Bulgarian Journal of Agricultural Science, 18 (No 2) 2012, 153-160
Agricultural Academy

OPTIMIZATION OF PROTEIN EXTRACTION FROM SUNFLOWER MEAL PRODUCED IN BULGARIA

P. IVANOVA[1], V. CHALOVA[1], L. KOLEVA[1], I. PISHTIYSKI[1] and M. PERIFANOVA - NEMSKA[2]
[1]*University of Food Technologies, Department of Biochemistry and Molecular Biology, BG - 4002 Plovdiv, Bulgaria*
[2] *University of Food Technologies, Department of Technology of Tobacco, Sugars, Vegetable and Essential Oils, BG - 4002 Plovdiv, Bulgaria*

Abstract

IVANOVA, P., V. CHALOVA, L. KOLEVA, I. PISHTIYSKI and M. PERIFANOVA – NEMSKA, 2012. Optimization of protein extraction from sunflower meal produced in Bulgaria. *Bulg. J. Agric. Sci.,* 18: 153-160

Sunflower meal obtained after defatting of sunflower seeds is a by-product, which has the potential to serve as an attractive source for the preparation of protein isolates for food purposes. Protein extraction depends on many factors. Sunflower proteins are commonly extracted under alkaline conditions due to their low solubility in mild-acidic and neutral conditions. However, the alkaline medium favors phenol-protein interactions leading to formation of dark-colored products, which are inappropriate for human consumption. The aim of this research was to identify the optimal conditions for protein extraction from sunflower meal produced in Bulgarian oil factory by investigating the effect of NaCl- and sunflower meal concentrations, pH, temperature, and extraction time on protein yield. It was established that increases of pH in the range from 2 to 10 constantly enhanced protein extractability. At pH 5-7, the protein extraction was dependent on NaCl concentrations. Extracting proteins with 7.5% -12.5% NaCl solutions and temperature 40ºC obtained greatest protein yields at pH6. The utilization of 2.5% sunflower meal appeared to be the most appropriate for the achievement of maximum protein extraction. The incremental increases of the sunflower meal from 5% to 15% did not substantially modulate the protein yields. The most efficient extraction occurred during the first 15 min but maximum protein yields were obtained after 60-75 min of extraction. Our study demonstrated that by extracting 10% sunflower meal with 10% NaCl, at pH6 and 40°C for the period of 60-75 min, a protein yield greater than 50% at diminished protein-phenol interaction could be achieved.

Key words: sunflower meal, proteins, extraction

Introduction

Sunflower is one of the most important oilseed crops cultivated in the world with an annual yield reaching 27.0 million tons in 2007 (FAO 2009). EU-27 (22%) and Russia (21%) appeared to be the most contributing countries to worldwide sunflower seed market followed by Ukraine (15%) and

Argentina (13%) (USDA, 2008). In Bulgaria, the production of sunflower seeds increased approximately 3-folds for the period from 2000 to 2008 (MAFS 2009).

Sunflower is predominantly used for extraction of edible oil (Lühs and Friedt, 1994) but is also a valuable source for a biofuel production (Kondili and Kaldellis, 2007). The remains of substantial amounts of sunflower meal accompany either for food or industrial purposes, the oil extraction process. For example, in Brasil, in 2003, 66 000 tons of sunflower grains used for oil extraction purposes resulted in the generation of 23 100 tons of sunflower meal (Carellos et al., 2005). Partially, this byproduct is used in animal nutrition as an alternative protein source. Its high protein content (40%) and relatively balanced amino acid composition make the sunflower meal an attractive supplement in animal diets (Meng and Slominski, 2005; Gandhi et al., 2008). However, its application in animal nutrition is limited by relatively high fiber content (Senkoylu and Dale, 2006**;** Raza et al., 2009). According to Carellos et al. (2005) and Tavernari et al. (2008) sunflower meal inclusion should not exceed 16% and 20% in broiler and swine diets respectively to avoid adverse performance effect. Thus, the overproduction and accumulation of excessive amounts of sunflower by-products may cause environmental problems associated with their disposal.

