International Food Research Journal 19(1): 341-346 (2012)

Optimization of extraction conditions by response surface methodology for preparing partially defatted peanut

[1*]Badwaik, L. S., [2]Prasad, K. and [1]Deka, S. C.

[1]Department of Food Engineering and Technology, School of Engineering, Tezpur University, Napaam, Assam, 784028, India
[2]Department of Food Engineering and Technology, Sant Longowal Institute of Engineering and Technology, Longowal, Punjab, 148106, India

Abstract: Suitability of different solvents like acetone, benzene, chloroform, hexane and petroleum ether was compared on the basis of oil recovery from splited peanut. Response surface methodology (RSM) was used to optimize the factors like seed to solvent ratio and extraction time based on maximum oil recovery and high sensory scores. A rotatable central composite design was used to develop models for the responses. The results showed that hexane was best suited for oil extraction from peanut considering its highest value of extraction constant (k=6.60 x 10^{-3} min^{-1}) with rapid, efficient and maximum extraction of oil than other solvents. Oil recovery found was linearly affected with seed to solvent ratio and time of extraction which was significant at P≤ 0.01 with a correlation coefficient (R^2) of 90%. Variation in the surface appearance, color and overall acceptability were found significant with respect to seed to solvent ratio and time of extraction at second order regression model. The optimum values for seed to solvent ratio and extraction time were found to be 1:6 and 5 hr respectively with an overall acceptability of 8.31 and oil recovery of 9.03%.

Keywords: Seed to solvent ratio, extraction time, response surface methodology, solvents, oil recovery

Introduction

Peanut (*Arachis hypogaea.*) is an important legume crop. It is rich both in oil and protein content and mostly grown and used for oil production (Zhang and Jiang, 1998). Oil is extracted from oilseed either mechanical or solvent extraction in a batch or continuous process. The high temperature in most of the efficient mechanical extractors damage the edible quality of obtained defatted materials and found not suitable for human consumption. The problem of this nature is not associated with solvent extraction. Solvent extraction is an efficient method of oil extraction with less damage to solid material. Various solvent were popularly used for extraction but extraction rate was found consistent for sunflower oil extracted using hexane (Durdev *et al*., 1982).

Recently, peanuts have aroused great interest as a source of low-cost protein to supplement human diets. In addition to the traditional food uses, peanut butter and roasted peanuts, have also been successfully utilized in supplemented foods such as bakery products, extenders in meat product formulations, in soups and desserts (Ismail *et al*., 1991; Wu *et al*., 2007). Also the peanut cake or meal was used as nutritional source for the manufacture of bakery products (Ory and Conkerton, 1983) like cookies (Tate *et al*., 1990), breads (Jan *et al*., 2003) and chapattis (Bhat, 1977), breakfast cereals (Coccodrilli *et al*., 1979), peanut butter (Lima *et al*., 2000) and popular drinks (Holsinger *et al*., 1978).

And recent studies have also demonstrated that oil extraction produces a protein-rich co-product which may be used for human consumption, if processed from edible-grade peanut seed by commercially accepted food processed (Cherry, 1990), generally, this material is available as flakes or grits and may be further processed to partially defatted peanut flour (DPF). DPF, as a protein-rich, inexpensive and underutilized product that offers the same health and dietary benefits of peanut with less fat (Liu *et al*., 1996), generally contains 47-55% high quality protein with high essential amino acid content (Basha and Pancholy, 1982) which lends itself being used in many food applications (Prinyawiwatkul *et al*., 1993).

Response surface methodology (RSM), as an effective tool for optimizing the process, is usually employed when many factors and interactions affect the desired response (Triveni *et al*., 2001). By using RSM, we could not only get information with less cost and short time, but also obtain rapid and efficient development of new products and processes (Pericin *et al*., 2008). Response surface experiments, whose aim is to identify the response that can be thought of as a surface over the explanatory variables experimental space, usually uses an experimental design such as central-composite rotatable design (CCRD) to fit an empirical, full second-order polynomial model. And generally, a CCRD coupled with a full second-order polynomial model, is a very powerful combination that usually provides an adequate representation of most continuous response surfaces over a relatively broad factor domain (Deming, 1990). RSM is an

*Corresponding author.
Email: *laxmikantbadwaik@gmail.com*
Tel: +91 9706 368117; Fax: +91 03712 267005

effective statistical technique for the investigation of complex processes. The main advantage of RSM is the reduced number of experimental runs needed to provide sufficient information for statistically acceptable result. It is a faster and less expensive method for gathering research result than the classical method. RSM has successfully been applied for the optimization of the different extraction conditions (Tiezheng *et al*., 2010, Yi *et al*., 2011, Wani *et al*., 2006, Li *et al*., 2005).

