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Amino acid composition and solubility of proteins isolated from sunflower meal produced in Bulgaria

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Abstract

Two protein isolates (PI1 and PI2) were prepared from sunflower meal produced in Bulgaria. They were obtained by using isoelectric or ethanol precipitation following the extraction of the proteins with 10% NaCl. Their amino acid composition and water solubility as a function of pH and presence of NaCl were investigated. Lysine was established as the first limiting amino acid in the protein isolates with amino acid scores of 35.81% (PI1) and 43.09% (PI2). Both protein isolates contained relatively high amounts of sulfur-containing amino acids with amino acid scores of 99.14%. Arginine reached 8.45 and 9.75 g/100 g protein in PI2 and PI1 respectively. Being obtained by isoelectric precipitation, PI1 was highly soluble in either acidic (pH ≤ 3.5) or alkaline (pH ≥ 7) medium. In contrast, PI2 was insoluble for each pH value below 5.5. The addition of 0.03 M NaCl did not change the solubility pattern of either PI1 or PI2. However, the presence of 0.25 M NaCl diminished the solubility of the protein isolates for the entire pH area from 2 to 8.5. PI1 and PI2 may serve as a valuable source of sulfur-containing amino acids and arginine to complement human diets deficient in these specific amino acids.

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Introduction

Sunflower meal is a by-product obtained after oil extraction from sunflower seeds. It contains relatively high amount of protein (30-50%) which may reach 66% depending on the efficiency of seed dehulling and defatting processes (Bau *et al*., 1983; Dorrell and Vick, 1997). Sunflower proteins are characterized with high nutritive value. They do not contain anti-nutritional compounds and exhibit well-balanced amino acid composition with the except for lysine which is the first limiting amino acid (Gassmann, 1983; González-Pérez and Vereijken, 2007). Sunflower proteins are rich in sulfur-containing amino acids which, in general, are deficient in most proteins with plant origin (Gassman, 1983; Ribarova *et al*., 1987; Canibe *et al.*, 1999).

Sunflower seeds contain 2 major protein groups, namely globulins and albumins. Sunflower albumins have low molecular weight and high solubility, which do not depend on pH and ionic strength of the solutions. The globulins, known as helianthinin, consist of oligomers with molecular weight from 300 to 350 kDa (11S globulins) which may dissociate to either oligomers with lower molecular weight (7S) or monomers (2S-3S). The dissociation and association of the helianthinin depends on pH, temperature and ionic strength of the solution (Sripad and Narasinga Rao, 1987; González-Pérez *et al.*, 2004). At low ionic strength, water solubility of the globulins is at minimum at their isoelectric points ranging from pH 4.0 to pH 6.0. At high ionic strength of the solution, the helianthinin is almost insoluble in acidic medium (Canella *et al*., 1985; Rossi *et al*., 1985; Vermeesch *et al*., 1987; González-Pérez *et al*., 2004, 2008).

The amino acid composition and solubility of the proteins in the sunflower meal, remaining after oil extraction, may vary and depend on the pre-treatment of the sunflower seeds and the procedure used for the oil production. Elevated temperature, which is frequently used to increase oil yield, favors protein denaturation and the formation of insoluble protein fraction (Lusas, 1985). In addition, it facilitates the protein interaction with other plant components leading to dark colored products which worsen the functional properties of the proteins (Parrado *et al*., 1993; Moure *et al*., 2006; González-Pérez and Vereijken, 2008; Salgado *et al.*, 2011).

Most of the studies related to sunflower protein isolates have been performed after defattining of the seeds under mild laboratory conditions where no change of the native structure and functionality of the proteins occurred. However, the industrial procedure of oil extraction from sunflower seeds may affect the amino acid composition, solubility and functional properties of the respective proteins. The protein isolates from industrially obtained sunflower meal, however, are of a higher practical interest since it is an alternative approach for a better and more complete use of this by-product for food application. For the

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purpose of their practical utilization, it is important to know the conditions which may affect the functional properties of the proteins. The purpose of this research was to establish the amino acid composition and water solubility of protein isolates, which were prepared from industrially produced sunflower meal, as a function of pH and NaCl concentrations. It was a continuation of a previous study where the optimal conditions for the extraction of the proteins from the same protein source have been established (Ivanova *et al*., 2011, 2012).

