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Compositional and functional characterisation of poppy seed (*Papaver somniferum* L.) press cake meals

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RESEARCH ARTICLE

Abstract

In this study, three registered poppy seed varieties (Ofis 3, Ofis 4 and Ofis 8) were used. The compositional and functional properties of the defatted meals (cakes) obtained from previously treated (pre-roasting and enzyme treatment against control) and cold pressed poppy seeds were determined. Moisture (11.01-16.21%), protein (26.87-35.14%), oil (2.32-4.28%), ash (8.70-11.14%), hunter lab L (40.63-68.63), a* (3.08-8.13), b* (8.46-22.62) and viscosity (132.5-1,084.5 cP) values were determined. Also phytic acid (9.96-15.08 mg P/g) and tannin (0.68-1.24 mg CE/g) were measured. Among the 11 minerals which were detected, K, Na, Ca, Mg and Zn were in higher concentrations. As functional properties water holding capacity (2.24-3.14 g/g), oil holding capacity (1.83-2.26 g/g), emulsifying activity (3.33-70.83%), emulsion stability (1.67-54.16%), foaming capacity (2.02-26.20%), foam stability (27.88-62.74%), and least gelation concentrations (around 16-18%) were measured. It was shown that for most functional properties, seed roasting caused some enhancements, while enzyme treatment affected negatively these properties. Defatted poppy meals can be utilised as human food due to their good nutritional and functional properties.

Keywords: poppy seed, roasting, enzyme, meal, functional properties

1. Introduction

Cold pressing has emerged as a simple, cheap and easily operated edible oil production technique without any refining processes. Its main drawback is the relatively limited oil yield that can be overcome by subsequent solvent extraction of the oily meal after cold pressing. Furthermore some pre-treatments like dehulling, crushing, limited heating, steaming, wetting, enzyme treatments can be applied to the seeds prior to pressing to enhance the yield. Hence, it could be expected that these treatments may cause some changes in technological and functional properties of the resulting defatted press cakes or flours and proteins extracted from them (Dündar Emir *et al.*, 2015; Yılmaz *et al.*, 2014).

In literature, although some of the functional properties of poppy seeds were identified (Eklund and Ågren, 1975; Srinivas and Narasinga Rao, 1986), the effects of roasting and enzyme pre-treatments on overall and nutritional properties of the seed meals have not been reported. It is possible to obtain different cakes or flours according to the pre-treatments and processing techniques applied to the seeds. Generally whole poppy seed contains 21-27% protein, while its meal contains 10.3% crude protein, 5.2% crude fat and 4.7% crude fibre (Eklund and Ågren, 1975). Poppy seed flour was shown to contain 8.08 mg/g phytate-P as well as 110.8 μM/g iron and 260.8 μM/g phosphorus (Eklund, 1975). Trypsin inhibitory activity and hemaglutinin were not detected in defatted poppy meals (DPMs) by Srinivas and Narasinga Rao (1981). Ebner *et al.* (1999) reported that, poppy seed contains 40 and 45 kDa glycoprotein allergen proteins which cause allergic symptoms. These local and allergic symptoms were reported to be very low in frequency.

There is only one study in the literature reporting the functional properties of DPM gained from the solvent extracted and defatted Dwahla chotta poppy seed variety (Srinivas and Narasinga Rao, 1986). In their study, poppy

seed meal was shown to have lower water absorption and higher fat absorption capacities than soybean meal. Furthermore, foam capacity and stability values were inferior to those of the soybean meal, while emulsification capacity was almost the same. It was also indicated that those functional properties were very dependent on sample pH, and salt addition usually improved them (Srinivas and Narasinga Rao, 1986). Unfortunately, there is no other study in literature reporting the general and functional properties of poppy seed meals or flours. In our previous study (Yılmaz and Dündar Emir, 2016), proteins from poppy meals were extracted and characterised. During protein extraction, some important portions of other edible materials are lost; hence, valorisation of defatted meal or flour could have technological importance for processed food applications beyond the special uses of the extracted proteins.

According to the 2014 yearly poppy sector report (TMO, 2015), Turkey produces annually around 20,000 tons poppy seed, and more than half of this production is cold pressed locally to produce edible poppy seed oil. The remaining oil containing meals are usually used in confectionary fillings and poppy spread formulations for local cuisine. Development of more efficient oil extraction techniques and meal utilisation studies could be important for the legal poppy producing countries listed by the United Nations.

Hence, in this study, our goal was to evaluate the physico-chemical properties, mineral composition and functional properties of the DPMs obtained after cold oil pressing. This study differ from literature by utilising three different poppy seed varieties, applications of the pre-treatments (roasting and enzyme treatments) prior to oil extraction, major oil extraction by cold pressing technique and full functional analysis. Especially information about the functional properties of the defatted meals may initiate new applications of the meals in processed food products such as, bakery and meat products, soups, sauces, and dry mixes.

2. Materials and methods

Materials

In this study, the defatted meals from three cold-pressed poppy seed varieties (Ofis 8, Ofis 4 and Ofis 3) were used. These poppy seed samples, also utilised in our previous studies for the production of cold pressed poppy seed oil (Dündar Emir *et al.*, 2014, 2015), were registered varieties by the Turkish Grain Board (TMO) and were harvested in 2011 season. Ferzim Hemicellulase (DSM, Heerlen, the Netherlands; 60,000 U/g activities at 55-65 °C and pH=4.0-6.5) and Alphamalt BK Quick Protease (Mühlenchemie GmbH Co, Ahrensburg, Germany; 12 U/g activities at 40-65 °C and pH=4.5-6.0) were bought from local chemical suppliers. All other chemicals and standards were of analytical grade and purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA).

