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International Journal of Current Research
Vol. 4, Issue, 11, pp.240-246, November, 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

APPLICATION OF RESPONSE SURFACE METHODOLOGY IN THE DEVELOPMENT OF OMEGA 3 RICH SNACK FOOD

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ARTICLE INFO

Article History:
Received 08th August, 2012
Received in revised form 07thSeptember, 2012
Accepted 10th October, 2012
Published online 23th November, 2012

Key words:
Roasted Linseed Powder,
Response Surface Methodology,
Omega 3 Fatty Acids.

ABSTRACT

In the present investigation attempts have been made to develop roasted linseed powder and analyzing its physiochemical properties and proximate composition and to optimize bengal gram flour, roasted linseed powder and gingelly seed powder with other ingredients using Response Surface Methodology (RSM) for acceptable omega 3 rich savory snack (Omapodi) preparation by considering carbohydrate, protein, omega 3 fatty acids and overall acceptability as a response variables. Results revealed that, Response Surface Methodology (RSM) was applied for optimization, the multiple regression was used to get optimum levels and it was found that desirable values of carbohydrate (69.78g), protein (16.35g), omega 3 fatty acids (7.77mg) and overall acceptability (8.93) was obtained for the corresponding optimum quantity of 64g of bengal gram flour 20g roasted linseed powder and 10g gingelly seed powder.

INTRODUCTION

Linseed (*Linum usitatissimum*) is an ancient crop cultivated in temperate zones of Asia and Europe since seven thousand years. Linseed finds a mention in the writings of Hippocrates and Theophratus recommended it for its medicinal properties. It belongs to the family Linaceae and genus Linum. Linseed in also known as Flax, Flaxseed, Flax weed, Lint bells, Toad flax, Winterlien etc. (Rickard and Thompson, 1997). Linseed is a treasure trove of nutrients and nutraceuticals, containing protein (20 %), and fat (45 %). It also contains fair amounts of B complex vitamins, Vitamin A and E and minerals such as potassium, magnesium and alike (Thompson, 1996). The omega-3 and omega-6 fatty acids constitute about 57 and 16 per cent of total fatty acid composition of linseed oil. Several sources of information suggest that human beings are evolved on a diet with a ratio of omega-6 to omega-3 essential fatty acids of approximately one.

But due to technological advancements coupled with shifts in dietary patterns in the recent past have contributed to a shift in the ratio of these essential fatty acids mainly due to either excessive amounts of omega-6 or due to deficiency of omega-3 fatty acids in the diets (Bozan and Temelli, 2008). Snack or snack foods represent a large group of food products. A variety of snack foods are available in the market. Although classification of the snack food is difficult, yet they can be categorized as sweet or savory. Sev (Omapodi) is one of the Indian traditional deep fried savory snack foods popular throughout the country. Traditionally, it is prepared from Bengal gram flour with additives. Demand and consumption of snack foods is increasing day by day. Hence, this commonly consumed savory has been selected for the formulation of omega 3 rich savory snack. With the background, the present investigation was undertaken to optimize of replacement of Bengal gram flour by omega 3 rich roasted flaxseed powder for the preparation of omapodi.

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MATERIALS AND METHODS

Materials

Linseeds, Bengal gram flour, gingelly seeds, oil, salt and chilli powder used for this investigation was purchased from local market. Linseeds were purchased and cleaned thoroughly. Heat a heavy, flat-bottomed skillet over medium high heat until very hot. Pour the cup of raw flaxseeds. Stir the seeds for 3 to 5 minutes in the skillet constantly until the seeds turn a uniform golden brown. Place them in a bowl for cooling. After that, put the roasted flaxseeds into a mixer and made intopowder.

Physiochemical properties of linseed and roasted linseed powder

Physical properties such as thousand seed weight and volume were calculated by the method of Ranganna, 1986. The chemical properties such as moisture, carbohydrate, protein, fat, fibre, vitamin C, iron, calcium and omega 3 fatty acids were determined using AOAC method. The carbohydrate was assessed using Anthrone method.

