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Preparation of cereal mix for nutri-composite bar development using response surface methodology

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ABSTRACT

The quantity of inclusion of three types of cereals for the preparation cereal mix as main component in Nutri-composite bar preparation such as whole wheat flour (WWF), proso millet flour (PMF), and germinated finger millet flour (GFMF) was optimized using central composite rotatable design for three variables with five level of each. The analyzed responses were carbohydrate, protein, fat, crude fibre, ash and moisture content. The influence of independent variables on responses and optimized level of incorporation was studied through second order polynomial quadratic regression models. The independent variables imposed significant effect on fat at linear level; carbohydrate, protein and crude fiber at quadratic level; moisture and crude fiber at interactive level. The optimum level of mixing of WWF, PMF and GFMF in cereal mix with validated means of response as 4.6g moisture, 71g carbohydrate, 11.18g protein, 1.7g fat, 2.7 g ash and 5.7 g crude fiber was 46.18 g, 35 g and 32.5 g respectively.

Introduction

While single crops such as rice and wheat can succeed in producing food security for India, millets produce multiple securities. They include securities of food, nutrition, fodder, fibre, health, livelihood and ecology. Millets are consumed as staple food (78%), drinks and other uses (20%). Feed use is still very small (2%). As food, they are nutritionally equivalent or superior to most cereals; containing high levels of methionine, cystine, and other vital amino acids for human health. They are also unique sources of micronutrients (Zn, Fe and Cu) which are especially high in finger millet. The finger millet yields carbohydrate74.0g, protein 7.3g, fat 4.8g, crude fibre 2.3g, ash 1.9g and food energy 1403 KJ per 100 g (Obilana and Manyasa, 2003**).** Millet protein has a beneficial influence on metabolism of cholesterol (Nishizawa and Fudamo, 1995). The caryopsis of proso millet is rich in protein, mineral substances, vitamins and its nutritive parameters are comparable or better than common cereals. The ration of nutrients is very similar to recommended ratio of protein, saccharide and lipids (Seetharam, 1999). The biological value of proso millet is on the level of bean and wheat flour (Becker, 1994; Dendy, 1995).

It will be of economic importance if local raw materials in India could be used in certain proportions to supplement wheat. This will however, not only minimize foreign exchange but will encourage farmers to produce or grow more of such crops (Aliyu and Sani, 2009). Although it is well known that no other crop can achieve the baking properties of wheat, composite flour introducing the possibility of replacing the wheat with tuberous plants, protein rich flours and other cereals including maize, rice, millet and sorghum became the subject of numerous studies (Berghofer, 2000; Bugusu et al., 2001). The extent to which wheat flour could be replaced by other cereal flours depends on the nature of the products to be prepared. Most of the composite flour research was carried out on cassava, a little on sorghum and very little on millets (Dendy, 1992). Keeping the above facts in view, the present study was done to optimize the level of blending of millet flours into whole wheat flour for the preparation of cereal mix which was the main component in preparation of nutri-composite bar.

Materials and Methods

Materials

It will be of economic importance if local raw materials in India could be used in certain proportions to supplement wheat. This will however, not only minimize foreign exchange but will encourage farmers to produce or grow more of such crops (Aliyu and Sani, 2009).

Whole wheat (*Triticum aestivum*), finger millet (*Eleusine coracana*) and proso millet (*Panicum miliaceum*) were procured from Salem local market. Millets were cleaned and sieved in 12”mesh (1.4mm) to get matured grains; and finger millet was germinated for two days in the germination container, dried, milled, sifted through 100 mesh sieve (150 micron) and kept in airtight containers for future use.

Experimental Design

The central composite rotatable design was used to determine the combination of three independent variables (Whole wheat flour, proso millet flour and germinated green gram flour). The coded level of each variables and experimental plan are shown in table 1.

