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Extraction and Functional Properties of Proteins from Pre-roasted and Enzyme Treated Poppyseed (*Papaver somniferum* L.) Press Cakes

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Abstract: In this study, proteins of the defatted meals obtained from cold-pressed poppyseed previously treated (pre-roasting and enzyme against control) were extracted and their compositional and functional properties were determined. Saline-alkaline extraction (pH 11-12, and 0.2-0.6 M NaCI) and isoelectric point (pH 4.0-5.5) precipitation technique showed that seed pre-roasting enhances protein yield while enzyme treatment reduces it. There were 7 bands on SDS-PAGE, and enzyme treated samples were weaker than control. While enzyme treatment decreased denaturation temperatures (T_d), roasting enhanced the enthalpy change (ΔH) values. Pre-treatments caused a decrease in protein least gelling concentration (LGC) values. Water and oil holding capacities (WHC and OHC) were found lower in enzyme treated and higher in pre-roasted samples. Similar effects were also determined for emulsifying activity (EA) and emulsion stability (ES) values. While foaming capacity (FC) in treated samples decreased, foam stability (FS) increased oppositely. In conclusion, poppyseed meals can be nutritionally good source for diet protein, and a limited pre-roasting can be very beneficial for enhanced protein extraction yield and desirable functional properties.

Key words: poppyseed, protein, extraction, functional property, thermal property

1 INTRODUCTION

Cultured poppy (*Papaver somniferum* L.) is an annual plant belonging to the *Papaveraceae* family under *Rhoedales* team. Since poppy capsules are the source of opium alkaloids, its cultivation has been regulated strictly by the United Nations (UN), and allowed in Turkey, India, Australia, France, Spain, and Hungary. In these countries, around 71.009 ha area was used for poppy cultivation in 2013, of which 47% of the area was in Turkey. In Turkey, the lands and even the individual producers are strongly controlled by government agencies (Turkish Grain Board-TMO) for poppy cultivation, harvesting and commerce[1,2].

After harvesting, the poppy capsules are broken and the seeds inside the capsules are separated. The capsules are used for the opium alkaloids production, while the seeds are mainly used as food materials[2]. It was indicated that poppyseeds and oils extracted from the seeds are free from the alkaloids, hence can be utilized safely as human food source, and in fact, has been consumed in the producing countries in large amounts. After oil extraction, the remaining poppyseed meal can be good source of protein for human nutrition[2,3].

It was indicated that protein from vegetable origin could be a valuable alternative to animal protein, since they are renewable, widespread, and in wide range of sources. However, some drawbacks also exist for these meals including presence of antinutritional factors (trypsin inhibitors, phytic acid and tannins), low protein solubility, and deficiency of sulfur amino acids. On the other hand, protein extracts or isolates from oilseed meals confer some functional (viscosity, water and oil holding, emulsification, whipping, etc.) properties, nutritional fortification, textural and organoleptic properties to the foods[4-6].

There are limited numbers of studies available on poppyseed proteins. In an early study[7], nutritive values of poppyseed meal and protein concentrates were analyzed. It was shown that seeds of white and blue poppies contained 27% and 21% protein, respectively. Also, amino acid compositions were similar and chemical scores were 60 and 66 for white and blue poppies, respectively. Sirinivas and Narasinga Rao[8] studied the poppy proteins in three seed varieties. The meal proteins had minimum solubility in water

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at around pH 6.6, and maximum solubility at around pH 9.2. In 1 M salt and 2% sodium hexametaphosphate solution, maximum solubility achieved was at pH 6.6. Gel electrophoresis showed one major and several minor bands, indicating that poppyseed contains mixture of high and low molecular weight proteins. There was no trypsin inhibitor and hemagglutinin activity. Glutamic acid and aspartic acid were the most abundant amino acids, while lysine content was low. The same research group[8] investigated 10S fraction of poppyseed proteins, and reported that its molecular weight was 215.000, with six nonidentical subunits. Sirinivas and Narasinga Rao[9] also studied the low molecular fraction of poppyseed proteins. It was shown that the proteins were heterogeneous and consisted at least five fractions. The molecular weight was determined as 14.500, with higher content of helical (35%) structures. The protein contained 3.1% carbohydrate and no phosphorus. 10S fraction of the protein was studied under pH gradient for molecular structure and stability[10]. Below pH 4.0 down to 2.5, protein dissociate into lower molecular weight fractions, suggesting denaturation, though at more acid milieu (pH 1.3), reassociation and refolding may occur, as indicated. In another study[11], some functional properties (water and oil absorption, emulsification and foam capacity and stability) of poppyseed meal was studied in comparison with soybean meal. Generally, oil absorption and foam capacity and stability of poppyseed meal were higher than those of the soybean meal. Unfortunately, there was no study reporting the functional properties of poppyseed protein extracts or isolates.

The objectives of this study were first extract poppyseed proteins with the highest possible yield from defatted meals gained from the cold-pressed three poppyseed varieties (ofis 8, ofis 4 and ofis 3), then characterize proteins by basic physico-chemical analyses and finally determine the functional properties of the extracted poppyseed proteins. There is no study in the literature about the functional properties of poppyseed proteins; hence the results of this study may aid utilization of poppyseed proteins as functional ingredients in various food applications.

2 EXPERIMENTAL

2.1 Materials

In this study, the defatted meals from the three cold-pressed poppyseed samples (ofis 8, ofis 4 and ofis 3) were used. The three poppyseed samples which were also utilized in our previous studies[12, 13] for the production of cold pressed poppyseed oil were registered varieties by Turkish Grain Board (TMO) and were harvested in 2011 season. To enhance oil yield, pre-roasting and enzyme incubation pretreatments were applied to the seeds prior to cold pressing against control group. Briefly, pre-roasting was applied by heating the seeds at 150℃ for 30 min in an oven. The enzymes, hemicellulase (60 U/g seed) and protease (0.012 U/g seed) were dissolved in 0.1 M Na_2PO_4 + 0.1 M citric acid buffer solution (pH 6.0), and the poppyseeds were mixed with the enzyme solution and incubated at 60℃ for 3 h, before heating up to 100℃ to reduce the moisture. Finally the seeds were cold-pressed under defined conditions[12, 13].

