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Isolation and characterization of protein isolates from apricot press cake

Anil Gupta, Devina Vaidya, Manisha Kaushal and Abhishek Thakur

Abstract
The apricot kernel deoiled press cake with good functional properties was used for extraction of protein isolate. Protein isolation conditions were optimized using response surface methodology (RSM) and 20 ^{o}C temperature with pH 10 was optimized on the basis of RSM desirability with 36.52 per cent yield, 14.25 per cent soluble protein and 90.15 per cent crude protein from apricot kernel press cake. The extracted protein isolate was light creamish in colour (L* 22.01, a* 6.45 and b* 17.85) contained 0.21 per cent crude fibre, 0.15 per cent crude fat with 362.51 Kcal/100g energy value. The apricot kernel protein isolate found to contain all essential amino acids. Further, the water absorption capacity (WAC) of 2.45ml/g, oil absorption capacity (OAC) of 2.52ml/g, emulsifying activity/capacity of 52.00 per cent, foaming capacity of 20.00 per cent with protein solubility of 88.00 per cent, which shows the better functional properties of the isolate. Thus, based on present studies the extraction of protein isolate from deoiled apricot press cake seems to be a profitable venture and provides many health benefits to the consumers.

Keywords: Deoiled press cake, protein isolate, protein solubility, essential amino acid.

Introduction
Protein isolates are the refined forms of protein, containing the greater amount of protein with greater digestibility. Protein isolates are the acceptable ingredient for food application, due to their fine particle size, dispersibility, emulsification and emulsion stability (Garba and Kaur, 2014) [10]. Proteins are of various origins classified into animal proteins (gelatins), vegetable proteins (e.g. peanut, soybean, wheat, almond, apricot, canola meal etc) and animal derived protein (e.g. milk proteins) (Ogunwolu *et al*. 2009) [16]. Protein isolate does not rely only in their nutritional value, but has some desirable functional properties and sensory attributes to facilitate processing and to develop the value added products. Functional properties of proteins and protein isolates are connected to the physico-chemical properties, which govern the behavior of protein in foods (Zhang *et al*. 2009) [28]. Functional properties affect the behaviour of various food products, including adhesiveness, coalescence and packaging. Emulsification capacity, water and lipid holding capacity, gelation capacity, foaming capacity and foaming stability are functional properties that enhance food sensory and organoleptic characteristics including colour, flavour, odour, texture or mouth feel (Elsohaimy *et al*. 2015) [9].
Apricot press cake is a by-product of kernels, during processing for extraction of oil from wild apricot. Nowadays, there is an increasing demand in engineering food process waste to reduce their pollution effects and to increase their value by conversion into useful by-products or even as raw material for other industries (Vaidya *et al*. 2017) [25]. According to Sharma *et al*. (2010) [22], the wild apricot kernel press cake is a rich source of protein and can be utilized for extraction of protein. Gupta *et al*. (2012) [11] have reported that apricot press cake contains 4.90-7.20 per cent moisture, 34.30-44.50 per cent crude protein, 5.40-9.70 per cent crude lipid, 4.90-5.10 per cent total ash, 7.00-10.80 per cent crude fibre, 27.50-32.70 per cent carbohydrates. Protein isolates are the acceptable ingredient for food application, due to their fine particle size, dispersibility, emulsification and emulsion stability. Further, the response surface methodology (RSM) is a statistical technique that helping in getting information with less cost and short time.

Further, the demand for the relatively cheap sources of protein that can be incorporated into value added food products is increasing worldwide (Gurpreet *et al*. 2006) [12]. Therefore, after extraction of oil from apricot kernel press cake can be a better alternative for protein isolates which contains higher amount of proteins.

Materials and Methods

Physico-chemical analysis: The chemical composition in terms of moisture, ash, fat, and crude fiber was determined as described by AOAC (2000) [3]. The crude protein Ranganna (2009) [19] based on nitrogen content (N % × 6.25), total carbohydrate content (Bhat and Bhat, 2013) [4], energy value (Kays and Barton, 2002) [15] were estimated by using standard methods. The protein content of press cake was calculated. Whereas, Colour of samples was measured in a Lovibond Colour Tintometer Model PFX-I in which RYBN colour units were obtained along with CIE readings i.e. L^* a^* and b^* values (Ranganna, 2009) [19]. Further, dry ashing method was used for mineral estimation (Rajasekaran *et al.* 2005) [18], the sample was digested with a mixture of HCl and nitric acid in the ratio 1:3. The digested sample was dissolved in 50 ml of distilled water and used for the assay of trace elements such as iron, calcium, zinc, copper, cobalt, manganese and magnesium through atomic absorption spectrophotometer.