Responses for Optimization

The cereal composite mix prepared as per the combination proposed by CCRD of 20 runs were analysed for its moisture and ash by AOAC method (Ranganna, 1977), protein by Microkjeldahl method, fat by Soxhlet method, total carbohydrate by Anthrone method and crude fibre by acid and alkali digestion method (Sadasivam and Manickam, 2005).

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Numerical Optimization and Point Prediction

A second order quadratic polynomial regression equation of the following form was fitted to the data of all responses for prediction.

$Y= \beta_0+ \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_1X_1+ \beta_{22}X_2X_2 + \beta_{33}X_3X_3$

Where β_0, β_1, β_2, β_3, β_{11}, β_{22}, β_{33}, β_{12}, β_{13}, β_{23} were regression coefficients; X_1, X_2, X_3 were the independent variables and Y was the dependent variable. The p values of regression coefficient, which in turn, are necessary to understand the pattern of mutual interactions between the best variables. The smaller the magnitude of 'p', the more significant is the corresponding coefficient (Kunamneni et al., 2005). The optimum level of each ingredient in cereal mix was obtained by combining set goals of all determined responses with maximum importance of 3. The quality of fit of second order equation was evaluated by the coefficient of determination R^2, model p value, lack of fit p value, coefficient of variation (CV) % and adequate precision levels. The individual and interactive effects of each independent variable were determined. The predicted response levels for optimum combination of three independent variables were validated through experimentation.

Statistical Analysis

All determinations were done in duplicate. Data were analysed statistically using design expert and SPSS software.

Results and Discussion

Estimated Response Levels of Variables

The estimated response levels of experimental variables were indicated in Table 2. The carbohydrate ranged from 57.96 to 74.4g%; protein from 5.25 to 12.25g%; fat from 1.3 to 1.75g%; ash from 1 to 3.5g%; moisture from 10.5 to 23g% on wet basis; and crude fibre was ranged from 2 to 5g%.

According to Food Safety and Standards Authority of India (2011), any whole meal/grain powder shall conform to moisture determined by heating at 130-133°C for 2 hours and total ash not more than 3.0% (on dry weight basis). The moisture and total ash content of composite cereal flour of 20 experimental runs was in accordance with the respective Food Safety and Standards Authority of India (FSSAI) standards.

Influence of Independent Variables on Response

The magnitude of the terms indicates the order of influence on each response and the difference in magnitude of the quadratic terms explains which variable was dominant for response (Karunanithy and Muthukumarappan, 2011).

The coefficient for the proposed quadratic model in terms of actual variables (Table 3) and the proposed model equations for actual terms of each response (Table 4) describe the influence of independent variables on responses.

As per the 'p' value of regression coefficients at the linear terms, all independent variables had significant effect on fat; PMF on carbohydrate and WWF on protein. The quadratic terms of PMF contributed significant influence on moisture, protein and crude fibre content of cereal mix. The significant interactive terms were noted for moisture (WWF and GFMF) and crude fiber (WWF and PMF; PMF and GFMF).

As evident from the proposed model equations for actual factors, both WWF and PMF had a positive influence on moisture, protein, fat, ash and crude fiber content; negative influence on carbohydrate content of cereal mix. The GFMF exhibited the positive influence on carbohydrate, protein and fat: negative influence on moisture, ash and crude fiber. According to difference in magnitude of quadratic terms, the dominate variables for moisture, carbohydrate, protein, fat, ash and crude fiber was GFMF, PMF, GFMF, all and WWF respectively.

In order to visualise the interactive effects of independent variables on each response, interaction cubical response surfaces are shown in fig1-6.

The moisture content of cereal mix gets reduced on reducing the level of PMF regardless of GFMF level. The saddle system of interaction was noted between PMF and GFMF. While increasing the level of PMF and WWF from centre point (25g and 50g respectively) the crude fiber content was increased linearly to the maximum of 6.2 to 6.5g%. If PMF and GFMF level was increased from 25g, the crude fibre content was drastically reduced to 2.5g%.