The application of the sunflower meal as an unconventional protein source for human consumption could be an alternative approach which leads to more complete use of this by-product. Except for lysine deficiency, nutritive value and functional properties of sunflower proteins are comparable to those of soy and other leguminous proteins (González-Pérez et al., 2005; González-Pérez and Vereijken, 2007). Additional advantage of the sunflower proteins is their low contents of anti-nutritional and allergen factors (González-Pérez and Vereijken, 2007). Currently, no genetically modified commercial sunflower varieties are available for field cultivation, which makes sunflower protein isolates useful and safe for the production of organic foods (Cantamutto and Poverene, 2007). Therefore, in addition to solving environmental issues, the more complete utilization of the sunflower meal as a source for inexpensive protein for human needs could also respond to continuously increasing worldwide demand for proteins. However, the optimal isolation of proteins from sunflower meal is very often impeded by decreased protein solubility due to denaturation occurring during oil production.

Materials and Methods

Materials

Sunflower meal was obtained from a local company. It was produced after oil extraction from heat-treated sunflower seeds. All reagents used in the study were of analytical grade and bought from Fillab (Sofia, Bulgaria).

Biochemical characterization of sunflower meal

Total protein content of sunflower meal (N x 5.6) was determined by Kjeldahl method, AACC 46-12 (AACC, 1995). Soluble protein was determined by Biuret method (AACC, 1983) after extraction with 0.4% Na_2CO_3. Bovine serum albumin was used to generate a standard curve. Crude fat content of the sunflower meal was determined by Soxhlet extraction method (ISO 7302:2003). Fiber and ash contents were determined by ICC Standard №156 and ICC Standard №104/1 respectively. The biochemical composition of the sunflower meal was expressed in percent on a dry weight basis.

Influence of NaCl concentrations and pH on protein extractability

A sample of 2.5 g ground sunflower meal was suspended in 40 ml aqueous solution of NaCl. The influence of NaCl on protein extractability was tested for concentrations varying from 5% to 20%

with an increment of 2.5. Each extraction was conducted at different pH values including 2.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 10.0. NaOH or HCl water solutions were used to adjust pH. Samples extracted without the addition of NaCl were used as controls. The extraction was performed for 1 h at room temperature (22°C) with constant agitation. To maintain the tested pH value, pH was measured (Hanna Instruments, Germany GmbH) in 20 minute intervals and re-adjusted if necessary. To quantify extracted protein, sunflower meal suspension was transferred into a 50 ml volumetric flask and leveled to the graduation mark with the respective NaCl solution. Solid particles were removed by paper filtration and the extracted protein was assayed by using Biuret method. Protein yield was calculated as a ratio of extracted and total protein and multiplied by 100 to express in percent. All experiments were performed in duplicates.

Influence of temperature, sunflower meal concentrations and extraction time on protein extractability

After identifying optimal pH and NaCl concentrations, additional experiments were carried out to evaluate the influence of temperature, sunflower meal concentrations, and extraction time on protein extractability. Protein extraction and protein quantification were conducted as described above but at different parameter combinations. The temperature was studied at 20ºC, 30ºC, and 40ºC that were maintained in a water bath shaker (New Brunswick, Edison, NJ, USA). The evaluation was performed at three NaCl concentrations (7.5%, 10.0%, and 12.5%) and two pH values (5 and 6). Meal-to-solvent ratio corresponding to 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, and 20.0% sunflower meal and extraction time varying from 15 to 90 min with an increment of 15 min were studied at pH 6.0 and 10% NaCl solution as a solvent. The extraction time study was performed with suspensions containing 2.5%, 5.0%, and 10.0% sunflower meal.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) program (IBM SPSS Stattistics, Somers, NY, USA). Presented results are averaged means of at least two independent experiments ± standard deviations. Mean differences and between-subject effect were established by one-way analysis of variance (ANOVA) using the general linear model procedure and Duncan's multiple comparison test. Statistical differences were considered significant at $p < 0.05$.