Process for isolation of protein isolate by isoelectric precipitation method: The defatted wild apricot kernel press cake mixed with the distilled water at ratio of 1:20 and the pH was adjusted using 0.1 N NaOH for maximum solubilization of proteins. The slurry was stirred in water bath for half an hour at 20-25ºC temperature followed by centrifugation at 4000 rpm for 20 minutes. The pH of the supernatant was adjusted at its isoelectric point (4.0) using saturated citric acid solution to precipitate the protein. The precipitated protein get collected and kept in dehumidifier for drying.

Optimization of process conditions for isolation of protein isolates through Response Surface Methodology (RSM): Response surface methodology (RSM) was used to determine the influence of independent variables and optimum conditions of protein isolates. The effect of the variables temperature (X_1) and pH (X_2) in the wild apricot kernel press cake protein isolation process was investigated.

Coded values of independent variables used for experimental design

Independent variable		Coded value		
		-1	0	+1
Real value	Temperature	20.00	35.00	50.00
	pH	9.00	10.50	12.00

Experiment plan for isolation of protein isolate as per the RSM design

Treatments	pH	Temperature
T_1	9	20
T_2	12	20
T_3	6	50
T_4	12	35
T_5	9	35
T_6	9	35
T_7	12	50
T_8	9	35
T_9	9	35
T_{10}	6	20
T_{11}	9	50
T_{12}	9	35
T_{13}	9	35

Independent variables = pH and temperature
Dependent variables = Yield (%), Soluble protein (%) and Crude protein (%)

The variables were coded according to the equation: Xi = (xi – xî)/ Δxi. Where, Xi was the dimensionless value of an independent variable, xi was the real value of an independent variable, xî was the real value of an independent variable at the center point and Δxi was the step change. The dependent variables were taken as yield (%), crude protein (%) and soluble protein (%). The quadratic model was used to analyze the responses, comprising of 13 experimental runs.

$$y = X_0 - X_1A_1 - X_2B_2 - X_{11}A_{11}^2 - X_{22}B_{22}^2 - X_{12}AB_{12} \quad \text{.....eq}_1.$$

From the equation 1, y was response variable, X_0 was intercept, X_1 and X_2 were linear coefficients, X_{11} and X_{22} were quadratic coefficients, X_{12} were interaction coefficients and A, B, A^2, B^2 and AB were the levels of independent variables (pH and temperature).To evaluate model adequacy, regression coefficient (R^2) and statistical significance, analysis of variance (ANOVA) was used. To visualize the relationship between the response and independent variables, surface response and contour plots of the fitted regression equations, optimal conditions for the targeted responses were generated. The adequacy of the model was determined using model analysis and coefficient of determination.

Water Absorption Capacity: Water absorption capacity (WAC) was determined by dissolving 0.5 g of the sample with 10 ml of distilled water in centrifuge tubes and vortexed for 30 s. The dispersions were allowed to stand at room temperature for 30 min, centrifuged at 3000 rpm for 25 min. The supernatant was filtered with Whatman No 1 filter paper and the volume retrieved was accurately measured. The difference between initial volumes of distilled water added to the sample and the volume obtained after filtration was calculated. The results were reported as mL of water absorbed per gram of sample.

Oil absorption capacity: Oil absorption capacity (OAC) was estimated by taking 1 g of the sample (W_0) into pre-weighed 15 ml centrifuge tubes. Mix thoroughly with 10 ml (V_1) of refined pure groundnut oil by using vortex mixer. Samples were allowed to stand for 30 min. The sample-oil mixture was centrifuged at 3000 rpm for 20 min. Immediately after

centrifugation, the supernatant was carefully poured into a 10 ml graduated cylinder, and the volume was recorded (V_2).

$$\text{Oil absorption capacity} = \frac{V_1 - V_2}{W_0}$$

Emulsification capacity/activity: To determine the emulsifying activity, sample (1-5g) is homogenized for 30 seconds in 50ml water using homogenizer at approximately 10,000rpm then corn oil (25ml) added to the mixture and again homogenized for 30 sec. The emulsion is divided into two equal volume aliquots and centrifuged at 1100 rpm for 5 minutes; the aliquot is heated for 15 minutes at 85°C. The ratio of the height of emulsion to the high of liquid layer is noted to calculate emulsion activity.