Materials and Methods

Preparation of protein isolates

Protein isolates were obtained from sunflower meal, provided by a local oil factory, after extraction with 10% NaCl as described by Ivanova *et al.* (2011, 2012). Protein isolate 1 (PI1) was prepared by isoelectric precipitation of the extracted proteins. After adjusting pH to 2.5, the protein precipitate was collected by centrifugation (6000 rpm), washed twice with HCl (pH 2.5) and dried by lyophilization. Protein isolate 2 (PI2) was obtained by addition of 96% ethanol to the sunflower protein extract. The precipitate was collected by centrifugation (6000 rpm), washed twice with 96% ethanol and dried at 40°C.

Amino acid analysis

Amino acid composition of protein isolates was determined by using an amino acid analyzer (T 339 M, Praha Czechoslovakia) after acidic hydrolysis with 6 N HCI at 105°C for 24 h. To determine sulfur-containing amino acids, the acidic hydrolysis of the protein isolate samples was preceded by a treatment with H_2O_2 and formic acid at 4°C for 12 h (BNS 11374, 1986; Amarakoon, 2012). Amino acid score was calculated as a ratio of the amount of each essential amino acid determined in a protein isolate and the amount of the respective amino acid in an "ideal" protein as stated by the Food and Agriculture Organization of the United Nations (FAO) and expressed in percent (FAO, 1970).

Solubility of protein isolates as a function of pH and NaCl concentrations

Solubility of the protein isolates was determined as described by González-Pérez (2003) with some modifications. Proteins were dispersed in water to a final concentration of 4 mg/ml. Variations in NaCl concentrations were achieved by adding NaCl. The influence of pH on protein solubility was tested by varying pH from 2 to 8.5 with an increment of 0.5 by using NaOH or HCl. After 2 h at room temperature, samples were centrifuged (6 000 rpm) for 15 min and supernatants were collected. Protein solubility was calculated as a ratio of the amount of the protein in a supernatant as determined by Biuret method (AACC, 1983) and the initial concentration of the protein used in the test system (4 mg/ml). The result was multiplied by 100 to express in percent. Complete solubility (100%) was assumed when no residue was observed after centrifugation. All experiments were performed in triplicates. Each datum on Figures represents an average mean of the results generated from three independent experiments ± standard deviations.

Results and Discussion

Amino acid composition of protein isolates

Numerous methods for preparation of protein isolates have been published. They include but are not limited to utilization of organic solvents, salts or/and reducing agents, and isoelectric precipitation (Rahma *et al*., 1981; Saeed *et al*., 1988; Pickardt *et al.*, 2009). Ethanol and isoelctric precipitation were chosen to prepare the sunflower protein isolates because of their potential practical application and advantages related to protein recovery and reduced protein denaturation (Fan *et al.*, 1976; Taha *et al*., 1981; Hewedi *et al.*, 1985).

Amino acid composition of protein isolates is a measure of their nutritive value. The concentrations of the amino acids in PI1 and PI2 did not differ from each other considerably (Table 1). It implies that the two approaches, namely ethanol and isoelectric precipitation, which were used for the preparation of the protein isolates, did not affect the amino acid profiles of the proteins. The amino acid contents of both protein isolates were comparable to those in the sunflower meal, used as the protein source in the study, with the except for lysine (Table 1, Ivanova *et al*., 2012). The concentrations of lysine in PI1 and PI2 did not exceed 1.97 g/100 g and 2.37 g/100 g protein respectively (Table 1), while the amount of this amino acid in the sunflower meal was determined to be 3.55 g/100 g protein (Ivanova *et al*., 2012). The decrease of the lysine amounts in the protein isolates is probably due to the interaction of the respective amino acid with other plant components during oil processing. As a result, lysine radicals form complex compounds which make the corresponding proteins insoluble in salt solution and slightly acidic medium which were used for the preparation of the protein isolates in our study. San Juan and Villamide (2001) who investigated the nutritional value of sunflower by-products also observed that lysine was the amino