Results and Discussion

Biochemical characteristics of sunflower meal

Biochemical composition of sunflower meal is an important characteristic, which determines its utilization in both food and feed industries. It varies depending on numerous factors including variety differences, growth conditions and applied agricultural practices (Senkoylu and Dale, 1999; Rosa et al., 2009). The sunflower meal used in our study contained high fiber concentration (43.46%) (Table 1). The insoluble fibers (40.8%) greatly exceeded the soluble part (2.66%) which limits the application of the sunflower meal as a feed ingredient in animal nutrition. In contrast, 96% out of the total protein (43.15%) was found soluble in

Table 1
Biochemical characteristics of sunflower meal

Biochemical component	Content. %
Dry matter	92.15
Total protein	43.15
Soluble protein*	41.3
Crude fat	0.76
Fibers:	43.46
· insoluble	40.80
· soluble	2.66
Ash	7.63

* - total protein soluble in 0.4% Na_2CO_3

0.4% Na_2CO_3. The relatively high protein fraction combined with low remains of crude fat (0.76%) demonstrated the potential of the studied sunflower meal to serve as an appropriate source for the preparation of protein isolates.

Influence of NaCl concentrations and pH on protein extractability

The influence of NaCl concentrations and pH on protein extractability from the studied sunflower meal was explored by a series of experiments including various concentrations of NaCl and pH. The protein extraction was conducted at pH ranging from 2 to 10 and NaCl concentrations varying from 0 to 20% at each pH value. The results obtained in our study demonstrated that the protein extractability increased with the increase of pH and reached maximum values in alkaline medium at pH 10 (Figure 1). Similar results were obtained by Gheyasuddin et al. (1970) and Pickardt et al. (2009). It is well known that sunflower seed proteins are mainly consisted of albumins and globulins, and small amounts of insoluble and alkali-soluble fractions. According to González-Pérez et al. (2004), helianthinin, which accounts for approximately 85% of the total proteins in the mature seed, is salt-soluble and is extracted at greatest extent at pH 8.5. However, oil production from sunflower seeds under industrial conditions may be associated with technological steps where changes in the fractional composition of seed proteins occur. For example, decreases in the amounts of albumins and globulins accompanied with an increase in glutelins may be observed as the proportional alternation among fractions depends on the type and the extent of the treatment. According to Marinchevski (1980) impeded by decreased protein solubility due to denaturation occurring during oil, untreated sunflower seeds contained 21.4% water- , 55.4% salt-, and 12.6% alkali-soluble and 10.6% insoluble nitrogen containing fractions. However, the salt-soluble nitrogen-containing fraction in sunflower meal produced after high temperature treatment of the seeds decreased to 15.2%, while alkali-soluble fraction increased to 29.5%. Insoluble protein fraction (41.8%) significantly increased as well. Therefore, the influence of NaCl concentrations and pH on protein extractability observed in our study may be explained by alternation in fractional protein profile caused by seed treatments preceding the industrial production of the sunflower meal.

