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Process optimization for extraction of protein from defatted safflower oil cake

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Abstract
Safflower (*Carthamus tinctorius* L.) is one of the oldest cultivated oilseed crops. Safflower meal is a source of high quality protein. Isolating safflower protein is one method of introducing this protein source into the human diet. Safflower meal protein was extracted with distilled water and the slurry was adjusted to pH 8, 9 or 10 with l.0 N NaOH. To study the effect of extraction process variables on protein isolate yield (% weight) and protein content, five levels of pH (7-11), extraction temperature (10-50 °C), extraction time (10-50 mins) and ratio of safflower meal to solvent (7-11w/v) using Design expert software were selected and evaluated. The moisture content and fat content of defatted safflower meal was 5.3 % fat content and moisture 12.74 % respectively. Extracted protein was precipitated at pH 5 with l.0 NHCl, and collected by centrifugation at 2000 rpm for 20 mins. Protein isolate yield and its protein content were estimated. In conclusion, response surface methodology technique was found to be very useful in determining the optimization conditions for extraction of protein isolate. The optimum extraction was achieved by extracting the meal at pH -9, temperature 30 °C, time 32mins and meal to solvent ratio 1: 9 w/v.

Keywords: Defatted safflower oil cake, protein isolates, protein content, sodium hydroxide, distilled water

Introduction
Safflower (*Carthamus tinctorius* L.) is one of the oldest cultivated oilseed crops. Originally grown for the dyestuff carthamin, this crop is being cultivated more recently for its polyunsaturated oil. Once the oil has been extracted, the remaining high protein meal is the raw material from which flours, protein concentrates, and isolates are derived. Safflower has some agronomic advantages over other oilseed crops, such as drought tolerance for arid or semi-arid countries [1].

Safflower meal is a source of high quality protein for animal feeds but has not been used for human consumption because it is bitter and mildly cathartic. Deleterious glucosides in the meal were removed or modified by extraction with either water at the isoelectric point or by processing to protein isolates [2].

Isolating safflower protein is one method of introducing this protein source into the human diet. Safflower protein isolates have been prepared from safflower kernels [3]. The influence of several variables upon the extractability of safflower proteins, which are mainly globulins and glutelins, has been reported by [4, 5]. have examined the amino acid composition of several protein isolates including safflower.

A simple one step alkaline method for protein extraction has shown reasonable yields, of about half of the original press cake protein [6]. Therefore, the aim of this work was to evaluate the possible applications of the alkaline protein extract as a food emulsifier, by investigating some of its functional properties. The experiments were conducted in dilute systems in a wide range of pH conditions using response surface methodology. RSM has important application in the design, development and formulation of new products, as well in the improvement of existing product design. RSM is a useful tool applied towards the optimization of several food processing operation [7].

Thus, the objective of this study was to investigate the influence of selected extraction and precipitation pH variables upon yield and protein content of safflower protein isolates.

Materials and methods
Safflower meal was purchased from the local market. 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 procedure.

Extraction of safflower protein

Safflower meal protein was extracted with distilled water and the slurry was adjusted to pH 8, 9 or 10 with l.0 N NaOH. Extraction was carried out at 20- 40 °C for 20-40 mins. The extracts were centrifuged at 2000 rpm for 20 mins. Supernatants were decanted to remove small quantities of hulls remaining on the surface of the supernatant. Extracted protein was precipitated at pH 5 with l.0 N HCl, and collected by centrifugation at 2000 rpm for 20 mins. The safflower protein isolates were washed with water adjusted to the pH of precipitation, and re centrifuged as previously described. The washed SPI was adjusted to pH 7 with NaOH prior to being dried. Yield was recorded [8] and the protein content of the isolates was determined by micro-kjehdahl method [9].

$$\text{Protein isolate yield (\% weight)} = \frac{\text{Weight of protein isolate}}{\text{Weight of original meal}} \times 100$$

Design of Experiment

To study the effect of extraction process variables on protein isolate yield (% weight) and protein content, five levels of pH (7-11), extraction temperature (10-50 °C), extraction time (10-50 mins) and ratio of safflower meal to solvent (7-11w/v) were selected and evaluated. The levels of pH, extraction temperature, extraction time and meal to solvent ratio were selected based on reviews and preliminary experiments. Since, extraction of protein involves many variables and levels an efficient statistical design called central composite design under response surface methodology for evaluation was employed for the experimentation. The design layout of RSM included 30 experiments with 6 replications at the centre points of the coded variables as shown in Table 1, to calculate the error sum of squares and lack of fit of the developed regression equation between the responses and independent variables.