Defatting of presscakes

The presscakes used in this study were obtained from our previous study (Dündar Emir *et al.*, 2014). To enhance oil yield, roasting and enzyme pre-treatments were applied to the seeds prior to cold pressing against control group. Briefly, roasting was applied by heating the seeds at 150 °C for 30 min in an oven (Lx3530; Luxell, Kumtel, Turkey). The enzymes, hemicellulase (60 U/g seed) and protease (0.012 U/g seed) solutions were mixed with the seeds and incubated at 60 °C for 3 h, and afterwards heating up to 100 °C was applied to inactivate the enzymes. Finally, the pre-treated and control seeds were cold-pressed under the defined conditions (Dündar Emir *et al.*, 2014, 2015).

The remaining oil from the poppy seed press cakes was removed according to the methods applied by Manamperi *et al.* (2007) and Onsaard *et al.* (2010) with slight modifications. In order to reduce the amount of oil remaining in the press cakes, the cake was slurred with hexane (1:4, w/v) and mixed at 150 rpm for 1 hour at room temperature in an orbital shaker (Unimax 2010; Heidolph Instruments, Schwabach, Germany). It was defatted in the same way 3 times, and then decanted. Hexane in the wet meal was removed in forced-air oven (Ecocell drying oven; MMM Medcenter Einrichtungen GmbH, Planegg, Germany) at 60 °C for 1 h. Finally, the oil free meals were put into fume hood to fly off any remaining solvent with natural air flow for 24 hours. The DPMs were ground (Warring blender 7011S; Warring Laboratory, Torrington, CT, USA) to pass through 75 mesh screen before filling into brown-coloured and capped glasses, and the samples were labelled and stored at -20 °C until analysis. The DPM samples produced in this study are shown in Figure 1.

Physico-chemical properties of defatted poppy meals

The moisture content of the DPM samples was measured with Ohaus MB45 (Ohaus, Nänikon, Switzerland) moisture analyser (1 g sample, 110 °C, 30 min). Crude protein content was measured by the Kjeldahl technique of method Aa 5-38 (AOAC, 1984); total ash by Ba 5a-49 method (AOCS, 1984) and oil content by Soxhlet according to AOAC 920.39 (AOAC, 2002).

The instrumental colour parameters (L, a* and b* values) of the DPM samples were measured with a Minolta CR-400 Reflectance colorimeter (Osaka, Japan) calibrated against white tile by CIE coordinates on at least ten different points of the dry DPM samples (Figure 1).

The apparent viscosities of the DPM samples were determined according to Khalid *et al.* (2003). First, 20%

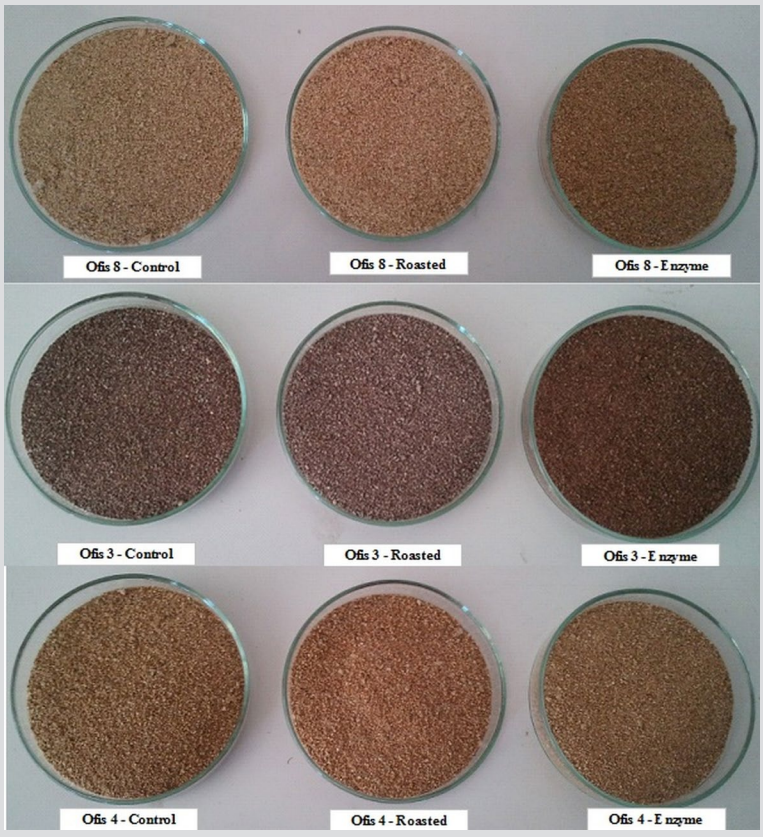

Figure 1. The defatted poppy meal samples.

(w/v) dispersions of the DPM samples were prepared and pH was adjusted to 7.0 using 1 N HCI or NaOH. Then, the viscosity was measured at 25 °C. The samples were set to 25 °C by inserting into a water bath circulating with 30 rpm speed of the spindle. Brookfield viscosimeter (model DV II. Pro with Rheocalc software; Brookfield Eng. Lab., Inc., MA, USA) equipped with LV-SC4-18 spindle was used for the measurements, and the apparent viscosity was recorded as cP value.

The phytic acid contents of the DPM samples were determined by the AOAC method 986.11 (AOAC, 1986). Briefly, 2 g of sample with 40 ml of HCl solution (2.4%) was shaked at 150 rpm at 25 °C for 3 hours and extracted by filtration through Whatman no. 1 filter paper. The ion-exchange column was prepared with Dowex® AG 1×4 chloride resin (100-200 µm), and 1:1 (v/v) Na_2EDTA-NaOH added extract diluted to 25 ml, and eluted on the column. After that, it was passed through the column following the assay protocol by 0.7 M NaCI solution, then 0.5 ml H_2SO_4 + 3 ml HNO_3 was added to the eluate containing phytate and burned by the Kjeldahl method. The phytate salts were dissolved in 100 °C water bath after adding 10 ml distillate water. Then, 2 ml molybdate and 1 ml sulfonic acid solutions were added and completed to 25 ml by distilled water to complete the reaction for 15 min. After the reaction, the spectrophotometric measurement was performed at 640 nm (Agilent 8453 UV-Visible Spectrophotometer; Agilent, Waldbronn, Germany). A standard curve was prepared with potassium acid phosphate, and the phytate content (mg P/g) was calculated.

