Full Paper

STATISTICAL MODELLING AND OPTIMIZATION OF EXTRACTION CONDITION FOR PROTEIN ISOLATE PRODUCTION FROM KERSTING'S GROUNDNUT USING RESPONSE SURFACE METHODOLOGY

O. R. Osungbade
Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Nigeria.

S. O. Gbadamosi
Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Nigeria.

A. V. Ikujenlola
Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Nigeria.
avjenlola@gmail.com

ABSTRACT

The study evaluated the effect of extraction conditions on the yield and protein concentration of protein isolate produced from Kersting's groundnut, and optimized the extraction conditions using response surface methodology. The extraction conditions were solid-solvent ratio (5, 10 and 15 g/ml), temperature (30, 40 and 50 °C) and time (3, 4 and 5 hours). The result revealed that increase in the protein isolate yield was associated with increased solid-to-solvent ratio and extraction temperature. The prediction accuracy of the RSM was supported by a non-significant lack-of-fit and R^2 of 0.9999 and 0.9972 for yield and concentration of the protein isolate respectively. Hence, optimal parameters obtained were 15 g/ml solid-solvent ratio, 30 °C extraction temperature and 3 hours extraction time. A yield of 20.05% protein isolate with 70.67 mg/ml concentration was obtained based on 100 g of Kersting's groundnut protein powder.

Keywords: Kersting's groundnut; Optimization; Protein isolate; Central composite design (CCD); Response surface methodology (RSM)

1. INTRODUCTION

Wide reports on new sources of cheap and locally available proteins have become necessary due to their importance in the human body and in their utilization as functional foods. The *leguminosae* or *fabacea* family has played a major role as an alternative source of protein especially in the developing world (Dairo *et al.*, 2007). However, the functional, sensory, nutritive and bioactive aspects of food proteins are better released upon extraction and isolation of these protein compounds from their native state involves a series of alternate solubilisation and precipitation on the basis of their physicochemical properties (Rodrigues *et al.*, 2012). Protein isolates are present as the most refined form of proteins. They are easily digestible and can also be introduced into other food products (Garba and Kaur, 2014).

Kersting's groundnut (*Macrotyloma geocarpum* Harms; Kerstingiella geocarpa Harms) is an underexploited West African grain legume (Obasi and Ezedinma, 1991). Commonly called ground beans or Hausa groundnut, it is a subterranean legume with cowpea-like seeds (Adu-Gyamfi *et al.*, 2012). Kersting's groundnut seeds have been reported to contain 21.3% crude protein, 6.2% crude fiber, 61.53% carbohydrate, 6.2% moisture, 0.98% fat and 3.8% ash (Oyetayo and Ajayi, 2009). Other reported protein values for the seeds are 24.70% (Duke *et al.*, 1977), 24.9% (Obasi and Agbatse, 2003) and 23.79% (Echendu *et al.*, 2009). These values show that the protein content of Kersting's groundnut is high and as such could serve as a source of cheap protein.

The need to improve process efficiency (such as protein extraction, starch hydrolysis, oil yield, vitamin retention, microbial load reduction, etc.) while minimizing cost and time remains an integral aspect in the food process industry. Hence a multivariate statistical technique called Response Surface Methodology (RSM) has been used in the optimization of food processes (Baş and Boyacı, 2007). The technique works by making statistical predictions based on a polynomial developed when a response or responses are affected by several variables (Kaushik *et al.*, 2006; Ghorbannezhad *et al.*, 2016).

Various works have reported the optimum extraction conditions for extracting proteins from Mung bean (Wang *et al.*, 2011), Mucuna *pruriens* (Blaise *et al.*, 2017), and soy bean (Kao *et al.*, 2011), among others. These studies have formed the basis for the industrial application of these proteins. However, there is a paucity of information on the optimum extraction conditions that can be applied to Kersting's groundnut. This work therefore aims to optimize the extraction conditions (solid/solvent ratio, temperature and time) for the production of Kersting's groundnut protein isolate.

2. MATERIALS AND METHODS

2.1. Material

Dried Kersting's groundnut seeds were purchased from a local market in Abakaliki, Ebonyi State, Nigeria and identified at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The seeds were thereafter screened for dirt, pebbles and other foreign materials.