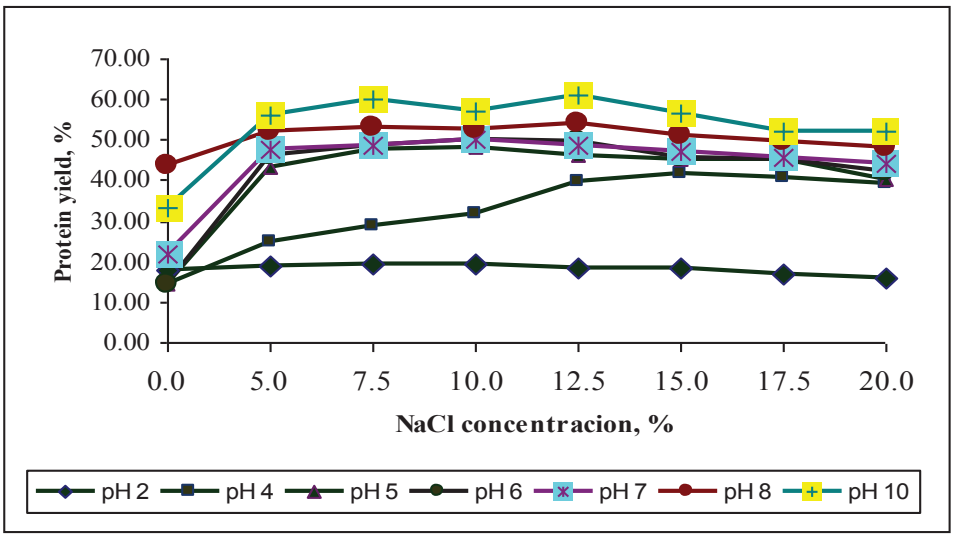

Fig. 1. Effect of NaCl concentrations on protein yield at different pH values

In our study, NaCl concentrations influenced protein extractability by a pH dependent manner (Figure 1). At pH 2, no significant differences in protein yields (20%) at the various NaCl concentrations were observed. At pH 4, protein yield increased with the increase of NaCl concentrations of up to 12.5% after which the protein yield remained unaltered. At the higher pH values, the extraction of the sunflower protein with 7.5% NaCl resulted in greatest yield. Further increases in NaCl concentrations did not affect the protein yield.

Highest protein yield (approximately 60%) was achieved at pH 10 and NaCl concentrations ranging from 7.5 to 12.5%. However, protein extracts obtained at high alkaline pH are dark-colored and inappropriate for human consumption, which is due to the oxidation of phenolic compounds and their interactions with proteins (Synge, 1975; Sahidi and Naczk, 2004). In our study, at pH 10, 35% from the much-extracted phenolic compounds co-precipitated with the proteins, while pH 6 favored the co-precipitation of 20% phenols only (data not given). In addition, higher NaCl concentrations prevent protein-phenol interactions, which lead to the production of higher quality protein, isolates (Pickardt et al., 2009). Although highest protein yield was achieved at pH 10, further experiments were performed at pH 5 and pH 6 in aqua solutions containing 7.5%, 10.0% and 12.5% NaCl to avoid the negative effects of the alkaline extraction. The protein recovery under the chosen conditions was lower (approximately 50%) but light colored protein isolates were obtained. Since the initial pH of the protein suspension was 5.8 ± 0.1, additional pH adjustment was not necessary.

Influence of temperature on protein extractability from sunflower meal

Protein extractability from sunflower meal was studied at 20°C, 30°C и 40°C. Higher temperatures were avoided to prevent further alternations in the fractional composition of the proteins. In addition, according to Marinchevski (1980), temperature increments in the range from 30°C to 60°C had negligible influence on sunflower protein extractability. In our study, for both pH (5 and 6), the protein yields obtained at 20°C and 30°C were not influenced either by temperature or NaCl concentrations (Table 2). Statistical enhancement of the protein yield was observed at 40°C but NaCl concentrations remained insignificant determinants of the protein extractability.

While at pH 6 the protein extraction with 7.5% and 10.0% NaCl reached 53.57% и 52.15% of the total protein content of the sunflower meal, the protein yield at pH 5 did not exceed 50%. Similar increases in protein yields at 40-45°C were reported by Gheyasuddin et al. (1970) and Pickardt et al. (2009).

Influence of sunflower meal concentrations on protein extractability

Optimal ratio between sunflower meal and an extracting agent is important for achieving high protein yield at a low cost of production while

Table 2
Influence of temperature on protein yield

NaCl,%	Protein yield, %					
	pH 5			pH 6		
	20ºC	30ºC	40ºC	20ºC	30ºC	40ºC
7.5	$43.28\pm0.73^{ab,B}$	$42.82\pm1.03^{b,B}$	$49.41\pm0.37^{a,A}$	$44.57\pm0.66^{b,B}$	$46.49\pm0.79^{a,B}$	$53.57\pm1.03^{a,A}$
10.0	$43.65\pm1.01^{a,B}$	$44.35\pm0.80^{a,B}$	$50.00\pm0.68^{a,A}$	$47.25\pm0.30^{a,B}$	$46.99\pm0.84^{a,B}$	$52.15\pm0.81^{ab,A}$
12.5	$42.12\pm0.88^{b,B}$	$42.67\pm0.53^{b,B}$	$49.13\pm1.53^{a,A}$	$45.45\pm1.78^{b,B}$	$42.53\pm0.61^{b,B}$	$50.03\pm2.16^{b,A}$

$^{a-b}$ Means within a column not sharing a common letter are significantly different ($p < 0.05$). $^{A-B}$ Means in a row followed by different capital letters are significantly different ($p < 0.05$).

avoiding a negative environmental impact. High sunflower meal concentrations do not ensure and may even interfere with the efficiency of protein extraction. The utilization of low amounts of sunflower meal for extraction purpose, however, requires large volumes of the extracting agent, which would increase the production cost of protein isolates. The resulting protein extract would be diluted and the subsequent step of precipitation impeded.