Preparation of savoury snack (omapodi)

Bengal gram flour + Gingelly seed powder

↓

Add Roasted linseed powder, salt and chilli powder

↓

Mix well

↓

Made into a thick paste

↓

Kept for half an hour

↓

Passed through "omapodi" mould into heated oil

↓

Fry till crisp and yellowish brown

Experimental design (Central Composite Rotatable esigns)

The Response Surface Methodology (RSM) is a widely adopted tool for the quality of optimizations processes (Nazni *et al.,* 2011). The RSM, originally described by Box and Wilson (1951) is effective for responses that affect many factors and their interactions. The Central Composite Rotatable Design (CCRD), (Box and Hunter, 1957) was adopted to predict responses based on few sets of experimental data in which all factors were varied within a chosen range. The experiment consisted of 8 factorial runs, 6 axial runs and 6 center runs. The 3 independent variables were bengal gram flour (g) (X_1), roasted linseed powder (g) (X_2) and gingelly seed powder (g) (X_3). Each variable was set at 5 levels and a total of 20 experiments were designed whereby formulation 15, namely the centre-point formulation, was repeated 6 times. The independent variables and their variation levels are shown in Table 1. The levels of each variable were established according to literature information and preliminary trials. The outline of the experimental layout with the coded and natural values is presented in Table 2.

Homogeneous variance is a necessary pre- requisite for (linear) regression models. Therefore, a reduction in variability within the objective response (dependent variables) was by transforming the data to standardized scores $Z = X-\bar{X} / S$ where $\bar{X}$ = dependent variable of interest; X = mean of dependent variable of interest and S = standard deviation). For each standardized scores, Analysis of Variance (ANOVA) was conducted to determine significant differences among the treatment combinations. Also, data were analyzed using multiple regression procedures (Design Expert Version 8.0). To estimate bengal gram flour, roasted linseed powder and gingelly seed powder effect on each objective response such as carbohydrate, protein, omega 3 fatty acids and overall acceptability the standardized scores were fitted to a quadratic polynomial regression model by employing a least square technique (Gacula and Singh, 1984; Wanasundara and Shahidi, 1996). The model proposed for each response of Y was:

$$Y= \beta_0+ \beta_1X_1+ \beta_2 X_2+ \beta_3X_3+ \beta_{11}X_1^2+ \beta_{22}X_2^2+ \beta_{33}X_3^2 + \beta_{12} X_1X_2+ \beta_{13}X_1X_3+ \beta_{23}X_2X_3$$

Where Y = the response, X_1= bengal gram flour, X_2 = roasted linseed powder, X_3 = gingelly seed powder, β_0= intercepts, β_1, β_2, β_3 are linear, β_{11}, β_{22}, β_{33}, = are quadratic and β_{12}, β_{13} and β_{23} are interaction regression coefficient terms, respectively. Coefficients of determination (R^2) were computed. The adequacy of the model was examined based on three criterion, F value, Lack of Fit (LoF) and adequate precision value. The optimization was done by numerical optimization. Constraints were set to get the optimized coded value of the variable between the upper and lower limits of the variable. For each response, response surface plots were produced from the equations, by holding the variable with the least effect on the response equal to a constant value, and changing the other two variables.

Table1. Independent Variables and Levels used for central composite rotatable design.