Response Surface Model Evaluation

The adjusted R^2 (Table 3) above 0.8 for fat (0.999) showed a good fit of the model with the experimental data, while R^2 value of more than 0.5 for moisture, carbohydrate and crude fibre indicated fair fit of the model with the experimental data. Coefficient of variation (CV) is another measure to evaluate the goodness of the model. As a general rule, the CV should not be greater than 10% (Cocharan and Cox, 1957; Linko et al., 1984; Vainionpaa and Malkki, 1987), considering the general rule, a low value of CV for fat (0.13%) and carbohydrate (4%) showed that the experiments conducted are precise and reliable.

Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The adequate precision value greater than 4 for moisture, carbohydrate, protein, fat and crude fibre indicated adequate signal for better the prediction/optimization. The proposed second order quadratic polynomial regression model was significant for moisture ($p<0.01$) and carbohydrate ($p<0.05$) content. The suggested fitted model for protein, fat, ash and crude fiber was linear, mean and quadratic polynomial equation respectively.

Lack of fit 'p' value for all determined responses except protein and ash predicted that the proposed quadratic model was probably appropriate and adequate for prediction/ optimization expect for protein and ash.

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 reported in Table 5 revealed that the optimum level of independent variables for cereal mix preparation with maximum carbohydrate, protein, fat, ash, crude fibre and minimum moisture content as set goals for optimization with desirability of 0.866 were 46.18g of WWF, 35g of PMF and 32.58g of GFMF.

The experimental value for protein, fat, ash and crude fiber were not significantly different from predicted levels. The predicted and experimental moisture, total protein (N*6.25) and total ash on dry basis levels of optimized cereal mix was able to meet the standards of malt based foods which should contain not more than 5% by weight of moisture, not less than 7% by weight of total protein and not more than 5% by weight of total ash specified by FASSI (2011).

Conclusion

The use of experimental design allowed the rapid screening of a large experimental domain in search of the best combination of cereals and millets. The cereal mix with maximum total carbohydrate, protein, fat, ash, crude fibre with minimum moisture content for maximum storage stability could be made from ratio of combination of WWF, PMF and GFMF at 46.18: 35: 32.58 respectively.

