SEQUENTIAL IMPLEMENTATION OF ISOELECTRIC PRECIPITATION FOLLOWED BY ULTRAFILTRATION FOR PRODUCTION OF SUNFLOWER PROTEIN ISOLATES

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ABSTRACT

The aim of this study was to improve the yield of protein isolates obtained from sunflower oil cakes by means of sequential implementation of isoelectric precipitation and ultrafiltration. The proteins were extracted from sunflower oil cake in mild acidic conditions (pH = 6 with solution of NaCl with concentration of 0.5, 1 and 2M). The first fraction of protein isolate was obtained by means of isoelectric precipitation at pH = 4, 3.5 and 3. Total protein, ash and dry matter contents, and residual chlorides of raw material and precipitates were determined. The second fraction was obtained from supernatant taken from the highest yield precipitate by means of ultrafiltration. The ultrafiltration experiments were conducted with an UF10-PAN membrane at a transmembrane pressure of 0.2, 0.35 and 0.5 MPa, and a volume reduction ratio (VRR) of 2, 3, 4, and 5. Total protein, ash, and dry matter contents of retentates and permeate were determined.

Keywords: protein isolates, isoelectric precipitation, ultrafiltration.

INTRODUCTION

Oilseeds are important agriculture feedstock in Bulgaria. They are consumed mostly processed in form of seed oil. The traditional approach of processing oilseeds is by cold or hot pressing, where the main product is vegetable oil, and remaining part from the seed is used mostly as animal feed. The sunflower takes most important place in terms of production volume in Bulgaria and is grown at 822 826 from 3 620 000 hectares in 2022. The pellets, expellers and meal left over from pressing are traditionally used as protein source in animal feed [1]. The processing of byproducts from the extraction of sunflower oil into protein isolates has great potential for added nutritional value and reducing carbon footprint. The protein isolates from sunflower seeds have valuable nutritional and technological benefits. Except for lysine deficiency, according to nutritive value and functional properties the sunflower proteins are comparable to those of soy and other leguminous proteins [2, 3]. They are technologically close to or even superior to soy protein, but their use in the past has been obstructed by lower sensory qualities such as dark colour, bitter and astringency taste due to phenolic compounds [4]. The sunflower isolate offers higher emulsifying and foaming properties, while its gel forming properties are relatively weak. The sunflower isolate shows a higher emulsifying capacity than wheat protein, skimmed milk powder or egg powder [5]. Its foaming properties are comparable with those of egg protein [5, 6]. The sunflower seeds contain 1 - 4 % phenolic compounds, which could interact with proteins by forming covalent bonds and the resulting compounds reduce the technological and sensory characteristics [7]. The reaction rate is increased at high pH, which is the most favourable for the extraction of proteins from

sunflower. This drawback of the raw material leads to slower implementation of the technology in practice and requires alternative approaches for extraction where the phenolic compounds contained in the sunflower are removed before the protein is extracted or extraction process is conducted under conditions reducing the possibility of reaction with phenolic acids [4, 8]. Our approach for dealing with this issue is based on extraction in a weakly acidic high salt solution, under which conditions the interaction between phenolic acids and proteins is strongly suppressed [3, 9].

The most frequently used technology for recovering the protein from the extracts is isoelectric precipitation. For sunflower protein, and yield is reported up to 55 % [3]. It is established that the precipitated fraction is consisted by globulin. Using the ultrafiltration technique for a recovery of water-soluble albumin can be reached [9].

Membrane processes are very efficient and have many advantages in comparison to other separation methods: keeping the nutritional and sensory properties of the products; yield's increase; obtaining high-quality, natural fresh-tasting and additive-free products; operation at room temperature with no heat application or the use of chemical agents, simple processing, etc. [10]. Ultrafiltration (UF) is a pressure-driven membrane process, and it is widely used for clarification of various plant products, for fractionation and for concentration [10 - 15]. The ultrafiltration membranes are used to retain large species such as microorganisms, lipids, proteins and colloids while small solutes such as vitamins, salts, and sugars passes through the membrane together with water [12]. In recent years ultrafiltration has gained a great interest for the separation and concentration of bioactive compounds from plant extracts and byproducts of agro-food industries [12, 15 - 18].

In our research we tested a combined method using sequential isoelectric precipitation followed by ultrafiltration for improving the yield of sunflower protein from extract obtained by mild acidic method.

EXPERIMENTAL

Materials

The sunflower meal was supplied from local company GreenGold Ltd. It was a byproduct of cold pressing of whole sunflower seeds, containing 25.3 % protein.