Foaming capacity/activity: The capacity of foams can be determined by dispersing 50ml of 3 per cent (w/v) of sample in distilled water and transfer immediately into a graduated cylinder, the volume should be recorded before and after whipping. The foaming capacity can be expressed as the percentage volume induced by whipping.

Protein solubility: To determine the protein solubility, the sample (0.5g) should be homogenized in 20 ml of 0.1 M NaCl at pH of 7.0 for 1h followed by centrifugation at 10,000 rpm for 30 min. Nitrogen content be determined in the soluble fraction and the solubility can be expressed as the percentage total nitrogen of the original sample to that of soluble fraction.

Statistical analysis: All the analytical parameters were recorded in triplicates and the mean was taken. The data of quantitative estimation of biochemical characteristics were assessed by RBD using two factors analysis of variance (ANOVA) with the help of OPSTAT software (Cochran and Cox, 1967) [6].

Results and Discussion

Isolation of Protein Isolates through Response Surface Methodology (RSM)

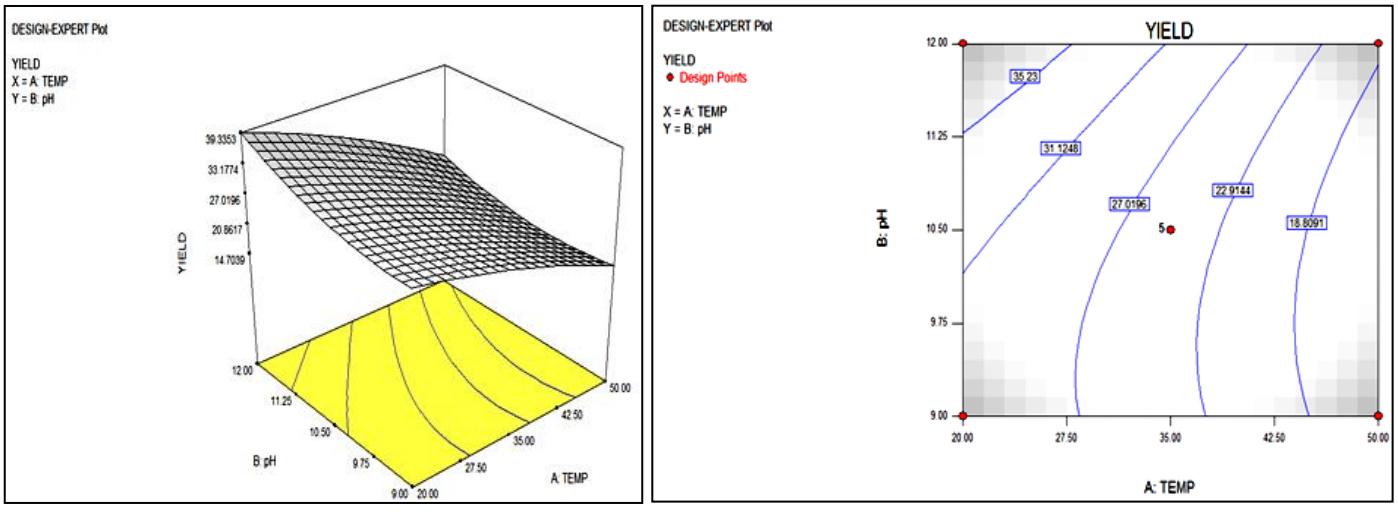
The relationship between the independent variables (temperature and pH) and yield of protein isolate from apricot press cake is mentioned in the equation (2).

$$Y = +25.00 - 8.50\,A + 3.35\,B - 1.41\,A^2 + 2.51\,B^2 - 1.38\,AB \quad \text{eq-2}$$
$$R^2 = 0.9350$$

Regression coefficient obtained from stepwise regression fitting model and the value of the determination coefficient (R^2) indicated that the model as fitted explained 93.50 per cent of variability in yield. The yield of protein isolate was significantly affected by independent variables.