Fig. 1-6: Interactive effect of independent variables on responses

Fig. 1

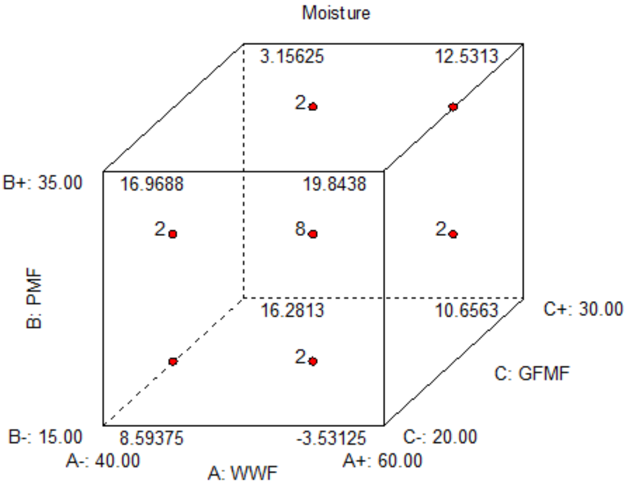
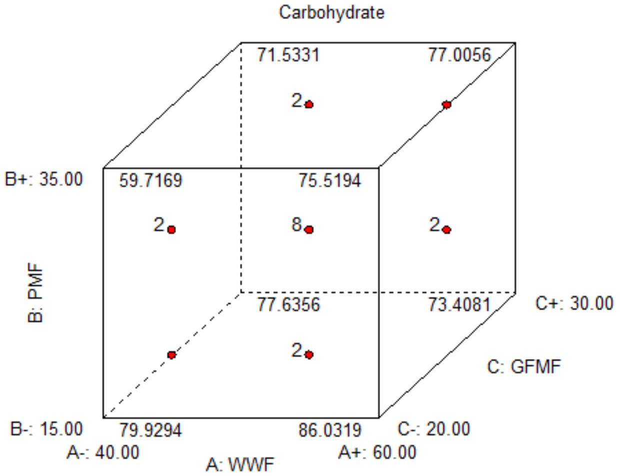
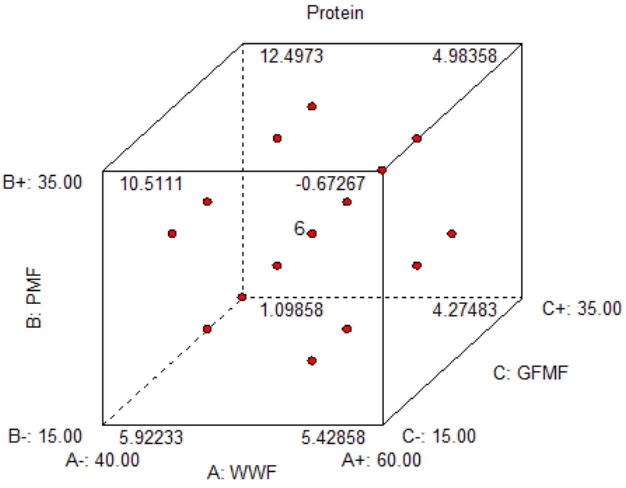
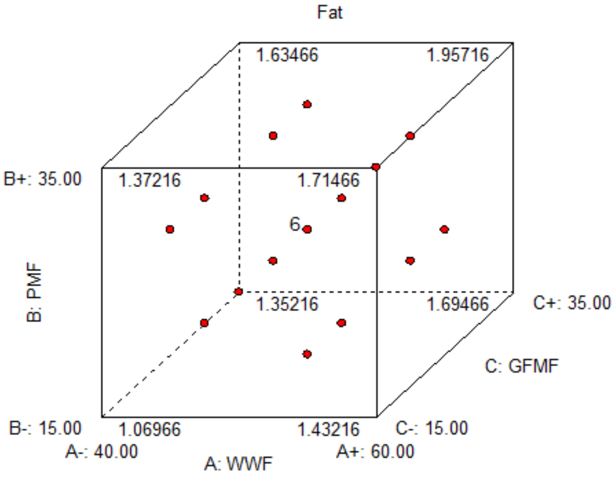
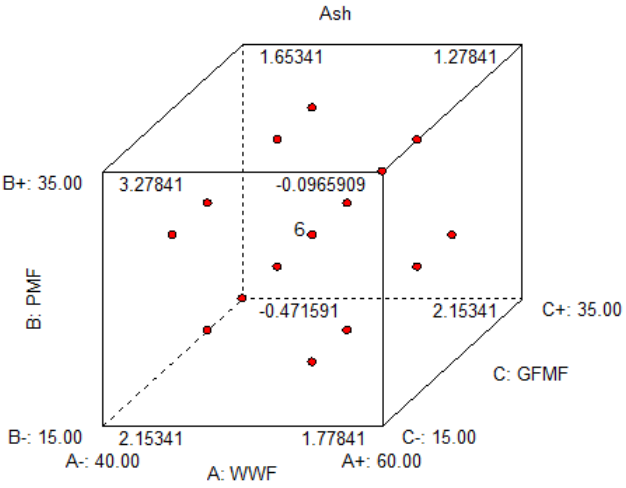