2.2 Defatting of the presscakes

The defatting procedures modified from Manamperi *et al.*[14] and Onsaard *et al.*[15] were used in this study. Each grounded presscake was slurred with *n*-hexane (1:4, w/v) and mixed at room temperature for 1 h at 150 rpm speed with a magnetic stirrer. The slurry was defatted in the same way 3 times and then decanted, and *n*-hexane in the wet meal was removed in a forced-air oven at 60℃ for 1 h. Finally, the meals were dried under an air hood overnight. Defatted poppyseed meals (DPM) were ground to pass through 75 mesh screen before filling in brown-colored and capped glasses.

2.3 Preparation of the protein extracts

In order to optimize protein extraction from the DPM, at first, the most suitable pH and salt concentrations were determined by the modified method of Achouri *et al.*[17]. Stock DPM suspensions (10%, w/v) were prepared in the presence of different amounts of dissolved salt (0, 0.2, 0.6 and 1.0 M), and mixed at room temperature at 150 rpm for 2 h. Then, these suspensions were aliquoted into test tubes (10 mL) and their pH was adjusted in the range of 2 to 12 with one unit increments using 1 N HCl or 1 N NaOH aqueous solution. The samples were sonicated for 30 min and stirred for another 30 min before centrifugation (Sigma 2-16K, Osterode, Germany) at 2060 × g for 30 min at 4℃. The protein contents of the supernatants were measured by Bradford method[16] and samples with maximum protein content were selected as the most suitable pH and salt concentration conditions to maximize protein extraction.

Since isoelectric point (pI) precipitation method was chosen for protein extraction, the pI of each sample was determined according to Manamperi *et al.*[14] in the following step. First, proteins were extracted into the supernatant fraction by the previously selected salt concentration and pH values. Each supernatant was then aliquoted into tubes (10 mL) and their pH was adjusted by 1 N HCl in the range of 3.5 to 6.0 by 0.5 unit increments. After 1 min vortexing, the solutions were centrifuged at 3000 × g for 20 min, and both supernatant and precipitates were collected. Protein content of the supernatants was measured by Bradford method[16] and supernatants with the lowest protein content were selected as the pI value for each sample.

Following the above described procedures, the most suitable pH values and salt concentrations for maximum

extraction, and the pI points for maximum protein precipitation were determined for each of the DPM samples. These parameters were subsequently used to extract proteins from the DPM samples. Protein extractions were achieved with 0.6 M NaCI and pH 11.0 for control and roasted poppyseeds, and 0.2 M NaCI and pH 12 for enzyme treated seeds. After 2 h mixing of the slurries, the supernatants were collected after centrifugation, and pI precipitation was done by adjusting the pH. Finally, the protein precipitates were collected after centrifugation as described above. Each protein extract was freeze-dried in a lyophilizer (Labfreeze FD-10 MR Bench-Top Freeze Dryer, Xiangtan city-Hunan, China), placed into amber-colored capped glass and kept at −20℃ until further analyses.

2.4 Physico-chemical properties of the protein extracts

2.4.1 pH-protein solubility

The modified method of Yin *et al.*[18] was followed for this purpose. Protein dispersion (1%, w/v) in deionized distilled (DI) water was prepared and stirred on a magnet plate for 30 min. Then, 10 mL of the solutions were aliquoted into tubes, and their pH values were adjusted to 2-12 by 1 unit increments using 1 N HCI or NaOH solutions. After vortexing samples for 2 min, the pH was checked again and corrected. Finally, the tubes were centrifuged at 2291 × g for 5 min, and the protein contents of the supernatants were measured according to the Bradford assay[16]. The protein solubility (PS) data was expressed as the mg soluble protein/mL sample solution by the pH values.

2.4.2 SDS-PAGE electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out following Laemmli method modified by Achouri *et al.*[17] and Yin *et al.*[18]. Ten mg of sample weighed into an eppendorf tube and 0.950 μL Laemmli buffer (65.8 mM Tris-HCl, pH 6.8, 26.3% (w/v) glycerol, 2.1% (w/v) sodium dodecyl sulfate-SDS, 0.01% (w/v) bromophenol blue - Biorad, USA) and 0.50 μL 2-mercaptoethanol (Biorad, USA) were added. The mixture was vortexed for 5 minutes and the dispersion was kept in a water bath at 100℃ for 5 min to denature the proteins before immediate cooling on ice. Finally the samples were centrifuged at 1000 × g at 10℃ in a refrigerated centrifuge (Sigma 2-16K, Osterode, Germany). Mini-protean precast gels (4-15%, USA) were used, and 10 μL of protein standard (Precision plus protein all blue standards, 10 - 250 kD, Biorad-USA) and each of the samples were loaded in the wells and gels were run at constant voltage (200 V) in buffer solution (10xTris/Glycine/SDS buffer, pH 8.3, Biorad-USA). Then, the gels were stained in 0.1% Coomassie brilliant blue R-250 solution for 4 hours. Finally, the gels were destained and washed in the fixing solution, and were dried at 45℃ for 2 h before casting into the cellophane membranes.

2.4.3 Apparent viscosity and color

Apparent viscosities of the protein extracts were determined according to Khalid *et al.*[19] and Kanu *et al.*[20]. Twenty % (w/v) dispersions of the lyophilized protein extracts were prepared and pH was adjusted to 7.0 using 1 N HCI or NaOH. Viscosities of the dispersions were measured at 40 and 60℃ set by a water bath circulating on Brookfield viscosimeter (model DV II. Pro with Rheocalc software, Brookfield Eng. Lab., Inc., MA, USA) equipped with LV-SC4-18 spindle at 30 rpm constant speed, and apparent viscosities as mPa.s values were recorded.