Table 1: Coded levels of independent variables and their values

Name	Symbols	-1 Level	+1 Level	0	-alpha	+ alpha
pH	A	8	10	9	7	11
Temperature (°C)	B	20	40	30	10	50
Extraction time (mins)	C	20	40	30	10	50
Ratio of Meal to Solvent (w/v)	D	1:8	1:10	1:9	1:7	1:11

Analysis of data

Design-Expert Version 7.0 was used for conducting the experimental design. The protein isolate yield and percentage of protein content were taken as dependent variables or responses. For predicting the optimum extraction process variables, the goals were set for maximum protein isolate yield and maximum protein content. The independent variables were kept within the experimental range. Subsequent confirmatory experiments were also carried out to validate the models.

Results and Discussion

The moisture content and fat content of defatted safflower meal was 12.74 % and 5.3 % respectively.

Experiments were conducted to optimize the extraction process and protein isolate yield and protein content were calculated and the results were analyzed statistically using Design Expert software. The effect of extraction process variables on the protein isolate yield and protein content are shown in Table 2.

Table 2: Central composite design (CCD) for the preparation of protein isolate and its responses

	Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2
Treatments	A:pH	B:Temperature (°C)	C:Extraction time(mins)	D: Meal to Solvent ratio(w/v)	Yield %	Protein %
1	9	30	30	1: 7	40.00	74.13
2	9	30	30	1: 9	40.50	80.73
3	8	20	20	1: 10	21.25	72.22
4	9	30	30	1: 9	40.50	80.73
5	8	20	40	1:10	22.50	80.32
6	11	30	30	1:9	44.25	62.74
7	9	30	30	1:9	40.50	80.73
8	9	50	30	1:9	38.75	70.19
9	10	40	20	1:10	40.50	68.47
10	10	20	20	1:8	36.25	72.76
11	9	30	50	1:9	38.75	71.65
12	7	30	30	1:9	05.00	56.19
13	9	30	30	1:9	40.50	80.73
14	9	30	30	1:9	40.50	80.73
15	8	40	20	1:8	26.57	74.25
16	8	40	40	1:8	15.00	90.60
17	9	10	30	1:9	28.00	79.23
18	8	40	20	1:10	20.00	84.77
19	8	20	40	1:8	10.00	86.30
20	10	20	20	1:10	38.75	70.22
21	9	30	10	1:9	33.75	75.04
22	10	20	40	1:8	38.75	73.72
23	9	30	30	1:9	40.50	80.73
24	9	30	30	1:11	40.50	75.50
25	10	40	40	1:10	42.00	69.97
26	10	20	40	1:10	42.50	65.52
27	8	40	40	1:10	18.33	83.88
28	10	40	40	1:8	38.00	67.75
29	10	40	20	1:8	40.00	66.20
30	8	20	20	1:8	18.00	81.01

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

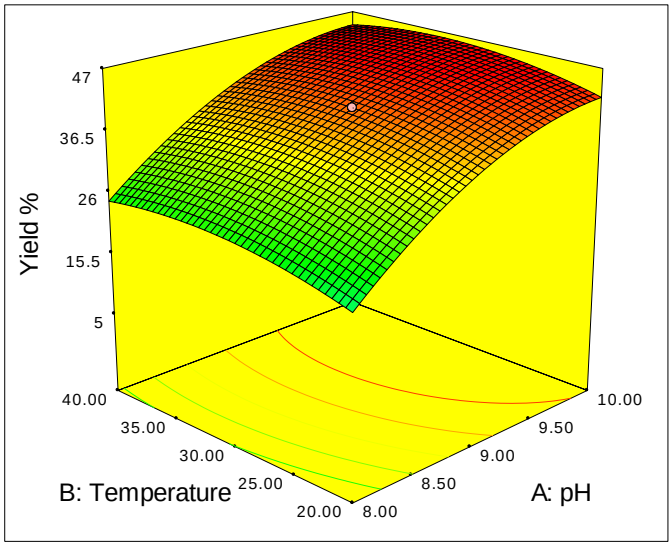
The relationship between extraction process variables and protein isolate yield is illustrated in three- dimensional response surface plots (Fig. 1). The response surface plots show that the protein isolate yield varied with different levels of the extraction process variables i.e., pH, temperature, extraction time and meal to solvent ratio. Using RSM, it was found that the relationship between the protein isolate yield and extraction process variables could be best explained using a second order polynomial model. Hence the experimental data was fitted to a second order polynomial model. The ANOVA was performed for the response surface quadratic model for protein isolate yield (Table 3). The ANOVA table indicated that the model was significant.