Variable	Symbols	Coded variable level				
		-1.68(α)	-1	0	1	1.68(α)
Bengal gram flour (g)	X_1	60.59	64	69	74	77.41
Roasted linseed powder (g)	X_2	6.59	10	15	20	23.40
Gingelly seed powder (g)	X_3	3.29	5	7.5	10	11.70

Table2. Experimental design in their natural units and coded form

Design points	Independent variables in Uncoded form			Independent variables in coded form		
	X_1	X_2	X_3	x_1	x_2	x_3
1	64	10	5	-1	-1	-1
2	74	10	5	1	-1	-1
3	64	20	5	-1	1	-1
4	74	20	5	1	1	-1
5	64	10	10	-1	-1	1
6	74	10	10	1	-1	1
7	64	20	10	-1	1	1
8	74	20	10	1	1	1
9	60.59	15	7.5	-1.682	0	0
10	77.41	15	7.5	1.682	0	0
11	69	6.59	7.5	0	-1.682	0
12	69	23.41	7.5	0	1.682	0
13	69	15	3.30	0	0	-1.682
14	69	15	11.70	0	0	1.682
15	69	15	7.50	0	0	0
16	69	15	7.50	0	0	0
17	69	15	7.50	0	0	0
18	69	15	7.50	0	0	0
19	69	15	7.50	0	0	0
20	69	15	7.50	0	0	0

X_1=Bengal gram flour, X_2 = Roasted linseed powder, X_3 = Gingelly seed powder

Proximate analysis

The developed roasted linseed powder incorporated omapodi were subjected to the proximate analysis such as moisture, carbohydrate, protein, fiber, vitamin, calcium, iron and omega 3 fatty acids. The standard procedures given by Ranganna (1986) were used for all the above determinations.

Statistical analysis

The analytical data obtained for roasted linseed powder omapodi were subjected to analysis of variance (ANOVA) (one way ANOVA) using complete randomized design. The critical difference at $p<0.05$ was estimated and used to find significant difference if any.

Findings

Physiochemical proprieties of linseed and roasted linseed powder

This quality is dependent on the size of embryo and reserved nutrients quantity used for sprouting and growth. Thousand grain weight 8.174g, thousand grain volume 8.4ml, hydration capacity and index 26.94g respectively. One thousand seed weight (1000m) increased linearly 250-295g, as moisture content increased from 15.4 to 32.4% (Saeed Firouzi, 2012); 4.22 to 4.62g when the moisture content was increased from 6.21 to 16.29% (Bo wang, 2007). The one thousand seed weight of linseed at different moisture content is lower than caper seed (Dursun and Dursun, 2005), similar to cumin seed (Singh and Goswami, 1996). The moisture content of the roasted linseed powder is 4.37%, carbohydrate 27.60g, protein 25.58g, fiber 2.62g, calcium 297.25mg, iron 14.01mg, vitamin C 500mg and omega 3 fatty acids 38g respectively.

The seed contains approximately 40% lipids, 30% dietary fibre and 20 % protein. The average protein content in flaxseed is 22 g 100 g-1 of seed (Rubilar, *2010)*. Lucas *et al.* (2004) found a reduction of cholesterol in the plasma and arteriosclerotic lesions after the incorporation of flax mucilage and α- linolenic acid into diet. (Guilloux, *et al.*, 2009). The endosperm contains only 23% of the lipids and 16% of protein (Daun *et al.*, 2003; Oohma, 2003).

Table 3. Physiochemical proprieties of linseed and roasted linseed powder

S.No	Physiochemical proprieties	Quantity (g/100g)
1	Thousand grain weight of linseed (g)	8.174
2	Thousand grain volume of linseed (ml)	8.4
3	Hydration capacity and index of linseed (g)	26.940
4	Moisture (%) of RLSP	4.37
5	Carbohydrate (g) of RLSP	27.60
6	Protein (g) of RLSP	25.58
7	Fibre (g) of RLSP	2.62
8	Calcium (mg) of RLSP	297.25
9	Iron (mg) of RLSP	14.01
10	Vitamin C (mg) of RLSP	500
11	Omega 3 fatty acids (g) of RLSP	38

RLSP- Roasted Linseed Powder

Optimization of roasted linseed powder incorporated omapodi

The omapodi prepared with the help of bengal gram flour, roasted linseed powder and gingelly seed powder was characterized for its proximate composition and organoleptic characteristics. Carbohydrate (Y_1), protein (Y_2), omega 3 fatty acids (Y_3) and overall acceptability (Y_4) was measured as response variables.

