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Research Article

Extraction Optimization of Red Kidney Bean (*Phaseolus vulgaris* L.) Protein using Response Surface Methodology

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ABSTRACT

Red kidney beans valued globally for their nutritional content, health benefits, and culinary versatility. Response surface methodology was used to determine the optimum conditions for the extraction of protein from red kidney bean seeds. By using a central composite design, the effects of three independent variables, namely pH (8, 9, 10, 11, and 12), temperature (20, 40, 60, 80, and 100^{o}C), and time (15, 30, 45, 60 and 75 min) were investigated on the selected response variable i.e. percent protein recovery. The second-order model obtained for protein recovery revealed a good coefficient of determination (98.79%). Maximum protein recovery was obtained when pH, temperature, and extraction time were 10.1, 31.4^{o}C, and 75 min, respectively. Additional experiments were performed at optimum conditions to confirm the suitability of the model. The results revealed that the range of protein recovery from red kidney bean flour was 68.7% to 83%, with temperature, pH, and extraction time having a significant impact. Strong agreement between predicted and experimental values was demonstrated by the second-order regression model, which had a high fitness (R2 = 98.79%). The highest protein recovery of 85.1% was obtained under ideal extraction conditions (pH 10.1, 31.4°C, 75 min). The experimental values for protein recovery were in close agreement with that of the predicted results, thus signifying the appropriateness of the model used. The study can instigate the production of protein isolates or concentrates from red kidney beans for use as promising food ingredients in the industry.

Keywords: Red Kidney Beans; Protein Extraction; Optimization; Response Surface Methodology.

INTRODUCTION

Proteins are vital constituents of the diet required for maintaining good health chiefly for their amino acid content and profile. Besides providing energy, these play an essential role in the proper growth and maintenance of body as well as in other regulatory functions (Jeejeebhoy, 2000; Salcedo-Chavez et al., 2002). Traditionally, milk, eggs, and meat are recognized as the best sources of high quality proteins. However, due to increased cost and limited supplies, proteins from animal sources are not accessible to large segments of the population especially in developing countries, resulting in the problems of protein-energy malnutrition. The quest for alternative sources of proteins has prompted researchers towards legume

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proteins because of their comparative cheapness, nutritive value and variety of sources (Eromosele et al., 2008; Boye et al., 2010).

Kidney beans known as “King of Nutrition” are produced all around the world, especially in Brazil. Locally, it is called Lobia and is known for its great nutritional importance. Kidney beans are rich in protein and it is cultivated in June and harvested in October/November (GOKP, 2023).

Red kidney bean is an extensively produced and consumed crop in Asian, South American, and African countries (Wani et al., 2010). Although, red kidney beans are a superior source of starch, dietary fiber, minerals, vitamins as well as some important phytochemicals; however the most important component from a nutritional point of view is their high protein content which is 2-3 times greater than cereal grains. The high protein content of beans (20-30%) is an excellent source of indispensable amino acids particularly lysine which is lacking in the cereal grains. Therefore, the nutritional complementation of beans and cereal proteins is an ideal strategy to ensure a balanced protein diet for the people of low income group in developing countries (Hayat et al., 2014; Mundi and Aluko, 2012). Although red kidney beans are widely produced in Indo-Pakistan regions, however, due to limited research work it is still underexploited as a source of protein. Kidney bean proteins can be more efficiently utilized by extracting them to prepare protein isolates or concentrates comprising of high protein content as well as eliminated antinutritional factors. Such protein based products exhibit better functional properties and have the potential to be utilized as promising food ingredients in the industry to improve the nutritional status of the diet (Yin et al., 2008). This could be of particular interest for country such as Pakistan where the problem of protein-energy-malnutrition is most common among the population.

Based on solubility behavior of proteins, various techniques have been reported for the extraction of proteins from different sources such as pumpkin seed (Quanhong and Caili, 2005), African yam bean (Eromosele et al., 2008), sunflower meal (Pickardt et al., 2009), bambara bean (Mune et al., 2010) and cowpea (Mune et al., 2008). The most simple and widely used method is the Alkaline extraction of proteins followed by isoelectric precipitation to get a relatively pure and high yield of protein. Different factors such as extraction time and temperature, pH, solvent type, solid-liquid-ratio and the presence of cross-linking components have a great influence on the solubility of protein as well as its functionality as a nutritional ingredient (Mizubuti et al., 2000).

