Volume 5, Issue 1, January 2015 ISSN: 2277 128X
International Journal of Advanced Research in Computer Science and Software Engineering
Research Paper
Available online at: www.ijarcsse.com

Optimisation of Conditions to Isolate Protein from Germinated Green Gram (Vigna radiata L.) using Response Surface Methodology

Anil Bukya*, T. Poongodi Vijayakumar
Department of Food Science and Nutrition
Periyar University, Salem,
Tamil nadu, India

Abstract: ***In order to increase the global utilization of green gram and availability of affordable, good quality protein for people, the study evaluated the optimum extraction conditions to extract protein from 12 hrs soaked and 24 hours germinated green gram using Central Composite Rotatable Design in Response Surface Methodology. The protein isolate was obtained by alkaline extraction-isoelectric precipitation method. The germinated green gram with shoot was sun dried and made into flour. The effect of extraction conditions as independent variables such as pH adjusted using NaOH (8.0 to 12.0), extraction time (30 min to 150 min) and isoelectric precipitate pH (3.8 to 4.6) on protein yield was studied by Central Composite Rotatable Design and second order quadratic polynomial regression equation. The alkaline pH revealed significant effect (p<0.05) on protein yield at quadratic level. As per the magnitude of terms, alkaline pH showed greater effect on protein yield than extraction time and isoelectric pH. The mean model is significantly suggested model. The determined optimum extraction conditions were alkaline pH of 11, 120 min of extraction time in alkali and isoelectric precipitation at ph 4.4, which predicated the protein yield of 18.7 g % with the desirability level of 0.7.***

Key words*: Green gram, Protein Isolate, Alkali extraction, Isoelectric precipitation, Response Surface Methodology, Central Composite Rotatable Design.*

I. INTRODUCTION

Green gram (*Vigna radiata* L.) commonly known as Mung bean belongs to the family Leguminoseae, is the third most important pulse crop of the thirteen different food legumes grown in India. It is widely cultivated throughout the Asia, including India, Pakistan, Bangladesh, Srilanka [1]. Green gram is a good source of vitamins, minerals, enzymes, complex carbohydrates and its protein quantity is better than others [2]. Proteins are essential in foods, not only for their nutritional value, but also as modulator of structure and perception of a food product. Proteins that are essential to growth and health are currently required more in the developing countries of the world, because of the chronic problem of protein-energy malnutrition [3]. Shortages and high prices have recently caused restriction of animal proteins in the diets of many families in the developing countries of the world. However, vegetable proteins which are cheaper and available are of great potentials as a direct food for human consumption [4]. The functional behaviour of a protein is inherently susceptible to physico-chemical conditions as pH, ionic strength, temperature, or pressure, making them also an unpredictable, and at the same time, opportune component in food production. Proteins are generally also industrially costly, and with increasing world population and welfare the pressure on protein-availability for food purposes gives rise to some concerns In view of increasing production of Green gram protein globally, there is need for increased utilization of Green gram, especially the nutritious germinated Green gram. It considered as a good source of protein Isolate for use in human food products. Hence the present study aimed to determine the appropriate extraction conditions to isolate protein from germinated Green gram using Central Composite Rotatable Design.

II. MATERIALS AND METHODS

Preparation of Germinated Green Gram Flour

The hybrid variety of Green gram purchased from Salem Super market, Tamil Nadu. Green gram seed were washed well to remove dust and stones, soaked for 12 hrs and germinated in a germination container for 24 hrs. Germinated seeds were sundried made in to flour and passed through 60 mesh (BSS unit) test sieve to get enhance the uniform in particle size and packed in an airtight container.

Preparation of Germinated Green Gram Protein Isolate

The Germinated Green Gram flour was defatted using hexane as solvent ratio of 1:3 at 250 rpm in a lab stirrer for 30 min and centrifuged at 5000 g for 10 min, at room temperature and was air dried over night, packed and stored at 5 ^{o}C [5]. The alkaline extraction followed by isoelectric precipitation method was used to isolate protein from germinated defatted green gram flour and distilled deionised water (1:4) was adjusted to 8-12 pH for 30 -150 min at room temperature. The slurry was centrifuged at 5000 g for 30 min. The pH of supernatant was adjusted to 3.8-4.6 (isoelectric precipitation) and centrifuged again at 5000 g for 30 min. The Precipitate was washed using water and brought to pH 7.0 using 1M NaOH, air dried at room temperature and stored at -5 ^{o}C [6].