The condensed tannins present in the DPM samples were determined according to Hagerman (2002) and Price *et al.* (1978). Each DPM sample (0.5 g) with 10 ml methanol was shaken at 150 rpm for 30 min at room temperature, before centrifugation (2-16K; Sigma, Osterode, Germany) at 2,291×*g* for 10 min. The collected extracts were placed into tubes, and 5 ml of 1% vanillin solution (dissolved in 8% HCI solution) was added, and incubated at 30 °C water bath in dark place for 20 min. The absorbance values were detected at 500 nm by spectrophotometer (Agilent 8453 UV-Visible Spectrophotometer). Catechin was used to prepare the standard curve for the calculations. Tannins were given as the catechin equivalents per gram sample.

The DPM samples were prepared by wet burning technique for mineral analyses. 0.5 g DPM sample was burned gradually with 100 ml HNO_3 (65%) on hot plate (90 °C – 30 min, 100 °C – 30 min, 150 °C – 30 min, 200 °C – 30 min, 300 °C until there is aclear colour) under a hood. Then the clear sample was diluted to 25 ml with distilled-

deionised (DI) water. Finally, the mineral contents were determined by inductively coupled plasma atomic emission spectroscopy (Varian Liberty II AX Sequential; Agilent Technologies, Mulgrave, Australia) with appropriate dilutions of AccuTrace reference standard stock (New Haven, CT, USA) used for curve calibration.

Functional properties of defatted poppy meals

Water holding capacity

5 g sample and 20 ml DI water (pH=7.0) were vortexed for 30 min and centrifuged (2,291×*g*, 15 min) according to Manamperi *et al.* (2007) and Moure *et al.* (2002). The supernatant was drained, tubes were inverted and weighed after 30 min. The difference between the initial weight and the final weight of the sample amount was determined by the proportion and the Water holding capacity (WHC) was expressed as g water/g sample.

Oil holding capacity

2 g sample and 10 ml sunflower oil were vortexed for 1 min and centrifuged (2,291×*g*, 15 min) according to Manamperi *et al.* (2007). The oil phase was drained, the tubes were inverted and weighed after 60 min. The difference between the initial weight and the final weight of the sample amount was determined by the proportion and the oil holding capacity (OHC) was expressed as g oil/g sample.

Emulsifying activity and emulsion stability

The emulsifying activity (EA) and emulsion stability (ES) of the samples were measured according to Wu (2001). DPM, water and sunflower oil were weighed based on the ratio of 7:100:100 (w:v:v) and homogenised at 10,000 rpm for 1 min. Finally, the mixture was centrifuged at 2,291×*g* for 5 min. The EA was calculated by the following formula:

EA = 100 × (height of emulsion layer) / (total height of mixture in tube)

To determine the ES, the emulsions prepared as described above were kept for 30 min at 80 °C in a water bath, then rapidly cooled with running tap water and ice. The samples were centrifuged again at 2,291×*g* for 5 min and the ES was determined by using the following formula:

ES = 100 × (height of remaining emulsified layer) / (total height of mixture in tube)

Foaming capacity and foam stability

DPM sample (3 g) was mixed with 100 ml DI water according to Cano-Medina *et al.* (2011) and Kanu *et al.* (2007). The pH values were adjusted to 7.0 with 1 N HCl or 1 N NaOH. After shaking with Warring blender 7011S (Warring Laboratory) at high speed for 3 min at room temperature, the samples were taken quickly to a 250 ml volumetric cylinder and the total volume was recorded, and the foaming liquid volume capacity (FC) was reported by the formula below;

FC(%) = (volume after agitation-volume prior to agitation) / (volume prior to agitation) × 100

After standing at room temperature for 30 min the remaining amount of foam was recorded again and foam stability (FS) was calculated by the following formula:

FS(%) = (residual foam volume) / (total foam volume) × 100

Least gelation concentration

The modified method of Moure *et al.* (2002) was followed. Stock DPM dispersion (20%, w/v) was prepared in DI water, and pH was adjusted to 7.0 by acid or alkali solutions. From this stock solution, DPM suspensions of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20% (w/v) were prepared. Preliminary analysis revealed that 2, 4, 6, 8% (w/v) sample ratios were not enough for coagulation, so sample rate from 10% was initiated, and 30, 34 and 38% concentrations was also tried for the enzyme-treated samples. Adjustment of the pH values was done with 1 N HCl or 1 N NaOH by mixing for 2 min. Each 5 ml of liquid suspensions was kept in water bath at 100 °C. After the samples were cooled under running tap water, the tubes were observed for solid gel structure that remains fixed in the tube wall.

Statistical analysis

The whole study was replicated twice, and the analyses in each replicate were done at least in duplicate, and the data were reported as mean±standard deviation. Evaluation of the data obtained from instrumental and physicochemical analysis and the differences between the groups were performed by two-way analysis of variance (ANOVA) and Tukey's multiple comparison tests. The statistical analyses were completed by using Minitab version 16.1.1 (Minitab, 2010) and SPSS professional statistics 10.1 (SPSS, 1994) package programs. For all statistical analyses, the level of confidence was at least 95% in this study.

