograms. Three polypeptide groups of helianthinin fraction were detected. Two of these were acidic (α, Mw = 36,800 - 42,900 Da and α', Mw = 31,000 - 35,300 Da), while one was basic (β, Mw=21,000 - 29,600 Da). The molecular weight of the 2S albumin proteins ranged from 11,500 to 20,100 Da. According to our results, there were significant differences among the seed and kernel protein contents. The 2S albumin content was significantly higher in kernels than in whole seeds of sunflower hybrids ($P<0.05$). By contrast, the 11S helianthinin content was significantly higher in seeds (where it ranged from 61.75 to 67.70% of totally extracted proteins) than in kernels (varied from 57.36 to 61.51% of totally extracted proteins) of sunflower hybrids ($P<0.05$).

Key words: sunflower, soluble protein fractions and subunits

INTRODUCTION

The sunflower is one of the four most important oil crops globally and is grown on over 21 million hectares worldwide (Škorić *et al.*, 2007). Seeds of sunflower are mainly used for their oil content, which accounts for 80% of the value of the sunflower crop. At the same time, there is an increasing interest in the use of sunflower protein (*Helianthus annuus* L.) in human nutrition. Sunflower seeds contain

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~ 20% of protein, whereas protein contents of the oil press cakes and extraction residues range from 30 to 50% (Dorrell and Vick, 1997). The techno-functional properties of sunflower proteins are comparable with those of soy and other leguminous proteins (González-Pérez *et al.*, 2005). Although lysine deficiency is a major drawback from the nutritional point of view, proteins from sunflower press cake are considered a valuable alternative as food ingredients, since they are low in antinutritional compounds and devoid of toxic substances (González-Pérez and Vereijken, 2007). The sunflower protein isolates can be subjected to hydrolytic treatments in order to produce protein hydrolysates that have improved functional and nutritional properties (Villanueva *et al.*, 1999). In addition, protein hydrolysates are also a source of bioactive peptides, which are short peptides and have a certain biological activity that may be beneficial for the organism (Megías *et al.*, 2008). Bioactive peptides with different impacts on the regulation of the gastrointestinal, nervous, cardiovascular and immune systems have been described (Korhonen and Pihlanto, 2006).

Composition and conformation are responsible for a sunflower protein's functionality. Compositional differences that may alter functionality include the ratio of protein fractions, variations in subunit concentrations within fractions, and differences in amino acid profiles. Sunflower proteins have two major salt-extractable fractions (2S, and 11S) that can be isolated on the basis of their sedimentation coefficients. The main storage proteins in sunflower make up about 85% of the total protein content. The 11S helianthinin belongs to the widely distributed legumin-like family of globulins and 2S albumins, which, in turn, belong to a larger protein family. The helianthinins are generally considered to be the major group of storage proteins, being reported to account for some 60% of the total proteins in the mature seed with the 2S albumins accounting for about 20% (Kortt and Caldwell, 1990). However, early studies of the salt-soluble proteins by sucrose gradient centrifugation gave different results, with 62% 2S albumins and 38% 11S globulins (Youle and Huang, 1981). González-Pérez and Vereijken (2007) reported that globulins constitute most of the sunflower proteins, ranging from about 40 to 90%, while albumins account for about 10-30% of the total proteins. Glutelins and, in particular, prolamins are only minor fractions. The proportions of proteins with different sedimentation coefficients depend largely on conditions such as type of buffer, pH, ionic strength, chemical, physical or enzymatic modification, *etc.* (Molina *et al.*, 2001). Therefore, literature data show considerable variation in the proportions of the different protein fractions according to the sedimentation coefficient. Also, sunflower proteins have two small amount salt-extractable fractions (6-9S) and high molecular weight fraction (15-18S). The latter fraction has been described as an aggregate of the 11S fraction (Guéguen *et al.*, 1988). In contrast to soybean globulins, sunflower globulins do not contain any genetically independent 7S constituent (Anisimova and Gavrilyuk, 1990). Nevertheless, various amounts of proteins with 7S sedimentation coefficient have been detected in sunflower seeds (Kabirullah and

Wills, 1983). These 7S constituents are most likely dissociation products of the 11S globulins, a dissociation which also occurs in soybean glycinin. In addition to the storage albumins and globulins, sunflower seeds also accumulate a third group of proteins. These are the oleosins, or oil body proteins, which are associated with the outer surface of the oil bodies and are thought to prevent coalescence (Ross and Murphy, 1992).