The instrumental color of the lyophilized dried protein extracts (Fig. 1) placed in a glass sample cup were measured with a colorimeter (CR-400, Konica Minolta Inc., Osaka, Japan) by CIE coordinates on at least five different points of the dry samples. The instrument was calibrated against white reference tile. Color parameters of L, a* and b* values were recorded.

2.4.4 Differential scanning calorimetry (DSC)

The method of Yin *et al.*[18] with some modifications was used. Ten % (w/v) dispersions of the protein extracts in 10 mM phosphate buffer (pH 7.0) were prepared. Ten mL of sample aliquots was hermetically sealed in aluminium sample pans. An empty pan was used as the reference sample. The instrument was previously calibrated with Indium and Zinc standards. The temperature programming of differential scanning calorimetry (Perkin Elmer, DSC 400 Series, Groningen, the Netherlands) was set to heating the sample from 25℃ to 120℃ by 10℃/ min heating rate. Finally, denaturation onset temperature (T_o), peak of denaturation temperature (T_d) and enthalpy change of denaturation (ΔH) were calculated from the thermograms by Pyris 1 Manager software of the instrument. All experiments

Fig. 1 The lyophilized poppyseed protein extracts.

were conducted in duplicate.

2.5 Functional properties of the protein extracts

2.5.1 Water holding capacity (WHC)

The method by Onsaard *et al.*[15] was used. First, 0.5 g of each protein extract was weighed and dispersed in 10 mL DI water, and pH was adjusted to 7.0 and then the mixture was vortexed. After keeping samples 30 min at room temperature, they were centrifuged at 2291 × g for 15 min. The supernatant was decanted; the tubes were inverted and awaited for 30 min for drainage. Finally, the amount of absorbed water was calculated from the weight differences between the initial and the end, and reported as g water/g protein.

2.5.2 Oil holding capacity (OHC)

The method adapted from Sharma *et al.*[5] and Manamperi *et al.*[14] was followed. Protein sample (0.5 g) was weighed into tube, and 5 mL of sunflowerseed oil was added onto it. After vortexing, the mixture was stanted for 30 min at room temperature, and then centrifuged at 2291 × g for 15 min. An additional 5 min centrifugation at 19890 × g was done before inverting the tubes to drain the free oil out for 1 hour. Finally, the absorbed oil was calculated from the weight difference and OHC was reported as g oil/g protein.

2.5.3 Emulsifying activity (EA) and emulsion stability (ES)

The method developed by Wu[21] was followed for these analyses. Protein extract (0.5 g) was dissolved in 10 mL DI water, and pH was adjusted to 7.0, before the addition of 10 mL sunflowerseed oil. After vortexing for 1 min, the mixture was centrifuged at 2291 × g for 15 min. Finally, the EA was calculated by the formulae given below;

$$EA = 100 \times (\text{height of emulsion layer}) / (\text{total height of mixture in tube})$$

To determine the ES, the tubes were first heated in a water bath at 80℃ for 30 min, and then cooled quickly under flowing tap water. Then, the tubes were centrifuged at 2291 × g for an additional 5 min. Finally, the ES was calculated using the following formulae;

$$ES = 100 \times (\text{height of remaining emulsified layer}) / (\text{total height of mixture in tube})$$

2.5.4 Foaming capacity (FC) and foam stability (FS)

FC and FS were determined according to the method modified from Cano-Medina *et al.*[22]. Briefly, 1% (w/v) protein extract dissolved in DI water (pH adjusted to 7.0) was whipped at high speed in a Warring blender and poured into 100 mL volumetric cylinder. FC was reported by the formula below;

$$FC(\%) = (\text{volume after agitation} - \text{volume prior to agitation}) / (\text{volume prior to agitation}) \times 100$$

The samples allowed to stand for 30 min at room temperature to estimate the FS given by the formula below;

$$FS(\%) = (\text{residual foam volume}) / (\text{total foam volume}) \times 100$$

2.6 Statistical analysis

The whole study was replicated twice with all analyses within each replicate performed at least twice. Data were reported as mean ± standard deviation. The minimum confidence level was 95% in all statistical analysis. The data were compared with two-way analysis of variance (ANOVA) and Tukey's multiple comparison tests by using Minitab v. 16.1[23] and SPSS software[24] programs.

3 RESULTS AND DISCUSSION

3.1 Protein extraction and solubility

The extracted proteins used in this study are pictured in Fig. 1. The maximum extracted protein contents with the selected extraction conditions were given in Table 1. Clearly, except the enzyme treated seeds, the optimum extraction pH was 11 for the samples. Similarly, the best extractions of proteins from control and roasted seeds were obtained at 0.6 M NaCI concentrations. Prior enzyme (hemicellulase and protease) treatment of the seeds caused some changes for the optimum extraction conditions (pH 12, 0.2 M NaCI). Once the optimum conditions for protein extraction were determined, we had isoelectric point precipitation for the protein gaining. For all control samples of the three seeds, pI was 5.0, whereas roasted and enzyme treated samples had pI of 4.0-4.5 and 5.0-5.5, respectively, depending on the seed type. The pI values of the samples are shown in Fig. 2. The poppyseed proteins from the de-

Table 1 Optimum pH and salt concentration values determined for protein extraction.