In our study, the influence of sunflower meal concentrations was studied in the range from 2.5% to 20.0%, at pH 6, 10.0% NaCl and room temperature. Data were presented in Figure 2. The highest (51.5%) - and the lowest protein yields (41.9%) were obtained at 2.5% and 20% sunflower meal respectively. The incremental increases of the sunflower meal from 5% to 15% did not substantially modulate the protein yields (48.2% to 46.9%). However, the highest protein concentration in the extract (33.35 mg/ml) was calculated when 20% sunflower meal was used, followed by 18.9 mg/ml and 5.12 mg/ml when 10% and 2.5% sunflower meal were extracted respectively. Therefore, the utilization of 10% sunflower meal may be considered appropriate and efficient for protein extraction since both the protein yield and the protein concentration in the extract were considerable. Other reports also recommended 5.0% (Taha et al., 1981; Pickardt et al., 2009) or 10.0% (Gheyasuddin et al., 1970; Marinchevski, 1980) sunflower meal when used for protein isolation purposes.

Influence of extraction time on protein yield

The influence of extraction time on protein yield was monitored for the period from 15 to 90 min with an increment of 15 min and 3 sunflower meal concentrations including 2.5%, 5% and 10% (pH 6, 10% NaCl). The most efficient extraction occurred during the first 15 min as indicated by the protein yields, which consisted approximately 90% of the total extractable proteins under studied conditions (Table 3). Enhancement of the protein yields with 10-12% was observed when the extraction was conducted for 60 min or 75 min where 2.5% and 5.0% or 10.0% sunflower meal were used respectively (Table 3). Marinchevski (1980) reported that 30-40 min were required for maximum extractability of sunflower proteins in alkaline medium, but in acidic medium, the extraction time was extended to 60 min to receive similar results which is close to the data obtained in our study.

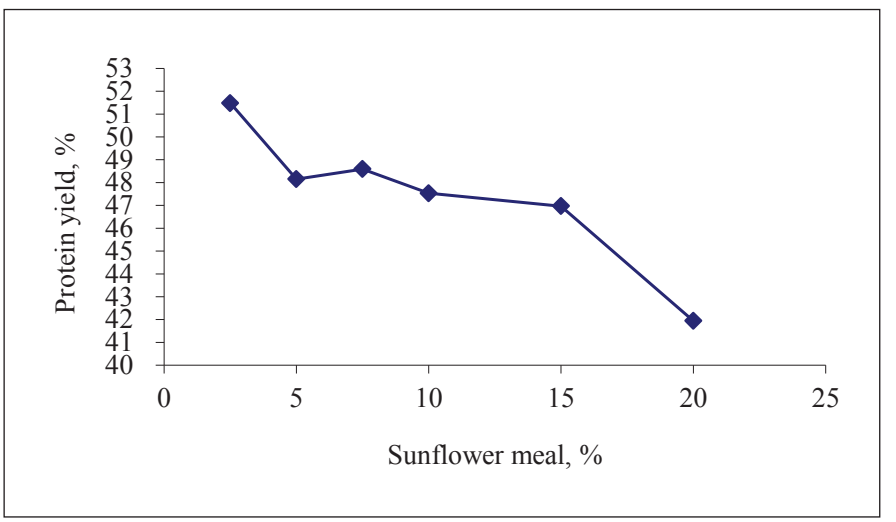

Fig. 2. Influence of sunflower meal concentrations on protein extrability. Standard deviations do not exceed ± 2.4

Rahma and Rao (1981) obtained maximum protein yields under similar conditions, namely 10% NaCI, 10% sunflower meal and 60 min extraction time. Gheyasuddin et al. (1970) established 15 min to be sufficient for the achievement of maximum protein yield. Based on our results, 60-75 min extraction time was required to obtain maximum protein yields from sunflower meal, which, however, could be reduced to 15 min if necessary.