Experimental Design

The Central Composite Rotatable Design (CCRD) with three independent variables was used to determine the optimum extraction conditions such as alkaline pH (8 to 12 pH), extraction time (30 to 150 min) and isoelectric precipitation (3.8 to 4.6 pH) for isolating protein. The coded level of each independent variables and experimental plan (Table 1) suggested 20 treatment combinations with 6 centre points for error correction.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$$

Table 1: Coded variables and number of experiments for the experimental design

Levels of independent variables						
Variables	**Code**	**Coded levels**				
		-2	-1	0	1	2
alkaline pH	X_1	8	9	10	11	12
extraction time	X_2	30	60	90	120	150
Isoelectric precipitation	X_3	3.8	4.00	4.2	4.4	4.6

Experimental plan			
X1	**X2**	**X3**	**Number of experiments**
±1	±1	±1	8
±2	0	0	2
0	±2	0	2
0	0	±2	2
0	0	0	6

The 'p' value of the regression coefficients explains the pattern of mutual interactions between the best variables. The smaller the magnitude of 'p', the more significant is the corresponding coefficient [7]. The optimum level of alkali pH, extraction time and isoelectric pH was obtained by maximum protein yield with maximum importance of 5 through numerical optimization. The quality of fit of second order equation was expressed by the coefficient of determination R^2 and its statistical significance determined by the F test. The individual and interactive effect of each independent variables was evidenced by 3 D surface graph with contour plots.

Protein Yield %

The protein yield % of protein isolate was determined as responses for optimisation and was calculated as [8]

$$\textit{protein yield}\ \% = \frac{\textbf{weight of protein isolate}}{\textit{weight of GGGF}}\ \textit{X}\ \mathbf{100}$$

Statistical Analysis

Analysis of variance (ANOVA) with DMRT technique was used to compare mean differences of experimental runs by Design Expert software Vs 8.0.0 samples.

III. RESULTS AND DISCUSSION

Estimated response levels of experimental variables

The estimated responses level of experimental variables (Table 2) suggested that maximum protein yield (21.18%) was observed when the alkaline pH set at 11, 120 min extraction time with isoelectric pH of 4.4. The results agreed with those reported by [9] found that protein content increased linearly from pH 7.5 to 11 and decreased at pH 12. At pH 12, the non-protein nitrogen components were soluble resulting in a reduction of protein purity extracted at this pH. The higher alkaline condition (experimental run 10) resulted in green gram protein isolate with strong brown colour be due to protein denaturation and hydrolysis at high pH, resulting in undesirable flavour and odour; increased Maillard reaction, leading to dark colored product; decreased nutritive value of protein, especially essential amino acid such as lysine; increased extraction of non-protein component, which also co-precipitated with protein and lower protein purity as indicated by [5]. In addition, at higher pH, some non-protein nitrogen may be a component accounting for high yield.

Influence of independent variables on responses

The magnitude of the terms indicates the order of influence on each response and difference in magnitude of the quadratic terms explains which variable was dominant for response [10]. The coefficients for the proposed quadratic model in terms of actual variables are given in Table 3. At quadratic level, Alkaline pH had significant positive influence on protein yield at $p<0.05$. The interactive effects of independent variables on response are visualized in Fig 1(a-c). As per the magnitude of terms the alkaline pH and extraction time showed positive influence on protein yield and isoelectric pH revealed a negative influence on protein yield.

The negative predicated R^2 implied that the overall mean is a better predictor of the protein yield than the second order Polynomial regression model. Adequate precision measures the signal to noise ratio. A ratio of 3.70 indicated an

inadequate signal and second order polynomial regression model should not be used to navigate the design space. According to R^2 value (R^2 = 0.459) 45.9% of variability was accounted for the data by the model second order polynomial regression model given in Table 4.

The second order polynomial regression model fitted for the protein yield was significant at 10 % level and had a low R^2 value and a relatively high CV. This may be attributed to difficulties in obtaining a representatives sample for protein yield measurement. The reproducibility of protein yield measurement was less than 80%. Based on the normal probability plot of the residual, plot of residual vs estimated values and plot of residual vs random order of runs the second order polynomial regression model was accepted though the lack of fit was significant.

A verification experiment at the optimum conditions, consisting of three runs, was performed. By using hypothesis testing technique [11], the difference between predicated and experimental levels of protein yield (21.18 ± 0.83) was shown to be nonsignificantly at $p<0.05$.

Model fitting

The application of response surface methodology (RSM) yields the regression Equation which represents an empirical relationship between the response (protein yield %) and the tested variables in coded units, as given in the Table 1.