From the derived equation (1) it was observed that, the temperature had negative linear and quadaratic effect on the yield of the protein isolates from apricot press cake, while pH had postive linear and quadaratic effect. The interaction of the temperture and pH had negative effect on the yield of the protein isolates. According to Jarpa *et al.* (2014) [14], the increase in protein yield leads to an enhanced solubility in the solvent system and ultimately increase in protein yield.

Three-dimensional response surface and two-dimensionalcontour plots are the graphical representations of regression equation. In the present study, two independent response surface plots and their respective contour plots weregenerated (Fig.1a-b).



A **B**

Fig 1: (a-b) Three dimensional response surface and two dimensional contour plots for yield percentage of protein isolates

The value of the coefficient of determination (R^2) explained 91.83 per cent variability in the crude protein of the protein isolates (eq. 3) from the wild apricot press cake.

$$CP = +89.72 - 2.84\,A - 1.23\,B - 3.02\,A^2 - 1.58\,B^2 + 1.16\,AB \quad \text{eq.3}$$
$$R^2 = 0.9183$$

Regression coefficient obtained from stepwise regression fitting model and the derived equation 3 clearly indicates that the temperature and pH had negative linear effect as well as quadaratic effect on the crude protein of the protein isolates, whereas the interaction showed the positive effect on the crude protein of the isolates from apricot press cake. Similarly, Vaidya *et al.* (2017) [25] observed significant (p<0.05) positive linear effect of pH on protein content of the custard apple protein isolate.

The protein content of protein isolate from apricot press cake was found increased with the increase in pH at low temperature, whereas a decrease in protein content was noticed with increase in pH at high temperature (Table 1).

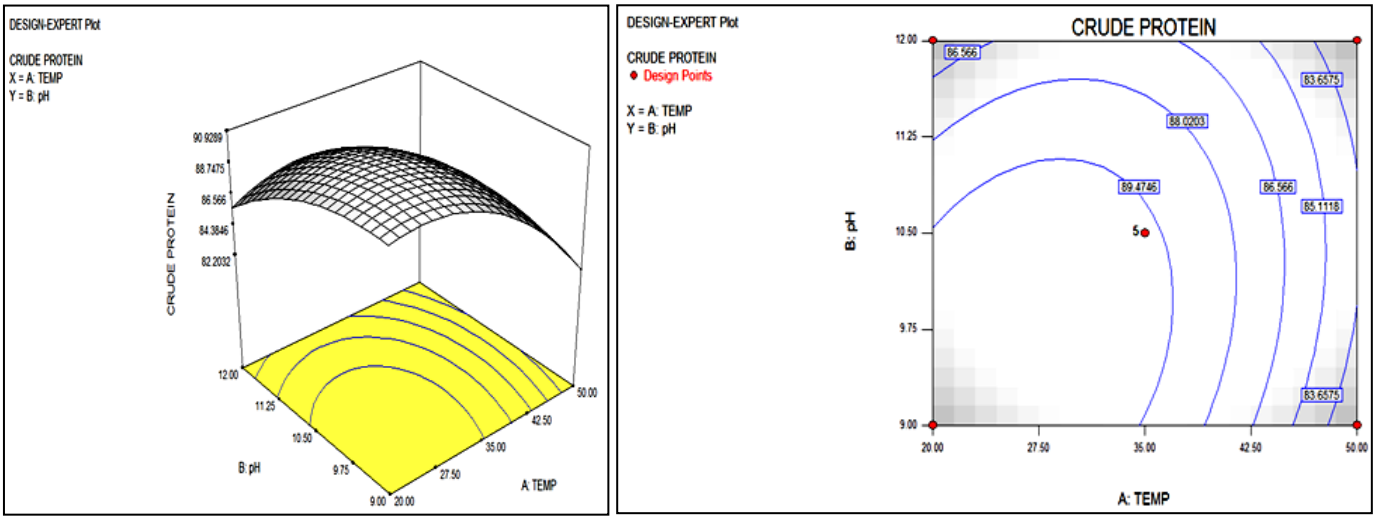
This might be due to the introduction of higher amount of starch into the concentrate as the starch granules, already

damaged by milling process, increased their solubility at high pH values. According to Han and Lim. (2004) [13], at low pH of the extract solution and at isoelectric point of proteins, some of the solubilized starch gets precipitated along with the protein.