2.2. Method

2.2.1. Preparation of defatted flour

Kersting's groundnut defatted flour was prepared using a modified method of Sathe (1994) as described. The cleaned seeds were milled using a laboratory blender (VLC sapphire grinder, IS-4930, England) at speed 1.25×10^{-2} ms^{-1} and sieved through a 600 µm sieve (Laboratory test sieve, ISO0330-1, Endecotts Ltd, London, England). The resulting flour was subsequently defatted with cold (4 ºC) acetone (flour-to-solvent ratio 1:5 w/v) with constant magnetic stirring provided. The slurry was filtered off and the defatted flour was desolventised by air-drying at room temperature. The dried flour was finally ground using a laboratory blender (VLC sapphire grinder, IS-4930, England) at speed 4 to obtain a homogenous defatted flour. The flour obtained was stored in an air-tight plastic bottle as defatted Kersting's groundnut flour and kept in a freezer at 4 °C until used.

2.2.2. Protein isolate preparation

Kersting's groundnut protein isolate was prepared by a method described by Gbadamosi *et al.* (2012). A known weight of the defatted flour was dispersed in an amount of distilled water to give the desired range of flour-to-liquid ratio (5 to 15 g/ml) for the RSM. The extraction proceeded with gentle stirring throughout the extraction time (3 to 4 hours) while maintaining constant temperature (30 to 50 °C) and pH 10 (most soluble pH). Non-solubilized materials were removed by centrifugation at 3500 × g for 10 min. The protein in the extract was precipitated by drop-wise addition of 0.1 N HCl with constant stirring until the pH was adjusted to pH 4.0 (least soluble pH). The mixture was then centrifuged at 3500 × g for 10 min using a centrifuge (MSE, Harrier 15/80, United Kingdom) in order to recover the protein. After separation of proteins by centrifugation, the precipitate was washed twice with distilled water. The precipitated protein was re-suspended in distilled water (two times the quantity of protein slurry) and the pH adjusted to 7.0 with 1 M NaOH, centrifuged and then freeze-dried as protein isolate.

2.3. Experimental design for isolation of proteins from Kersting's groundnut

Response Surface Methodology (RSM) was used to estimate the effect of three independent variables (solid-solvent ratio of 5, 10 and 15 g/ml; extraction temperature of 30, 40 and 50 ºC and extraction time of 3, 4 and 5 hours) on the conditions for obtaining optimum protein yield and concentration. The rotatable Central Composite Design (CCD) was employed to vary the independent variables at 5 levels (Table 1). The experiment generated 20 runs (8 factorial runs, 6 star runs and 6 centre points) in a completely randomized order. The experimental runs in coded forms as well as the responses are shown in their standard form in Table 2. Each experiment was done in triplicate and the response values were fitted by second order polynomial quadratic regression model (equation 1) so as to relate the responses to the independent variables.

$$Y = b_o + b_1A + b_2B + b_3C + b_{12}AB + b_{23}BC + b_{13}AC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 \quad (1)$$

where; Y represents the dependent variable; b_o, is the constant regression coefficient; b_1, b_2, and b_3 are the linear regression coefficients; b_{11}, b_{22}, and b_{33} are the quadratic regression coefficients; b_{12}, b_{23}, and b_{13} are the cross-product regression coefficients; while A, B and C represent the coded values of the independent variables (solid-solvent ratio, extraction temperature and extraction time, respectively). To obtain the regression coefficients, analysis of variance (ANOVA) was carried out using Stat Ease Design-Expert version 10 software package, USA.

Table 1: Experimental Process Variables and Levels for the Extraction of Protein Isolates from Kersting's Groundnut

Independent variables	Code	Range of values				
		$-\alpha$	-1	0	+1	$+\alpha$
Solid/solvent ratio (g/ml)	A	1.59	5	10	15	18.41
Temperature (°C)	B	23.18	30	40	50	56.82
Time (h)	C	2.318	3	4	5	5.68

3. RESULTS AND DISCUSSION

3.1. Experimental design and statistical analysis

The results from the Central Composite Design showed that the variation in the experimental factors influenced both the experimental and predicted protein yield as well as the experimental and predicted protein concentration (Table 3). The values ranged between 16.93 to 21.00% and 16.92 to 21.00% for experimental and predicted yields respectively, while the experimental and predicted protein concentration recorded a range of 52.06 to 72.86% and 52.1 to 72.84% respectively. Wang *et al.* (2011) reported a much higher yield value of 77.60% for mung bean protein isolate. This could be due to differences in the anatomical structure of the two legumes. A close range between the experimental and predicted responses indicates the suitability of the polynomial to predict the extraction of protein isolate from Kersting's groundnut.