3. Results and discussion

Physico-chemical properties of defatted poppy meals

The physico-chemical properties and anti-nutritional factors such as phytic acid and tannin contents of the DPM samples obtained from the cold press cakes of the

Table 1. Physico-chemical properties of the defatted poppy meals.[1]

Property	Treatment	Ofis 8 (white)	Ofis 3 (blue)	Ofis 4 (yellow)
Moisture (%)	Control	12.16±0.05 Ab	12.50±0.33 Ab	12.73±0.33 Ab
	Roasted	13.16±0.19 Ab	12.69±0.36 Ab	11.01±0.04 Bc
	Enzyme	16.21±0.06 Aa	15.45±0.29 Aa	14.91±0.06 Aa
Protein (%) (× 6.25)[2]	Control	31.88±0.76 Ab	28.39±0.09 Bb	32.87±0.12 Ab
	Roasted	33.66±0.48 Aa	31.70±0.05 Ba	35.14±0.74 Aa
	Enzyme	31.37±0.72 Bb	26.87±0.99 Cb	33.48±0.33 Ab
Oil (%)[2]	Control	3.45±0.53 Aa	3.09±1.27 Aa	3.52±0.02 Aa
	Roasted	3.55±0.62 Aa	2.32±0.19 Aa	4.28±0.15 Aa
	Enzyme	4.07±0.21 Aa	3.39±1.32 Aa	2.75±0.11 Aa
Ash (%)[2]	Control	8.70±0.91 Aa	9.63±0.30 Aa	10.03±1.11 Aa
	Roasted	10.74±0.34 Aa	9.73±0.97 Aa	8.86±0.80 Aa
	Enzyme	11.08±1.22 Aa	11.14±0.99 Aa	9.82±0.78 Aa
L	Control	68.63±0.16 Aa	47.56±1.39 Cab	59.30±0.03 Ba
	Roasted	65.99±0.62 Aa	48.88±1.08 Ca	57.87±0.05 Ba
	Enzyme	55.79±0.83 Ab	40.63±1.46 Bb	58.11±0.26 Aa
a*	Control	3.08±0.26 Aa	5.37±0.23 Ab	5.26±0.01 Aa
	Roasted	3.51±0.42 Aa	3.84±0.06 Ab	5.50±0.01 Aa
	Enzyme	5.46±0.08 ABa	8.13±0.54 Aa	4.96±0.03 Ba
b*	Control	19.46±0.70 Aa	9.91±0.38 Bb	22.33±0.01 Aa
	Roasted	21.41±0.17 Aa	8.46±0.31 Bb	22.52±0.00 Aa
	Enzyme	22.62±0.07 Aa	15.03±0.43 Ba	21.89±0.06 Aa
Viscosity 25 (°C, cP)	Control	590.0±33.9 Aa	1,022.5± 6.4 Aa	976.5±43.1 Aa
	Roasted	548.5±2.1 Ba	1,084.5±3.5 Aa	946.5±4.9 ABab
	Enzyme	349.5±0.7 Aa	132.5±4.9 Ab	521.5±10.6 Ab
Phytic acid (mg P/g)	Control	12.34±1.58 Aa	13.43±0.46 Aa	14.16±0.88 Aab
	Roasted	11.54±1.56 Ba	11.57±0.87 Bab	15.08±0.86 Aa
	Enzyme	12.32±0.02 Aa	9.96±0.17 Ab	11.40±0.97 Ab
Tannins (mg CE/g)	Control	0.78±0.02 Bc	1.03±0.01 Ab	0.68±0.02 Bc
	Roasted	1.23±0.02 Aa	1.24±0.01 Aa	1.13±0.01 Aa
	Enzyme	0.99±0.02 Ab	1.03±0.00 Ab	0.87±0.02 Ab

[1] Means followed by lower case letters within the same column represent significant differences (P<0.05) between treatments in the same seed variety; means followed by capital case letters within the same row represent significant differences (P<0.05) between seed varieties in the same treatment group.
[2] Protein, oil and ash contents are given on dry matter basis. Values are mean ± standard deviation.

three poppy seed varieties (Ofis 8, Ofis 3 and Ofis 4) are presented in Table 1.

The DPM samples of three different seed varieties differ for the majority of the examined physical and chemical properties. The differences between the treatments and varieties are statistically significant (P≤0.05). The moisture contents of the samples ranged approximately from 11% to 16%. Generally, the enzyme treated samples had higher moisture content than the others.

The remaining oil of the meals was eliminated by solvent extraction and the solvent was evaporated. Because a further drying process was not applied, the moisture of the cake was found to remain substantially. The protein contents of the DPMs were in the range of 26.87-35.14%, and from the perspective of all the treatment groups, the highest protein content (32.87-35.14%) was observed in Ofis 4-yellow while the lowest protein content (26.87-31.70%) was in the treatment groups of the Ofis 3-blue variety (Table 1). Hence, the protein content is affected by both seed variety and treatment type. In fact, in our previous study done with the seeds of these samples, the protein contents of the seeds were from the highest to the lowest in the orders of Ofis 8, Ofis 4 and Ofis 3 (Dündar Emir *et al.*, 2015). Clearly, the protein content of the DPM samples was not affected extensively by cold pressing and defatting procedures. This situation can be accounted well in terms of the nutritional quality of the DPM samples. Eklundand Ågren (1975) found 40.6% crude protein in the

press cake obtained from white poppy variety while 27.4% crude protein was detected in the cake obtained from the blue variety and these data is consistent with the results of our study. Rapeseed press cake (Purkayastha *et al.*, 2014) was analysed for proximate composition, and 44.8 g crude protein, 11.4 g oil and 6.4 g ashes per 100 g meals were reported. It was indicated that the cake is a good source of protein to valorise. In another study (Zhong and Zhao, 2015), soy processing by-products (soy hull, okara and molasses) were analysed for nutritional composition. It was shown that okara had the highest protein, while soy hull was the best source of dietary fibre. Generally, agro-food by-products are suggested as good sources of some nutrients. The results of our study concur with literature.

The remaining amount of oil in the cake is related to the effectiveness of the applied solvent extraction conditions. Overall oil contents of the DPM samples were reduced to less than 4.5%, and defatting these amounts of oil might be possible by increasing the repetition and duration of the extraction. However, this level was considered sufficient for our subsequent analytical requirements. In addition, this much oil remaining in the DPM samples can be considered valuable from nutritional point of view, it would also be satisfactory for the oxidative stability of the meals. The ash contents of DPM samples ranged from 8 to 11%. Compared to the analytical results (Dündar Emir *et al.*, 2015), of the same seeds (Ofis 8, Ofis 3 and Ofis 4), there is a proportional increase of the protein and ash contents in the DPM samples, after oil extraction and defatting procedures. According to the Turkish Standard TS 319 for poppy meal (poppy seed meal/cake), the meals obtained from regularly extracted, expeller extracted and press extracted meals must contain 35.0, 33.0 and 30.0% crude protein (w/w), 3.0, 6.0, and 9.0% crude oil (w/w), and 9.0, 9.0, and 9.0% crude ash (w/w), respectively (TSE, 2003). Since our samples were obtained from cold pressed seeds and were defatted, the oil contents differ, but the protein and ash contents are usually in agreement.

