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Extraction of protein from sunflower Deoiled cake

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Abstract
Sunflower seeds (*Helianthus annuus* L.) are among the world's most important oilseeds. The techno functional properties of sunflower proteins are comparable with those of soy and other leguminous proteins. Sunflower meal is a source of high quality protein. Preliminary experiment on extraction of protein was carried out with sodium chloride (NaCl concentration of 1-3 mol/L) using 1.0 N NaOH for pH adjustment. Three levels of pH (6-8), extraction temperature (15-45 °C), NaCl concentration of 1-3 mol/L and ratio of sunflower meal to solvent (10-20 w/v) were selected and evaluated to study the effect of extraction process variables on protein isolate yield (% weight) and protein content (%). The results revealed that the protein isolate yield was less than 15% and were also dark in colour. Hence, the treatments were modified by changing NaCl concentration to 8.0% minimum to 12.0% maximum and Meal % (8 to 12%). The temperature (30 °C) and extraction time (30 min) were kept constant for all treatments for better extraction. Extracted protein was precipitated at pH 4.5 with 1.0 N HCl, and collected by centrifugation at 5000 rpm for 30 mins. The sunflower protein isolates (SPI) were washed with water and adjusted to the pH of precipitation, and again re-centrifuged. The washed SPI was adjusted to pH 7 with NaOH prior to being dried. In conclusion, response surface methodology technique was found to be very useful in determining the optimization conditions for extraction of protein isolate. The optimum protein extraction was achieved by extracting the cake at the extraction conditions of: pH-9, NaCl % -9, Meal % - 10.

Keywords: Defatted sunflower oil cake, protein isolates, protein content, sodium chloride, and sodium hydroxide.

Introduction
Sunflower seeds (*Helianthus annuus* L.) is most important oilseed compared to others in the world. Though it contains about 20% of protein, the protein content of the oil press cake and extraction residues however, range from 30 to 50% (Dorrell & Vick, 1997) [4]. The techno functional properties of sunflower proteins are comparable with those of soy and other leguminous proteins (Gonza´ lez-Pe´ rez *et al*., 2005) [8]. Although lysine deficiency is a major drawback from the nutritional point of view, proteins from sunflower press cake are considered a valuable alternative as food ingredients, since they are low in antinutritional compounds and devoid of toxic substances (Gassmann, 1983; Gonza´ lez-Pe´ rez & Vereijken, 2007) [5, 2].

The application of the sunflower meal as an unconventional protein source for human consumption could be an alternative approach which leads to more complete use of this by-product. Except for lysine deficiency, nutritive value and functional properties of sunflower proteins are comparable to those of soy and other leguminous proteins. Additional advantage of the sunflower proteins is their low contents of anti-nutritional and allergen factors. Currently, no genetically modified commercial sunflower varieties are available for field cultivation, which makes sunflower protein isolates useful and safe for the production of organic foods (Cantamutto and Poverene, 2007) [3]. Therefore, in addition to solving environmental issues, the more complete utilization of the sunflower meal as a source for inexpensive protein for human needs could also respond to continuously increasing worldwide demand for proteins. However, the optimal isolation of proteins from sunflower meal is very often impeded by decreased protein solubility due to denaturation occurring during oil production. Like proteins of other oilseeds, those of sunflower seeds mainly consist of globulins. Thus, the majority of the proteins is insoluble in water but can be extracted with 5% NaCl (Gheyasuddin *et al*., 1970) [6]. It is well known that the addition of salts enhances protein extractability, especially close to the isoelectric point. The pH region of minimum solubility (pH 4–6) is shifted towards lower pH values upon salt addition (Pawar *et al*., 2001) [10]. The effects of pH and various salt concentrations on protein extractability from sunflower meal have been studied by different authors. Additionally, Gheyasuddin *et al*. (1970) [6] investigated the effects of temperature and the meal-to-solvent ratio (MSR) on protein extractability.

An alternative process for the production of light-coloured sunflower protein isolates has recently been proposed using mild-acidic protein extraction and high salt concentrations to enhance the protein solubility to exploitable levels (Pickardt *et al*., 2009) [11].