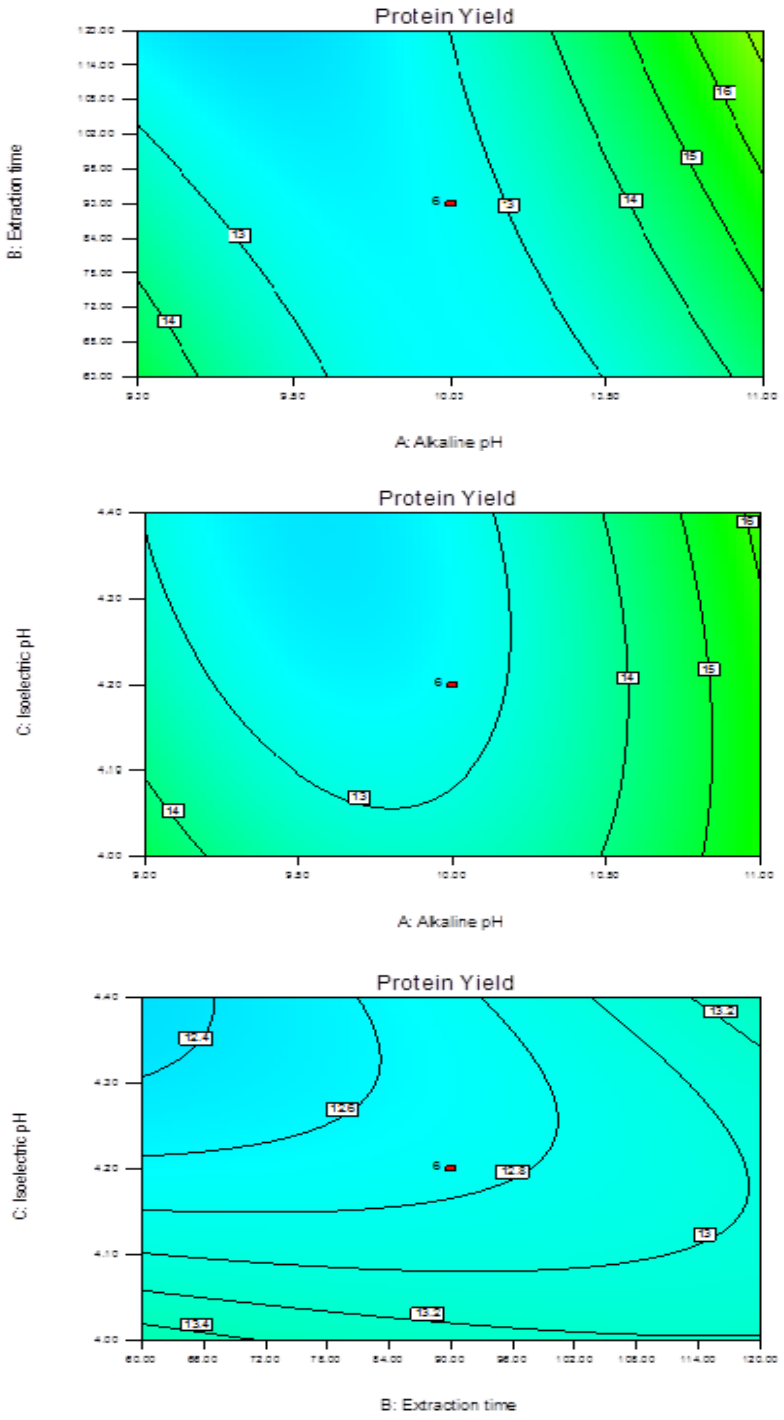

Fig 1 (a-c): Interaction effect of three independent variables on protein yield

Table2. Percent yield of Germinated Green gram Protein Isolate extracted under different conditions

Runs	Experimental Values			protein yield %
	Alkaline pH	Incubation Time(Min)	Isoelectric pH	
1	9	60	4	19.546
2	11	60	4	16.63
3	9	120	4	17.5
4	11	120	4	19.47
5	9	60	4.4	17.24
6	11	60	4.4	16.125
7	9	120	4.4	16.21
8	11	120	4.4	21.18
9	8	90	4.2	17.575
10	12	90	4.2	20.61
11	10	30	4.2	10.44
12	10	150	4.2	9.81
13	10	90	3.8	11.18
14	10	90	4.6	10.55
15	10	90	4.2	14.695
16	10	90	4.2	11.73
17	10	90	4.2	11.41
18	10	90	4.2	11.12
19	10	90	4.2	11.14
20	10	90	4.2	10.48

Table 3: Proposed model (2^{nd} order polynomial regression) equation for response

Protein yield	$\mathbf{Y = 12.746 + 1.123\,x\,A + 0.187\,x\,B - 0.264\,x\,C + 1.30\,x\,AB + 0.529\,x\,AC + 0.333\,x\,BC + 1.869\,x\,A2 + 0.082\,x\,B2 + 0.267\,x\,C2}$

Where, Y is the predicted response (Protein yield %); A is the alkaline pH; B is the Incubation time (min); C is the Isoelectric pH.