Although not reported in the previous studies on poppy meal in literature, the instrumental colour data of the DPMs measured in this study is coherent to name with the colour of the seeds (Figure 1). In general, the highest value of L (clear) (55.79-68.63) among all treatment groups were observed in white seeds. In the CIE colour measuring system with L=0 (black), L=100 (white), a * value (+a * =red, -a *=green) and b *value (+b *= yellow, -b *=blue) is determined (Pomeranz and Meloan, 1994). Roasting and enzyme treatments applied prior to cold pressing were very effective on the colour characteristics of DPMs of all varieties ($P \leq 0.005$). When the control groups were examined, the highest value of redness (a*=5.37) was detected in Ofis 3-blue variety and highest value of yellowness (b*=22.33) was measured in Ofis 4-yellow variety (Figure 1).

Viscosity values were measured in DPM dispersions (20%) at room temperature. In terms of consistency, they did not show great changes in the apparent viscosity as a result of roasting, while enzyme treatment resulted in significant decreases in the viscosity values. Ofis 3-blue variety has the highest viscosity values measured (132.5-1,084.5 cP) and also for this variety the sharpest decline after enzyme treatment was detected (Table 1). It might be quite possible that proteolytic and hemicellulotic enzyme activity applied to the seeds prior to cold pressing (Dündar Emir *et al.*, 2015) caused some significant hydrolysis in the protein and carbohydrate polymers to result in a significant decrease in the viscosity of the meals obtained from the seeds. When the DPM samples are considered as food ingredient in some applications, the observed effect of enzyme treatment on the meals must be taken into account, and a judgment could be achieved whether or not to apply enzyme treatment, depending on the final purpose of oil and meal production. Inyang and Nwadimkpa (1992) reported that viscosities of oil-free sesame flours of 1% and 10% dispersions are 2.5 and 7.0 cP, respectively. These values are rather low when compared to those of the DPM samples (Table 1). The materials nature and ratio of dispersion (20% in our study) might have caused the difference.

Phytic acid contents of the poppy varieties are usually close to each other (9.96-15.08 mg P/g) (Table 1).While phytic acid contents of the Ofis 8-white samples (11.54 to 12.34 mg P/g) showed minor changes by the pre-treatments, Ofis 3-blue (9.96-13.43 mg P/g) and Ofis 4-yellow (11.40 to 15.08 mg P/g) were affected more. Eklund (1975) found 8.08 mg/g level of phytate-P in poppy seed flour, and it is seen that our values are slightly higher than this amount of phytic acid. Phytate-P contents of rapeseed, sunflower seed and nigerseed were also reported as 6.89, 8.80, and 8.35 mg/g (Eklund, 1975). Clearly, the phytate content of the DPM samples is higher than that of those seeds, indicating some nutritional considerations for bio-efficiency of some minerals and proteins.

The tannins ranged from 0.68 to 1.24 mg CE/g (catechin equivalents) in the DPM samples. The tannin contents of Ofis 3-blue variety (1.03-1.24 mg CE/g) were found to be the highest for all pre-treatment groups (Table 1). Overall higher tannin levels (1.13-1.24 mg CE/g) were obtained in the groups administered roasting. In the literature, no data on the tannin content of poppy seeds was found. Tannin content of proteins obtained from different oilseeds was determined by Sharma *et al.* (2010). The tannin contents were found as 0.7 mg/g for almond, 0.3 mg/g for Brazil nut, 0.3 mg/g for chestnut, 0.9 mg/g for hazelnuts, 0.3 mg/g for the macadamia nut, 0.0 mg/g for the pine nuts, 1.3 mg/g for the pistachio, 0.2 mg/g for soybean variety W82, and it was observed that the tannin contents of DPMs were much higher. However, it is also possible that the loss of seed tannins during the protein extraction might have occurred.

Mineral contents of defatted poppy meals

The mineral contents of DPMs were measured and are given in Table 2. The mineral substances specified in poppy meals are: Zn (6.25-18.30 mg/kg), Ba (0.05-0.61 mg/kg), Fe (0.22-0.79 mg/kg), B (0.53-1.29 mg/kg), Mn (0.89-1.01 mg/kg), Mg (49.89-67.00 mg/kg), Ca (263.65-289.10 mg/kg), Cu (0.38-0.59 mg/kg), Al (0.48-0.84 mg/kg), Na (97.8-3,507 mg/kg), K (1,163.0-5,469 mg/kg). Heavy metals (lead, cobalt, cadmium, nickel and chromium) were analysed, but not detected in the DPM samples. The impact of seed variety and treatments on the amount of mineral content were statistically significant, as expected. When the DPM samples from all treatment groups were analysed for mineral content, it was found that poppy seed is rich in especially calcium (268.55-289.10 mg/kg), magnesium (49.89-67.00 mg/kg), sodium (97.8-3,507.0 mg/kg) and potassium (1,163.0-5,469.0 mg/kg), but it is poor in terms of iron (0.22-0.79 mg/kg) (Table 2). In the study of Eklund (1975) iron was found as 110.8 μM/g and phosphorous as 260.8 μM/g in poppy flour. The ferrous content of our poppy varieties used in this study is lower than their finding.