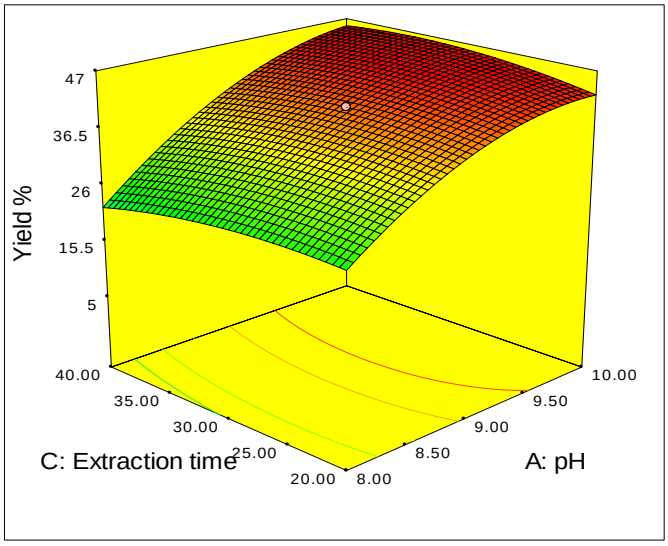
Table 3: ANOVA for response surface quadratic model for protein isolate yield (%weight)

Source	Sum of square	df	Mean square	F value	P- value Prob>F
Model	3370.53	14	240.75	19.31	<0.0001
A- pH	2462.40	1	2462.40	197.51	<0.0001
B- Temperature	46.48	1	46.48	3.73	0.0726
C- Time	0.94	1	0.94	0.075	0.7878
D- Meal to solvent ratio	23.52	1	23.52	1.89	0.1898
AB	0.72	1	0.72	0.058	0.8130
AC	43.03	1	43.03	3.45	0.0829
AD	0.099	1	0.099	7.959E-003	0.9301
BC	10.14	1	10.14	0.81	0.3813
BD	25.60	1	25.60	2.05	0.1724
CD	37.21	1	37.21	2.98	0.01046
A^2	602.95	1	602.95	48.36	<0.0001
B^2	171.57	1	171.75	13.76	0.0021
C^2	87.13	1	87.13	6.99	0.0184
D^2	16.79	1	16.79	1.35	0.2641
Std. Dev.	3.53		R- squared	0.94	
Mean	32.70		C.V. %	10.80	

ANOVA indicated that the model is statistically acceptable at 1 % level and possessed an insignificant lack of fit. The protein isolate yield was influenced mostly by the pH, which accounts for 73.05 % of the total sum of squares, followed by temperature, meal to solvent ratio and the least by extraction time.

The interactions of these parameters had no significant effect on protein isolate yield. In this case, A, A^2, B^2, C^2, are significant model terms. A high R^2 value of 0.94 and a low coefficient of variation of 10.80 % 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. The CV of the model was 10.80%. It means that the model was quite reproducible. It was also found that the estimated values are close to the actual values as evident from high R^2 value (0.94) and the close to unity slope of straight line fit between them.