Conclusion

Sunflower meal produced after industrial oil extraction from heat-treated sunflower seeds was used in our study. It was established that 96% of the total protein content of the sunflower meal (43.15%) was soluble in 0.4% Na_2CO_3 that makes it suitable for the preparation of protein isolates. Our study demonstrated that by extracting 10% sunflower meal with 10% NaCl, at pH6 and 40°C for the period of 60-75 min, a protein yield greater than 50% at diminished protein-phenol interaction could be achieved.

Table 3
Influence of extraction time on protein yield

Time durability, min	Protein yield, %		
	2.5% meal	5.0% meal	10.0% meal
15	49.48 ± 0.20^{d}	41.58 ± 0.35^{d}	45.50 ± 0.90^{d}
30	51.00 ± 0.39^{bcd}	44.56 ± 0.23^{bc}	46.40 ± 0.86^{cd}
45	51.60 ± 0.09^{bc}	44.40 ± 0.51^{b}	47.91 ± 1.76^{bc}
60	54.92 ± 0.24^{a}	46.38 ± 0.26^{a}	48.69 ± 1.58^{ab}
75	52.08 ± 0.24^{b}	45.20 ± 0.56^{ab}	49.80 ± 0.33^{a}
90	50.16 ± 0.46^{cd}	43.74 ± 0.46^{c}	48.20 ± 0.69^{ab}

$^{a-d}$ Means within a column not sharing a common letter are significantly different ($p < 0.05$).

References

AACC, 1995. Approved methods of the American Association of Cereal Chemists. The American Association of Cereal Chemists Inc., St. Paul, MN, USA.

AACC, 1983. Method 46-15: Crude protein – 5-minute Biuret method for wheat and other grains. Approved Methods of the AmericanAssociation of Cereal Chemists, The American Association of Cereal Chemists, St. Paul, MN, USA.

Abo-Tarboosh, H. M., 1995. Factors affecting protein extractability of defatted Karkade (*Hibiscus sabdariffa*)seed flour. *J. King Saud. Univ. Agric. Sci.,* **7**: 179-185.

Cantamutto, M. and M. Poverene, 2007. Genetically modified sunflower release: Oportunities and risks. *Field Crops. Res.,* **101**: 133-144.

Carellos, D. C., J. A. F. Lima, E. T. Fialho, F. de Freitas, H. O. Silva, P. A. C. Branco, Z. A. de Souza and J. V. Neto, 2005. Evaluation of sunflower meal on growth and carcass traits of finishing pigs. *Ciênc. Agrotec.,* **29**: 208-215.

FAO, 2009. FAOSTAT-Agriculture, last update 11 June 2008. FAO Statistics Division 2009. Available at: http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID¼567#ancor. Accessed: 02/2011

Gandhi, A. P., K. Jha and V. Gupta, 2008. Studies on the production of defatted sunflower meal with low polyphenol and phytate contents and its nutritional profile. *ASEAN Food J.,* **15**: 97-100.

Gheyasuddin, S., C. M. Cater and K. F. Mattil, 1970. Effect of several variables on the extractability of sunflower seed proteins. *J. Food Sci.,* **35**: 453-456.

González-Pérez, S., J. M. Vereijken, K. B. Merck, G. A. van Koningsveld, H. Gruppen and A. G. J. Voragen, 2004. Conformational states of sunflower (*Helianthus annuus*) helianthinin: Effect of heat and pH. *J. Agric. Food Chem.,* **52**: 6770-6778.

González-Pérez, S., G. A. van Konignsveld, J. M. Vereijken, K. B. Merck, H. Gruppen and A. G. J. Voragen, 2005. Emulsion properties of sunflower (*Helianthus annuus*) proteins. *J. Agric. Food Chem.,* **53**: 2261-2267.