Table 2. Mineral contents of the defatted poppy meals.[1,2]

Minerals (mg/kg)	Treatment	Ofis 8 (white)	Ofis 3 (blue)	Ofis 4 (yellow)
Zn	Control	11.60±0.00 Ab	6.25±0.07 Cb	9.60±0.00 Bb
	Roasted	11.35±0.35 Ab	6.25±0.07 Cb	9.40±0.28 Bb
	Enzyme	13.60±0.14 Ca	18.30±0.00 Aa	16.95±0.21 Ba
Ba	Control	0.06±0.00 Bb	0.07±0.00 Ba	0.57±0.00 Ab
	Roasted	0.055±0.07 Bb	0.07±0.00 Ba	0.55±0.00 Ac
	Enzyme	0.11±0.00 Ba	0.06±0.00 Ca	0.61±0.01 Aa
Fe	Control	0.23±0.00 Bb	0.22±0.07 Bb	0.39±0.00 Aa
	Roasted	0.23±0.00 Bb	0.22±0.07 Bb	0.38±0.07 Aa
	Enzyme	0.79±0.07 Aa	0.26±0.07 Ba	0.27±0.00 Bb
B	Control	1.29±0.02 Aa	0.47±0.01 Cb	1.23±0.07 Ba
	Roasted	1.26±0.02 Aa	0.46±0.00 Bb	1.23±0.07 Aa
	Enzyme	0.75±0.07 Ab	0.61±0.07 Ba	0.53±0.07 Cb
Mn	Control	1.01±0.07 Aa	0.92±0.01 Ba	0.90±0.00 Bab
	Roasted	0.99±0.01 Aa	0.91±0.00 Ba	0.89±0.07 Bb
	Enzyme	0.94±0.07 Ab	0.86±0.01 Bb	0.92±0.07 Aa
Mg	Control	58.29±1.58 Ba	50.97±0.95 Ca	62.29±1.75 Ab
	Roasted	58.26±1.53 Ba	50.91±0.86 Ca	62.13±1.52 Ab
	Enzyme	53.79±0.57 Bb	49.89±0.04 Ca	67.00±0.89 Aa
Ca	Control	281.75±1.77 Ba	279.65±2.19 Ba	289.10±3.25 Aa
	Roasted	280.00±0.71 Ba	277.85±0.35 Ba	288.85±2.90 Aa
	Enzyme	268.55±2.05 Bb	263.65±0.92 Bb	285.80±0.99 Aa
Cu	Control	0.51±0.00 Ba	0.43±0.07 Ca	0.59±0.00 Aa
	Roasted	0.51±0.07 Ba	0.42±0.00 Ca	0.59±0.00 Aa
	Enzyme	0.50±0.01 Aa	0.38±0.00 Bb	0.51±0.07 Ab
Al	Control	0.62±0.07 Bb	0.68±0.00 Ab	0.48±0.07 Cb
	Roasted	0.61±0.00 Bb	0.68±0.00 Ab	0.48±0.07 Cb
	Enzyme	0.83±0.07 Aa	0.84±0.01 Aa	0.75±0.07 Ba
Na	Control	288.0±9.1 Ab	310.2±2.5 Ab	101.2±2.0 Bb
	Roasted	298.4±5.6 Ab	301.7±14.4 Ab	97.8±6.8 Bb
	Enzyme	776.7±15.1 Ca	3,507.0±2.8 Aa	2,875.5±2.1 Ba
K	Control	2,620.0±116.0 Ba	3,640.5±753.1 Ab	1,163.5±13.4 Cb
	Roasted	1,563.5±36.1 Bb	5,439.0±468.0 Aa	1,163.0±14.1 Bb
	Enzyme	2,206.5±3.5 Ba	5,469.0±36.8 Aa	5,305.0±46.7 Aa

[1] Means followed by lower case letters within the same column represent significant differences (P<0.05) between treatments in the same seed variety; means followed by capital case letters within the same row represent significant differences (P<0.05) between seed varieties in the same treatment group.
[2] Values are given on dry matter basis as mean ± standard deviation.

Functional properties of defatted poppy meals

Different seed treatment processes have significant effects on the functional properties of the obtained DPM samples (Table 3). The effects of pre-treatment and seed variety on WHC of the poppy meal was not significant. The WHC values ranged from 2.24 to 3.14 g/g and the highest value of 3.14 g/g was determined in control Ofis 8-white sample. The water holding capacity of the white varieties showed some decrease with the roasting process though slight increase was observed for Ofis 3-blue and Ofis 4-yellow varieties. The enzyme treatment applied to all poppy varieties caused a small drop in the WHC. The lowest WHC values were determined in the enzyme treatment group for all seed varieties. The lowest WHC value (2.24 g/g) was measured in the blue variety of the enzyme treatment groups. Water and OHCs of the proteins are relative to the polar and non-polar amino acid composition (Martinez-Flores *et al.*, 2002). Srinivas and Narasinga Rao (1986) found the WHC of the oil free poppy flour as 1.24 g/g and the values of our study are significantly higher than their results. Adebowale *et al.* (2005) determined the WHCs of 6 different 'mucuna' bean varieties in the range of 1.40-2.20 g/g and the data of our study is above these values. Water holding capacity is an important functional property of the protein in foods such as bakery products, soups, sauces and pasta (Foegeding and Davis, 2011; Moure *et al*., 2006). The water holding capacity of the crude linseed flour was found as 3.45 g/g, while it was measured 4.43 g/g in the heat applied samples (Madhusudhan and Singh, 1985). In this respect, DPM is a suitable source for this type of foods in terms of WHC property.