3.2. Analysis of the model

The proposed quadratic equations of the regression models for the yield and concentration of Kersting's groundnut protein isolate are shown in equations 2 and 3, respectively.

Table 2: Design in Coded Units and Responses for Extracting Protein Isolates from Kersting's Groundnut

	FACTORS			RESPONSES	
Sample (Runs)	Solid / solvent ratio (g/ml)	Extraction Temperature (°C)	Extraction time (hr)	Average yield	Protein concentration (mg/ml)
1	-1	-1	-1	18.47	72.86
2	1	-1	-1	20.05	70.56
3	-1	1	-1	19.27	68.57
4	1	1	-1	21.00	52.06
5	-1	-1	1	17.41	61.26
6	1	-1	1	17.64	72.35
7	-1	1	1	18.05	70.41
8	1	1	1	18.41	66.84
9	-1.682	0	0	18.94	68.78
10	1.682	0	0	20.60	64.09
11	0	-1.682	0	17.39	70.74
12	0	1.682	0	18.71	62.87
13	0	0	-1.682	19.95	65.71
14	0	0	1.682	16.93	67.16
15	0	0	0	18.20	65.71
16	0	0	0	18.25	65.71
17	0	0	0	18.19	66.29
18	0	0	0	18.19	65.71
19	0	0	0	18.20	65.88
20	0	0	0	18.19	65.71

3.3. Analysis of the model

The proposed quadratic equations of the regression models for the yield and concentration of Kersting's groundnut protein isolate are shown in equations 2 and 3, respectively.

$$\text{Yield} = +18.20 + 0.49\text{A} + 0.39\text{B} - 0.90\text{C} + 0.036\text{AB} - 0.34\text{AC} - 0.045\text{BC} + 0.55\text{A}^2 + 0.054B^2 + 0.084C^2 \quad (2)$$

$$Concentration = +65.83 - 1.40\text{A} - 2.37\text{B} + 0.68\text{B} - 3.61\text{AB} + 3.29\text{AC} + 3.30\text{BC} + 0.27\text{A}^2 + 0.40\text{B}^2 + 0.27\text{C}^2 \quad (3)$$

where A indicates solid/solvent ratio (g/ml); B indicates extraction temperature (ºC); and C indicates extraction time (hour). The regression equation illustrates the influence (either positively or negatively) each variable had on the selected response. Variables with positive signs influenced the response positively while those with negative signs influenced the responses negatively. Hence, from the equation, variable C (extraction time) had the most significant influence on the yield of the protein isolate, although the negative sign indicates an inverse relationship. That is, the yield of protein isolate reduced as the extraction time increased. For the concentration on the other hand, one of the interactive terms, that is, AB (combination of solid/solvent ratio and extraction temperature) had the most significant effect but also in the opposing direction.

On a general note, the linear terms (A, B and C) had a stronger effect on the yield of the protein isolate than the interactive (AB, AC and BC) and the quadratic terms (B^2 and C^2), while the interactive terms (AB, AC and BC) had the strongest effect on the protein concentration of the isolate than the linear (A, B and C) and the quadratic (A^2, B^2 and C^2) terms.

The analysis of variance (ANOVA) generated for the multiple regression models is presented in Table 4. The fitness and adequacy of the models were judged based on coefficient of determination (R^2) and the significance of lack-of-fit. From this study, the R^2 and adjusted R^2 (Adj R^2) values obtained for protein yield were 0.9999 and

Table 3: Effect of Processing Variables on the Experimental and Predicted Responses of Kersting's Groundnut Protein Isolate Extraction

	FACTORS			RESPONSES			
Sample runs	Solid / solvent Ratio (g/ml)	Extraction temperature (°C)	Extraction time (hr)	Experimental yield (%)	Predicted yield (%)	Experimental Protein concentration (mg/ml)	Predicted Protein concentration (mg/ml)
1a	10	40	4	18.19	18.20	65.71	65.83
2	10	56.82	4	18.71	18.71	62.87	62.96
3	15	30	3	20.05	20.05	70.56	70.67
4a	10	40	4	18.19	18.20	66.29	65.83
5	10	40	5.68	16.93	16.92	67.16	67.72
6	5	50	5	18.05	18.04	70.41	70.09
7	10	40	2.32	19.95	19.96	65.71	65.45
8a	10	40	4	18.19	18.20	65.71	65.83
9	5	30	3	18.47	18.46	72.86	72.84
10	15	50	5	18.41	18.42	66.84	66.64
11	5	50	3	19.27	19.26	68.57	68.71
12	10	23.18	4	17.39	17.39	70.74	70.94
13	15	30	5	17.64	17.65	72.35	72.00
14	15	50	3	21.00	21.00	52.06	52.10
15a	10	40	4	18.20	18.20	65.88	65.83
16	5	30	5	17.41	17.42	61.26	61.01
17	18.41	40	4	20.60	20.59	64.09	64.22
18	1.59	40	4	18.94	18.94	68.78	68.95
19a	10	40	4	18.20	18.20	65.71	65.83
20a	10	40	4	18.25	18.20	65.71	65.83