Sample	pH	NaCI (M)	Max. Extracted Protein (mg/mL)
Ofis 8-Control	11	0.6	21.54 ± 0.56
Ofis 8-Roasted	11	0.6	21.22 ± 0.11
Ofis 8-Enzyme	12	0.2	20.50 ± 0.01
Ofis 3-Control	11	0.6	19.01 ± 0.33
Ofis 3-Roasted	11	0.6	18.13 ± 0.22
Ofis 3-Enzyme	12	0.2	16.30 ± 0.22
Ofis 4-Control	11	0.6	21.30 ± 0.01
Ofis 4-Roasted	11	0.6	20.27 ± 0.33
Ofis 4-Enzyme	12	0.2	20.67 ± 0.22

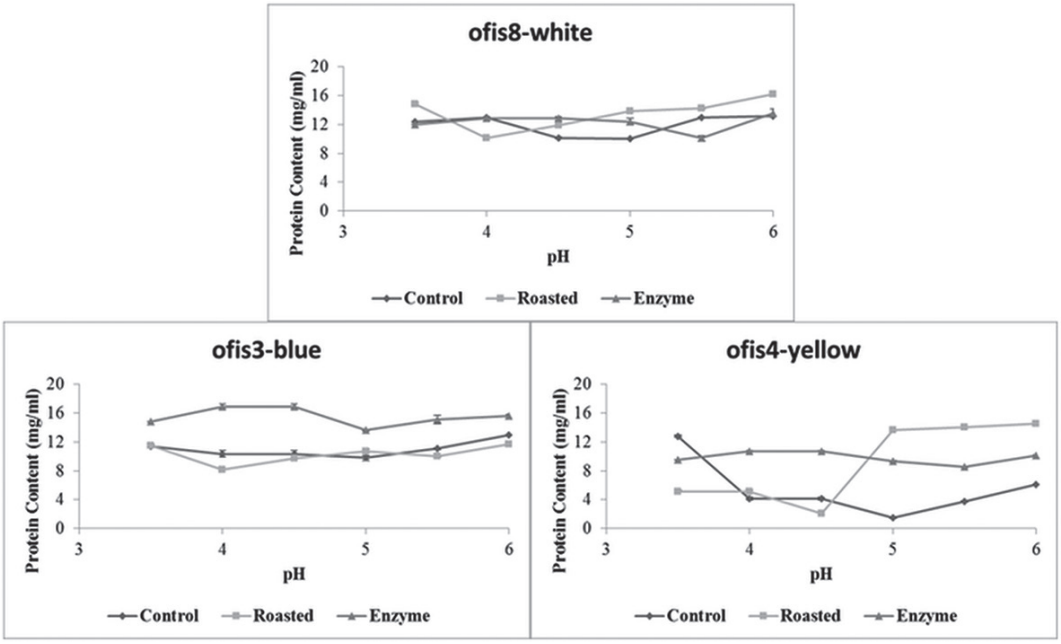

Fig. 2 The isoelectric points (pI) of the poppyseed proteins extract.

Table 2 Protein yield values of the poppyseed protein extracts.

Sample	Protein yield (%)		
	Control	Roasted	Enzyme
Ofis 8 (white)	61.34 ± 3.52 A**a*	43.24 ± 3.00 Ba	10.72 ± 1.43 Cb
Ofis 3 (blue)	44.00 ± 3.55 Ac	33.39 ± 1.06 Bb	16.06 ± 3.34 Cab
Ofis 4 (yellow)	52.02 ± 0.34 Ab	26.51 ± 1.59 Bc	19.77 ± 1.01 Ca

* Means followed by small letters represent significant differences between seed varieties in the same treatment group ($p \leq 0.05$).

** Means followed by capital letters represent significant differences between treatment groups in the same seed variety ($p \leq 0.05$).

fatted poppy meal (DPM) samples were hence, extracted by alkaline solution with some added salt and pI precipitation technique. The protein yield (%) values of the applied extraction protocol were presented in Table 2. The yield values were calculated over the protein content values of the DPM samples. For all three seed types, protein extraction yield was the highest in the control, and the lowest in the enzyme treated samples. Seed type and treatments were significantly effective ($p = 0.001$ and $p = 0.001$) on protein yields. In an early study on poppyseed proteins[7], the best extraction conditions determined as 1 M NaCl and pH 9.0 values with 5.0 pI value for precipitation. In another study, sesame proteins were extracted by alkaline solution and precipitated by pI precipitation method, and protein extraction yield was around 47%[25]. Sharma *et al.*[5] extracted 10 different edible oilseed proteins using borate saline buffer (0.1 M, pH 8.45) with yield values ranging from 10.6 to 27.4% from the defatted meals. Achouri *et al.*[17] achieved the maximum sesame protein extraction at pH 7-10 and in the presence of 0.6-1 M NaCl. Recently, Hojilla-Evangelista *et al.*[26] compared alkaline solution (pH 10) and saline procedure (0.1 M NaCl) for protein extraction from pennycress press cake. The yields and protein purity values were 23-90% and 45-67%, respectively. In our study, poppyseed proteins from the DPMs were successfully extracted using alkaline-saline procedure and isoelectric point precipitation technique. However, previous enzyme treatments and roasting to some extent had caused some losses in seed proteins.

Solubility of the extracted-lyophilized proteins as a function of pH was also studied (Fig. 3). Protein dispersions were prepared at pH 2-12 by 1 unit increments, and protein content in the supernatants was measured[18]. Clearly, protein solubility was lowest at the pI and near pH ranges, but enhanced as drifted apart from pI points. Eklund and Ågren[7] previously reported that poppyseed protein extract nitrogen solubility was around 81% at pH 10, and 20% at pH 3-6. In another study[8], solubility of de-