Diagnostic checking of fitted model and surface plot for all Y responses

Regression analysis indicated that the fitted quadratic model accounted that above 99% for carbohydrate, 75% for protein, 100% for omega 3 fatty acids and 58% for overall acceptability of the developed roasted linseed powder incorporated omapodi. The values of regression coefficients, sum of squares, F values and P values for coded form of process variables are presented in Table 4.

Carbohydrate

The carbohydrate of the developed omapodi was ranged from 66.73 to 76.00g. The coefficient of determination R^2 was 99% of the regression model. The F value of the model is 6.59 and lack of fit is significant. The developed model for omapodi in the form of uncoded (actual) process variables is as follows:

$$Y_1 \text{ (Carbohydrate)} = -31.514+0.536X_1-0.069X_2-0.483X_3 - 5.037X_1^2-8.643X_2^2-6.572X_3^2 +4.275X_1X_2 +8.550X_1X_3+8.550X_2X_3$$

Table 4. Regression coefficient from quadratic model and their significance

Coefficients	Carbohydrate (g)	Protein(g)	Omega 3 fatty acids (mg)	Overall acceptability
Model	71.02**	9.14*	5.88**	8.33
X_1	2.97**	0.78	5.56**	-0.12
X_2	1.32**	0.33	1.90**	0.00
X_3	0.56**	-0.77	0.00	-0.12
X_1^2	-0.013	3.51**	2.45**	0.09
X_2^2	-0.022	2.27*	6.82	-0.08
X_3^2	-4.108	2.67*	6.82	0.09
$X_1 X_2$	0.11	0.00	0.00	-0.50*
$X_1 X_3$	0.11	0.00	0.00	0.00
$X_2 X_3$	0.11	0.00	0.00	0.00
F-value	6.59	3.35	6.68	1.55
R^2	0.998	0.750	1.000	0.58
Adj R^2	0.997	0.526	1.000	0.20
Pred R^2	0.986	-0.886	1.000	-0.47
Adeq precision	90.74	4.9	31568.02	4.71
Lack of Fit	0.25	104.96	8.198	0.67

** - 1% significant level, * - 5% significant level

In coded form of process variables, the model equation is as follows:

Y_1 (Carbohydrate) = $71.02+2.97x_1+1.32x_2+0.56x_3-0.0137x_1^2-0.022x_2^2-4.108x_3^2+0.11x_1x_2+0.11x_1x_3+0.11x_2x_3$

The magnitude of P and F value in Table 4 indicates the positive contribution in the bengal gram flour, roasted linseed powder and gingelly seed powder. The quadratic terms have negative effect on carbohydrate. The interactions of X_1 X_2, X_1 X_3 and X_2 X_3 have the positive effect on carbohydrate. The effect of bengal gram flour, roasted linseed powder and gingelly seed powder on the carbohydrate has been shown in Figs 1 to 3.

Carbohydrate

The carbohydrate of the developed omapodi was ranged from 66.73 to 76.00g. The coefficient of determination R^2 was 99% of the regression model. The F value of the model is 6.59 and lack of fit is significant.

The developed model for omapodi in the form of uncoded (actual) process variables is as follows:

Y_1 (Carbohydrate) = $-31.514+0.536X_1-0.069X_2-0.483X_3-5.037X_1^2-8.643X_2^2-6.572X_3^2+4.275X_1X_2+8.550X_1X_3+8.550X_2X_3$

In coded form of process variables, the model equation is as follows:

Y_1 (Carbohydrate) = $71.02+2.97x_1+1.32x_2+0.56x_3-0.0137x_1^2-0.022x_2^2-4.108x_3^2+0.11x_1x_2+0.11x_1x_3+0.11x_2x_3$

The magnitude of P and F value in Table 4 indicates the positive contribution in the bengal gram flour, roasted linseed powder and gingelly seed powder. The quadratic terms have negative effect on carbohydrate. The interactions of X_1 X_2, X_1 X_3 and X_2 X_3 have the positive effect on carbohydrate. The effect of bengal gram flour, roasted linseed powder and gingelly seed powder on the carbohydrate has been shown in Figs 1 to 3.