a = centre points

Table 4: ANOVA for Response Surface Quadratic model of Kersting's Groundnut Protein Isolate Extraction

	Yield of Isolate (%)				Protein Concentration of Isolate (mg/ml)			
Source	Sum of Squares	Mean Square	F Value	P-value Prob > F	Sum of Squares	Mean Squares	F Value	P-value Prob > F
Model	22.12	2.46	7480.88	< 0.0001*	391.91	43.55	395.76	< 0.0001*
A	3.29	3.29	10019.5	< 0.0001*	26.95	26.95	244.94	< 0.0001*
B	2.12	2.12	6441.13	< 0.0001*	76.86	76.86	698.51	< 0.0001*
C	11.16	11.16	33971.16	< 0.0001*	6.26	6.26	56.86	< 0.0001*
AB	0.01	0.01	31.28	0.0002*	104.26	104.26	947.53	< 0.0001*
AC	0.92	0.92	2798.03	< 0.0001*	86.63	86.63	787.3	< 0.0001*
BC	0.016	0.016	48.94	< 0.0001*	87.33	87.33	793.64	< 0.0001*
A^2	4.41	4.41	13428.66	< 0.0001*	1.02	1.02	9.29	0.0123*
B^2	0.041	0.041	126.24	< 0.0001*	2.26	2.26	20.5	0.0011*
C^2	0.1	0.1	308.26	< 0.0001*	1.03	1.03	9.32	0.0122*
Residual	3.29E-03	3.29E-04			1.1	0.11		
Lack of Fit	5.98E-04	1.20E-04	0.22	**0.9376****	0.83	0.17	3.09	**0.1208****
Pure Error	2.69E-03	5.37E-04			0.27	0.054		
Cor Total	22.12				393.01			
R^2	**0.9999**			**R^2**	**0.9972**			
Adj R^2	0.9997			Adj R^2	0.9947			
Pred R^2	0.9996			Pred R^2	0.9829			

0.9997 respectively while the R^2 and adjusted R^2 values obtained for concentration were 0.9972 and 0.9947, respectively. The nearness of the R^2 values to unity shows the statistical significance ($p < 0.05$) of the model (Kumar *et al*., 2016). A lower R^2 value of 0.95 was reported by Kalaydzhiev *et al.* (2019) for the extraction of rapeseed protein. Also, in agreement with the fitness of the model is the closeness between the R^2 and adjusted R^2 (which is the adjusted value for R^2 after removal of irrelevant terms). The lack-of-fit values (a measure the model's accuracy) obtained in this study were non-significant values of 0.9376 and 0.1208 for the protein yield and concentration, respectively. This also indicates the desirability and appropriateness of the model in describing the response surface (Kao *et al.*, 2011).