In this study, the OHCs of DPMs were found to be affected by both kinds of the pre-treatments and seed varieties (Table 3). The OHCs of the samples (1.83-2.26 g/g) are generally in line with the data reported by Srinivas and Narasinga Rao (1986), in which, the OHC of oil-free poppy flour was determined as 2.5 g/g flour. The highest OHCs (2.06-2.25 g/g) were found in the roasted seeds. Adebowale *et al.* (2005) determined the OHCs of 6 different 'mucuna' bean varieties in the range of 2.1-2.6 g/g and the DPMs values were found to be close to these data. The OHC of crude and heat applied linseed flour samples were 2.36 and 1.37 g/g, respectively. It was also indicated that while WHC increases by heat application, OHC decreases (Madhusudhan and Singh, 1985). In another study, water and OHCs of heat treated bean flour were reported as 38% and 57%, respectively (Narayana and Narasinga Rao, 1982). Abbey and Ibeh (2006) reported that the WHC and OHC of chickpea flour increased from 2.4 g/g to 3.6 g/g and from 2.9 g/g to 3.2 g/g with heat treatment, respectively. In our study, roasting caused a less drop in the WHC of Ofis 8-white while slight increase was observed in Ofis 3-blue and Ofis 4-yellow samples so it can be said that heat treatment improves the OHC properties of the DPM samples. WHC of blue and yellow seed varieties were

Table 3. Functional properties of the defatted poppy meals.[1,2]

Property	Treatment	Ofis 8 (white)	Ofis 3 (blue)	Ofis 4 (yellow)
Water holding capacity (g/g)	Control	3.14±0.23 Aa	3.03±0.29 Aa	2.80±0.31 Aa
	Roasted	2.86±0.37 Aa	3.07±0.23 Aa	3.22±0.22 Aa
	Enzyme	2.48±0.32 Aa	2.24±0.17 Aa	2.66±0.52 Aa
Oil holding capacity (g/g)	Control	2.26±0.10 Aa	1.98±0.03 Bab	1.92±0.04 Bb
	Roasted	2.25±0.12 Aa	2.06±0.06 Ba	2.13±0.02 ABa
	Enzyme	1.89±0.02 Ab	1.83±0.03 Ab	2.00±0.05 Aab
Emulsifying activity (%)	Control	50.00±0.00 Ab	16.66±0.00 Cb	25.00±0.00 Bb
	Roasted	70.83±5.89 Aa	33.33±0.00 Ca	54.16±5.89 Ba
	Enzyme	4.17±0.00 Ac	4.17±1.17 Ac	3.33±0.00 Ac
Emulsion stability (%)	Control	41.66±0.00 Aa	16.66±0.00 Ba	25.00±0.00 Ba
	Roasted	54.16±5.89 Aa	16.66±11.78 Ca	37.49±5.89 Ba
	Enzyme	1.67±0.00 Ab	4.16±1.17 Aa	3.33±0.00 Ab
Foaming capacity (%)	Control	19.41±2.19 Aa	22.79±2.58 Aa	18.33±0.00 Aa
	Roasted	17.91±5.82 Ba	26.20±1.10 Aa	16.96±1.26 Ba
	Enzyme	2.02±0.01 Ab	3.88±0.05 Ab	5.31±0.64 Ab
Foam stability (%)	Control	46.92±9.79 Aa	34.13±4.75 Ab	45.45±12.85 Aa
	Roasted	27.88±4.07 Ba	62.74±5.54 Aa	36.67±4.71 ABa
	Enzyme	50.00±0.00 Aa	50.00±0.00 Aab	35.00±11.21 Aa

[1] Means followed by lower case letters within the same column represent significant differences (P<0.05) between treatments in the same seed variety; means followed by capital case letters within the same row represent significant differences (P<0.05) between seed varieties in the same treatment group.
[2] Values are mean ± standard deviation.

enhanced with roasting but for white seed this property was decreased by heat. The OHCs of the DPM samples were observed in a similar pattern. An increase was observed for blue and yellow varieties with roasting but it is almost the same as the value for the white variety.

Being important functional properties, EA and ES of the samples were measured (Table 3) and both pre-treatment and seed variety effects on EA with ES were found statistically significant. The best emulsifying activity, when analysed in terms of all the treatment groups, belongs to Ofis 8-white (4.17-70.83%) variety. The data obtained are consistent with earlier studies on oil-free poppy flour (Srinivas and Narasinga Rao, 1986). ES values were measured after leaving the samples in 80 °C water bath for 30 min and varied in the range of 1.67-54.16%. Ofis 8-white variety exhibited the highest ES in control (41.66%) and roasted (54.16%) groups while in the enzyme-treated samples Ofis 3-blue (4.16%) were found to be more resistant.

There was no study on ES of oil-free poppy flour or poppy protein in the literature. Heat treatment had been reported to cause reduction in the emulsion and FC of flax seed flour (Madhusudhan and Singh, 1985). In the study of Narayana and Narasinga Rao (1982), decrease in emulsion and FC of bean flour by heat treatment was reported with values of 35% and 18%, respectively. Martinez-Flores *et al.* (2002) reported the ES of flax seed protein concentrates as 88.4% for pH=8.0 and 50% for pH=4.0. The ES of the DPM samples (1.67-54.16%) were measured at pH=7.0 and said to be similar to flaxseed in terms of this functional property. EA and ES values were adversely affected by the enzyme treatment. The highest EA and ES results were observed in roasted seed samples. Denaturation increases the emulsification properties of the proteins due to the increase of the hydrophobic domain and developing flexibility (Moure *et al.*, 2006). Particularly, DPMs obtained from roasted seeds can be used in many foods for good emulsifying properties.