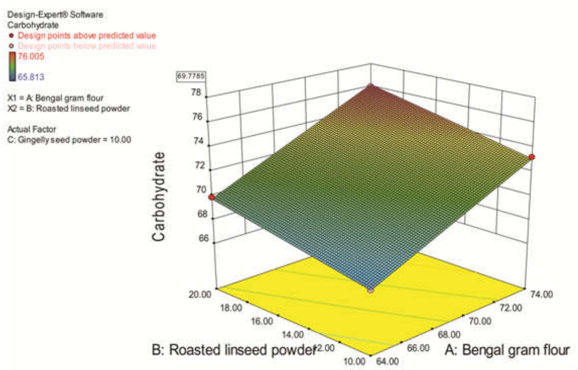

Fig. 1: Effect of bengal gram flour and roasted linseed powder on carbohydrate content of omapodi

Protein

The protein of the developed omapodi was ranged from 9.27 to 21.87. The coefficient of determination R^2 was 75% of the regression model. The F value of the model is 3.35 and lack of fit is significant.

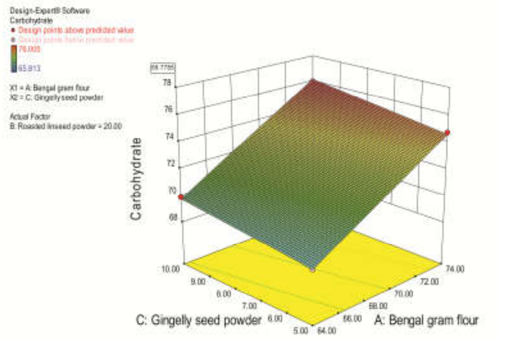

Fig. 2: Effect of bengal gram flour and gingelly seed powder on carbohydrate content of omapodi

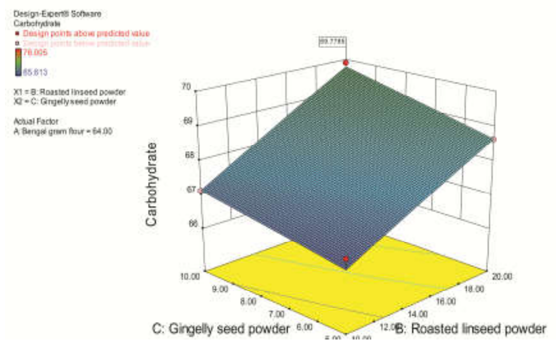

Fig. 3: Effect of roasted linseed powder and gingelly seed powder on carbohydrate content of omapodi

The developed model for omapodi in the form of uncoded (actual) process variables is as follows:

Y_2 (Protein) = $711.99-19.203X_1-2.653X_2-6.709X_3+0.140X_1^2+0.090X_2^2+0.426X_3^2+2.135X_1X_2+2.838X_1X_3-7.536X_2X_3$

In coded form of process variables, the model equation is as follows:

Y_2 (Protein) = $9.14+0.78x_1+0.33x_2-0.77x_3+3.51x_1^2+2.27x_2^2-2.67x_3^2+0.00x_1x_2+0.00x_1x_3+0.00x_2x_3$

The magnitude of P and F value in Table 4 indicates the positive contribution in the bengal gram flour and roasted linseed powder, negative contribution in the gingelly seed powder. The quadratic terms have positive effect on protein but X_2^2 has the negative effect on protein content. The interactions of X_1 X_2 have the negative effect where as X_1 X_3 and X_2 X_3 has the positive effect on protein content. The effect of bengal gram flour, roasted linseed powder and gingelly seed powder on the protein level has been shown in Figs 4 to 6.