Helianthinin has been reported to be present as a globular oligomeric protein with a molecular weight (Mw) of 300-350 kDa. The currently most accepted model of helianthinin (11S), at neutral pH, consists of an arrangement of six spherical subunits into a trigonal antiprism (Plietz *et al.*, 1983). The monomeric subunits consist of an acidic (32-44 kDa) and a basic (21-27 kDa) polypeptide linked by a disulfide bond (Dalgalarrondo *et al.*, 1984). Sunflower albumins are basic proteins with a molecular mass in the 10-18 kDa range (Kortt and Caldwell, 1990). Two types of albumin are distinguished: methionine-rich and methionine-poor albumins. In contrast to 2S seed albumins from other species that consist of two chains linked by disulfide bonds, sunflower albumin consist of a single polypeptide chain (Pandya *et al.*, 2000).

Sunflower protein contains low levels of lysine, whereas it is relatively rich in sulphur-containing amino acids compared with other oil seeds (Canibe *et al.*, 1999). These authors reported that the average contents of lysine, threonine, cystine and methionine in twelve sunflower genotypes were 3.72, 3.65, 1.63 and 2.33 g/16 gN, respectively. Glutamic acid was the amino acid present at the highest concentration (19.18 g/16 gN).

The purpose of the present study was to employ analytical methods to determine differences among investigated sunflower genotypes and whether the analyzed hybrids could be sources of specific proteins. A more detailed knowledge of the variability of protein and protein subunit accumulation among ZP sunflower genotypes could facilitate ongoing efforts to improve both quantity and quality of sunflower protein. Seed storage proteins may be used to determine the cultivar trueness and genetic purity of the sample.

MATERIAL AND METHODS

Plant material

The three oilseed hybrids of sunflower (*Helianthus annuus* L.) selected for this investigation were Es Petunija, Allium and Albatre. All three are adapted to European conditions and have been developed in cooperation between the Maize Research Institute, Zemun Polje, (MRIZP), Serbia and the French agribusiness cooperative *Euralis*. The three hybrids all have a high yield potential and are characterized by high tolerance to lodging and *Phomopsis* spp. The hybrid Allium has significant tolerance of *Sclerotinia* spp, while Albatre, as a stay-green hybrid, is tol-

erant of drought. Seeds were collected at full maturity from plants grown in a field-trial at the MRIZP during the 2009 growing season.

The defatted 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. Defatting was carried out by diethyl ether extraction at 35°C in a Soxhlet extractor.

Albumin, globulin, prolamin and glutelin contents

Different protein fractions were obtained by successive extractions of defatted sunflower flour with a series of solvents (in a ratio 1:10 w/v) according to a modified Landry and Moureaux (1970) method. Distilled water, 0.5 M NaCl, 70% ethanol, and 0.2 M NaOH were used to extract albumin, globulin, prolamin, and glutelin fractions, respectively. Extraction of each protein fraction was done by repeated stirring tree times for 30 min at 4^{o}C, followed by centrifugation at 20,000 g for 15 min. Protein content was calculated in each fraction from the nitrogen content determined by the micro Kjeldahl method using 5.50 as the conversion factor. The results are given as percentage of dry matter (d.m.) as well as percentage of total protein (protein solubility index-NSI).