Present study was carried out for selection of suitable solvent for oil extraction on the basis their extraction rate as well as RSM was used to optimize and to study the effect of seed to solvent ratio and extraction time for maximum oil recovery and high sensory score.

Materials and Methods

Materials

Punjab-1 peanut verity was procured from the market of Ludhiana, India. The peanuts were dried in laboratory scale hot air oven (Microsil, India) for 8 hr at 70°C temperature. Then peanuts skin was separated by rubbing between hands. The obtained splited peanuts were passed through seed grader (Agrosaw Seed Grader, Model-Junior) to obtain the seeds of 6.5 to 7.5 mm size for further investigations. The solvents (Acetone, Benzene, Chloroform, Hexane and Petroleum Ether), chemicals and reagents used in the present investigation were of analytical reagent grade.

Solvent Selection

Acetone, benzene, chloroform, hexane and petroleum ether as solvents were used for extraction of oil using SOCS PLUS apparatus (model SCS-6, Make Pelican, Chennai, India). Ten gram split peanuts were taken for extraction of oil as per condition (Table 1). Suitability of solvents for oil extraction was compared on the basis of oil recovery.

The oil extraction kinetics for different solvents was calculated using equations 1.

$$Y_t = Y_0 e^{kt} \quad (1)$$

Where, Y_t is the percent extracted oil content at time *t*; and Y_o is the percent unextracted oil at time zero, *t* is the time of extraction (min); *k* is the extraction constant. The solvent suitability was identified on the basis of oil recovery and extraction constant (*k*).

Standardization of oil extraction variables

Seed to solvent ratio and time were selected as process parameters for the preparation of partially defatted peanuts using hexane as solvent. The experiment was carried out as per central composite rotatable design (CCRD). Process parameters at the design center point were as follows.

X_1 (Seed: Solvent Ratio) = 1:6

X_2 (Time, hr) = 5 hrs

The design depended upon the symmetrical selection of variations increment about the center point composition (Table 2). The range of levels of process parameters were chosen with the criteria of responses in the reasonable range.

Table 1. Condition maintained at the time of extraction

Solvent	Boiling Point (°C)	Heating Plate Temp. (°C)	Seed: solvent Ratio (wt/vol)	Time of extraction (Min)	Time Interval (Min)
Acetone	56-56.5	100	1:10	360	60
Benzene	79-80	150	1:10	360	60
Chloroform	63-66	140	1:10	360	60
Hexane	63-70	140	1:10	360	60
Pet. Ether	40-60	110	1:10	360	60

Table 2. Experimental increments values of coded levels for preparing partially defatted peanuts

Symbol	Levels				
Coded	-1.414	-1	0	1	1.414
X_1 (Seed: Solvent Ratio)	1:2	1:3.175	1:6	1:8.828	1:10
X_2 (Time, hr)	2.00	2.88	5.00	7.12	8.00

The process parameters were optimized on the basis of percent oil recovery and sensory quality using Response Surface Methodology (RSM). Sensory analysis of partially defatted peanut was analyzed on a nine point hedonic scale (Ranganna, 1986) by semi trained panel of 15 members of the department. Various parameters like color, appearance, texture and overall acceptability (OA) were taken for analysis. All the experiments were carried out thrice.

Statistical analysis

A central composite rotatable design (CCRD) was used to evaluate the combined effect of different variables on its product. The design matrix (Table 3) is a 2^2 factorial design combined with 5 central points and 4 axial points where one variable is set at an extreme level (±1.414) while other variables are set at their central points (Montgomery, 1997).