Fig.2









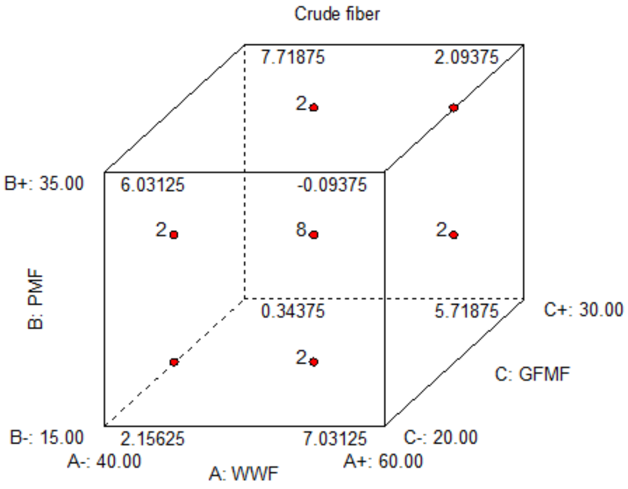


Table 1: Independent variables and their levels in central composite rotatable design

A. Levels of independent variables						
Independent variables	**Symbol**	**Coded levels**				
		-2	**-1**	**0**	**1**	**2**
Whole wheat flour (g)	X_1	40	45	50	55	60
Proso millet flour (g)	X_2	15	20	25	30	35
Germinated finger millet flour (g)	X_3	15	20	25	30	35

B. Experimental plan			
X_1	X_2	X_3	**Number of experiments**
±1	±1	±1	8
±2	0	0	2
0	±2	0	2
0	0	±2	2
0	0	0	6

Table 2: Estimated response levels (in g %) of experimental runs

Runs	**Protein**	**Fat**	**Ash**	**Moisture**	**Fibre**	**Carbohydrate**
1	07.87±0.02	1.30±0.07	2.0±0.01	04.5±0.42	3.5±0.04	70.8±0.70
2	06.13±0.06	1.48±0.01	3.0±0.01	02.5±0.70	4.5±0.21	74.4±0.70
3	10.59±0.07	1.45±0.04	3.5±0.02	10.5±0.06	4.0±0.08	58.0±0.03
4	05.25±0.07	1.62±0.02	1.5±0.01	11.2±0.23	2.0±0.14	66.6±0.35
5	08.75±0.06	1.44±0.03	1.5±0.01	07.5±0.05	3.0±0.16	67.8±0.49
6	07.00±0.12	1.61±0.04	1.0±0.00	07.5±0.10	4.0±0.07	68.9±0.13
7	12.25±0.03	1.58±0.02	1.0±0.01	05.5±0.00	5.0±0.00	64.7±0.04
8	08.75±0.04	1.75±0.03	2.0±0.01	08.5±0.14	3.5±0.07	65.5±0.30
9	10.59±0.04	1.36±0.04	1.5±0.00	04.0±0.10	4.5±0.07	68.1±0.00
10	08.75±0.42	1.70±0.02	1.0±0.00	03.5±0.03	4.5±0.21	72.6±0.50
11	07.87±0.00	1.39±0.03	3.0±0.01	02.5±0.00	3.5±0.00	73.7±0.10
12	09.62±0.05	1.67±0.03	3.0±0.00	02.1±0.21	4.0±0.21	70.7±0.02
13	08.75±0.04	1.40±0.04	2.0±0.00	09.5±0.02	3.0±0.00	63.4±0.03
14	06.13±0.03	1.66±0.02	3.0±0.00	11.2±0.03	3.0±0.70	63.2±0.02
15	09.62±0.06	1.53±0.02	2.5±0.01	07.5±0.07	3.0±0.70	65.9±0.07
16	10.59±0.03	1.53±0.02	2.5±0.01	04.0±0.70	4.5±0.70	66.9±0.05
17	09.62±0.03	1.53±0.02	2.5±0.01	03.5±0.00	4.0±0.70	68.9±0.07
18	09.62±0.03	1.53±0.02	2.0±0.00	08.5±0.07	4.5±0.42	63.9±0.02
19	10.59±0.01	1.53±0.02	2.0±0.00	09.5±0.07	4.5±0.21	61.9±0.05
20	08.75±0.06	1.53±0.21	3.0±0.01	05.1±0.16	3.5±0.14	68.2±0.02