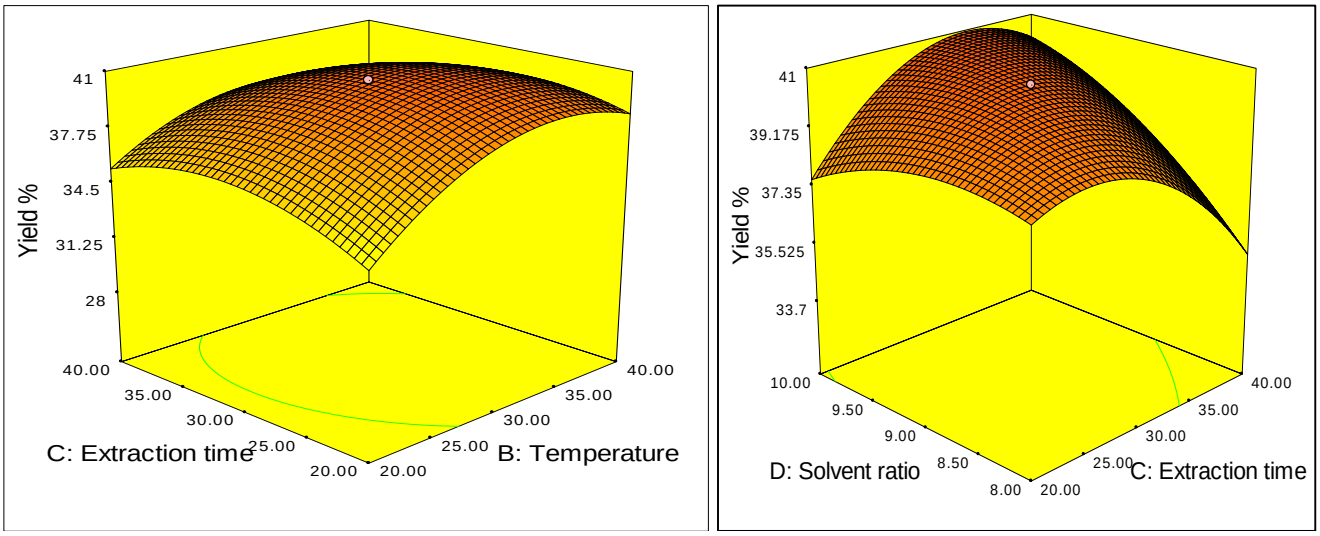


Fig 1: Response surface plots for protein isolate yield as a function of pH, extraction temperature, extraction time and meal to solvent ratio

Effect of extraction process variables on protein content

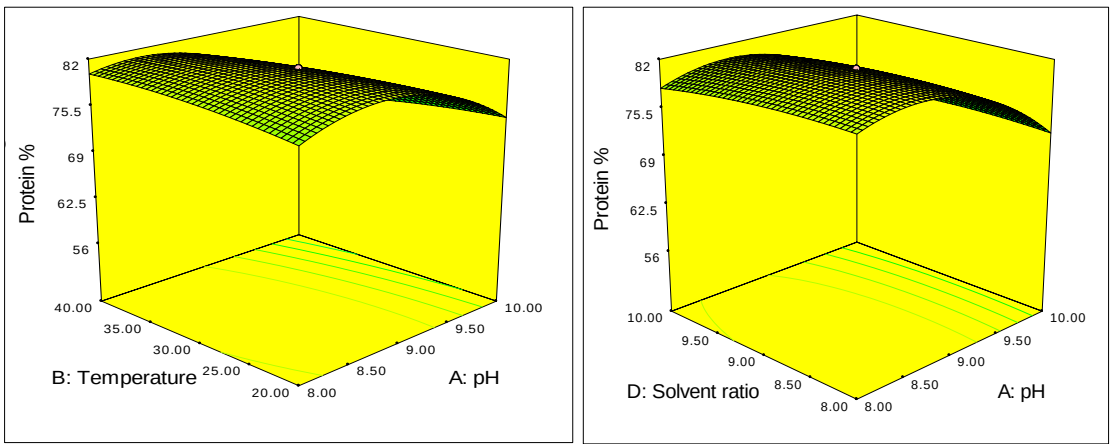
The relationship between extraction process variables and protein content is illustrated in three- dimensional response surface plots (Fig. 3). The response surface plots show that the protein content varied with different levels of the extraction process variables i.e., pH, temperature, extraction time and meal to solvent ratio. The ANOVA was performed for the response surface quadratic model for protein content (Table 4). The ANOVA table indicated that the model was not significant.

Table 4: ANOVA for response surface quadratic model for protein content

Source	Sum of square	df	Mean square	F value	P- value Prob>F
Model	1092.83	14	78.06	1.97	0.1030
A- pH	305.59	1	305.59	7.70	0.0142
B- Temperature	8.47	1	8.47	0.21	0.6507
C- Time	19.05	1	19.05	0.48	0.4991
D- Meal to solvent ratio	8.74	1	8.74	0.22	0.6457
AB	34.46	1	34.46	0.87	0.3662
AC	54.54	1	54.54	1.37	0.2594
AD	1.39	1	1.39	0.035	0.8539
BC	4.91	1	4.91	0.12	0.7300
BD	71.40	1	71.40	1.80	0.1998
CD	25.35	1	25.35	0.64	0.4366
A^2	554.76	1	554.76	13.98	0.0020
B^2	12.91	1	12.91	0.33	0.5769
C^2	28.95	1	28.95	0.73	0.4065
D^2	11.94	1	11.94	0.30	0.5914
Std. Dev.	6.30		R- squared	0.64	
Mean	75.23		C.V. %	8.37	

Table 4 shows that the protein content was not significantly ($p < 0.001$) affected by all extraction process variables. The protein content was influenced mostly by the pH, which accounts for 28 % of the total sum of squares, followed by time, temperature and the least by meal to solvent ratio. The interactions of these parameters had no significant effect on protein content.