Response surface methodology (RSM) is the most efficient mean that can be used to improve and optimize processes when numerous variables influence the characteristics of a product or process. RSM is the grouping of statistical and mathematical techniques that enable us to get the required information with minimum resources and shorter time thus ensuring fast and efficient development of new processes or products (Bera et al., 2008). RSM suggests appropriate designs for ample representation of most continuous response surfaces over a comparatively wider spectrum (Myers and Montgomery, 2002). The prime purpose of this study was to apply RSM to standardize the extraction parameters such as temperature, pH, and time for maximum protein recovery from red kidney bean seeds.

MATERIALS AND METHODS

Raw material collection preparation

The seeds of the indigenous cultivar of red kidney beans (Accession No. 027076) were obtained from NARC Islamabad, Pakistan. The seeds were screened and rinsed in deionized water to remove the extraneous material. After manual dehulling and drying the seeds at room temperature (28±2^{o}C); these were ground and passed through a screen to get uniform size flour which was stored in air-tight polyethylene bags at 10^{o}C until use.

Protein extraction

Red kidney bean flour was extracted for protein with different levels of independent variables, such as pH (8-12), temperature (20-40^{o}C) and time (15-75min) as per central composite design. The dispersions of bean flour in distilled water (10% w/v) were adjusted to desired pH and temperature values and continuously stirred in a magnetic stirrer for selected period. The dispersions were then centrifuged at 10,000g for 20 minutes at 4^{o}C. The supernatants were collected and the residues were re-extracted twice to get maximum extraction. The supernatant fluids were filtered through Whatman filter paper No 1 and their protein contents were determined by Auto kjeldhal (BUCHI Autokjeldhal Unit K-370) using 6.25 as conversion factor (AOAC, 2000). The protein recovery percentage was determined by the following equation:

$$R(\%) = \frac{Ns}{No} \times 100$$

where R% is the percentage of protein recovery while Ns and No are the amounts of nitrogen in supernatant and original sample, respectively.

Experimental design and statistical analysis

The optimum combination of three independent variables was determined to study the response pattern by using the central composite rotatable design. This design enables to locate the optimum point from unidentified area of interest by permitting uniformity of the extent of prediction error at the same radial distance for all points from the central point. The independent variables optimized were pH, temperature and time, each at five coded levels as shown in Table 1. The coding of variables was performed by using the following equation:

$X_i = (x_i - \bar{x_i})/\Delta x_i$ (1)

Where $\bar{x_i}$ is the real central value of an independent variable, Δx_i is the step change while X_i and x_i are the coded and real values of an independent variable, respectively.

Based on equation 1, equations 2, 3 and 4 were derived to code the levels of pH, temperature and time, respectively. Coded and uncoded levels of independent variables are depicted in Table 1.

Table 1.The independent variables with their coded and uncoded levels.

Independent variables	Symbol		Levels				
	Codified	Uncodified	−2	−1	0	+1	+2
pH	X_1	x_1	8	9	10	11	12
Temperature	X_1	x_1	20	40	60	80	100
Time	X_1	x_1	15	30	45	60	75

$X_1(pH) = x_1 - 10/1$ (2)

$X_2(Temperature) = x_2 - 60/20$ (3)

$X_3(Time) = x_3 - 45/15$ (4)

The response function investigated was Y i.e. percent recovery of proteins from the bean seed flour. By fitting the Central composite design, an appropriate regression model was obtained (Table 2).

Table 2. Central composite design arrangement for independent variables with predicted and experimental values for protein recovery.

Treatments	Variable levels			Protein recovery(%)		(Y0−Yi)
	X_1	X_2	X_3	Experimental (Y0)	Predicted (Yi)	
1	−1	−1	1	82.0±1.4	81.51	0.48
2	−1	−1	−1	72.2±1.15	72.25	−0.05
3	1	−1	−1	76.1±1.16	75.28	0.81
4	0	2	0	68.7±1.38	68.93	−0.23
5	0	−2	0	74.9±1.2	75.40	−0.50
6	1	−1	1	83.0±1.55	82.50	0.49
7	−1	1	−1	74.1±0.8	73,86	0.23
8	−1	1	1	75.8±1.01	75,87	−0.07
9	2	0	0	74.9±1.17	75.70	−0.80
10	0	0	2	82.5±1.30	82,98	−0.48
11	0	0	−2	73.5±1.27	73,75	−0.25
12	1	1	1	75.2±1.28	74.41	0.78
13	1	1	−1	74.7±1.05	74.45	0.24
14	−2	0	0	74.2±1.02	74.13	0.06
15	0	0	0	79.5±0.69	79.62	−0.12
16	0	0	0	78.7	79.62	−0.92
17	0	0	0	80.2	79.62	0.58
18	0	0	0	78.9	79.62	−0.72
19	0	0	0	79.2	79.62	−0.42
20	0	0	0	80.4	79.62	0.78