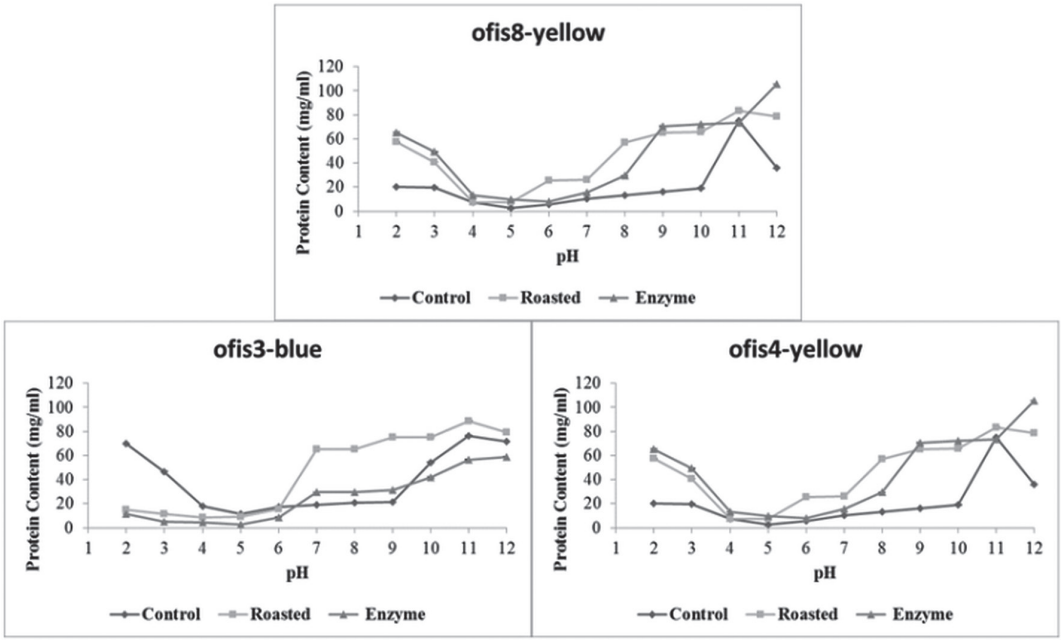

Fig. 3 Solubility properties of the poppyseed protein extracts as function of pH value.

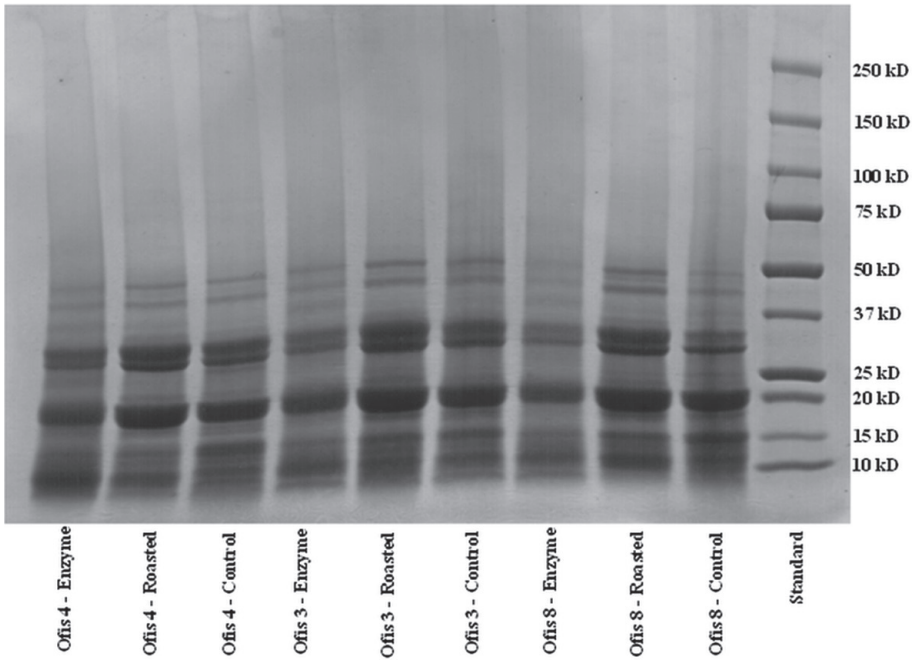

Fig. 4 The SDS-PAGE electrophoresis bands of poppyseed protein extracts.

fatted poppyseed flours were determined in water, 1 M salt and 2% sodium hexametaphosphate solutions. Maximum nitrogen solubility in water, salt solution and sodium hexametaphosphate solution were around pH 9.3, 2.0 and 7.0, respectively. Obviously, nitrogen solubility or protein solubility is affected by both pH gradient and presence of solutes. Similarly, Martinez-Flores *et al.*[27] found that linseed protein solubility enhances as pH value goes beyond the pI point of 5.0 towards the maximum at around pH 10. Our findings are in good agreement with literature.

3.2 SDS-PAGE results

SDS-PAGE bands are shown in Fig. 4. There are 7 bands separated on the gel for all nine samples. Two weak bands were identified with molecular weights just below 50 kD, compared to the protein standard. Two other darker bands were located between 37 and 25 kD reference ranges. One major dark and wide band was located on the 20 kD region.

Similarly, two other major bands at around 15 kD and 10 kD were identified. There was no band above 50 kD molecular weight. For all three type seeds, the enzyme treated samples had bands with less darkness and wideness, indicating some loss of proteins by the prior enzyme treatments. Since previous enzyme treatments had accomplished by both hemicellulase and protease, it would be quite possible that the proteins were digested and lost to some degrees. Contrarily, bands in the roasted samples were darker than the control samples. This might be caused by the aid of roasting to the protein agglomeration. Most importantly, there was no single distinct and different band among the samples. Hence, the treatments had some effects on protein quantity or agglomeration but have not created new or smaller peptides. Sirinivas and Narasinga Rao[8] have studied electrophoresis of three poppyseed varieties, and identified one major and several minor bands. Two of the bands were of low mobility and five were high mobility. There was no molecular weight marker in that study. In another study[28], high molecular weight fraction of poppyseed proteins was isolated and electrophoresis at basic (8.3) and acidic (4.5) pHs were conducted. One single band with low mobility at basic pH, and a single band with high mobility and also a slow moving faint band at acidic pH were identified. Sirinivas and Narasinga Rao[9] have also studied the low molecular weight proteins of poppyseeds. They have separated the 10S fraction of the proteins by chromatography and performed gel electrophoresis. Three high mobility bands at around 13, 16, and 18 kD were determined. Furthermore, the molecular weight of 0.8S fraction of poppy protein was reported as 14.5 kD in that study. In one study (Sharma *et al.*[5], proteins of ten selected edible oilseeds were determined to have molecular mass range between around 3-200 kD. In a recent study[26], electrophoresis of pennycress press cake proteins indicated nine bands resolving between 6.5 and 98 kD. Our results under reducing conditions concur with previous results.