Table 1: Optimization of conditions for isolation of protein isolates from apricot press cake through Response Surface Methodology (RSM)

Runs	Independent Variables		Responses		
	Temperature (A)	pH (B)	Yield (Y) (%)	Crude protein (CP) (%)	Soluble protein (SP) (%)
1	20.00	9.00	32.66	90.15	14.25
2	35.00	8.38	25.00	89.72	14.00
3	56.00	10.50	13.40	80.62	8.72
4	35.00	10.50	25.00	89.72	14.00
5	14.00	10.50	30.32	86.52	13.25
6	35.00	10.50	25.00	89.72	14.00
7	35.00	10.50	25.00	89.72	14.00
8	35.00	10.50	25.00	89.72	8.72
9	35.00	12.62	34.40	83.20	10.15
10	50.00	12.00	17.40	82.62	9.50
11	20.00	12.00	42.20	87.50	13.28
12	35.00	10.50	25.00	89.72	14.00
13	50.00	9.00	13.40	80.62	8.72

In this study contour plot was drawn to visualize the effect of pH on protein content of the protein isolates from apricot press cake (Fig 2a-b).



A **B**

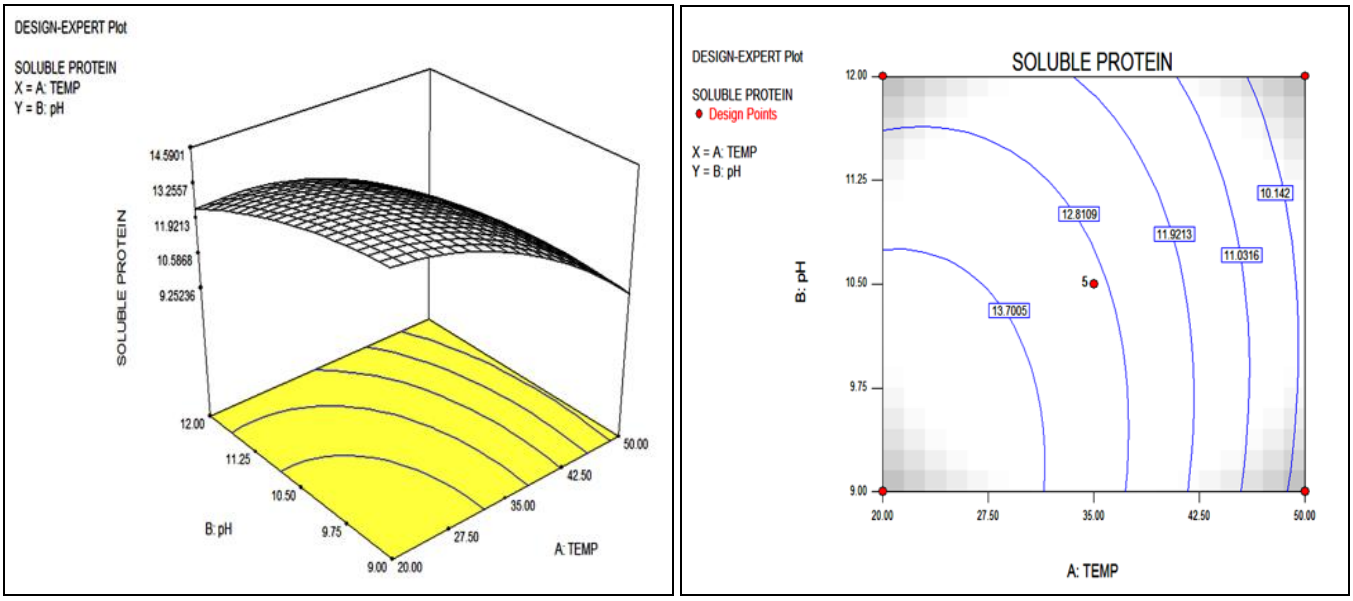
Fig 2: (a-b): Three dimensional response surface and two dimensional contour plots for crude protein percentage of protein isolates