Table 1. Amino acid composition of sunflower protein isolates

Amino acids	"Ideal" protein*	Protein isolate 1		Protein isolate 2	
	Amino acid, g/100 g protein	Amino acid, g/100 g protein	Amino acid score, %	Amino acid, g/100 g protein	Amino acid score, %
Essential amino acids					
Lys	5.5	1.97	35.81	2.37	43.09
Met+Cys	3.5	3.47	99.14	3.47	99.14
Thr	4.0	3.39	84.75	3.13	78.25
Ile	4.0	3.16	79.00	2.61	65.25
Leu	7.0	6.18	88.29	5.64	80.57
Phe+Tyr	6.0	8.56	142.67	7.86	131.00
Val	5.0	3.58	71.60	3.16	63.20
Trp	1.0	ND**		ND**	
Nonessential amino acids					
Asp		10.50		9.76	
Ser		4.50		5.33	
Glu		26.91		25.39	
Pro		5.11		5.65	
Gly		4.88		8.34	
Ala		4.98		5.46	
His		3.06		3.38	
Arg		9.75		8.45	

*Amino acid composition of an "ideal" protein as published by FAO (1970)
**Not determined

acid to be affected by sunflower oil processing the most. Valine and isoleucine appeared to be the second and third limiting amino acids in our protein isolates as the decreases in the amino acid scores for PI2 (63.20% and 65.25%) was more prominent than for PI1 (71.60% and 79.00%) when compared to the respective amino acids in the sunflower seeds (115.40% and 119.25%) (Ribarova *et al*., 1987).

Despite of the deficiency of lysine, valine and isoleucine, PI1 and PI2 have the potential to be used in food industry because of their relatively high amount of sulfur-containing amino acids (Table 1). Sulfur-containing amino acids i.e. methionine and cystine limit the nutritive value of numerous plant proteins. A study on protein and amino acid composition of wild and cultivated genotypes of Phaseolus species by Baudoin and Maquet (1999) implied that both plant protein sources should be mixed with other plant species which are rich in methionine and cystine to overcome the deficiency of these amino acids. According to Burstin *et al.* (2011), legume seeds such as pea, fababean, lentil and cowpea, which are commonly used in human nutrition, should be combined with other plant species to balance the diet with respect to methionine and cystine. In addition, both protein isolates exhibited relatively high amounts of arginine which in PI1 and PI2 reached 9.75 and 8.45 g/100 g protein respectively (Table 1). Arginine is considered a semi-essential amino acid for humans and participates in numerous biochemical processes such as ammonia detoxification, hormone secretion, and immune modulation (Appleton, 2002). Humans can synthesize it de novo but there is no compensatory mechanism in case of depletion and dietary supplementation appears to be the way to replenish the plasma concentrations of this amino acid (Castillo *et al.*, 1994). Although containing relatively small amounts of histidine, PI1 (3.06 g/100 g protein) and PI2 (3.38 g/100 g protein) may also potentially serve as a source of this amino acid which is considered essential for children. If compared to data on amino acids in Bulgarian food products which were investigated by Ribarova *et al.* (1987), the levels of arginine and histidine in PI1 (9.75 and 3.06 g/100 g protein) and PI2 (8.45 and 3.38 g/100 g protein) exceeded the respective amino acids in egg protein (6.09 and 2.4 g/100 g protein) and milk (3.28 and 2.45 g/100 g protein).