3.3 Viscosity of protein aqueous dispersion and color

Apparent viscosities of the 20% protein dispersions at 40 and 60℃ were measured (Table 3). Enhancement of temperature caused significant increases in viscosity, while sample type was not a significant factor. In all samples, viscosity increased by roasting treatment, and decreased by enzyme treatment. These results confirm the SDS-PAGE findings, that previous protease treatment caused some protein loss, while pre-roasting agglomerated proteins to enhance viscosity. Kanu *et al.*[20] showed that viscosities of sesame proteins increased significantly from around 50 mPa.s to 150 mPa.s when solution temperature increased from 40℃ to 70℃.

Color of the lyophilized dry protein extracts were also measured (Table 3). There were significant differences among the samples and treatments for the color compo-

Table 3 Physical properties of poppyseed protein extracts.

Property	Treatment	Ofis 8 (white)	Ofis 3 (blue)	Ofis 4 (yellow)
Viscosity (40℃, mPa.s)	Control	32.35 ± 1.62 A**a*	27.85 ± 1.62 Bb	31.40 ± 1.27Aa
	Roasted	31.75 ± 1.76 Aa	32.45 ± 2.47 Aa	31.90 ± 1.55 Aa
	Enzyme	26.05 ± 0.35 Ab	24.15 ± 1.06 Ab	24.95 ± 0.35 Ab
Viscosity (60℃, mPa.s)	Control	58.40 ± 2.12 Aa	52.75 ± 2.05 Aa	59.25 ± 2.89 Aa
	Roasted	57.50 ± 3.67 Aa	53.85 ± 2.89 Aa	59.95 ± 3.32 Aa
	Enzyme	42.45 ± 1.76 Ab	43.50 ± 1.27 Ab	41.95 ± 2.05 Ab
L	Control	66.75 ± 0.03 Bb	64.27 ± 0.01 Cb	74.14 ± 0.04 Ab
	Roasted	79.28 ± 0.02 Ca	79.87 ± 0.03 Ba	80.37 ± 0.07 Aa
	Enzyme	55.81 ± 0.00 Bc	45.68 ± 0.15 Cc	69.38 ± 0.08 Ac
a*	Control	1.84 ± 0.00 Bb	4.29 ± 0.01 Ab	1.19 ± 0.01 Cb
	Roasted	1.10 ± 0.01 Bc	1.62 ± 0.00 Ac	0.60 ± 0.01 Cc
	Enzyme	4.20 ± 0.00 Ba	7.94 ± 0.01 Aa	2.29 ± 0.02 Ca
b*	Control	13.94 ± 0.02 Cb	14.05 ± 0.01 Bb	14.39 ± 0.00 Ab
	Roasted	11.55 ± 0.00 Bc	9.92 ± 0.00 Cc	12.23 ± 0.00 Ac
	Enzyme	18.87 ± 0.01 Ba	18.96 ± 0.03 Aa	17.07 ± 0.03 Ca

* Means followed by small letters in the same column represent significant differences between treatments in the same seed variety ($p \leq 0.05$).

** Means followed by capital letters in the same row represent significant differences between seed varieties in the same treatment group ($p \leq 0.05$).

nents of L, a* and b* values (Fig. 1). Generally, roasted samples were lighter than control samples, whereas enzyme treated samples were much darker than the control samples. It would be quite possible that these pretreatments had caused some effects on natural pigments or created new color active compounds. Sharma *et al.*[5] indicated that color of isolated nut seed proteins is a result of the combination of the quantity of total phenolics, presence of nonprotein components (pigments and minerals) and type of phenolic compounds. Color of the dry protein extracts can be an important factor during different food products applications.

3.4 Protein thermal properties

Protein denaturation initial temperature (T_o), denaturation peak temperature (T_d) and enthalpy change of denaturation (ΔH) were assessed by differential scanning calorimetry (DSC), and the results are presented in Table 4. It was stated[29] that T_d indicates the level of structural disruption maintaining tertiary and quaternary conformations of protein, and this disruption is usually related to the hydrogen bonds breakings. Similarly, ΔH indicates the proportion of undenatured protein or proportion of ordered structure in the sample. In fact, higher T_o and T_d values may indicate that heat sensitive proteins are present in the sample, and they are denatured easily during the heat application. The endothermic peak in DSC curves indicates that protein absorbs heat energy during the process of heating, and higher peaks indicate thermal stability. Hence, larger ΔH values show the proportions of undenatured proteins[30]. Thermal properties of the poppyseed protein samples showed some significant variation depending on the sample and treatment types (Table 4). Both denaturation onset temperature (T_o) and denaturation temperature (T_d) of the enzyme treated samples were significantly lower than those of the control and roasted samples. This might be due to the initial disruption of the the proteins by the protease, which readily unfolds the proteins to some degree to denature more easily. Among the three type seeds, ofis 8-white seed proteins showed the highest denaturation temperature, followed by ofis 3-blue and ofis 4-yellow seeds. Furthermore, pre-roasting of the seeds had caused some enhancement of denaturation temperature in the blue and yellow seed samples. Generally, pre-roasting application decreased ΔH significantly, indicating that during roasting some proteins had already denatured. Thermal study for soapnut seed proteins[18] indicated that acidic or alkali treatment during processing causes some loss of the ordered structure of protein. Our results have revealed that enzyme or heat treatment change the denaturation temperature and enthalpy of the samples.