Table 3. Central composite rotatable design (CCRD) for the preparation of partially fatted peanuts and their responses

Sr.	Coded Values		Oil Recovery#	Sensory Responses#			
No	Seed: Solvent	Time	(%)	Appearance	Texture	Color	OA
1	-1	-1	5.55 ± 0.79	7.25 ± 0.29	7.75 ± 0.65	7.38 ± 0.63	7.46 ± 0.37
2	1	-1	5.33 ± 0.69	7.75 ± 0.96	7.63 ± 0.63	7.50 ± 0.82	7.58 ± 0.42
3	-1	1	17.11 ± 1.52	7.38 ± 0.75	7.88 ± 0.63	7.13 ± 1.18	7.13 ± 0.83
4	1	1	17.36 ± 0.56	7.50 ± 0.68	7.13 ± 0.85	7.05 ± 1.22	7.88 ± 1.34
5	-1.414	0	6.71 ± 1.03	6.63 ± 0.41	7.13 ± 1.03	7.50 ± 0.71	7.42 ± 1.19
6	1.414	0	8.21 ± 0.39	6.88 ± 0.50	7.63 ± 0.75	7.50 ± 0.91	7.29 ± 1.20
7	0	-1.414	3.98 ± 0.39	6.25 ± 0.96	6.38 ± 1.60	6.38 ± 1.25	7.03 ± 0.93
8	0	1.414	15.40 ± 0.96	7.25 ± 0.50	7.25 ± 0.65	7.88 ± 0.63	7.10 ± 0.52
9	0	0	9.07 ± 0.18	8.38 ± 0.63	7.88 ± 0.48	8.25 ± 0.54	8.51 ± 0.23
10	0	0	9.04 ± 0.11	8.50 ± 0.25	8.00 ± 0.41	8.15 ± 0.67	8.03 ± 0.56
11	0	0	9.00 ± 0.23	8.63 ± 0.28	7.75 ± 0.29	8.50 ± 0.14	8.63 ± 0.34
12	0	0	9.04 ± 0.14	8.50 ± 0.21	7.75 ± 0.29	8.33 ± 0.45	8.13 ± 0.83
13	0	0	9.02 ± 0.13	8.50 ± 0.41	8.00 ± 0.41	8.25 ± 0.67	8.28 ± 0.44
		R^2	**90.05**	**82.24**	**53.51**	**80.00**	**84.55**

#Results are mean ± SD of three individual responses

Response surface methodology (RSM) was used to determine the effect of independent variables on product qualities. A second degree polynomial equation (Eqn. 2) was fitted in each response to study the effect of variables and to describe the process mathematically.

$$Y = a_o + \sum_{i=1}^{n} a_i x + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} a_{ij} x_i x_j + \sum_{i=1}^{n} a_{ii} x_i^2 \quad (2)$$

Where, a_o, a_i, a_{ii} and a_{ij} are the regression coefficients and x_i, x_j are the coded levels of independent variables i and j. Model adequacy was evaluated using F ratio and coefficient of determination (R^2) represented at 1, 5 and 10 % level of significance accordingly.

Results and Discussions

Selection of solvents for oil extraction

The oil recovery data with respect to time is represented in Table 4. The maximum oil recovery obtained was 7.27, 20.03, 19.96, 20.05 and 6.54 percent for acetone, benzene, chloroform, hexane and petroleum ether respectively. The oil extraction rate was found maximum for benzene and chloroform. Hexane showed a slow oil extraction rate initially and increased exponentially afterward. The oil extraction kinetics revealed that the extraction constant (*k*) for different solvents were 4.71 x 10^{-3}, 4.92 x 10^{-3}, 4.04 x 10^{-3}, 6.60 x 10^{-3} and 3.88 x 10^{-3} min^{-1} for acetone, benzene, chloroform, hexane and petroleum ether, respectively (Table 4). So it was interpreted that hexane had highest diffusivity than that of other solvents. On the basis of extraction rates, hexane could be considered as suitable solvent for oil extraction than other solvents. The same finding for sunflower oil was reported by Durdev *et al*. (2008).