3.4. Effects of Process Variables on the Protein Yield and Concentration of Kersting's Groundnut

3.4.1. Single factor effects

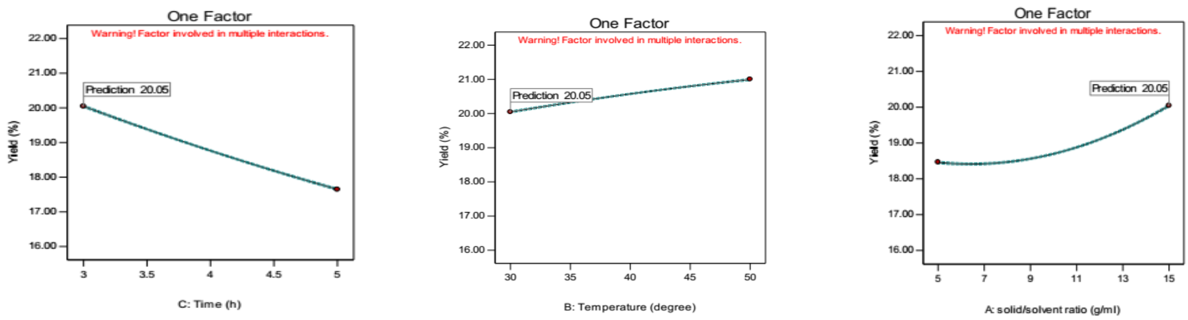
Although the variables were involved in multiple interactions with one another, Figures 1(a to c) and 2 (a to c) explain the individual effect of a single variable on the response. From the model, extraction time (1a) has an inverse relationship with the protein yield of Kersting's groundnut while extraction temperature (1b) and solid-to-solvent ratio (1c) show a direct effect on the yield.

Figures 2a to 2c show the single effect of each of the variables on the protein concentration of Kersting's groundnut. The protein concentration of Kersting's groundnut increased with increasing extraction time (2a), while the concentration reduced as extraction temperature (2b) solid-to-solvent ratio (2c) increased.

3.4.2. Interactive effects

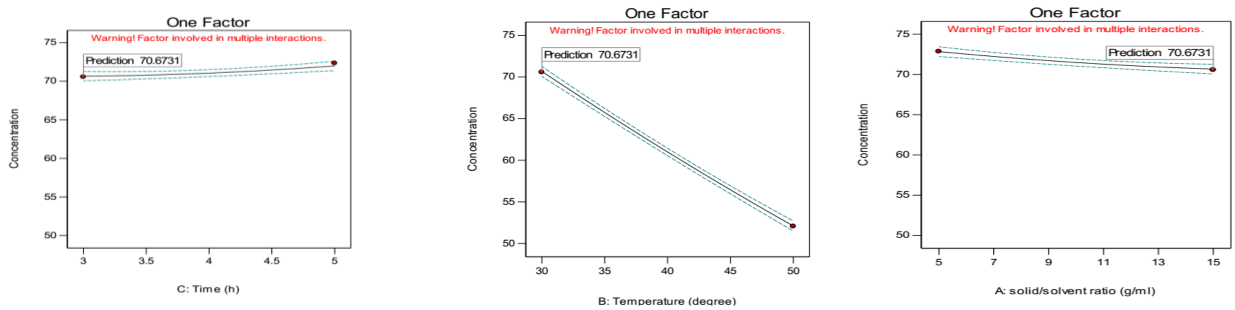
The contour plots were used to show the influence of the independent variables on the yield (Figures 3a, 3b and 3c) and concentration (Figures 4a, 4b and 4c) of Kersting's groundnut protein isolate. The plot revealed the effects of two of the independent variables on each of the dependent variables while the third was held constant. The yield of Kersting's groundnut protein isolate increases as the combined effect of temperature and solid-to-solvent ratio increases (Figure 3a). Figure 3b and 3c also depict increased yield in protein isolate as the interaction between time and temperature as well as that between time and solid-to solvent ratio decreased.

Figure 4a shows that the interaction between solid-to-solvent ratio and temperature (4a) had a negative effect on the concentration of Kersting's groundnut protein isolate. Also, the concentration of Kersting's groundnut protein isolate exhibited a direct relationship. That is, increases as the combined effect of temperature and time (Figure 4b) as well as that of time and solid-to-solvent ratio (4c) increases.



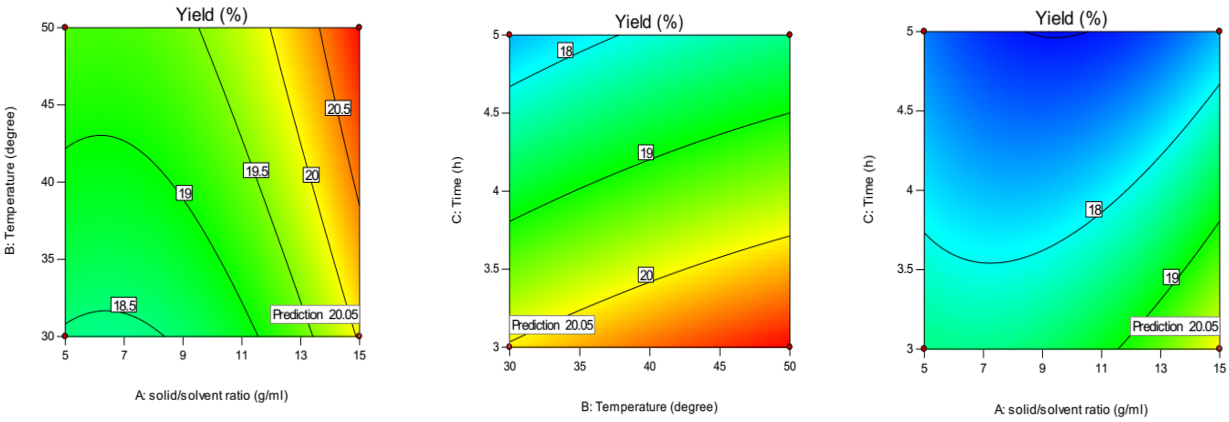
(a) (b) (c)