3.5 Functional properties

Water holding capacity (WHC) of the samples is reported in Table 5 ranging from 1.67 to 2.47 g water/g protein. Clearly, pre-roasting has enhanced the WHC of samples, whereas enzyme treatment caused some reduction. It was indicated that WHC is a measure of water retention against gravity, and includes bound, hydrodynamic, capillary and physically entrapped waters. Furthermore, it was stated that WHC is closely related to the amino acid profile and number of charged residues, conformation, hydrophobicity, temperature, pH and ionic strength and protein concentration[4]. Unfortunately, we could not find any study reporting the poppy protein WHC value, but in one study[11], water absorption capacity (WAC) of poppyseed meal was mea-

Table 4 Thermal properties of the poppyseed protein extracts.

Property	Treatment	Ofis 8 (white)	Ofis 3 (blue)	Ofis 4 (yellow)
T_o (℃)	Control	80.37 ± 0.88 A**a*	75.43 ± 1.05 Bb	62.95 ± 0.06 Cb
	Roasted	53.26 ± 0.30 Cb	98.83 ± 0.09 Aa	68.76 ± 0.97 Ba
	Enzyme	54.60 ± 0.92Ab	30.15 ± 0.58 Cc	38.28 ± 0.53 Bc
T_d (℃)	Control	93.41 ± 0.00 Aa	84.75 ± 0.06 Bb	67.92 ± 0.00 Cb
	Roasted	64.01 ± 0.00 Ca	99.79 ± 0.00 Aa	76.44 ± 0.06 Ba
	Enzyme	67.86 ± 0.29 Ab	44.82 ± 0.06 Cc	51.75 ± 0.05 Bc
ΔH (J/g)	Control	36.86 ± 5.34 Aa	33.01 ± 5.67 Aa	30.80 ± 0.95 Ab
	Roasted	34.62 ± 1.41Ba	12.96 ± 3.42 Ca	13.89 ± 1.93 Aa
	Enzyme	36.72 ± 0.14 Aa	31.27 ± 3.07 Aa	25.15 ± 1.32 Ab

T_o: initial denaturation (on-set) temperature, T_d: thermal denaturation temperature, ΔH: enthalpy change of major endotherm.

* Means followed by small letters in the same column represent significant differences between treatments in the same seed variety ($p \leq 0.05$).

** Means followed by capital letters in the same row represent significant differences between seed varieties in the same treatment group ($p \leq 0.05$).

sured. In that study, WAC value of 124% of its weight was reported for the poppy meal and compared with soybean meal (149%). Although, material and method used in that study were different, it was clear that WAC of poppy meal was lower than that of the soybean meal. Water absorption capacity (g/g) of different seeds and kernels proteins was reported to be between 0.34 and 6.60 g water/g protein[4]. It was also indicated that the value increased with germination, fermentation, soaking and thermal treatments. In another study[26], pennycress press cake proteins WHCs were measured, and indicated that saline extracted and acid precipitated samples were identical. Our results mostly agree with the literature indicating that poppyseed protein extracts can be utilized in foods (comminuted meat products, bakery, food gels etc.) where higher water holding is expected.

Oil holding capacity (OHC) values of the samples were also measured (Table 5). In all samples, pre-roasting has significantly increased OHC, compared to control samples. Enzyme treatment of the seeds had not created any significant effect on the OHC of the protein extracts. Diverse values of oil absorption capacity between 0.36 and 5.87 g oil/g protein were reported for many oilseeds and kernels proteins[4]. In the study of Sharma *et al.*[5], ten selected oilseed proteins (almond, Brazil nut, cashew, hazelnut, macadamia, pine nut, pistachio, Virginia peanut, Spanish peanut and soybean) were analyzed and the highest oil absorption was measured in soybean proteins. Their values were ranging from 2.8 to 7 g oil/g protein. Our results are mostly within the reported ranges in the literature. Similar to WHC, the OHC of the pre-roasted samples were higher, indicating some positive effects of the heat treatment. Partial heat denaturation may unfold protein to some extent, and this might result more exposure of the buried amino acids to the milieu to enhance the binding capacities[5]. Khattab and Arntfield[31] reported that roasting and boiling of canola seeds had caused significant increases in their proteins WHC and OHC values. Overall, our results on WHC and OHC concur with literature.

Being important functional properties, emulsifying activity (EA) and emulsion stability (ES) of the samples were measured (Table 5). The EA values were significantly affected by both sample and treatment types. The highest EA values were observed in ofis 8-white and control samples. In all three types, roasting has significantly enhanced the EA values. Contrarily, enzyme treatment caused significant reductions in the EA values of all samples. The same effects were observed on the emulsion

Table 5 The functional properties of the poppyseed protein extracts.