The estimation of pure sum of squares was carried out by using five replicates (16-20) at the centre of design. The randomization of the experiments was carried out to maximize the effects of unexplained variability in the observed response due to extraneous factors. Following model was proposed for the response (Y).

$$Y = b_0 + \sum_{n=1}^{3} b_n X_n + \sum_{n=1}^{3} b_{nn} X^2_n + \sum_{n<m}^{3} b_{nm} X_n X_m$$

Where b_0 is the value of fixed response at the central point of the design while b_n, b_{nn} and b_{nm} are the linear, quadratic and interaction regression coefficients, respectively. The Student *t*-test was used to determine the significance of each test and the multiple coefficient of determination was used to assess the adequacy of the polynomial model while the optimization process was carried out by using the graphical technique (Floros and Chinan, 1988). The optimum conditions were verified by conducting additional experiments and comparing the experimental results with the model predictions. The computer software used for this study was Minitab-14.2 (Minitab Inc, USA).

RESULTS AND DISCUSSION

Effect of independent variables on protein recovery

Red kidney bean flour was extracted for its protein following 20 combinations of three independent variables, namely pH, temperature and time as per experimental design. These independent variables have an individual as well as interactive effect on the protein recovery. The results regarding the individual effect of pH on protein recovery are depicted in figure. 1. These results revealed an increasing trend in protein recovery with an increase in pH from 8 to 10, beyond that subsequent rise in pH decreased the recovery of protein. Extreme pH values cause denaturation of proteins which may lead to agglomeration thus resulting in marked reduction of protein solubility as well as extractability (Fennema, 1993).

Variations in temperature levels also exerted significant effect on the extraction of protein from red kidney bean flour. Protein recovery was increased with rise in temperature upto 40^{o}C after that a gradual decline was noticed up to 60^{o}C while a sharp fall of protein recovery was observed by increasing the temperature from 60 to 100^{o}C (Figure 2) The rise in temperature promotes mass transfer rate by faster movement of molecules as well as reduces the viscosity of the solution, thus increasing solubility and extractability of proteins (Zang, 2009; Roy et al., 2021). As the temperature exceeded 40-50 ^{o}C, the amount of molecular movement attained by the protein become sufficient to disrupt its secondary and tertiary structure causing disentanglement of the native protein or denaturation (Kanu et al., 2007). Another possible reason for low protein recovery at higher temperature may be the formation of ionic bonds within protein molecules or between adjacent protein molecules causing aggregation of the protein molecules (De-Wit and Hontelex-Backx, 1988).

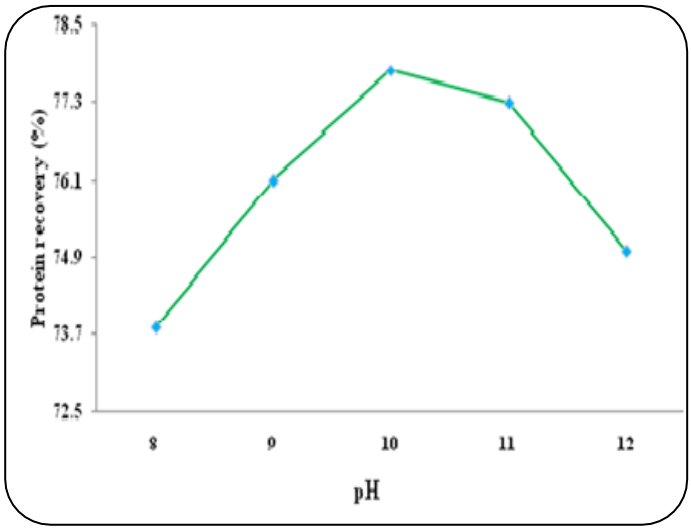

Figure 1. Protein recovery from red kidney bean flour as influenced by different pH levels.