The value of the coefficient of determination (R^2) explained 61.78 per cent variability in the soluble protein of the protein isolates (eq. 4)

$$SP = +12.94 - 1.96\,A - 0.70\,B - 1.00\,A^2 - 0.46\,B^2 + 0.44\,AB \quad \text{eq.4}$$

$$R^2 = 0.6178$$

The derived equation 4 clearly indicates that the temperature and pH had negative linear as well as quadaratic effect on the soluble protein of the protein isolates, whereas the interaction showed the positive effect on the soluble protein of isolates (Fig 3 a-b). Durmus and Evranuz (2010) [7] used response surface methology (RSM) to study the effect of temperature and pH on the soluble protein content of red pepper seed meal and observed significant model with regression coefficient (R^2) 96.70 per cent.



A **B**

Fig 3: (a-b): Three dimensional response surface and two dimensional contour plots for soluble protein percentage of protein isolates.

The three dimensional surface plots were drawn to illustrate the main and interactive effects of the independent variables (temperature and pH) on the dependent one (soluble protein). During present study, contour plot was drawn to visualize the effect of temperature and pH on protein content (soluble protein) of the apricot protein isolates.

Optimization of conditions through desirability of responses of wild apricot kernel press cake protein isolate

Factors and responses	Goal	Lower limit	Upper limit	Importance	Optimized	Predicated value
Temperature (A)	In range	20.00	50.00	3	20.00	-
pH (B)	In range	9.00	12.00	3	10.00	-
Y	In range	13.40	42.20	3	36.52	25
CP	Target	80.62	90.15	3	90.15	89.72
SP	Target	8.72	14.25	3	14.26	12.94

The result obtained from the RSM plot indicated the dependence of combination of independent variables (temperature and pH) on the yield, crude protein and soluble protein of the apricot protein isolate. Response surface plots were taken into account in the optimization, considering that the optimal solution arises from interactions among the different responses.

Optimization was performed on the basis of a multiple response method called desirability and the response predicted by the design expert-6 software. The difference between the predicated and actual optimized values calculated below 5% is desirable. The closeness of the actual values of protein yield 36.52 per cent, crude protein 90.15 per cent and soluble protein 14.26 per cent and predicated values of protein yield 25 per cent, crude protein 89.72 per cent and soluble protein 12.94 per cent confirms the validation of response surface methodology (RSM) model. The desirability scores for extraction of protein isolates was 1.00, which was found within the most acceptable limits.

Quality characteristics of apricot kernel press cake and protein isolate

Physico-chemical composition: The physico-chemical composition of apricot kernel press cake and extracted protein isolate are presented in Table 2. The protein isolate found to contain 8.20 percent moisture, water activity 0.88, crude protein 90.15 percent and 14.65 percent soluble protein, 0.21 per cent crude fibre, 0.15 per cent crude fat, and 362.51 Kcal/100g energy value with no traces of HCN. Gupta *et al.* (2012) [11] and Sharma *et al.* (2010) [22] reported similar results in apricot kernel press cake protein isolate.

Table 2: Physico-chemical composition

Parameters	Observations (Mean ± SE)	
	Wild apricot press cake	Protein isolate
Moisture (%)	8.89± 0.01	8.20± 0.02
Water activity	0.85± 0.03	0.88± 0.01
Total ash (%)	2.67± 0.02	1.15± 0.02
Crude protein (%)	33.60± 0.03	90.15± 0.03
Soluble protein (%)	5.65± 0.02	14.65± 0.03
Crude fibre (%)	9.50± 0.03	0.21± 0.01
Crude fat (%)	9.48± 0.01	0.15± 0.03
HCN (mg/100g)	0.77± 0.02	-
Carbohydrate (%)	-	0.14 ± 0.01
Energy value (Kacl/100g)	-	362.51± 1.22

Colour value: Color or appearance properties are critical parameters in defining the uses and acceptability of products. The apricot kernel press cake was light brown with L * 25.45, a* 6.27 and b* 25.91 whereas; the protein isolate was creamish in colour with lightness (L *) value 22.01, redness (a*) 6.45 and yellowness (b*) 17.85. Similar Lab colour value reported by Viskelis *et al.* (2017) [26] in raspberry press cake Whereas, Alabi and Falade (2017) [2] had reported lightness (L *) 89.84, redness (a*) 0.95 and yellowness (b*) 15.27 in groundnut protein isolate.