Property	Treatment	Ofis 8 (white)	Ofis 3 (blue)	Ofis 4 (yellow)
WHC-Water holding capacity (g/g)	Control	1.87 ± 0.25 A**ab*	2.04 ± 0.36 Aa	2.20 ± 0.09 Aa
	Roasted	2.47 ± 0.32 Aa	2.35 ± 0.31 Aa	2.25 ± 0.28 Aa
	Enzyme	1.67 ± 0.04 Ab	1.95 ± 0.04 Aa	1.76 ± 0.05 Aa
OHC-Oil holding capacity (g/g)	Control	1.68 ± 0.01 Ab	1.87 ± 0.14 Aa	2.17 ± 0.05 Aa
	Roasted	2.44 ± 0.13 Aa	2.27 ± 0.20 Aa	2.45 ± 0.28 Aa
	Enzyme	1.67 ± 0.03 Ab	1.91 ± 0.50 Aa	1.41 ± 0.45 Ab
EA-Emulsifying activity (%)	Control	21.87 ± 2.65 Aa	10.00 ± 3.53 Bab	8.75 ± 1.76 Ba
	Roasted	28.75 ± 1.76 Ab	22.50 ± 3.53 Aa	21.25 ± 1.76 Aa
	Enzyme	2.50 ± 0.00 Ac	4.37 ± 0.88 Ab	3.75 ± 1.76 Aa
ES-Emulsion stability (%)	Control	10.63 ± 0.88 Aa	6.25 ± 1.76 Aab	5.62 ± 0.88 Aa
	Roasted	11.50 ± 3.53 Aa	8.75 ± 1.76 Aa	5.62 ± 2.65 Aa
	Enzyme	1.87 ± 0.88 Ab	2.50 ± 0.00 Ab	1.87 ± 0.88 Aa
FC-Foaming capacity (%)	Control	15.04 ± 1.06 Aa	19.26 ± 7.04 Aa	17.06 ± 0.56 Aa
	Roasted	14.31 ± 2.86 Aa	15.31 ± 1.07 Aab	14.28 ± 0.00 Aa
	Enzyme	13.21 ± 1.75 Aa	9.45 ± 1.78 Ab	9.37 ± 0.86 Aa
FS-Foam stability (%)	Control	23.61 ± 1.96 Aa	21.87 ± 9.25 Aa	19.19 ± 1.42 Aa
	Roasted	20.78 ± 9.18 Aa	29.86 ± 9.18 Aa	34.37 ± 3.25 Aa
	Enzyme	46.43 ± 5.05 Aa	36.67 ± 4.71 Aa	35.00 ± 7.07 Aa

* Means followed by small letters in the same column represent significant differences between treatments in the same seed variety ($p \leq 0.05$).

** Means followed by capital letters in the same row represent significant differences between seed varieties in the same treatment group ($p \leq 0.05$).

stability (ES) values, where roasting enhanced ES significantly and enzyme treatment reduced it. There is one study with poppyseed meals[11] dealing with emulsification capacity. It was shown that emulsification capacity versus pH profile resembled nitrogen solubility versus pH profile. Furthermore, addition of 0.1-0.4 M NaCI improved emulsion capacity. In our study, the EA value at pH 7.0 without salt addition was measured. Kanu *et al.*[20] showed that sesame proteins exhibit high emulsion activity at pH 2-7 and 7-10 range, but it was lower at pH values near pI point. Emulsion activity index (%) values ranging from 2.20% to 100% were reported for various seed and kernel proteins. Similarly, emulsion stability (%) values from 7.64% to 100% were reported[4]. In another study[26], saline extracted pennycress press cake proteins were shown to have exceptionally high emulsion activity index (m^2/g) values increased at alkaline pH ranges. On the other hand, acid-precipitated counterparts were superior in the emulsion stability index (minute) values. Since different studies used different measurement techniques and reported their results with different units, it would not be possible to compare them directly. Generally, both EA and ES values of the poppyseed protein extracts in this study could be considered lower to intermediate levels. It was indicated that emulsification properties of proteins can be affected by their molar mass, level of hydrophobicity and solubility, conformation, charge status as well as physico-chemical factors like pH, ionic strength, temperature, and presence of interfering agents[4, 26].

For some food applications, foaming is an indispensable property for food proteins. Foaming capacity (FC) and foam stability (FS) are important functional properties and they were measured and results are presented in Table 5. Basically, foams are gas in liquid or gas in solid dispersions. For many foods, foam is an air bubble dispersed and surrounded by a continuous aqueous phase. Proteins usually located at the air-water interface by forming a film to reduce the interfacial tension to enhance the foam stability. The thickness, gas permeability and viscoelasticity of proteins at the interface determine its functionality. Good foaming proteins must rapidly adsorb, rearrange as thin film and form a viscoelastic cohesive durable film. Protein hydrophobicity, net charge and charge distribution and hydrodynamic properties usually determine its foaming properties[4, 32]. When the FC of samples are scrutinized (Table 5), it could be clearly seen that both roasting and enzyme treatment had negative effects on foaming capacity. As opposed to FC, the foam stability (FS) of samples had a different trend. Enzyme treatment and roasting (to some limited extent) improved the FS of the samples. FC as percent volume change and FS as mL in 1-120 minutes were reported for proteins extracted with different techniques from seeds and kernels. FC and FS values ranged from 10-295% and 7-600 mL[4]. Foaming capacity (% overrun) and stability (< 1 h) of ten selected edible oilseed proteins[5] indicated that they were usually poor in these properties. Studies with sesame proteins[20] indicated that both FC and FS were better at pH values far from the isoelectric point. Generally, literature suggests that FC and FS are dependent not only on protein type, but also its level of denaturation, pH value and presence of ions. Usually, in addition to proper protein source selection, partial denaturation and pH values above or below pI are suggested for better foaming properties. In this respect, poppyseed protein extracts can be considered as poor foaming proteins.

4 CONCLUSIONS

Saline-alkaline extraction and isoelectric precipitation method found suitable for protein extraction from poppyseeds. It was shown that pre-enzyme treatment caused some protein loss while, pre-roasting improved both protein extraction yield and bands on electrophoresis. Therefore, a limited pre-roasting before oil extraction can be suggested for subsequent protein extractions. Enzyme treatment decreased proteins denaturation temperatures, while pre-roasting caused enthalpy to decrease. Least gelling concentrations of all samples decreased by the treatments. For both water and oil holding capacity values, pre-roasting had positive effects whereas enzyme treatments decreased them. Similarly emulsion activity and stability measurements indicated enhancing effects of roasting, as opposed to enzyme treatments. While foaming capacity values were decreased by both treatments, foam stabilities were increased. From all of the findings of this study, it can be concluded that, seed pre-roasting can be a positive factor for effective protein extraction from the meals as well as for better functional properties. While enzyme treatments, including protease might be harmful for protein yield, quality and functionality, cold pressed poppyseed meals can be a good source for dietary proteins.

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