Table 4. Effect of solvent on oil recovery

Time (min)	Oil Recovery (%)*				
	Acetone	Benzene	Chloroform	Hexane	Pet. Ether
60	1.78±0.41	4.44±0.19	5.45±0.20	2.59±0.17	1.97±0.51
120	2.55±0.29	7.83±0.17	7.79±0.13	4.61±0.18	2.97±0.50
180	3.48±0.26	11.85±0.17	9.56±0.15	7.81±0.19	3.56±0.30
240	4.46±0.25	13.85±0.13	11.98±0.13	10.41±0.28	4.16±0.10
300	6.08±0.57	18.85±0.19	14.03±0.10	14.02±0.13	5.74±0.40
360	7.27±0.66	20.03±0.13	19.96±0.13	20.05±0.15	6.54±0.29
k (min^{-1})	4.71 x 10^{-3}	4.92 x 10^{-3}	4.04 x 10^{-3}	6.60 x 10^{-3}	3.88 x 10^{-3}

* Results are mean ± SD of three individual experiments, *k* is extraction constant

Standardization of solvent extraction variables

Responses for all the experimental design are shown in Table 3. Linear, quadratic and interaction effects were observed for each model. The correlation coefficients for each model are shown in Table 5. The correlation coefficients for the responses oil recovery, surface appearance, sensory color and OA (R^2 = 90.05%, 82.24%, 80.03% and 84.55%) are quite high for response surfaces and indicate that fitted quadratic model accounted for more than 80% of the variance in the experiment data which were found to be highly significant. However seed to solvent ratio doesn't have significant effect on texture. Based on t-statistics, the only regression coefficient significant at 95 and 99% probability levels were selected for developing the model given below.

Oil recovery (Y_1) = 9.03 + 4.97 X_2
(d.f.= 12 , R^2 = 0.900)

Surface Appearance (Y_3) = 8.50 – 0.69 X_1^2 – 0.70 X_2^2
(d.f.= 12, R^2 = 0.822)

Sensory Color (Y_4) = 8.32 - 0.38 X_1^2- 0.52 X_2^2
(d.f.= 12 , R^2 = 0.800)

Overall acceptability (Y_5) = 8.31 - 0.40 X_1^2 - 0.55 X_2^2
(d.f.= 12 , R^2 = 0.845)

Table 5. Coefficients of oil extraction responses for partially defatted peanuts

Factors	Coefficients				
	Oil Recovery (%)	Appearance	Texture	Color	OA
Intercept	9.030	8.500	7.88	8.320	8.310
A	0.270	0.120	-0.020	0.030	0.080
B	4.970***	0.160	0.110	0.065	0.015
A2	-0.096	-0.690***	-0.120	-0.38***	-0.400***
B2	1.020	-0.700***	-0.410**	-0.52***	-0.550***
AB	0.120	-0.095	-0.160	0.045	0.170

*** coefficient are significant at 99 % level, ** coefficient are significant at 95 % level.

The variation in the oil recovery was significantly affected by the time of oil extraction. But other response variables were not significant at second order polynomial model. The response surfaces were obtained by plotting graphs between two variables. The effect of seed to solvent ratio and time on oil recovery, appearance, color and overall acceptability of partially defatted peanut are presented in Figures 1 to 4. The oil recovery was found maximum with increased time of extraction (Figure 1). Sensory appearance, color and overall acceptability were found acceptable near to design center point and then it decreased because of increased time of extraction (Figure 2, 3 & 4).

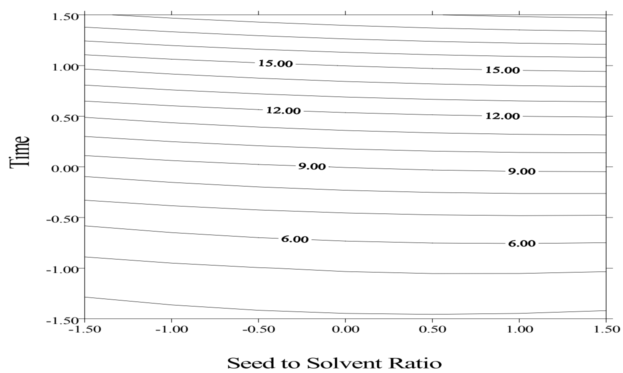

Figure 1. Effect of seed to solvent ratio and time on oil recovery from peanut

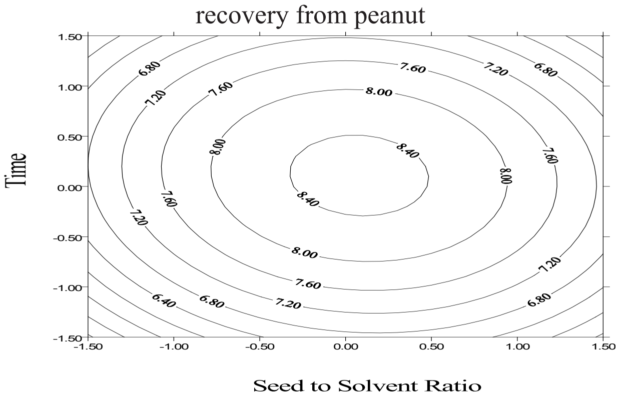

Figure 2. Effect of seed to solvent ratio and time on sensory appearance of defatted peanut