Sodium dodecyl sulfate-polyacrilamide gel electrophoresis

Soluble protein composition of the defatted samples was detected by the sodium dodecyl sulfate-polyacrilamide gel electrophoresis (SDS-PAGE) performed according to Fling and Gregerson (1986) on 12.5% gel in vertical electrophoretic unit (LKB, Sweden). Defatted flour was extracted for 120 min at room temperature with Tris-HCl buffer pH 8.0 in the ratio 1:20 and centrifuged at 17,000 g for 15 min (Thanh and Shibasaki, 1976). The protein content in the supernatant was determined according to the method of Bradford (1976) using bovine serum protein (BSA, Sigma, USA) as a standard. Prior to electrophoresis, soluble proteins have been diluted to 2 mg/cm^3 with the sample buffer (0.055 M Tris-HCl, pH 6.8, 2% (w/v) sodium dodecyl sulfate (SDS), 7% (v/v) glycerol, 4.3% (v/v) β-mercaptoethanol, 0.0025% (w/v) bromophenol blue), heated at 90°C for 5 min and cooled at room temperature. Twenty five μl sample was loaded per well. Gels were run at 30 mA for 6 hours, fixed and stained with 0.23% (w/v) Coomassie Blue R-250 dissolved in 3.9% (w/v) trichloroacetic acid (TCA), 6% (v/v) acetic acid and 17% (v/v) methanol for 45 min. Destaining was performed with 8% acetic acid and 18% (v/v) ethanol. Molecular weights of the polypeptides were estimated by using low molecular weight standards (Pharmacia, Sweden): phosphorylase B (94.0 kDa), bovine albumin (67.0 kDa), ovalbumin (43.0 kDa), carbonic anhydrase (30.0 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa). The protein bands on the destined gel were quantitated using SigmaGel software version 1.1 (Jandal, San Rafael, CA).

The kernel/achene ratios were 3.02, 2.76 and 2.62 in the sunflower hybrids Es Petunija, Allium and Albatre, respectively. However, the content of protein frac-

tions, subunits and different polypeptides are expressed as percentage of total extracted protein. Therefore, our data are a result of differences among kernel and seed protein, not of kernel to achene ratio.

Statistical analyses

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

RESULTS AND DISCUSSION

Sunflower protein applications refer mainly to the fortification of foods by sunflower meal, especially meat and milk products, infant formulae, bakery products, and pasta products.

Data in Table 1 indicate that no significant differences in total protein content were detected among either the seeds or the kernels of the sunflower genotypes analyzed ($P<0.05$). The results showed that the total protein content was significantly higher (by about 42%) in the kernels than in the seeds of the sunflower hybrids studied. The content of total proteins was 25.03, 23.84, and 23.84% in the seeds and 43.42, 43.02, and 42.73% in the kernels of the hybrids Es Petunija, Allium and Albatre, respectively.

Table 1: The content of soluble protein fractions in defatted sunflower flour. The results are presented as % of dry matter (1) and % of total protein (2)

Hybrids	Protein	Extracting solution							
		Water		NaCl		C_2H_5OH		NaOH	
	(1)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Es Petunia (seed)	25.03^{c}	10.98^{d}	43.87^{a}	3.08^{d}	12.30^{e}	0.24^{b}	0.96^{bc}	4.62^{e}	18.45^{c}
Allium (seed)	23.84^{d}	9.48^{e}	39.77^{c}	3.28^{d}	13.76^{d}	0.38^{a}	1.61^{a}	5.59^{c}	23.32^{a}
Albarte (seed)	23.84^{d}	9.32^{f}	39.09^{d}	2.80^{d}	11.74^{e}	0.24^{b}	1.01^{b}	4.15^{f}	17.41^{d}
Es Petunia (kernel)	43.43^{a}	18.60^{a}	42.83^{b}	8.75^{c}	20.14^{c}	0.36^{a}	0.77^{c}	5.35^{d}	12.32^{f}
Allium (kernel)	43.02^{ab}	16.58^{c}	38.52^{e}	10.7^{a}	24.87^{a}	0.34^{a}	0.84^{bc}	9.68^{a}	22.50^{b}
Albatre (kernel)	42.73^{b}	18.24^{b}	42.69^{b}	9.89^{b}	23.14^{b}	0.36^{a}	0.84^{bc}	7.05^{b}	16.50^{e}
$LSD_{0.05}$	0.486	0.047	0.141	0.520	0.667	0.047	0.199	0.176	0.518

$^{a-f}$ Means followed by the same letter within the same row are not significantly different ($P<0.05$)