Solubility of PI1 and PI2 as a function of pH and NaCl concentrations

Solubility of proteins depends on numerous factors such as amino acid composition, pH, presence of salts, interaction with other matrix components and denaturation (Kinsela *et al.*, 1985). PI1 and PI2 did not differ substantially in their amino acid composition (Table 1). Therefore, the amino acid profile of both isolates would not be expected to be a dominant factor influencing their solubility. According to Damodaran (1994), the solubility of proteins is highly dependent on their conformation. By studying the conformational states of sunflower proteins, González-Pérez *et al.* (2004) demonstrated that helianthinin conformation was modulated by pH, salt concentrations and thermal denaturation.

The influence of pH on the solubility of PI1 and PI2 is presented on Figure 1. Both isolates exhibited similar pattern of solubility at pH $\geq$ 4. PI1 was insoluble in pH ranging from 4.0 to 5.5. Out of this range, the solubility gradually increased by reaching maximum values at pH 7 and pH 2. The solubility pattern exhibited by PI1 as a function of pH is similar to the solubility of helianthinin and other sunflower protein isolates at low ionic strength (Rossi *et al.*,

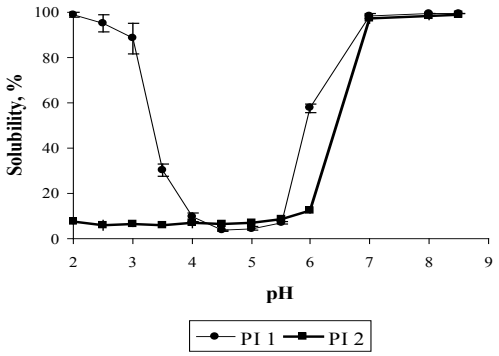

Figure 1. Influence of pH on solubility of protein isolate 1 (PI1) and protein isolate 2 (PI2)

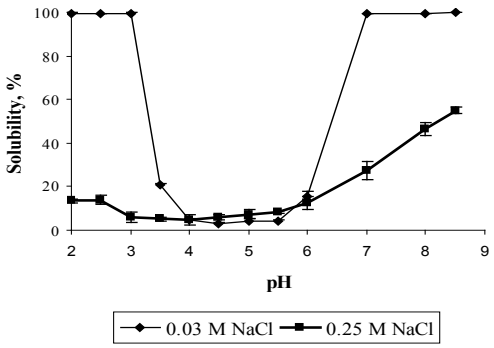

Figure 2. Influence of pH on solubility of protein isolate 1 in the presence of different concentrations of NaCl

1985; Vermeesch *et al.*, 1987; Saeed and Cheryan, 1988; González-Pérez, 2003; González-Pérez *et al.,* 2004). PI2 was almost insoluble at pH ≤ 5.5 but reached its complete solubility at pH ≥ 7. Similar behavior of solubility of sunflower protein isolates was observed by González-Pérez (2003) when 250 mM NaCl were added to protein suspensions. The data on PI1 and PI2 solubility presented on Figure 1 imply that in addition to pH other factor(s) which modulate their solubility may exist. The differences in solubility pattern of PI1 and PI2 are probably due to their different chemical composition. PI1 was characterized with higher protein content (94.25%) than PI2 (75.34%) (Ivanova *et al.*, 2011). However, PI2 exhibited approximately 10-fold higher ash content (13.26%) than PI1 (1.34%) which probably influenced the lower solubility of this isolate at pH ≤ 5.5.

Numerous studies have been published on sunflower proteins. However, most of them are related to undenatured helianthinin and protein isolates (Canella, 1985; Vermeesch, 1987; González-Pérez, 2003). Since industrially produced sunflower meal was used as a protein source in our study, it could be expected that the isolated proteins were pre-denatured at some extend and would have different solubility pattern in salt solutions when compared to undenatured proteins. Two levels of salt concentrations (0.03 and 0.25 M NaCl) were used to investigate the solubility of PI1 and PI2 in the presernce of salts. No change in