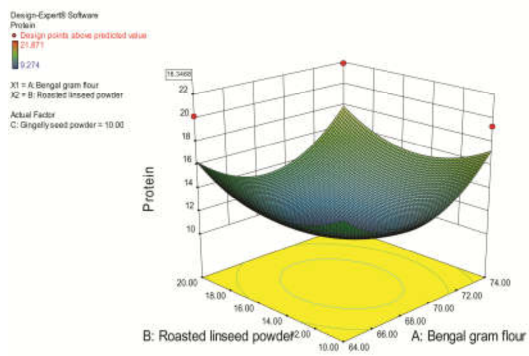

Fig. 4: Effect of bengal gram flour and roasted linseed powder on protein content of omapodi

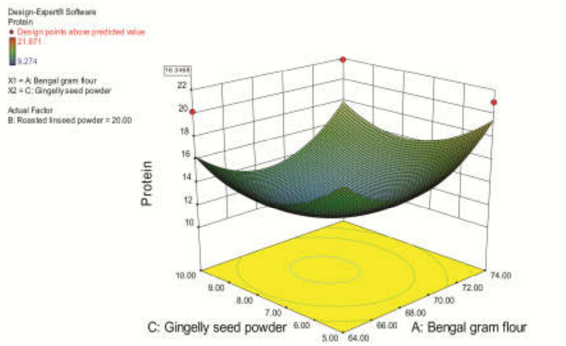

Fig. 5: Effect of bengal gram flour and gingelly seed powder on protein content of omapodi

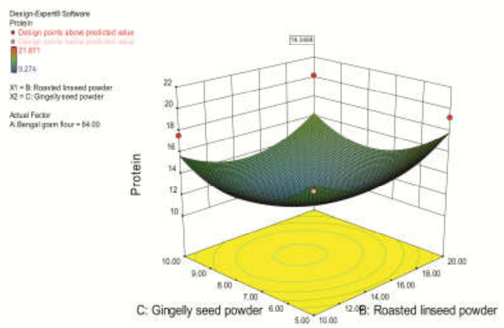

Fig. 6: Effect of roasted linseed powder and gingelly seed powder on protein content of omapodi

Omega 3 fatty acids

The omega 3 fatty acids of the developed omapodi were ranged from 2.68 to 9.07. The coefficient of determination R^2 was 100% of the regression model. The F value of the model is 6.68 and lack of fit is significant.

The developed model for omapodi in the form of uncoded (actual) process variables is as follows:

Y_3 (Omega 3 fatty acids) = $0.147-2.399X_1+0.379X_2-1.636X_3+9.799X_1^2+2.728X_2^2+1.091X_3^2+4.126X_1X_2+7.371X_1X_3+8.633X_2X_3$

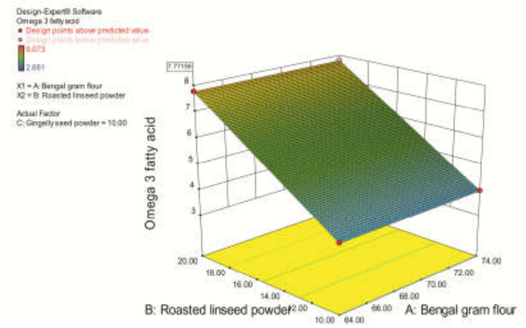

Fig. 7: Effect of bengal gram flour and roasted linseed powder on omega 3 fatty acids of omapodi

In coded form of process variables, the model equation is as follows:

Y_2 (Omega 3 fatty acids) = $5.88+5.56x_1+1.90x_2+0.00x_3+2.450x_1^2+6.821x_2^2+6.821x_3^2+0.00x_1x_2+0.00x_1x_3+0.00x_2x_3$

The magnitude of P and F value in Table 4 indicates the positive contribution in the bengal gram flour, the roasted linseed powder and gingelly seed powder. The quadratic terms have positive effect on omega 3 fatty acids. The interactions of X_1 X_2, X_1 X_3 and X_2 X_3 have the positive effect on omega 3 fatty acids. The effect of bengal gram flour, roasted linseed powder and gingelly seed powder on the omega 3 fatty acids level has been shown in Figs 7 to 9.