González-Pérez, S. and J. M. Vereijken, 2007. Sunflower proteins: overview of their physicochemical, structural and functional properties. *J. Sci. Food Agric.,* **87**: 2173-2191.

ICC Standard №104/1, Approved 1960, Revised

1990. Determination of ash in cereals and cereal products.

ICC Standard №156, Approved 1994. Determination of total dietary fiber.

ISO 7302:2003, Cereals and cereal products -Determination of total fat content.

Kaldmäe, H., R. Leming, M. Kass, A. Lember, S. Tölp and O. Kärt, 2010. Chemical composition and nutritional value of heat-treated and cold-pressed rapeseed cake. *Vet. Med. Zoot.,* **49**: 55-60.

Kondili, E. M. and J. K. Kaldellis, 2007. Biofuel implementation in East Europe: Current status and future prospects. *Ren. Sust. Energy Rev.,* **11**: 2137-2151.

Lühs, W., and W. Friedt, 1994. The major oil crops. In: D. J. Murphy (Editor) Designer Oil Crops. Breeding, Processing and Biotechnology. *VCH*, Weinheim, Germany, pp. 5-58.

MAFS, 2009. Ministry of Agriculture and Food Supply, Bulgaria. Agricultural report 2009. Available at: http://www.mzh.government.bg/mzh/bg/Documents/areport.aspx. Accessed: 02/2011

Marinchevski, I. D., 1980. Research on sunflower seeds as a source for preparation of protein concentrates and isolates for food purposes. Dissertation (Bg).

Meng, X. and B. A. Slominski, 2005. Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. *Poult. Sci.,* **84**: 1242-1251.

Pickardt, C., S. Neidhart, C. Griesbach, M. Dube, U. Knauf, D. R. Kammerer and R. Carle, 2009. Optimisation of mild-acidic protein extraction from defatted sunflower (*Helianthus annuus* L.) meal. *Food Hydrocoll.,* **23**: 1966-1973.

Rahma, E. H. and M. S. N. Rao, 1981. Isolation and characterization of the major protein fraction of sunflower seeds. *J. Agric. Food Chem.,* **29**: 518-521.

Raza, S., M. Ashraf, T. N. Pasha and F. Latif, 2009. Effect of enzyme supplementation of broiler diets containing varying level of sunflower meal and crude fiber. *Pak. J. Bot.,* **41**: 2543-2550.

Rosa, P. M., R. Antoniassi, S. C. Freitas, H. R. Bizzo, D. L. Zanotto, M. F. Oliveira and V. B. R. Castiglioni, 2009. Chemical composition of brazilian sunflower varieties. *Helia,* **32**: 145-156.

Sahidi, F. and M. Naczk, 2004. Phenolics in food and neutraceuticals. CRC Press, Boca Raton, Fl.

Senkoylua, N. and N. Dale, 1999. Sunflower meal in poultry diets: a review. *World's Poult. Sci. J.,* **55**: 153-174.

Senkoylu, N. and N. Dale, 2006. Nutritional evaluation of a high-oil sunflower meal in broiler starter diets. *J. Appl. Poult. Res.,* **15**: 40-47.

Synge, R. L. M., 1975. Interactions of polyphenols with proteins in plants and plant products. *Plant Foods Hum. Nutr.,* **24**: 337-350.

Taha, F. S., M. Abbasy, A. S. El-Nockrashy and Z. E. Shoeb, 1981. Countercurrent extraction-isoelectric precipitation of sunflower seed protein isolates. *J. Sci. Food Agric.,* **32**: 166-174.

Tavernari, F. C., L. F. T. Albino, R. L. Morata, W. M. Dutra Júnior, H. S. Rostagno and M. T. S. Viana, 2008. Inclusion of sunflower meal, with or without enzyme supplementation, in broiler diets. *Rev. Bras. Cienc. Avic.,* **10**: 233-238.

USDA Foreign Agricultural Services, 2008. Production, supply, and distribution database. Available at: http://www.fas.usda.gov/psdonline/psdHome.aspx. Accessed: 02/2011

Received July, 2, 2011; accepted for printing February, 2, 2012.