All chemical reagents were analytical grade, purchased from Merck (Darmstadt, Germany) and Sigma Aldrich (Steinheim, Germany).

Instrumentation

Janetzki K23 centrifuge with 30 cm rotor was used for centrifugal separation.

Velp Scientifica heating digested DK6 and destilation unit UDK 127 were used for protein determination.

Methods

Isoelectric precipitation

Two series of experiment were performed: the first series were for optimization of parameters, the second were in larger scale for producing quantity of extract sufficient for ultrafiltration experiment. The first series were performed in 50 cm^3 flacons. In 6 flacons 5 g from homogenized sunflower meal were weighted, and 45 cm^3 from solvent solution containing 7, 10 or 13 % NaCl were added. The mixture was homogenized on vortex and pH was adjusted with 10 % HCl, or 10 % Na_2CO_3. After pH adjustment, the flacons were homogenized on vortex and left to rest 1h. The flacons were centrifuged at 3000 rpm for 2 min and the supernatant was collected and combined. The solution was filtered on laboratory vacuum system with paper filter, pH was adjusted to isoelectric point and left for 1h under stirring, distributed evenly between 6 flacons, and centrifuged at 3000 rpm for 2 min. The precipitate was separated from the supernatant, and recovered with 7cm^3 HCl solution with pH adjusted to isoelectric point with vortex, the slurry was combined in flacon and centrifuged at 3000 rpm for 2 min. The supernatant was removed, and the flacon was dried under vacuum at 60°C until constant weight.

The second series were performed following the same steps at pH for extraction at 6 and pH for isoelectric precipitation set to 4. Larger test tubes were used with 600 cm^3 batches. The supernatant from centrifugation after isoelectric precipitation was collected and stored in refrigerator at 4 ± 1°C. Ten liters accumulated supernatant were used for ultrafiltration. The protein precipitate accumulated was dried under vacuum and analysed for protein and salt content.

Ultrafiltration

The membrane filtration was performed on a laboratory system with a plate and frame membrane

module with a membrane area of 1250 cm^2, shown in Fig. 1. Polyacrylonitrile membrane UF10-PAN with molecular weight cut-off (MWCO) of 10 kDa was used for ultrafiltration. The volume of the feed solution (V_F) was 6 L. The working conditions during ultrafiltration were: transmembrane pressure of 0.2; 0.35 and 0.5 MPa; temperature of 20°C, feed flow rate of 330 $dm^3 h^{-1}$, volume reduction ratio (VRR) of 2, 3, 4, and 5. The membrane system worked with recirculation of the feed solution. The membranes were cleaned using NaOH 0.5 %, a temperature of 50°C, a pressure of 0.2 MPa, and a circulation time of 30 min followed by a final rinsing with distilled water.

Calculation of main characteristics of ultrafiltration process

- Volume reduction ratio

The VRR was calculated as follows:

$$VRR = \frac{V_F}{V_R} \quad (1)$$

where: V_F was the volume of the feed solution, dm^3; V_R was the volume of the retentate obtained during ultrafiltration, dm^3.

- Transmembrane pressure

The transmembrane pressure (p, MPa) was calculated by the following formula:

$$p = \frac{p_1 + p_2}{2} - p_3 \quad (2)$$

where: p_1 was the feed stream's inlet pressure, Pa; p_2 was the retentate stream pressure, Pa; p_3 was the permeate stream pressure, Pa.

- Permeate flux

The permeate flux (J, L m^{-2} h^{-1}) characterizes the volume of permeate generated per unit area of membrane per unit time:

$$J = \frac{V}{A.t} \quad (3)$$

where V was the volume of collected permeate, L; A was the membrane area, m^2; t was the time, h.

- Selectivity (rejection)

The selectivity (rejection R, %) of the membrane was calculated as follows:

$$R = \left(1 - \frac{C_p}{C_R}\right) 100,\% \quad (4)$$

where: C_P was the content of the compound in the permeate, %; C_R was the content of the compound in the retentate, %.