Water soluble proteins, determined by the Landry and Moureaux (1970) method, were the dominant protein fraction in all the genotypes. The NSI of water soluble proteins was 43.87% in the whole seed of the hybrid Es Petunija. In the other sunflower samples, the content of water soluble proteins was lower by 2.5-12.2%, ranging from 42.83% in the kernel of the hybrid Es Petunija to 38.52% in the kernel of the hybrid Allium (Table 1). The salt soluble protein content was significantly higher in the kernels than in the seeds of the hybrids ($P<0.05$). The ker-

nel of the sunflower Allium had the highest content of salt soluble proteins by a significant margin (24.87% of the total protein content). The lowest content of salt soluble proteins was found in the seed of the hybrid Albatre (11.74% of the total protein content). The protein fraction with the lowest NSI (0.77-1.61%) was soluble in alcohol in all analyzed samples (Table 1). The NSI of base soluble proteins was very high in all analyzed samples and ranged from 16.50% in the kernel of Albatre to 23.32% in the seed of Allium. Considering that most water soluble proteins are also soluble in salt solution, the literature notes the helianthinin fraction as the major storage protein component in sunflower that accounts for approximately 40 to 90% of the total storage proteins in sunflower seed (González-Pérez and Vereijken, 2007).

Table 2: The polypeptides composition in seed and kernel of sunflower hybrids (% per total soluble proteins)

Polypeptides	Whole seed			Kernel			$LSD_{0.05}$
	Es Petunia	Allium	Albarte	Es Petunia	Allium	Albarte	
94,500 Da	0.57^{b}	1.01^{a}	0.40^{c}	1.01^{a}	1.04^{a}	0.97^{a}	0.124
89,500 Da	0.15^{e}	0.66^{c}	0.26^{d}	0.95^{b}	1.56^{a}	0.67^{c}	0.047
66,970 Da	1.11^{c}	1.63^{a}	1.29^{b}	0.72^{f}	0.97^{d}	0.88^{e}	0.081
58,850-63,300 Da	1.71^{e}	2.22^{bc}	2.92^{a}	1.97^{d}	2.38^{b}	2.08^{cd}	0.235
49,800-54,800 Da	3.61^{ab}	3.22^{cd}	3.77^{a}	3.08^{d}	3.41^{bc}	3.30^{bcd}	0.300
46,200 Da	3.08^{a}	2.43^{c}	2.77^{b}	1.53^{e}	2.13^{cd}	2.26^{c}	0.271
42,900 Da	0.58^{d}	n.d.	1.30^{b}	1.54^{a}	1.39^{b}	1.12^{c}	0.110
	n.d.	1.06^{a}	0.90^{b}	0.90^{b}	0.72^{c}	0.92^{b}	0.047
	n.d.	0.75^{a}	0.73^{ab}	0.77^{a}	0.63^{c}	n.d.	0.032
36,800-38,180 Da	9.02^{a}	7.62^{d}	8.19^{c}	7.52^{d}	8.82^{ab}	8.55^{b}	0.332
35,300 Da	9.19^{b}	7.34^{d}	9.64^{a}	7.70^{d}	7.54^{d}	8.35^{c}	0.395
33,800 Da	3.54^{cd}	2.96^{e}	3.24^{de}	3.77^{bc}	4.26^{a}	3.88^{b}	0.300
	n.d	1.52^{a}	0.79^{c}	n.d.	n.d.	0.94^{b}	0.039
32,950 Da	3.20^{a}	1.33^{d}	1.11^{e}	3.07^{ab}	3.00^{b}	2.35^{c}	0.176
31,600 Da	8.74^{a}	8.29^{b}	8.66^{ab}	7.13^{d}	6.78^{d}	7.72^{c}	0.417
	2.39^{a}	1.98^{b}	n.d.	1.97^{b}	1.96^{b}	n.d	0.135
29,600 Da	2.27^{c}	2.99^{b}	3.26^{b}	1.60^{d}	n.d.	3.67^{a}	0.369
25,100-25,800 Da	15.89^{a}	14.00^{c}	14.80^{b}	14.87^{b}	11.39^{e}	12.72^{d}	0.748
23,600 Da	5.13^{a}	4.84^{a}	4.08^{b}	3.96^{b}	4.16^{b}	3.84^{b}	0.565
22,800 Da	5.54^{a}	5.00^{c}	5.69^{a}	4.34^{d}	4.23^{d}	5.23^{b}	0199
	2.21^{bc}	2.07^{c}	2.49^{a}	2.37^{ab}	2.48^{a}	2.07^{c}	0.188
20,100 Da	6.88^{b}	6.32^{c}	6.05^{c}	6.83^{b}	7.54^{a}	7.39^{a}	0.412
	n.d.	1.32^{b}	1.12^{c}	1.47^{a}	1.21^{c}	1.49^{a}	0.089
	1.75^{d}	2.11^{b}	1.76^{d}	2.04^{bc}	5.35^{a}	1.77^{cd}	0.289
16,800 Da	2.24^{e}	3.25^{cd}	3.12^{d}	3.67^{c}	7.97^{a}	4.64^{b}	0.499
14,800 Da	6.60^{c}	7.57^{d}	8.10^{b}	8.31^{b}	1.61^{e}	9.10^{a}	0.503
11,500 Da	3.99^{ab}	3.68^{ab}	3.55^{b}	4.14^{a}	3.89^{ab}	4.08^{a}	0.469