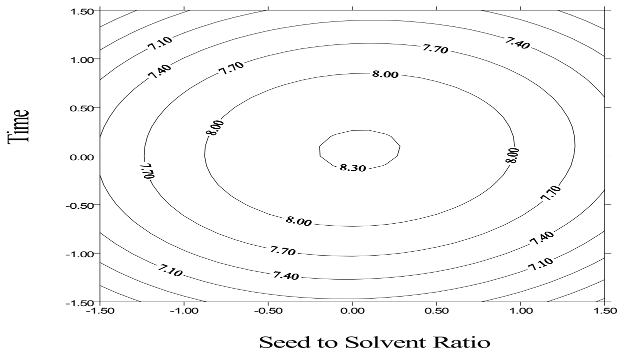

Figure 3. Effect of seed to solvent ratio and time on sensory color of defatted peanut

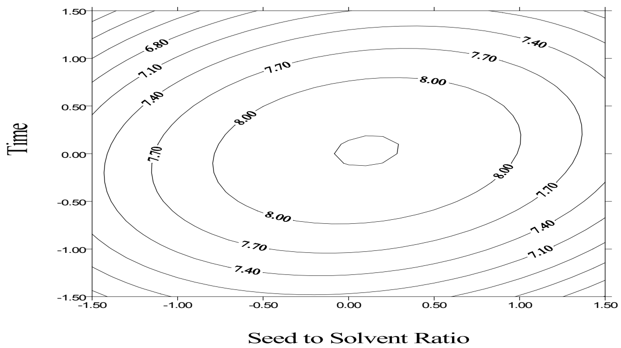

Figure 4. Effect of seed to solvent ratio and time on overall acceptability of defatted peanut

Analysis of variance

When a model had been selected, an analysis of variance was calculated to assess how well the model represented the data. An analysis of variance for all the responses is presented in Table 6. The F value for oil recovery (32.59%) was significant at 99% probability level and for surface appearance, color and overall acceptability (6.48, 4.60 and 7.38 scores respectively) was significant at 95% level. On this basis it can be concluded that the selected models adequately represent the data for oil recovery, surface appearance, color and overall acceptability. There was no outlier in the regression model.

Table 6. Analysis of variance for different models

Responses	Sources of Variation	d. f.	Sum of squares	Mean square	F
Y_1	Regression	2	197.99	99.00	32.59#
	Residual	10	30.38	3.04	
	Total	12	228.37		
Y_3	Regression	5	6.32	1.26	6.48*
	Residual	7	1.36	0.19	
	Total	12	7.68		
Y_4	Regression	5	3.51	0.70	4.60*
	Residual	7	1.07	0.15	
	Total	12	4.58		
Y_5	Regression	5	3.05	7.38	7.38*
	Residual	7	0.58	1.70	
	Total	12	3.63		

$P \leq 0.01$, * $P \leq 0.05$

Optimization of extraction variables

The overall acceptability was taken for the optimization of process variables as oil recovery had shown a linear relation with time for oil extraction (Eqn. 4.1). The criteria of maximum overall acceptability with other variables in the range were kept to obtain the process conditions suitable to obtain defatted peanuts.

Seed to solvent ratio and time found were 1:631 and 5.064 hrs respectively with maximum overall acceptability value of 8.31. At the optimized maximum OA level the oil recovery was 9.21% and sensory texture, color and appearance values found were 7.90, 8.32 and 8.51, respectively. The value at assumed optimum for seed to solvent ratio 1:6 and time 5hr resulted in overall acceptability value is 8.31 and oil recovery is 9.03% (Insignificantly different with the found optimum condition).