Materials and methods

Sunflower meal was purchased from Bijapur oil mill in Karnataka state. The meal was passed through a 44 mesh (350 microns) to remove most of the hull fragments. Estimation of fat and moisture content was carried out using standard procedures

Plate 1: Sunflower seed and de-oiled cake

Based on reviews, preliminary experiment on extraction of protein was carried out with sodium chloride (NaCl concentration of 1-3 mol/L) using 1.0 N NaOH for pH adjustment (Gheyasuddin *et al*., 1970) [6]. Three levels of pH (6-8), extraction temperature (15-45 °C), NaCl concentration of 1-3 mol/L and ratio of sunflower meal to solvent (distilled water-10-20 w/v) were selected and evaluated to study the effect of extraction process variables on protein isolate yield (% weight) and protein content. Extraction was carried out at 15-45°C for 30 mins. The extracts were centrifuged at 5000 rpm for 30 mins. Extracted protein was precipitated at pH 4.5 with 1.0 N HCl, and collected by centrifugation at 5000 rpm for 30 mins. The sunflower protein isolates (SPI) were washed with water and adjusted to the pH of precipitation, and again re-centrifuged as previously described. The washed SPI was adjusted to pH 7 with NaOH prior to being dried. Yield was recorded (Betschart and Saunders, 1978) [2] and the protein content of the isolates was determined by Micro-Kjehdahl method (AOAC, 2012) [1].

Design of experiments

The extraction of protein involves many variables and levels; an efficient statistical design called Box-Behnken design under response surface methodology was employed for the experimentation. The design layout of RSM included 29 treatments with 5 replications at the centre points of the coded variables as shown in Table 1.

Table 1: Coded levels of independent variables and their values

Varible Name	Symbols	-1 Level	+1 Level
pH	A	6	8
Temperature (°C)	B	15	45
NaCl concentration (mol/L)	C	1	3
Ratio of Meal to Solvent (w/v)	D	1:10	1:20

The extraction of protein was carried out based on the aforesaid design and the protein yield was noted. The results are as given in Table 2. The moisture content and fat content of deoiled sunflower cake was found to be 9.2% and 4.25%, respectively. The results revealed that the protein isolate yield was less than 15% even with the tested high concentration of NaCl and high temperature. The isolates were also dark in colour. Hence, the treatments were modified by changing NaCl concentration to 8.0% minimum to 12.0% maximum and Meal % (8 to 12%) according to the results concluded by Ivanova *et al*. (2012) [12]. Second set of experiments with Box-Behnken design was planned with three variables each at three levels with a total of 17 treatments. The temperature (30 °C) and extraction time (30 min) were kept constant for all treatments for better extraction as mentioned above.

Table 2: Box-Behnken design used for the preparation of protein isolate with four variable and its responses

	Factor1	Factor 2	Factor 3	Factor 4	Response 1
Treatments	A:pH	B:Temperature (°C)	C:NaCl Concentration (mol/L)	D: Meal to Solvent ratio(w/v)	Protein Isolate Yield (% weight)
1	6	30	1	1:15	6.00
2	7	45	3	1:15	13.22
3	8	45	2	1:15	8.85
4	8	30	3	1:15	7.40
5	8	30	1	1:20	7.84
6	7	15	3	1:15	11.75

Results and Discussion

Effect of extraction process variables on protein isolate yield (%weight)

The protein isolate yield for various treatment combinations are presented in Table 3. It revealed that the protein isolate yield ranged from 22.00 to 32.64% when subjected to different extraction process variables. The maximum protein isolate yield was found for treatment combination of pH -9, NaCl % -10 and Meal % -10. The minimum protein isolate yield was found for treatment combination of pH -10, NaCl % - 10 and Meal % -12.

The effect of pH, NaCl % and Meal % was significant and the ANOVA table also indicated that the model was statistically acceptable at 1% level. The protein isolate yield was influenced mostly by the NaCl % followed by pH and the least by Meal %. The interactions of these parameters had little significant effect on protein isolate yield. In this case, B, B^2, C^2 and BC are significant model terms. The R^2 value of 0.91 and a low coefficient of variation of 5.42% suggest that the second order polynomial model was adequate for predicting the protein isolate yield. The coefficient of variation (CV) is the ratio of the standard error of estimate to the mean value of observed response expressed as a percentage. It is a measure of reproducibility of the models.

Effect of extraction process variables on protein content of extracted isolate

The protein contents for various treatment combinations are presented in Table 3. It revealed that the protein content was observed to be ranging from 56.00 to 74.00%. The maximum protein content was found for treatment combination of pH -9, NaCl % -8 and Meal % -12. The minimum protein content was found for treatment combination of pH -8, NaCl % - 8 and Meal % - 10.