$^{a-f}$ Means followed by the same letter within the same row are not significantly different ($P<0.05$), n.d.- not detected

To identify variants of storage proteins in sunflower seeds and kernels, protein extracts were analyzed by SDS-PAGE. Electrophoresis patterns of total proteins from the analyzed genotypes are shown in Figure 1. The content of total extracted proteins, determined by the Bradford (1976) method, was 7.49, 6.53, and 5.44 mg/ml in the seeds and 10.04, 7.62, and 7.71 mg/ml in the kernels of the hybrids Es Petunija, Allium and Albatre, respectively. However, 25 μl of the solution containing 50 μg of protein were loaded per well. The quantification of the 11S and 2S fractions and the other detected polypeptides was done by densitometric analysis of the gel protein fraction (Table 2). The SDS-PAGE profiles of the seed and kernel proteins in the crude extracts for all the genotypes showed a very similar number of protein bands (thirty two) in the electrophoretograms (Figure 1). The highest molecular weight protein observed in the seed and kernel was 94,500 Da and the lowest 11,500 Da. Jiang *et al.* (1994) reported that sunflower proteins showed bands at 21,000-57,000 Da.

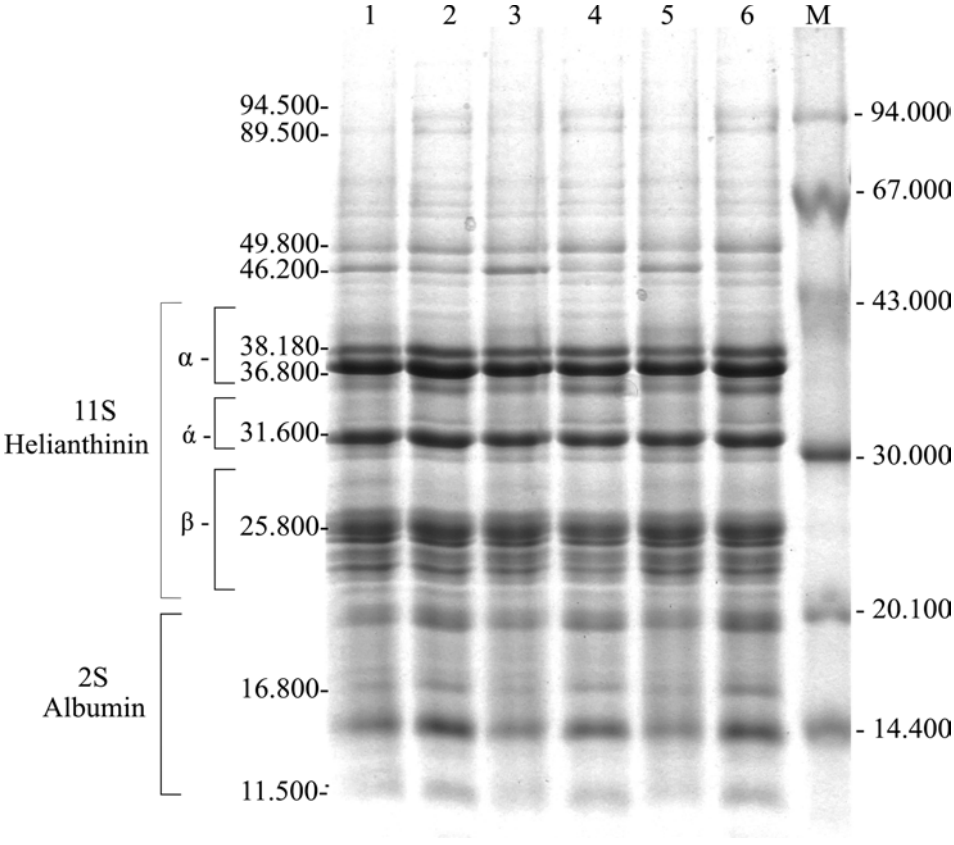

Figure 1: SDS-PAGE patterns of proteins from sunflower hybrids. α', α and β indicate acidic and basic polypeptides of 11S helianthinin, respectively.
1-Albatre (seed), 2-Albatre (kernel), 3-Es Petunija (seed), 4-Es Petunija (kernel), 5-Allium (seed), 6-Allium (kernel), M-molecular weight standards

A treatment with the reduction agent β-mercaptoethanol induced dissociation of the helianthinin fraction to polypeptides whose spectrum included three groups of components, two of which were acidic (α, Mw=36,800-42,900 Da and α', Mw=31,000-35,300 Da) and one basic (β, Mw=21,000-29,600 Da) (Figure 1). The results of Anisimova *et al.* (2004) showed that the standard helianthinin spectrum included eight to ten major components and a number of minor variants. Association and dissociation phenomena are a common feature of many 11S seed globulins (Marcone, 1999). Like other 11S seed globulins, helianthinin seems to dissociate into subunits according to the following scheme: 11S → 7S → 3-2S (González-Pérez *et al.