Graphical optimization was carried out by superimposing the contour plots (Figure 5) which shows that X_1 could be used from -0.80 to 0.66 level while X_2 from -0.60 to 0.70 level. Range of seed to solvent ratio from 1:3.738 to 1:7.866 and time from 3.728 hr to 6.484 hr resulted in recovery range from 6.048% to 12.51% with OA more than 8.

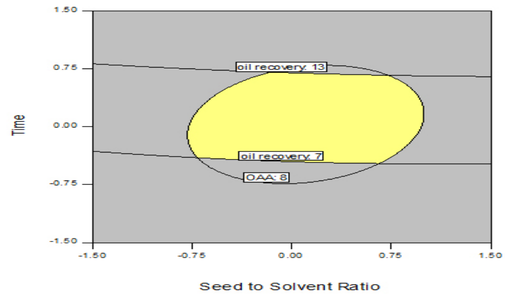

Figure 5. Superimposed counter plots for all the responses affected by seed to solvent ratio and time

Conclusions

The defatted peanut was prepared effectively by optimizing the ingredient levels using RSM with a minimum number of experiments. During this study it was observed that hexane is suitable solvent for oil extraction from peanut since it has highest value of extraction constant (k=6.60 x 10^{-3} min^{-1}) with rapid, efficient and maximum extraction of oil than other solvents. Oil recovery found was linearly affected with the time of extraction which was significant at P≤ 0.01 and R^2 of 0.90. Five hours extraction time was found suitable for the preparation of defatted peanuts with 8.31 overall acceptability score and 9.21% oil recovery.

References

Basha, S. M. and Pancholy, S. K. 1982. Composition and characteristics of basic proteins from peanut (*Arachis hypogaea* L.). Journal of Agricultural and Food Chemistry 30:1176-1179.

Bhat, C. M. 1977. Effect of incorporation of soy flour, peanut flour and cottonseed flour on the acceptability and protein quality of chapattis. Dissertation Abstracts International 38(2):615.

Cherry, J. P. 1990. Peanut protein and product functionality. Journal of the American Oil Chemists' Society 67(5): 293-301.

Coccodrilli, G., Shah,N., Sommer, S. and Ali, R. 1979. Bioavailability of zinc from ready-to-eat breakfast cereals, wheat bran, and defatted peanut flour. Federation Proceedings 38(3):558.

Deming, S. N. 1990. Quality by design-part 5. Chemtech 20: 118-120.

Durdev, S., Perdih, A., Turkulov, J. and Djurdjev S. 1982. Quality of sunflower oil obtained by continuous pressing and solvent. Fabrik Ulja "INUS", sombor, Yugoslavia 23(11/12):245-246.

Holsinger, V. H., Sutton, C.S., Vettel, H.E., Allen, C., Talley, F.B. and Woychik, J.H. 1978. A beverage base from cheese whey and peanut flour. Peanut Science 5(2): 97-101.

Ismail, Y. S., Rustom, M. H., López-Leiva and Baboo, M. N. 1991. A study of factors affecting extraction of peanut (*Arachis hypogaea* L.) solids with water. Food Chemistry 42: 153-165.

Jan, M., Mahmood, F., Zeb, A. and Chaudry, M. A. 2003. Nutritional and technological evaluation of wheat bread supplemented with peanut and soybean flours. Pakistan Journal of Scientific and Industrial Research 46(1): 68.

Li, Q. H. and Fu, C. L. 2005. Application of response surface methodology for extraction optimization of germinant pumpkin seeds protein. Food Chemistry 92: 701-706.

Lima, I.M., Guraya, H.S. and Champagne, E.T. 2000. Improved peanut flour for a reduced fat peanut butter product. Journal of Food Science 65(5): 854-861.

Liu, D. C., Hu, X. H., Zhang, W. N., Wang, Y. and Liu, Y. F. 1996. Research on preparation and functional properties of peanut flour and peanut protein concentrate. China Oils and Fats 21(3): 5-7.

Montgomery, D. C. 1997. Introduction to Statistical Quality Control (3rd Eds), John Wiley and Sons, New York.

Ory, R.L. and Conkerton, E.J. 1983. Supplementation of bakery items with high protein peanut flour. Journal of the American Oil Chemists' Society 60(5): 986-989.