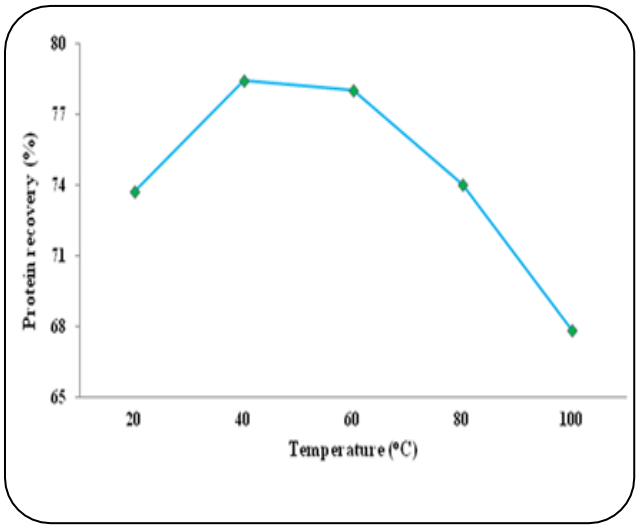

Figure 2. Protein recovery from red kidney bean flour as influenced by different temperature levels.

The influence of extraction time on protein recovery of kidney bean flour is illustrated in figure 3 which clearly elucidated that protein recovery increased linearly by increasing the extraction time. This increase in protein recovery may be attributed to the fact that maximum protein was solubilized as the extraction time prolonged

resulting in better extractability as well as recovery of protein

Fitting the models

The results of the protein recovery obtained by conducting experiments on the proposed experimental model are depicted in Table 2. It is evident from the results that the experimental protein recovery from red kidney bean flour varied from 68.7% to 83%. By analyzing the dependent and independent variables, a model regression equation was created, which can be used to envisage the response under the specified ranges. This regression equation is a pragmatic relationship between the test variables and protein recovery and is given as follows:

$$Y = 79.6227 + 0.3938X_1 - 1.6188X_2 + 2.3063X_3 - 1.1761X_1^2 - 1.8636X_2^2 - 0.3136X_3^2 - 0.6125X_1X_2 - 0.5125X_1X_3 - 1.8125X_2\,X_3 \quad (5)$$

It is obvious from the results that the experimental values for protein recovery were in close agreement to the predicted values. The significance of each coefficient as determined by using Student *t* test and *p* value also elucidated that the effects of linear, quadratic as well as interaction terms were significant ($p<0.05$) (Table 3). The level of significance depend upon the computed *t* and *p* values as the variables with larger *t* value and smaller *p* value were highly significant (Amin and Anggoro, 2004; Quanhong and Caili, 2005; Bai et al., 2024). Among the variables, the linear term of time **(**X_3) exerted the largest effect on protein recovery followed by the quadratic term of temperature(X_2X_2). The *t* values of X_3 and X_2X_2 were 16.203 and 16.414, respectively with corresponding *p* values of 0.0001 each. The value of multiple coefficient of correlation R was 0.9939 which signify a close concurrence between the predicted and observed values for protein recovery. Similarly, the value for total determination coefficient (R^2) was found to be 98.79% indicating the aptness of the model used (Table 3). The value of R^2 was well within the range of 80-99.3% as previously reported for protein recovery from germinant pumpkin seed (Quanhong and Caili, 2005) and bambara bean (Mune et al., 2010).

Table 3. Significance of regression equation coefficients for protein recovery.

Variables	Regression coefficient	Standard error	Computed *t* value	Significance level, *p* value
Constant	79.6277	0.2271	350.634	0.000
Linear				
X_1	0.3938	0.1423	2.766	0.020
X_2	−1.6188	0.1423	−11.373	0.000
X_3	2.3063	0.1423	16.203	0.000
Quadratic				
X_1X_1	−1.1761	0.1135	−10.359	0.000
X_2X_2	−1.8636	0.1135	−16.414	0.000
X_3X_3	−0.3136	0.1135	−2.762	0.020
Interaction				
X_1X_2	−0.6125	0.2013	−3.043	0.012
X_1X_3	−0.5125	0.2013	−2.546	0.029
$X_2X_{3\backslash}$	−1.8125	0.2013	−9.005	0.000
R	0.9939			
R^2	0.9879			