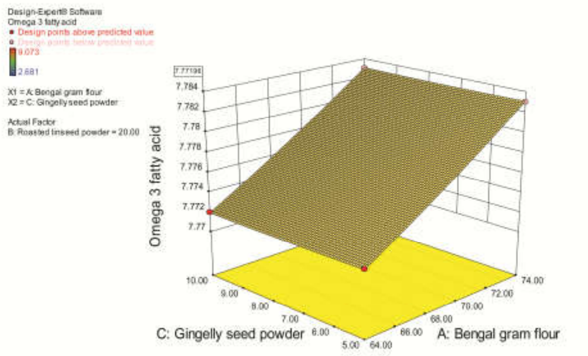

Fig. 8: Effect of bengal gram flour and gingelly seed powder on omega 3 fatty acids of omapodi

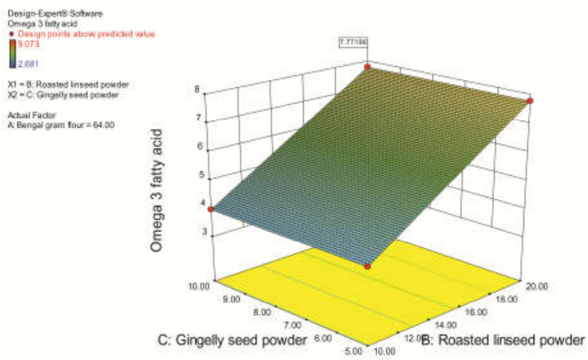

Fig. 9: Effect of roasted linseed powder and gingelly seed powder on omega 3 fatty acids of omapodi

Overall acceptability

The overall acceptability of the developed omapodi was ranged from 8 to 9. The coefficient of determination R^2 was 58% of the regression model. The F value of the model is 1.55 and lack of fit is not significant.

The developed model for omapodi in the form of uncoded (actual) process variables is as follows:

Y_4 (Omega 3 fatty acids) = $-0.244+1.479X_1-0.275X_2-0.020X_3+3.763X_1^2-3.307X_2^2+0.015X_3^2-0.02X_1X_2+3.360X_1X_3+7.913X_2X_3$

In coded form of process variables, the model equation is as follows:

Y_4 (Omega 3 fatty acids) = $8.33-0.12X_1+0.00X_2-0.12X_3+0.094x_1^2-0.083x_2^2+0.094x_3^2-0.50x_1x_2+0.00x_1x_3+0.00+x_2x_3$

The magnitude of P and F value in Table 4 indicates the positive contribution in the roasted linseed powder, negative contribution in the bengal gram flour and gingelly seed powder. The quadratic terms have negative effect on overall acceptability but X_2^2 has the positive effect on overall acceptability. The interactions of X_1 X_2 and X_2 X_3 have the positive effect where as X_1 X_3 has the negative effect on overall acceptability. The effect of bengal gram flour, roasted linseed powder and gingelly seed powder on the omega 3 fatty acids level has been shown in Figs 10 to 12.

Table 5. Criteria of optimum value for the responses

Process parameters	Target	Experimental range		Importance	Optimum values	Desirability
Bengal gram flour	Is in range	64	74	3	64	
Roasted linseed powder	Is in range	10	20	3	20.00	
Gingelly seed powder	Is in range	5	10	3	10.00	
Responses						
Carbohydrate	Maximum	66.7	76.0	3	69.77	
Protein	Maximum	9.27	21.87	3	16.34	
Omega 3 fatty acids	Maximum	2.68	9.07	3	7.77	0.635
Overall acceptability	Maximum	8	9	3	8.93	