In this case, A, A^2, is only the significant model terms. R^2 value of 0.64 and a low coefficient of variation of 8.37 % suggest that the second order polynomial model was adequate for predicting the protein isolate yield. The CV of the model was 8.37 % means that the model was quite reproducible.



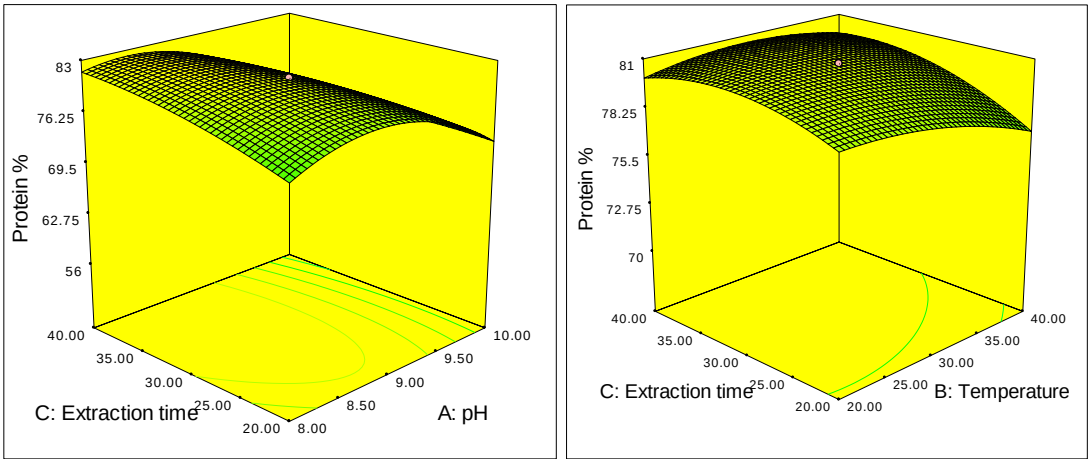


Fig 2: Response surface plots for protein content as a function of pH, extraction temperature, extraction time and meal to solvent ratio

Effect of pH on protein isolates yield and protein content

Results showed that with increase in pH there is an increase in both yield and protein content. As reported by Betschart and Saunders, 1978, % weight yield of safflower protein isolate increases with more alkaline extraction where as the protein content decreases as pH increases due to Maillard reaction and decreases the nutritive value of protein especially essential amino acid such as lysine, while it increases the extraction of non-protein component, which co precipitates with protein leading to lower protein purity [10]. Maximum yield was observed at pH 11 and minimum was noticed at pH 7 where as the maximum protein content was observed at pH 8 and minimum was at pH 11.

Effect of extraction temperature on protein isolates yield and protein content

The highest protein isolate yield was observed at 30 °C where as the highest protein content was observed at 40 °C. There is no much effect of temperature on both the responses. Graphical representation shows that a good yield and better protein content can be achieved at around 30-40 °C.

Effect of extraction time on protein isolates yield and protein content

Similar trend was noticed as above graph. The highest protein isolate yield was observed at 30 mins where as the highest protein content was observed at 40 mins.

Effect of meal to solvent ratio on protein isolates yield and protein content

The highest protein isolate yield was observed at 1:9 where as the highest protein content was observed at 1:8. It shows that the effect of other variables have also influenced the responses.

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

The optimization 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 5.

Table 5: Criteria for optimization of extraction process variables

Factors	Goal	Lower limit	Upper limit	Importance
pH 3	is in range	8	10	-
Temperature	is in range	20	40	-
Extraction time	is in range	20	40	-
Meal to solvent ratio	is in range	8	10	-
Yield %	maximize	5.00	44.25	3
Protein %	maximize	56.19	90.6	5

A total of 2 solutions were obtained from optimization step. 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 9.04, temperature of 28.7 °C, time of 32.5 mins and meal to solvent ratio of 1: 8.74w/v were found to be optimal for better protein isolate yield and protein content. At this optimized conditions, the yield and protein content as predicted by the software are: Protein isolate yield – 40.1% and Protein content - 80.1 %.

In order to validate the optimum parameters, the experiment confirmation was conducted in triplicates at optimum parameters (pH -9, temperature 30 °C, time 32mins and meal to solvent ratio 1:9 w/v). 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 6: Predicted and observed responses at optimum parameters

Responses	Predicted value	Observed value
Protein isolate yield %	40.1	40.5
Protein content %	80.1	81.0

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 safflower 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 extraction was achieved by extracting the meal at pH -9, temperature 30 °C, time 32 mins and meal to solvent ratio 1: 9 w/v.

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