Table 3: Coefficient values of the fitted model for determined responses

Coefficients	**Moisture**	**Carbohydrate**	**Protein**	**Fat**	**Ash**	**Crude fibre**
β_0	16.682*	65.768	9.691	1.530	2.352	3.977
β_1	-0.344	1.447	-1.001**	0.086*	-0.094	-0.094
β_2	1.281	-2.077**	0.662	0.071*	0.031	0.031
β_3	0.094	-0.208	0.104	0.066*	-0.156	0.094
β_{12}	0.938	0.606	-0.668	-0.001	-0.188	-0.688
β_{13}	0.813**	-1.291	0.229	-0.001	0.188	0.063
β_{23}	-2.688	1.764	0.426	-0.001	0.063	0.438
β_{11}	-0.722	1.018	-0.086	0.000	-0.324	0.114
β_{22}	-1.221**	1.499**	-0.317	-0.000	0.114	-0.074
β_{33}	1.653**	-0.737	-0.644**	-0.000	-0.011	-0.261**
Adj. R^2	0.577	0.577	0.387	0.999	-0.102	0.7644
Model p value	0.0079	0.0231	0.1785	0.9798	0.2061	0.0635
Lack of fit p value	0.3953	0.485	0.0276	-	0.0192	0.9168
CV%	16.26	4.00	15.62	0.13	36.95	13.21
Adequate precision value	7.533	8.089	4.572	321.64	3.519	7.393

*-Significant at $p<0.01$, **-Significant at $p<0.05$

Table 5: Validation of optimum level of independent variables

Response	Predicted value	Experimental value
Moisture	4.62	04.49±0.01*
Carbohydrate	71.01	74.23±0.35*
Protein	11.18	11.30±0.46^{NS}
Fat	1.70	01.55±0.22 ^{NS}
Ash	2.75	02.74±0.05 ^{NS}
Crude fiber	5.73	05.69±0.19 ^{NS}

*Significant at $p<0.01$, ^{NS}- Not Significant

Table 4: Proposed model (2^{nd} order polynomial regression) equation for responses

Moisture	$= -27.81 + 0.68X1 + 3.12X_2 - 2.226X_3 + 0.0375X_1X_2 + 0.0325X_1X_3 - 0.1075X2X3- 0.028864X_1^2 - 0.048864X_2^2 + 0.066136X_3^2$
Carbohydrate	$= 193.35 - 3.097X_1 - 6.3896X_2 + 2.253X_3 + 0.0243X_1X_2 - 0.0517X_1X_2 + 0.071X_2X_3 - 0.041X_1^2 + 0.060\ X_2^2 - 0.0295X_3^2$
Protein	$= -28.014 + 0.5813X_1+1.677X_2 + 0.424X_3 - 0.02673X_1X_2 + 0.009175X_1X_3 + 0.017X_2X_3 - 0.0343X_1^2 - 0.0127X_2^2 - 0.0258X_3^2$
Fat	$= -0.181 + 0.0201X_1 + 0.0181X_2 + 0.0171X_3 - 0.00005X_1X_2 - 0.00005X_1X_3 - 0.00005X_2X_3 - 0.0000045X_1^2 - 0.0000045X_2^2 - 0.0000045X_3^2$
Ash	$= -24.352 + 1.277X_1 + 0.0915X_2 - 0.4460X_3 - 0.0075X_1X_2 + 0.0075X_1X_3 + 0.0025X_2X_3 - 0.01296X_1^2 + 0.00454X_2^2 - 0.000454X_3^2$
Crude fiber	$= -13.0398 + 0.15170X_1 + 1.0915X_2 - 0.0210X_3 - 0.0275X_1X_2 + 0.0025X_1X_3 + 0.0175X_2X_3 + 0.00454X_1^2 - 0.002955X_2^2 - 0.0105X_3$

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