*, 2004). The molecular weight of the 2S albumin proteins ranged from 11,500 to 20,100 Da (Figure 1). According to Kortt *et al.* (1991) the sunflower 2S protein fraction contains at least eight distinct proteins, and two of these are sulfur-rich with 8% of residues cysteine and 16% methionine.

Besides major protein subunits, polypeptides with molecular weight 94.5 kDa, 89.5 kDa, 66.97 kDa, 58.85 to 63.3 kDa, 49.8 to 54.8 kDa and 46.2 kDa were detected by SDS-PAGE in all analyzed samples. These polypeptides could be a result of partial helianthinin dissociation (Figure 1, Table 2).

According to our results, there were significant differences among the seed and kernel protein contents. In our study, the 2S albumin content was significantly higher in kernels than in seeds of sunflower hybrids ($P<0.05$). The kernels of Albatre had the highest content of 2S protein (28.48% of the total extracted protein) by a significant margin. In the seed, the 2S content ranged from 21.46 to 24.50% of the total extracted proteins (Table 3). In all analyzed samples, the content of 11S protein was more than two times higher than that of 2S protein. In contrast with 2S albumin, 11S helianthinin content was significantly higher in seeds than in kernels of the sunflower hybrids ($P<0.05$). The 11S content ranged from 61.75 to 67.70% of the total extracted proteins in the seeds and from 57.36 to 61.51% in the kernels of the hybrids. Also, the content of total acid and basic subunits of helianthinin was significantly higher in the seeds than in the kernels of the hybrids ($P<0.05$). Among the detected proteins, acidic (α + α') subunits of 11S protein were dominant and ranged from 32.85 to 36.66% of the total extracted proteins in the seeds and from 33.83 to 35.10% in the kernels of the hybrids. Of particular note was hybrid Allium, which had a significantly higher content of acid (α + α') subunit in the kernel than in the whole seed (35.10% of total extracted proteins, as opposed to 32.85%) ($P<0.05$). Also, this genotype had the lowest content of basic (β) subunit of helianthinin in the kernel (22.26% of total extracted proteins) (Table 3).