Optimization of the extraction conditions

The extraction conditions can be optimized by using counter plots, surface graphs and steepset ascent techniques (Wani et al., 2006; Wanasundara and Shahidi, 1996). However, in the present study, the main as well as interactive effects of independent variables on protein recovery were illustrated by using 3D surface plots. For this purpose, one variable was fixed at the coded zero level while the remaining two variables were varied within the experimental range to predict the response variable. The influence of temperature and pH on protein extraction is illustrated in figure 4 which indicated that both these variables exerted a quadratic effect on protein recovery.

The influence of pH and time is illustrated in figure 5 indicating a quadratic effect of pH whereas time exerted a linear effect on protein recovery. The influence of time and temperature is illustrated in Fig. 6 which indicated linear effect of time while that of quadratic effect of temperature on protein recovery.

Precise coordinates of maximum were achieved by defining obligatory circumstances. By setting the partial derivative

of regression equation (5) to zero, following equations can be constructed:

$0.1968 - 0.5880X_1 - 0.1513X_2 - 0.1281X_3 = 0$ (6)

$-0.8093 - 0.9318X_1 - 0.1531X_2 - 0.4531X_3 = 0$ (7)

$1.1531 - 0.1568X_1 - 0.1281X_2 - 0.4531X_3 = 0$ (8)

By solving the equations 6-8, following codified optimum conditions can be achieved:

X_1 = 0.10 X_2 = −1.43 X_3 =2

By using equations 2-4, following extraction conditions for maximum recovery of protein can be obtained:

x_1 =10.1 x_2 =31.4°C x_3 = 75 minutes

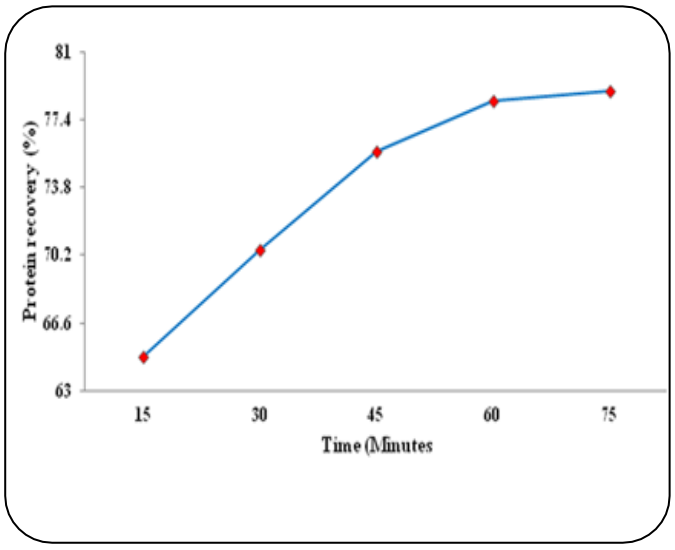

Figure 3. Protein recovery from red kidney bean flour as influenced by different time intervals.

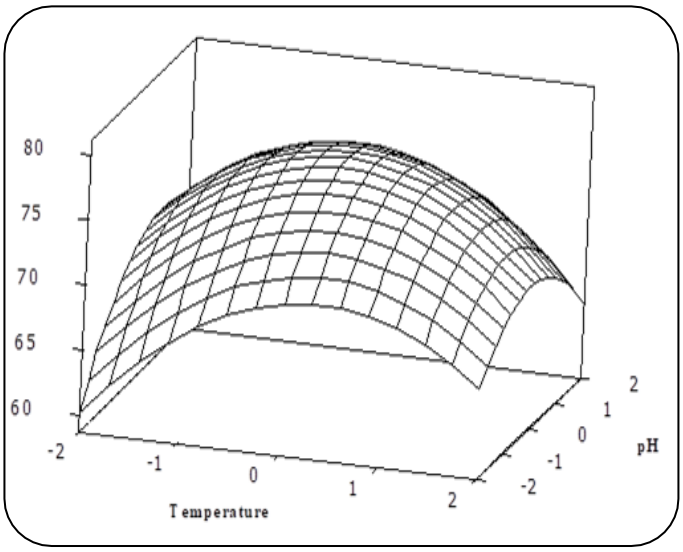

Figure 4. 3D graphic surface optimization of protein recovery versus temperature and pH.