Figures 1 (a - c): Single Factor Effect of Process Variable on Protein Yield of Kersting's Groundnut Protein Isolate



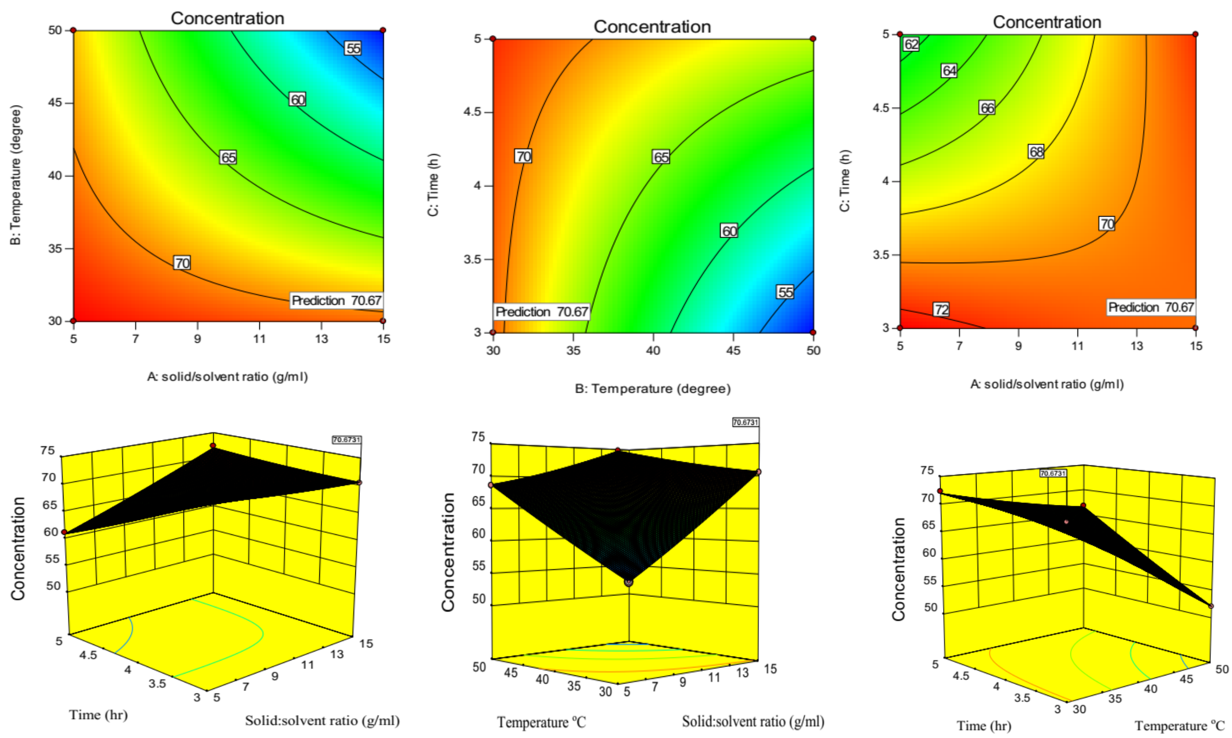
(a) *(b)* *(c)*

Figures 2 (a - c): Single Factor Effect of Process Variable on Protein Concentration of Kersting's Groundnut Protein Isolate



(a) *(b)* *(c)*

Figures 3a-c: Contour Plots for the Interactive Effect of Process Variable on the Yield of Kersting's Groundnut Protein Isolate



(a) *(b)* *(c)*

Figures 4a-c: Contour Plots for the Interactive Effect of Process Variable on of the Concentration of Kersting's groundnut Protein Isolate

Using the central composite design expert based on the values from the quadratic regression model, a selection of process variables that maximized the protein yield and content of Kersting's groundnut protein isolate is presented in Table 5. It shows the respective variable combination; their yield, protein content and the degree of desirability. The highlighted process conditions (Serial No. 1 in Table 5) which correlates with experimental run No. 3 (from Table 3) recorded the highest degree of desirability (0.828).