- Concentration factor

For calculating the concentration factor (CF) the following equation was used:

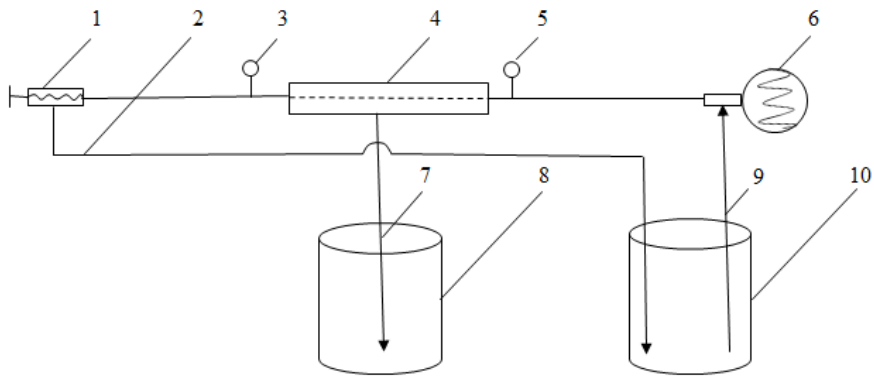

Fig. 1. Scheme of laboratory membrane system with a replaceable plate and frame membrane module: 1 - Pressure regulator; 2 - pipeline for retentate; 3 - manometer (0-1 MPa); 4 - replaceable plate and frame membrane module; 5 - manometer (0 - 1 MPa); 6 - 3-frame piston pump; 7 - pipeline for permeate; 8 - tank for permeate; 9 - pipeline for feed solution/retentate; 10 - tank for feed solution/retentate.

Table 1. Protein yield dependence from extraction pH and salt concentration of solvent at precipitation pH of 3.5.

NaCl, %	Yield		
	pH 5	pH 6	pH 7
7	37.3 ± 0.4 %	39.1 ± 0.4 %	38.8 ± 0.4 %
10	38.9 ± 0.4 %	41.2 ± 0.4 %	39.8 ± 0.4 %
13	38.9 ± 0.4 %	41.3 ± 0.4 %	39.9 ± 0.4 %

Table 2. Protein yield dependence from precipitation pH.

pH	Yield
4	38.3 ± 0.4 %
3.5	41.2 ± 0.4 %
3	41.9 ± 0.4 %

Table 3. Protein content in raw material and in precipitate

	Oilcake	Protein precipitate
Protein content, %	25.3 ± 0.3 %	63.9 %
Salt content, %	-	16.8 ± 0.2 %
Yield, g	-	41.2 %

$$CF = \frac{C_R}{C_O} \quad (5)$$

where: C_R was the content of the compound in the retentate, %; C_O was the content of the compound in the feed solution, %.

Statistical analysis

The results of this study were given as the averages of at least three determinations. One-way ANOVA in Microsoft Excel 2010 was used to compare the averages, utilizing Fisher least significance test at a 95 % confidence level.

RESULTS AND DISCUSSION

Optimization of parameters for isoelectric precipitation

The protein yield was measured gravimetrically, and the results are shown in Tables 1 and 2.

The results showed that the optimal pH for extraction is 6. There was a trend for increasing the yield at higher salt concentration, but above 10 % NaCl it was statistically insignificant.

Table 2 shows that lowering pH at stage of protein precipitation led to increasing the yield. The pH below 3 led to a protein coagulation and lowered its technology value, therefore for the main experiment a value of pH of 3.5 was chosen.

Isoelectric precipitation of sunflower protein

The main experiment was performed with 10 % solution of NaCl at pH = 6 for protein extraction. Precipitation was performed at pH = 3.5. The protein content was measured in raw material and in the dried final product. The supernatant was used for next stage - ultrafiltration.

The results showed that the single washing of the protein precipitate in isoelectric point solution was insufficient for removing NaCl from the extraction solution.

Ultrafiltration of water-soluble fraction of sunflower protein

Fig. 2 presents the permeate flux at different operating conditions. The permeate flux decreased with the VRR increase ($p < 0.05$). This could be explained with the concentration increase of the solutes which leads to an increase in the dynamic viscosity and thus the flux decreases [12]. Cai established that the flux reduction is due to the cake layer formation during ultrafiltration [19]. The effect of the transmembrane pressure was positive on the permeate flux ($p < 0.05$). This could be explained with the fact that the pressure provokes a recirculation velocity increase which improves the hydrodynamical conditions leading to a reduction of the concentration polarization's effect [10]. Fig. 2 also shows that the biggest flux reduction was from the beginning of the process until VRR 2 for all transmembrane pressure. The flux slightly decreased from VRR 2 to VRR 5. Generally, the biggest flux was obtained at the beginning of the process (VRR 1) for a transmembrane pressure of 0.5 MPa (16 L m^{-2} h^{-1}), the

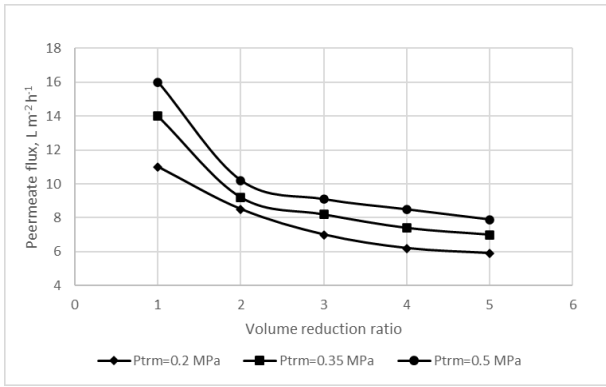

Fig. 2. Permeate flux at different operating conditions.