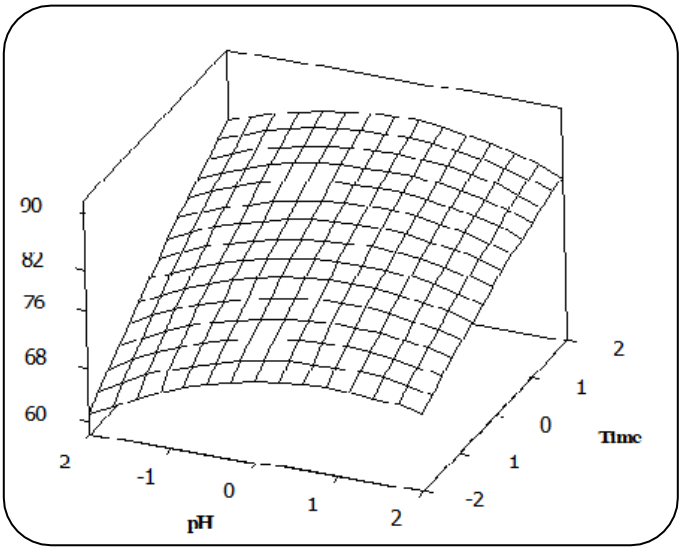

Figure 5. 3D graphic surface optimization of protein recovery versus pH and time.

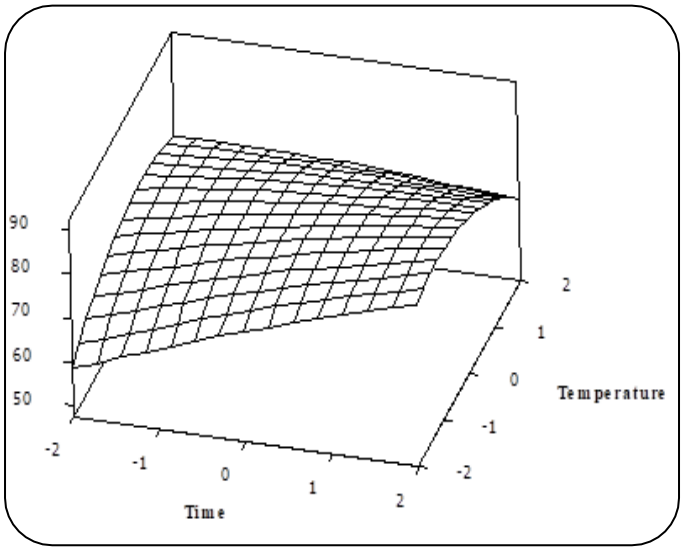

Figure 6. 3D graphic surface optimization of protein recovery versus time and temperature.

Confirmative studies

Additional experiments were performed at the optimum conditions to confirm the appropriateness of the model equation. The incorporation of the optimum values of independent variables into the regression equation predicted protein recovery of 86.68% while the experiments conducted at optimum conditions resulted in a protein recovery of 85.1±1.6% (Table 4). The predicted value of protein recovery from the regression equation was found to be in close agreement with the experimental value, which validates the suitability of the experimental model.

Table 4. The predicted and experimental values for protein recovery from red kidney bean flour at optimum conditions.

Variables	Coded levels	Actual levels
pH	0.10	10.1
Temperature	−1.43	31.4 ^{o}C
Time	2	75 min
Response	Predicted value	Actual value
Protein recovery (%)	86.68	85.1±1.6

CONCLUSION

Response surface methodology was proved to be an effectual tool for optimizing the protein extraction process from a limited number of experiments. The experimental values of protein recovery from red kidney bean flour varied from 68.7% to 83% by using 20 selected combinations of pH, temperature and time. Higher values of multiple coefficient of correlation as well as coefficient of determination for the developed second-order model signified the aptness of the experimental model. Maximum protein recovery of 85.1% can be obtained from red kidney bean flour by extracting at pH 10, temperature of 31.6^{o}C for 75 minutes extraction time. By using these optimum conditions, protein isolates or concentrates can be produced from red kidney beans for utilization as promising food ingredients in the industry.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to this research.

COMPETING OF INTEREST

The authors declare no competing interests.

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