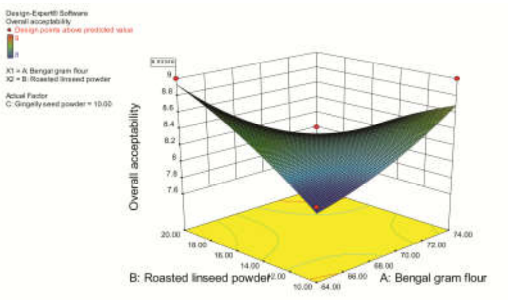

Fig. 10: Effect of bengal gram flour and roasted linseed powder on overall acceptability of omapodi

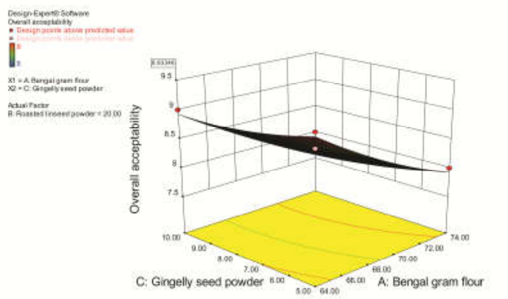

Fig. 11: Effect of bengal gram flour and gingelly seed powder on overall acceptability of omapodi

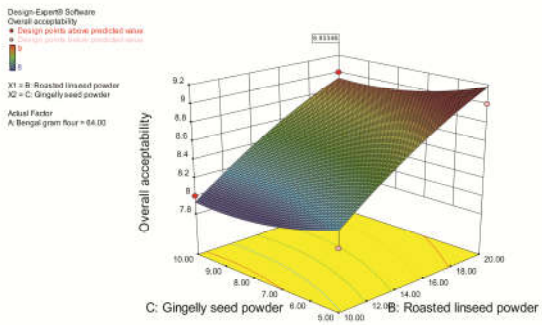

Fig. 12: Effect of roasted linseed powder and gingelly seed powder on overall acceptability of omapodi

Criteria of optimum value for the responses

For the optimization variables, the responses, that is carbohydrate, protein, omega 3 fatty acids and overall acceptability were selected on the basis that these responses had direct effect on the acceptability and quality of omapodi. To consider all the responses simultaneously for optimization, the multiple regression was used to get compromise optimum conditions and it has found that the scores were 69.77, 16.34, 7.77 and 8.93 for carbohydrate (g), protein (g), omega 3 fatty acids (mg) and overall acceptability respectively, corresponding to the optimum condition of bengal gram flour 64g as X_1, roasted linseed powder 20g as X_2 and gingelly seed powder 10g as X_3.

Nutrient content in roasted linseed powder incorporated omapodi

Moisture, carbohydrate, protein, fiber, vitamin C, iron and calcium content of roasted linseed incorporated omapodi were analyzed. The results revealed (table.6) that the moisture content 11.86%, carbohydrate 69.889g, protein 20.157g, fiber 4.313g, calcium 221.611mg, iron 7.42mg, vitamin C 103.42mg and omega 3 fatty acids 7.772mg respectively.

Table 6. Nutrients content in roasted linseed powder incorporated omapodi

Sl.No	Nutrient	Amount
1	Moisture (%)	11.86
2	Carbohydrate (g)	69.889
3	Protein (g)	20.157
4	Fiber (g)	4.313
5	Calcium (mg)	221.611
6	Iron (mg)	7.42
7	Vitamin C (mg)	103.42
8.	Omega 3 fatty acids (mg)	7.77

Conclusion

Response Surface Methodology (RSM) was used successfully to optimize the level of bengal gram flour, roasted linseed powder and gingelly seed powder for the development of omapodi. The incorporation of roasted linseed powder had an impact on the physicochemical and sensory properties of noodles. The incorporation of roasted linseed powder significantly increased (P<0.05) in the appearance, colour, flavor, texture, taste, overall acceptability, weight and omega 3 fatty acids. The sensory evaluation result showed there were only slight differences between the values of sensory attributes with the increasing incorporation of roasted linseed powder. In conclusion, this study provides useful functional information for the future development of linseed powder-based food products.

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