Functional properties of protein isolate: The functional properties of protein are presented in Table 3. Water absorption capacity (WAC) of 2.45ml/g was noticed during

the present studies which improves mouthfeel and flavour retention of food. The results are in confirmatory with the findings of Ogunwolu *et al*. (2009) [16], Tounkara *et al*. (2013) [23] and Saranya (2013) [21] in different plant protein isolates. The high water absorption of protein isolates help to reduce moisture loss in package products (Butt and Batool, 2010) [5]. Protein isolate had oil absorption capacity (OAC) 2.52ml/g which were in confirmation to results reported by Saranya (2013) [21], Onsaard *et al*. (2010) [17] and Sharma *et al*. (2010) [22] in soyprotein isolate (2.94ml/g), faba bean protein isolate (1.60ml/g) and apricot protein isolate (1.40ml/g), respectively.

Table 3: Functional properties of protein isolates

Parameters	Observation (Mean±SE)
Water absorption Capacity (g/g)	2.45 ± 0.20
Oil absorption Capacity (g/g)	2.52 ± 0.10
Emulsion activity/Capacity (%)	52.00 ± 1.10
Protein solubility (%)	88.00 ± 1.25
Foaming Capacity (%)	20.00 ± 0.30

Further, the protein isolate had emulsifying activity/capacity 52 percent, protein solubility 88 percent and foaming capacity of 20 percent. Our results are in confirmatory with the findings of Ogunwolu *et al*. (2009) [16], Abbas *et al*. (2015) [1] and Ulloa *et al*. (2017) [24].

Amino acid estimation: The apricot kernel press cake protein isolate contained all the nine essential amino acids determined based on their R_f values. The calculated R_f value for methionine, arginine, tryptophan, threonine, valine, isoleucine, phenylanine, lysine and histidine were 0.40, 0.17, 0.56, 0.23, 0.36, 0.45, 0.52, 0.19 and 0.23 respectively and was within the range of R_f values of standard amino acids. Elijah *et al*. (2016) [8] estimated sulphur containing amino acids in soybean products by thin layer chromatography (TLC).

The amino acid profile of apricot kernel press cake protein isolate is presented in Table 4. Our body needs 20 different amino-acids to grow and function properly, 17 amino-acids are present in apricot kernel press cake protein isolate and out of them 9 are essential amino-acids. The maximum concentration among essential amino acids was of arginine (2504mg/100g), followed by leucine (1766mg/100g) and phenylalanine (1518mg/100g). The essential aliphatic amino-acids (EAAA), isoleucine and valine which constitute the hydrophobic regions of protein were also abundantly present (973mg/100g and 1196mg/100g) in apricot kernel protein isolate. The maximum concentration among non-essential amino acids was of glutamic acid (5934mg/100g), followed by aspartic acid (2602mg/100g) in apricot kernel protein isolate.

Table 4: Total amino acids present in protein isolate

Sr No	Essential amino acids	Quantity (mg/100g)
1	Arginine	2504
2	Valine	1196
3	Tryptophan	NF
4	Isoleucine	973
5	Leucine	1766
6	Lysine	740
7	Methionine	152
8	Phenylalanine	1518
9	Threonine	726
10	Histidine	606
Non-essential amino acids		
11	Tyrosine	764
12	Cysteine	118
13	Serine	1038
14	Alanine	1268
15	Glutamic Acid	5934
16	Glycine	1066
17	Aspartic Acid	2602
18	Proline	1362

NF- Not Found

Conclusion

The apricot kernel press cake is nutritionally dense and contained protein, minerals and amino acids. Hence, it can be used for extraction of protein isolate (32% protein) by isoelectric precipitation method. The protein isolate found rich in essential amino acids and having better functional properties thus can be further utilized for supplementation in value added food products. Study concluded that the isolation of protein isolate from apricot press cake seems to be a profitable venture by utilizing the waste and boosting the income of the farmers, apart from providing many health benefits to the population.

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