FC at pH=7.0 and FS after 30 min at room temperature of the samples were also tested. The FCs of the DPM samples ranged between 2.02 and 26.20% (Table 3). The highest FC values belonged to Ofis 3-blue variety in the control (22.79%) and roasted (26.20%) samples so that treatment and variety affected this feature significantly. The FCs of poppy seed oil-free flour was determined as 104% at pH=6.5 and 106% at pH=8.0 by Srinivas and Narasinga Rao (1986). The values obtained in our study are lower than these data and this result may be considered to arise from the influence of the difference in variety and oil extraction procedures. However, Sharma *et al.* (2010) reported low FC and stability for protein isolates derived from almonds, chestnuts and soy despite high protein (92.72, 92.29 and 94.80%, respectively) contents. In that study (Sharma *et al.*, 2010), the foaming capacities of almonds, chestnut, Brazil nuts, hazelnuts, macadamia nuts, pine nuts, pistachio nuts, soy beans W82 variety were determined above 40%. Besides the protein content, the kind of material was also indicated as important factor for the foaming properties of proteins (Sharma *et al.*, 2010). FS (pH=7.0) is expressed as a percentage of the remaining part of the initial amount of foam after 30 min at room temperature. In a previous study, FS at pH=8.0 was found 40% after 30 min (Srinivas and Narasinga Rao, 1986), and this finding is consistent with our values (Table 3). In the study of Adebowale *et al.* (2005), FC of 6 variety of 'mucuna' bean ranged between 50.0 and 84.3% and FS after 30 min was found to be 74.0-94.0%. These measurements are very high compared to our poppy meal values. Hrčková *et al*. (2002) studied on commercial defatted soy flour and the samples were treated with Flavourzyme (exopeptidase and endoprotease complex), Novozym (proteolytic enzymes), and Alcalase (endopeptidases). Enzyme treatment caused increase in FC, but significant reduction in FS. It was reported that partially hydrolysed protein is needed for stable foaming and some large molecules in order to provide the necessary protein components for foam stabilisation (Foegeding and Davis, 2011; Hrčková *et al*., 2002; Moure *et al.*, 2006). Generally, DPMs can be considered low in FC and better in FS.

The gelling properties of DPMs were tested at pH=7.0 and the least gelation concentrations (LGC) were stated as the ratio (%) of solid gel formation observed (Table 4). In preliminary tests, concentration of the sample under 10% resulted only in clot formation, so that the test plan is prepared with concentrations above 10% dispersions. Stable solid gels were observed between 16-20% dispersions and were quite high. Roasting had a negative effect on the gelation property for all seed varieties. The LGC values of the roasted samples (20%) were higher than the values of the control group (16-18%). Gelation properties of the enzyme treatment groups were extremely affected adversely, even full clot formation was not observed in 20% of sample concentration; hence, up to 38% concentrations were tested and at this concentration, only a coagulum was observed (Table 4). In the literature there was no study on the gelling properties of poppy flour or poppy protein isolates to compare with our results. Adebowale *et al.* (2005) reported the LGC of 6 different varieties of 'mucuna' bean as 14-20%. Gelation properties are in parallel with the water holding capacity and meals that have low WHC are known to form in sufficient gels. Gelation occurs more easily as a result of the increase in intermolecular contacts in high protein concentration during heating. High protein solubility is always indicated to be necessary for gelation (Adebowale *et al.*, 2005). Sharma *et al.* (2010) reported the LGCs for almonds, Brazil nuts, chestnuts, hazelnuts, macadamia nuts, pine nuts, pistachios, soy protein concentrates as 6, 8, 8, 12,20, 12, 10, 16%, respectively. In another study minimum gelation concentration of oil-free pumpkin seed meal was reported as 8% (Lazos, 1992). Studies on chickpea flour showed that while the minimum gelation concentration

Table 4. Least gelation concentrations of the defatted poppy meals.

Seed Variety	Treatment	Concentration								
		10%	12%	14%	16%	18%	20%	30%	34%	38%
Ofis 8 (white)	Control	liquid+clot	liquid+gel	liquid+gel	Solid gel	Solid gel	Solid gel	–	–	–
	Roasted	liquid+clot	liquid+clot	liquid+gel	liquid+gel	liquid+gel	Solid gel	–	–	–
	Enzyme	liquid	Liquid	liquid	liquid	liquid+clot	liquid+clot	liquid+clot	coagulum	coagulum
Ofis 3 (blue)	Control	liquid+clot	liquid+clot	liquid+gel	liquid+gel	Solid gel	Solid gel	–	–	–
	Roasted	liquid+clot	liquid+clot	liquid+clot	liquid+gel	liquid+gel	Solid gel	–	–	–
	Enzyme	liquid	Liquid	liquid	liquid	liquid+clot	liquid+clot	liquid+clot	coagulum	coagulum
Ofis 4 (yellow)	Control	liquid+clot	liquid+clot	liquid+clot	liquid+clot	Solid gel	liquid+clot	–	–	–
	Roasted	liquid+clot	liquid+gel	liquid+gel	liquid+gel	liquid+gel	Solid gel	–	–	–
	Enzyme	liquid	Liquid	liquid	liquid	liquid+clot	liquid+clot	liquid+clot	coagulum	coagulum

of the crude flour was 16%, as a result of heat treatment that increases to 18% (Abbey and Ibeh, 2006). Ingyang and Nwadimkpa (1992) determined the minimum gelation concentration of sesame flour as 6% with weak gel and a strong gel structure was formed with concentrations above 10%. Hrčková *et al.* (2002) treated commercial defatted soy flour with Alcalase for 4 hours, and observed minimum gelling concentration at 2%, but the gel was observed to slide on the test tube wall. A weak gel was formed with 6% Novozym sample concentration. Flavourzyme samples exhibited solid gel structure and the formation of gelling at 2% concentration was apparent. Gelling properties of oil-free seed flours and protein extracts as well as DPM may be an important feature for foods like processed meat and bakery products.

4. Conclusions

Poppy seed press cakes (meals) obtained by cold pressing were good enough quality materials in terms of composition and some functional properties. Besides, it was determined that these meals have the potential to be used as human food. Low yield of oil and the remaining high amount of oil in the meal is the fact of cold pressing method. Therefore, the press cake prior to consumption as food or additives and protein isolation techniques, an additional solvent defatting can be made. It was determined that DPMs are very nutritious with protein contents of 26.87-35.14%, and low tannins. They are also good source of potassium, calcium and magnesium minerals. Some compositional and functional properties of the DPM samples were affected by previous roasting and enzyme treatment applications. Generally, enzyme (hemicellulase and protease) treatments reduced, and roasting enhanced the functional properties. Water holding capacities as well as viscosity are important functional properties of the protein in foods such as bakery products, soups, sauces and pasta. In this respect, DPM can be suggested as a suitable source for this type of foods. The results of this study indicate that DPMs can be safe, nutritious and partially functional ingredient for processed food applications.

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