Table 3: Box Behnken design for the preparation of protein isolate with three variables and its responses

Treatments	Factor 1	Factor 2	Factor 3	Response 1	Response 2
	A:pH	B:NaCl %	C: Meal %	Protein Isolate Yield %	Isolate Protein Content %
1	8	8	10	29.70	56.00
2	10	8	10	30.00	70.15
3	10	10	8	28.11	70.00
4	9	10	10	32.64	68.00
5	9	10	10	32.00	70.00
6	9	8	8	24.00	65.00
7	10	10	12	22.00	69.00
8	8	12	10	28.50	60.21
9	8	10	12	28.95	64.15
10	9	10	10	32.64	67.45
11	8	10	8	29.25	56.00
12	9	8	12	30.00	74.00
13	9	10	10	32.64	68.00
14	9	10	10	32.00	70.00
15	9	12	8	25.50	62.55
16	9	12	12	22.50	58.00
17	10	12	10	26.50	66.00

Modelling of protein isolate yield content as a function of extraction process variables (pH, NaCl %, Meal %)

The ANOVA (Table 4) indicated that the model was significant. The effect of pH and Meal % was significant. The protein content of the isolate was mainly influenced by pH and meal % which accounts to be 80 % sum of square. R^2 value of 0.89 suggests that the second order polynomial model was adequate for predicting the protein content. The CV of the model was 4.07 % means that the model was quite reproducible. The protein content varied from 56 to 74%.

Table 4: ANOVA for response surface quadratic model for protein isolate yield (%weight)

Source of variation	Sum of square	df	Mean square	F value	P- value Prob>F
Model	177.05	9	19.67	8.16	0.0057
A- pH	11.98	1	11.98	4.97	0.0610
B- NaCl %	14.31	1	14.31	5.94	0.0450
C- Meal %	1.45	1	1.45	0.60	0.4629
AB	1.32	1	1.32	0.55	0.4830
AC	8.44	1	8.44	3.50	0.1035
BC	20.25	1	20.25	8.40	0.0230
A^2	4.78	1	4.78	1.98	0.2018
B^2	29.42	1	29.42	12.20	0.0101
C^2	75.72	1	75.72	31.41	0.0008
Std. Dev.	1.55		R- squared	0.91	
Mean	28.64		C.V. %	5.42	

Table 5: ANOVA for response surface quadratic model for protein content (%)

Source	Sum of square	df	Mean square	F value	P- value Prob>F
Model	409.06	9	45.45	6.39	0.0115
A- pH	188.08	1	188.08	26.43	0.0013
B- NaCl %	42.27	1	42.27	5.94	0.0449
C- Meal %	16.82	1	16.82	2.36	0.1681
AB	17.47	1	17.47	2.46	0.1611
AC	20.93	1	20.93	2.94	0.1300
BC	45.90	1	45.90	6.45	0.0387
A^2	34.20	1	34.20	4.81	0.0645
B^2	31.84	1	31.84	4.47	0.0722
C^2	4.66	1	4.66	0.66	0.4448
Std. Dev.	2.67		R- squared	0.89	
Mean	65.56		C.V. %	4.07	

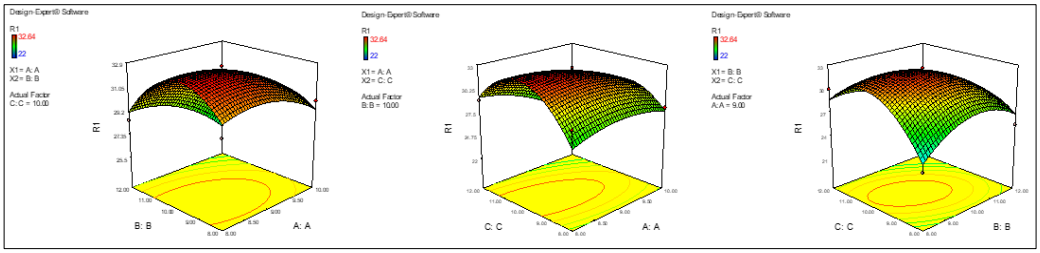

Fig 1: Response surface plots for protein isolate yield (%) as a function of pH, NaCl % and Meal %