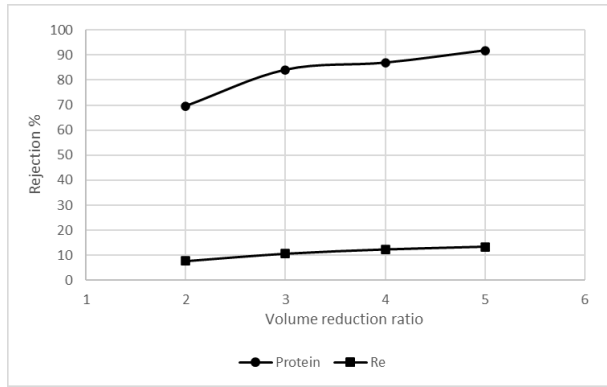

Fig. 3. Effect of the VRR on the rejection (R, %).

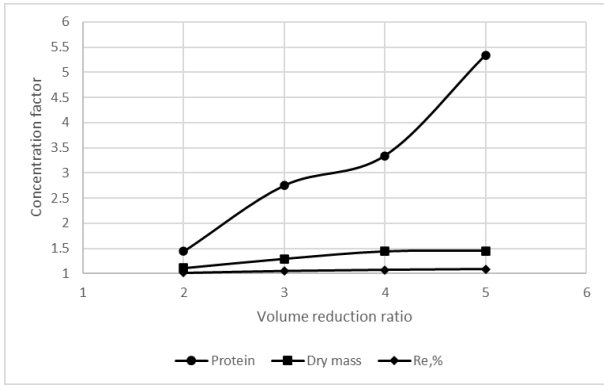

Fig. 4. Effect of the VRR on the concentration factor (CF).

lowest - at VRR 5 and pressure 0.2 MPa (5.9 L m^{-2} h^{-1}). The increased transmembrane pressure can be applied when the flux decreased due to VRR rise.

Fig. 3 shows the rejection of protein and dry solids (Re) during ultrafiltration of sunflower protein isolate. It could be seen that the rejection of both studied compounds increased with the VRR rise ($p < 0.05$) as the rejection rise of proteins was more significant than dry solids (Re, %). This shows the possibility of application of ultrafiltration and this membrane to retain and concentrate the proteins in sunflower isolate. The highest values of rejection of proteins and dry solids were obtained at VRR 5 - 91.8 % and 13.3 %, respectively. The high rejection of proteins shows the suitability of the chosen concentration level (VRR 5).

Fig. 4 presents the concentration factor of proteins and dry mass determined by two different methods. It could be seen that the concentration factor of all investigated compounds increased with the VRR rise ($p < 0.05$) as the rejection rise of proteins was more significant than the dry mass. The protein's concentration was significantly increased from 1.43 to 5.34 which shows the possibility of application of ultrafiltration and the 10 kDa membrane to concentrate the proteins in sunflower isolate. Experimental data for application of ultrafiltration to concentrate proteins in other food products were published from other researchers [20 - 23].

CONCLUSIONS

Sunflower protein isolates of light colour were produced from cold pressed press cake by the simplified process that consists in mild-acidic protein extraction at pH 6 without removal of phenolic compounds from the protein crude extracts. The protection effect originating from acidity and salt content proved to be sufficient for protection of extracted protein from reaction with extracted polyphones during stages of extraction, precipitation and drying. The product obtained had a protein content leaning toward concentrates range. The concentration of NaCl during extraction had small effect in studied ranges. The pH for precipitation had a higher

impact, with decreasing the pH yield increases.

During ultrafiltration, the highest values of rejection of proteins and dry solids were obtained at VRR 5 - 91.8 % and 13.3 %, respectively. The highest value of protein concentration factor was obtained at VRR 5 - 5.34. The results for rejection and concentration factor for proteins showed the possibility of application of ultrafiltration and this membrane to retain and concentrate the proteins in sunflower isolate.

Both protein types yielded from isoelectric precipitation and from ultrafiltration are food grade products has potential for use as an ingredient in foods or in biodegradable or edible food packaging materials.

Acknowledgements

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