Table 4: Coefficients of the fitted model for determined response

Coefficients	Protein yield (%)
β_0	12.75
β_1	1.123
β_2	0.187
β_3	-0.264
β_{12}	1.30
β_{13}	0.529
β_{23}	0.333
β_{11}	1.869
β_{22}	0.082
β_{33}	0.267
R2	0.459
Model 'p' value	0.5298
Lack of fit p value	0.0073**
CV%	26.853
Adequate precision value	3.699

**Significant at $p<0.05$,

Optimization and Validation

Numerical multi-response optimization was adopted to determine the optimum level of each independent variable and the respective predicted level of responses as per the set goals with maximum desirability function. The optimum alkaline

pH, extraction time and isoelectric pH for protein isolation with maximum protein yield as set goals for optimization with desirability of 0.7. For 2 gm sample 8 ml of water to adjust 11 pH 0.1 N NaOH 2.7 ml required, for iso electric precipitation 4.4 pH to adjust 2.4 ml of 0.1 N HCL required. The determined experimental value of each response based on optimal condition was in comparison with predicted levels at $p<0.05$.

Optimised experimental procedure

The defatted germinated green gram flour (2 g) and deionised water (8 ml), [1:4] was adjusted to pH 11 by adding 2.7 ml of 0.1 N NaOH and stirred for 120 min at room temperature. The slurry was centrifuged at 5000 g for 30 min. The pH of supernatant was adjusted to 4.4 by adding 2.4 ml of 0.1 N HCl and centrifuged again at 5000 g for 30 min. The precipitate was washed using water and brought to pH 7 using 1 M NaOH, air dried at room temperature and stored at -5 ^{o}C until used further.

IV. CONCLUSIONS

The Central Composite Rotatable Design in Response Surface Methodology determined the optimum conditions for isolation of protein from Germinated green Gram flour using Alkaline extraction method which could most probably adopted by Indian food industries. The quality of protein present in germinated green gram flour was 52.537% determined by the micro kjeldhal method at convertion factor of 6.25. While conducting this, the amount of protein extracted using suggested alkali isoelectric precipitation method (hot air oven dried) was only 35.59 %.

ACKNOWLEDGEMENT

The author expresses immense thanks to the Periyar University, Salem, Tamil Nadu, India for providing the funding assistance under the University Research Fellowship programme.

REFERENCES

[1] C.P. Chandrasekher, and J.Ghosh, "Food economy in disarray. Indian Food Ind", Vol. 21, 2002, pp.10-14.

[2] A, Kataria, B.M. Chauhan, and Punia, "Antinutrients and protein digestibility (in vitro) of mangbean as affected by domestic processing and cooking", Food Chem., 32, 1989, pp 9-17.

[3] A. Moure, J. Sineiro, H. Dominguez and J. S. Parajo, "Functionality of oilseed protein products: A review", Nutr Res. Vol 11, 2003, 1147 – 1154.

[4] J.C. Cherry, K. H. McWatters and M.R Holmes, "Effects of moist heat on solubility and structural components of pea nut protein", J. Food. Sci., Vol 40, 1975, pp. 1199-1205.

[5] M. Wang, N.S. Hettiarchchy, M. Qi, W. Burks, and T. Siebenmorgen, "Preparation and functional properties of rice bran protein isolate", J. Agric. Food Chem., Vol 47, 1999, pp 411-416.

[6] R. Gnanasambandam, and N.S. Hettiarachchy, "Protein concentrates from unstabilized and stabilized rice bran preparation and properties", J. Food Sci., Vol 60, no 5, 1995, pp 1066-1069.

[7] A, Kunamneni, K.S. Kumar and S. Singh, "Response surface methodological approach to optimize the nutritional parameters for enhanced production of α-amylase in solid state fermentation by Thermomyce lanuginosus", African Journal of Biotechnology, Vol 4 , no7, 2005, pp 708-716.

[8] S. Jiamyangyuen, V. Srijesdaruk, and W.J. Harper, "Extraction of rice bran protein concentrate and its application in bread", Songklanakarin J. Sci. Technol., Vol.27, no.1, 2005, pp55-64.

[9] L. Chin and D.F.Houston, "solubilisation and recovery of protein from defatted rice bran", AACC meeting Chicago, Vol.42, 1970, pp 72-79.

[10] C. Karunanithy, and K. Muthukumarappan, "Optimization of alkali concentration and extruder parameters for maximum sugar recovery from prairie cord grass using response surface methodology", *Biochemical Engg J.*, 2011, DOI:10.1016/j.bej.2011.02.001,1369-703X%,

[11] D.C. Montgomery, "Design and analysis of experiments", 1984, 2nd ed. John wiley & Sons, Inc; Singapore.