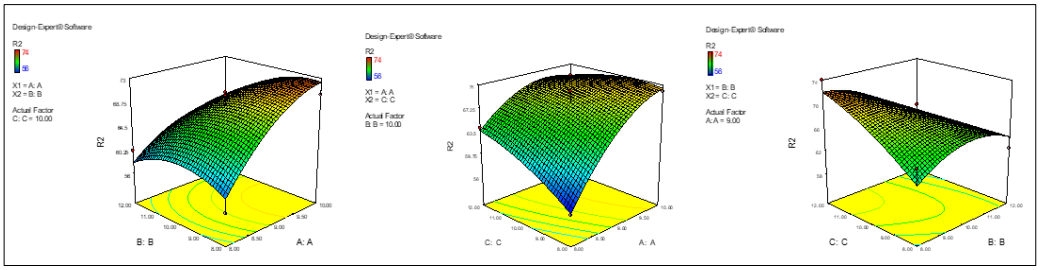

Fig 2: Response surface plots for protein content (%) as a function of pH, NaCl % and Meal %

Effect of pH on protein isolate yield (%weight) and protein content (%)

The protein extraction was conducted at pH ranging from 8 to 10. At pH 8, there was a slight variation in the extraction of protein isolates. However, it increased at pH 9 and there was a decline in the yield at pH 10. The results obtained in our study demonstrated that the protein extractability increased with the increase of pH and reached maximum values in alkaline medium at pH 9 (Figure 1). Similar results were obtained by Gheyasuddin *et al*. (1970) [6] and Pickardt *et al*. (2009) [11]. It is well known that sunflower seed proteins mainly contained albumins and globulins, and small amounts of insoluble and alkali-soluble fractions. According to González-Pérez *et al*. (2004), helianthinin which accounts for approximately 85% of the total proteins in the mature seed, is salt-soluble and is extracted to a greater extent at pH 8.5. In this study, the protein isolate yield varied from 22 to 32.64% and the protein content of isolates varied from 56.00 to 74.00%. The lowest protein content was observed for pH 8 and the highest was observed for pH 9.

Plate 1: Sunflower defatted cake Sunflower defatted cake powder Sunflower protein isolate

Effect of NaCl % on protein isolate yield (%weight) and protein content (%)

Protein isolate yield increased with the increase of NaCl concentrations up to 10%. Further increase in NaCl concentration showed decrease in protein yield. At the higher pH values, the extraction of the sunflower protein with 10% NaCl resulted in greatest yield. The protein content was maximum at 8% NaCl.

Effect of Meal % on protein isolate yield (%weight) and protein content (%)

The protein isolate yield (% weight) was maximum for Meal 10 % and decreased further as the meal % increased. Whereas the protein content decreased initially and increased further as meal % increased.

Optimization of the extraction process variables for better yield and protein content

Optimization of the extraction process variables was carried out using response surface methodology in Design Expert 7.00 software. The numerical optimization involves application of desirability function method in which weights were assigned to the goals to adjust the shape of their respective desirability functions. The criteria used to optimize the extraction process variables for better protein isolate yield and protein content are listed in Table 6.

Table 6: Criteria for optimization of extraction process variables

Factors	Goal	Lower limit	Upper limit	Importance
pH3	is in range	8	10	-
NaCl %	is in range	8	12	-
Meal %	is in range	8	12	-
Yield %	maximize	22.00	32.64	5
Protein %	maximize	56.00	74.00	3

The solution having highest desirability value was given priority and the factor combination obtained in the corresponding solution was selected as optimal. Thus, the pH of 9.24, NaCl % of 9.19 and Meal % of 10.23 were found to be optimal for better protein isolate yield and protein content. At this optimized condition, the yield and protein content as predicted by the software are:

Protein isolate yield - 32.14 %

Protein content - 70.63 %

In order to validate the optimum parameters, the experiment confirmation was conducted in triplicates at optimum parameters (pH -9.00, NaCl % - 9.00, Meal % - 10.00). The values of responses predicted by the model were compared with the value observed. The experiment validation showed that under the optimum parameters, the experimental values for extraction process variables were in close agreement with

the predicted value which confirmed the adequacy of the model developed by RSM.

Table 7: Predicted and observed responses at optimum parameters

Responses	Predicted value	Observed value
Protein isolate yield %	32.14	30.00
Protein content %	70.63	70.15

Conclusion

In conclusion, response surface methodology technique was found to be very useful in determining the optimization conditions for extraction of protein isolate. Protein isolate was extracted from defatted sunflower meal that remained after oil extraction. The quadratic model developed exhibited a non-significant value for lack of fit and high value for the coefficient of determination. The optimum protein extraction was achieved by extracting the cake at the extraction conditions of: pH-9, NaCl % -9, Meal % - 10.

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