Pericin, D., Radulovic, L., Trivic, S. and Dimic, E. 2008. Evaluation of solubility of pumpkin seed globulins by response surface method. Journal of Food Engineering, 84: 591-594.

Prinyawiwatkul, W., Beuchat, L. R. and McWatters, K. H. 1993. Functional property changes in partially defatted peanut flour caused by fungal fermentation and heat treatment. Journal of Food Science 58: 1318-1323.

Ranganna, S. 1986. Proximate Constituents. In Ranganna, S. (Eds). Handbook of Analysis and Quality Control for Fruits and Vegetable Products, p.1-30. Tata Mc Graw Hill Publishing Co. Ltd., New Delhi.

Tate, P.V., Chavan, J.K., Patil, P.B. and Kadam, S.S. 1990. Processing of commercial peanut cake into food-grade meal and its utilization in preparation of cookies. Plant Food for Human Nutrition 40(2): 115-121.

Tiezheng, M., Qiang, W. and Haiwen, W. 2010. Optimization of extraction conditions for improving solubility of peanut protein concentrates by response surface methodology. LWT - Food Science and Technology 43: 1450-1455.

Triveni, R., Shamala, T. R. and Rastogi, N. K. 2001. Optimized production and utilization of exopolysaccharide from *Agrobacterium radiobacter*. Process Biochemistry 36: 787-795.

Wani, A. A., Kaur, D., Ahmed, I. and Sogi, D. S. 2008. Extraction optimization of watermelon seed protein using response surface methodology. LWT-Food Science and Technology 41(8): 1514-1520.

Wani, A. A., Sogi, D. S., Grover, L. and Saxena, D. C. 2006. Effect of temperature, alkali concentration, mixing time and meal solvent ratio on the extraction of watermelon seed proteins-response surface approach. Biosystems Engineering 94(1): 67-73.

Wu, H. W.,Wang, Q. and Zhou, S. M. 2007. Research progress on peanut protein and its functional properties. China Oils and Fats 32(9): 7-11.

Yi, S., Bingjian, D., Ting, Z., Bing, H., Fei, Y., Rui, Y., Xiaosong, H., Yuanying, N. and Quanhong, Li. 2011. Optimization of extraction process by response surface methodology and preliminary structural analysis of polysaccharides from defatted peanut (*Arachis hypogaea*) cakes, Carbohydrate Research 346: 305-310

Zhang, X. L., Ni, P. D. and Jiang, Z. W. 1998. Peanut and progress in technology of preparation of peanut protein. China Oils and Fats 23(4): 3-5.beans during drying. Journal of Food Engineering 99: 276-283.

Rodriguez-Campos, J., Escalona-Buendía, H.B., Orozco-Avila, I., Lugo-Cervantes, E. and Jaramillo-Flores, M. E. 2011. Dynamics of volatile and non-volatile compounds in cocoa (*Theobroma cacao* L.) during fermentation and drying processes using principal components analysis. Food Research International 44: 250-258.

Sanagi, M. M., Hung, W. P. and Md Yasir, S. 1997. Supercritical fluid extraction of pyrazines in roasted cocoa beans – Effect of pod storage period. Journal of Chromatography 785: 361-367.

Schwan, R. F. and Wheals, A. E. 2004. The microbiology of cocoa fermentation and its role in chocolate quality. Critical Review in Food Science and Nutrition, 44, 205 -221.

Schwan, R. F., Rose, A. H. and Board, R. G. 1995. Microbial fermentation of cocoa beans, with emphasis on enzymatic degradation of the pulp. Journal of Applied Bacteriology 79: 96S– 107S.

Thompson, S. S., Miller, K. B. and Lopez, A. S., 2007. Cocoa and coffee. In: Doyle, M.P., Beuchat, L.R., Montville, T.J. (Eds.), Food Microbiology Fundamentals and Frontiers. ASM Press, Washington, DC, pp. 837-850.

Voigt, J., Biehl, B., Heinrichs, H., Kamaruddin, S., Gaim Marsoner, G. and Hugi, A., 1994. *In vitro* formation of cocoa-specific aroma precursors: aroma-related peptides generated from cocoa seed protein by co-operation of an aspartic endo proteinase and a carboxy-peptidase. Food Chemistry 49: 173-180.

Wood, G.A.R. and Lass, R.A. 1985. Cocoa, 4th edition. Longman Group, London.