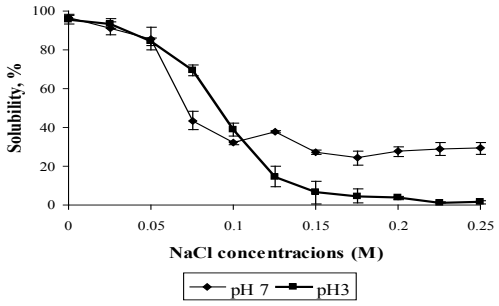

Figure 3. Solubility of protein isolate 1 as a function of different NaCl concentrations at pH 3 and pH 7

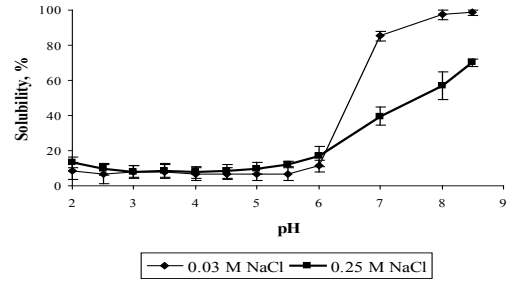

Figure 4. Influence of pH on solubility of protein isolate 2 in the presence of different concentrations of NaCl

the solubility pattern of PI1 after the addition of 0.03 M NaCl occurred (Figure 2). However, the solubility of PI1 highly decreased in the presence of 0.25 M NaCl and reached only 55% of its capacity at pH 8.5. The isolate remained insoluble at each pH value lesser than 6. Since the most profound modulation in the solubility of PI1 occurred at pH 3 and pH 7, it was investigated in more details for both pH values in the presence of NaCl which concentrations were varied from 0 to 0.25 M with an increment of 0.05 (Figure 3). The results indicated that relatively low concentrations of NaCl limited the solubility of PI1 as more noticeable changes occurred in the range from 0.05 to 0.15 M NaCl. At 0.15 M NaCl, PI1 exhibited 30% solubility in neutral medium (pH 7) and remained insoluble at pH 3. In contrast, at the same salt concentration, undenatured protein isolates expressed 80% solubility at pH 3 and remained as high as 90% soluble at pH 7 even when higher concentrations of NaCl were added (González-Pérez, 2003). The addition of 0.03 M NaCl did not change substantially the solubility profile of PI2 (Figure 1 and Figure 4). However, the supplementation of the solution with the higher level of NaCl (0.25 M) diminished the maximum solubility of the isolate with approximately 25%.

As a whole, the solubility of both protein isolates was influenced by the addition of NaCl. Even relatively small amounts of salts decreased the solubility of PI1 at pH 3 as well as pH 7. It is probably due to the partial denaturation of the proteins in the sunflower meal that was used for the preparation of the protein isolates. Elevated temperature used during

oil extraction and the production of sunflower meal probably exerted denaturation effect on sunflower seed proteins. Most probably, lysine radicals from the proteins of the sunflower meal interacted with other seed components thus diminishing the positive charge of the protein molecules in acidic medium. Therefore, only small amounts of chloride anions would be sufficient to neutralize the positive charge of the protein molecules after which the latter become capable in aggregating and coagulating. In alkaline medium, the proteins are charged negatively and cations have low affinity to bind (Schnepf, 1992). Thus, salts appear to be less powerful modulator of protein solubility in alkaline medium.

Conclusions

In summary, PI1 and PI2 differed from each other in their solubility profiles, which was probably due to the differences in their chemical contents. The addition of 0.03 M NaCl did not change the solubility pattern of either PI1 or PI2. However, 0.25 M NaCl decreased the solubility of both protein isolates in the entire pH scale ranging from 2 to 8.5. Our study indicated that PI1 and PI2 could be potentially used as valuable additives in food industry due to their amino acid composition. They could supplement human diet with sulfur-containing essential amino acids which, in general, are deficient in most proteins with plant origin. The relatively high amounts of histidine and arginine additionally increased the nutritive value of PI1 and PI2.

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