In other words, for optimum extraction of Kersting's groundnut protein isolate, a combination of 15 g/ml solid-solvent ratio, 30 °C extraction temperature and 3 hours extraction time generated the highest level of desirability value of 0.828. This implies a lower limit for extraction temperature, lower limit for extraction time and upper limit for solid-solvent concentration favoured the protein yield and concentration. That is, the optimization of the extraction process is based majorly on energy and time conservation. An upper limit flour-to-solvent ratio has also been reported to favour the optimization of defatted custard apple seed (Kumar *et al*., 2016) while Blaise *et al*. (2017) stated that lower limit temperature (27 ºC) resulted in optimized protein yield (40.07%) for *Mucuna pruriens*. Wang *et al*. (2011) also reported optimized conditions of 10% solid-liquid ratio, 31.74% extraction temperature and 33.24 mins extraction time for mung beans protein isolate. Also reported by Meshkani *et al*. (2016) for tomato seed are optimization conditions of 1:40 solid-powder ratio, 37.73 °C extraction temperature and 60 mins extraction time.

3.5. Validation of the model

In order to test the suitability of the developed model, an experiment was carried out (in triplicate) using the optimum process conditions of the variables to validate the experimental and predicted values of the responses. The experimental procedure resulted in protein isolate yield of 19.94% and concentration of 69.61 mg/ml both of which are in close range with those from the model (20.05% and 70.67 mg/ml) as shown in Table 5. Hence, the model is reliable.

4. CONCLUSION

The extraction of protein isolate from Kersting's groundnut is dependent on ratio of flour-to-water, extraction time and extraction temperature used in the process. According to the model, extraction of protein isolate with optimal yield and concentration from Kersting's groundnut can be achieved through a combination of 15 g/ml solid-solvent ratio, 30 °C extraction temperature and 3 hours extraction time. A yield of 20.05% protein isolate with 70.67 mg/ml concentration was obtained based on 100 g of Kersting's groundnut protein powder.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife for the support received in terms of laboratory facilities.

CONFLICT OF INTERESTS

Authors have declared no conflicting interests.

FUNDING/GRANTS

This research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

REFERENCES

Adu-Gyamfi, R., Israel, K. D. and Juliana, L., Evaluation of growth and yield potential of genotypes of Kersting's groundnut (*Macrotyloma geocarpum* harms) in Northern Ghana. International Research Journal of Agricultural Science and Soil Science. 2(12):509-515, 2012.

Baş, D., and Boyacı, İ. H., Modeling and optimization I: usability of response surface methodology. Journal of Food Engineering, 78(3), 836–845, 2007.

Blaise, K., Clémence, B., Nicolas, N. Y. and Richard K., Production of *Mucuna pruriens* (Var. *Utilis*) proteins isolates using central composite design and effect of drying techniques on

Table 5: Numerical Optimisation Solution for Kersting's Groundnut Protein Isolate

No	Solid:solvent ratio (g/ml)	Extraction temperature (°C)	Extraction time (h)	Gross Yield (%)	protein concentration (mg/ml)	Desirability
1	15.00	30.00	3.00	20.05	70.67	0.828*
2	14.96	30.00	3.00	20.03	70.68	0.826
3	15.00	30.14	3.00	20.06	70.54	0.826
4	15. 00	30.01	3.01	20.03	70.67	0.826
5	14.91	30.00	3.00	20.01	70.68	0.823
6	15.00	30.32	3.00	20.07	70.35	0.823
7	15.00	30.00	3.032	20.00	70.68	0.822
8	14.85	30.00	3.00	19.99	70.69	0.820
9	15.00	30.09	3.03	20.01	70.59	0.820
10	15.00	30.52	3.00	20.08	70.15	0.820
11	15.00	30.00	3.06	19.97	70.68	0.818
12	14.77	30.00	3.00	19.96	70.70	0.817
13	15.00	30.00	3.07	19.96	70.68	0.816
14	15.00	30.00	3.07	19.95	70.68	0.815
15	14.63	30.00	3.00	19.91	70.72	0.810