Variations were found in the mean protein ratio of 11S and 2S proteins among the seeds and kernels of the hybrids under study. The ratio of 11S/2S proteins varied from 2.08 to 3.15 among the genotypes (Table 3). According to Mazhar *et al.* (1998), the mean 11S helianthinin to 2S albumin ratio was 2:1. This ratio is known to influence the protein quality of sunflower and greatly affects the functional properties of food products made from sunflower (González-Pérez *et al.*, 2005). Kortt *et*

al. (1991) reported that the sunflower 2S proteins are resistant to degradation by rumen bacteria. This indicates that these sulfur-rich proteins may be ideal candidates for improving the nutritive content (with respect to the sulfur amino acids) of the seed and vegetative tissue of plants destined for ruminant feeding. However, sunflower seed has been responsible for serious anaphylactic reactions in some allergic individuals. The nature of the major allergen remains unknown, but some IgE-binding proteins have been identified, including the 2S methionine-rich albumin protein (SSA). Preliminary findings indicated the possibility that SSA possesses linear epitopes (Kelly and Hefle, 2000). The globulins (helianthinin) have typically been obtained as a secondary by-product from the processing of the seed. The protein isolates in human foods continue to be used as a meat substitute in products such as hamburgers and sausages and have also been used as whipping agents, emulsifiers and binding agents in a variety of food products such as bakery products and dairy analogues in order to replace more expensive animal-based protein ingredients derived from eggs and milk (Fukushima, 1991).

Table 3: Concentration of 2S and 11S fractions in seed and kernel of sunflower hybrids (% per total soluble proteins)

Polypeptides	Whole seed			Kernel			$LSD_{0.05}$
	Es Petunia	Allium	Albarte	Es Petunia	Allium	Albarte	
Total 2S (Albumin)	21.46^{f}	24.25^{d}	23.70^{e}	26.46^{c}	27.54^{b}	28.47^{a}	0.248
Total 11S (Helianthinin)	67.70^{a}	61.75^{c}	64.88^{b}	61.51^{c}	57.36^{d}	61.36^{c}	0.427
Total acid subunit of helianthinin ($\alpha + \alpha'$)	36.66^{a}	32.85^{e}	34.56^{c}	34.37^{c}	35.10^{b}	33.83^{d}	0.437
Total basic subunit of helianthinin (β)	31.04^{a}	28.90^{c}	30.32^{b}	27.14^{d}	22.26^{e}	27.53^{d}	0.395
11S/2S ratio	3.15^{a}	2.55^{c}	2.74^{b}	2.32^{d}	2.08^{e}	2.15^{e}	0.141

$^{a-e}$ Means followed by the same letter within the same row are not significantly different ($P<0.05$)

There is not much data in the literature on the content of sunflower protein fractions, so it was difficult to compare our results. Different problems arose during the isolation and purification step of the major protein fraction of sunflower seeds (Durante *et al.*, 1989). One of the reasons was the presence of relatively high amounts of phenolic compounds, especially chlorogenic acid. Phenolic compounds interact and form complexes with proteins, thereby reducing both their digestibility and their functionality (Sastry and Rao, 1990). Many methods have been proposed for isolating sunflower protein and removing phenolic compounds from sunflower seeds. Also, one of the main factors that has complicated the purification and characterization of globulins (helianthinin) is that they are known to dissociate with very small shifts in pH (Wright, 1987). It therefore appears that pH dependent dissociation of globulins is a common physicochemical characteristic.

Our data provide increased knowledge of the sunflower proteins.

CONCLUSION

Currently, limited information is available on the biochemical and genetic mechanisms that regulate high-proteins. Essentially, two avenues of improving sunflower protein have to be utilized. The first is through traditional breeding using high-protein germplasm and the second is the use of biotechnology.

The results showed that the protein bands were similar among all the sunflower samples. Three polypeptides groups of helianthinin fraction were detected. Two of these were acidic (α, Mw=36,800-42,900 Da and α', Mw=31,000-35,300 Da), while one was basic (β, Mw=21,000-29,600 Da). The molecular weight of the 2S albumin proteins ranged from 11,500 to 20,100 Da.

However, the contents of 2S and 11S proteins were statistically different among the sunflower hybrids as well as among the seeds and kernels of sunflower. The 2S albumin content was significantly higher in kernels than in seeds of sunflower hybrids. In contrast, the 11S helianthinin content was significantly higher in seeds than in kernels of the hybrids.

The highest content of 2S albumin was found in the kernel of the hybrid Albatre, while the highest content of 11S helianthinin was observed in the seed of the hybrid Es Petunija. The latter sample had the highest content of acid and basic subunits of 11S helianthinin.

Our data provide increased knowledge of the variability of protein and protein subunit accumulation among Serbian hybrids, which will facilitate ongoing efforts to improve both the quantity and quality of sunflower protein.

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