**= Selected*

some properties. Chemical Science International Journal 20(1): 1-12, 2017.
Dairo, F. A. S., Aye, P. A. and Oluwasola, T. A., Some functional properties of Loofah gourd (*Luffa cylindrical* L., M.J. Roem) seed. Journal of Food Agriculture and Environment. 5(1): 97-101, 2007.
Duke, J. A, Okigbo, B. N., Reed, C. F., Under-exploited Legumes; Grain legume. In: Musah, A. J. (Eds.), Savanna Biodiversity Conservation Project Newsletter 1(2): 4-7, 1977.
Echendu, C. A. Obizoba, I. C. and Anyika, J. U., Effects of heat treatments on chemical composition of ground bean (*Kerstingiella geocarpa harm*) Pakistan Journal of Nutrition 8(12): 1877-1883, 2009.
Garba, U. and Kaur, S., Review- protein isolates: production, functional properties and application. International Research Journal of Chemistry *(IRCJ)*. 4: 22-36, 2014.
Gbadamosi, S. O., Abiose, S. H. and Aluko, R. E., Amino acid profile, protein digestibility, thermal and functional properties of conophor nut (*Tetracarpidium conophorum*) defatted flour, protein concentrate and isolates. International Journal of Food Science and Technology. 47, 731-739, 2012.
Ghorbannezhad, P., Bay, A., Yolmeh, M., Yadollahi, R., and Moghadam, J. Y., Optimization of coagulation–flocculation process for medium density fiberboard (MDF) wastewater through response surface methodology. Desalination and Water Treatment, 1–16, 2016.
Kao, F., Tsou, M., Kao, H. and Chiang, W., Optimization of Enzymatic Hydrolysis Conditions for Producing Soy Protein Hydrolysate with Maximum Lipolysis-Stimulating Activity. Journal of Food and Drug Analysis, 19(2):197-201, 2011.
Kaushik, R., Saran, S., Isar, J., and Saxena, R., Statistical optimization of medium components and growth conditions by response surface methodology to enhance lipase production by Aspergillus carneus. Journal of Molecular Catalysis B: Enzymatic, 40(3):121–126, 2006.
Kalaydzhiev, H., Brandão, T. R. S., Ivanova, P., Silva, C. L. M. and Chalova, V. I., A two-step factorial design for optimization of protein extraction from industrial rapeseed meal after ethanol-assisted reduction of antinutrients. International Food Research Journal. 26(4): 1155-1163, 2019.
Kumar, R., Sharma, R., Srivastava, T. and Saxena, D. C., Response surface methodology for optimization of protein isolate from defatted custard apple seed (*Annona squamosa*). International Journal of Computer Applications (0975 – 8887). International Conference on Advances in Emerging Technology *(ICAET 2016)*.2016.
Meshkani, S. M., Mortazavi, S. A., Elhami Rad, A. H. and Beigbabaei, A., Optimization of protein extraction and evaluation of functional properties of tomato waste and seeds from tomato paste plants. Biosciences Biotechnology Research Asia. 13(4), 2387-2401, 2016.
Obasi, M. O. and Ezedinma, F. O. C., Evaluation of the growth and chemical composition of Kersting's groundnut (*Kerstingiella geocarpa* Harms). Indian Journal of Agricultural Science, 61(11): 811–814, 1991.
Obasi, M. O. and Agbatse, A., Evaluation of nutritive value and some functional properties of Kersting's groundnut seeds for optimum utilization as a food and feed resource. East Africa Agricultural For. Journal. 68: 173-181, 2003.
Oyetayo, F. L. and Ajayi, B. O., Chemical profile and zinc bioavailability on "Hausa groundnut" (*Kerstingiella geocarpa*). Bioscience and Biotechnology Research Asia. 3:47–50, 2005.
Sathe, S.K., Solubilisation and electrophoretic characterization of cashew nut (*Anacardium occidentale*) proteins. Food Chemistry 51: 319-324, 1994.
Rodrigues, I. M., Coelho, J. F., Carvalho, M. G. V., Isolation and valorisation of vegetable proteins from oilseed plants: Methods, limitations and potential. Journal of Food Engineering. 109(3):337-346, 2012.
Wang, M., Jiang L., Li, Y., Liu, Q., Wang, S. and Sui, X., Optimization of extraction process of protein isolate from mung bean. Procedia Engineering 15:5250 – 5258, 2011.