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**Research Articles**

# Development of barnyard millet based nutri-functional snack food: A response surface methodology approach

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ABSTRACT

Response surface modeling approach was applied to optimize the ingredients level for barnyard millet based Nutri-Functional Snack (NFS) food using a D-optimal mixture design. The effect of ingredients level of barnyard millet (50–70%), quality protein maize (20–50%) and flax seed (0–20%) with constant level of skim milk powder (5%) on the physical and functional properties of NFS such as bulk density, colour, expansion ratio,water absorption index, water solubility index, crispiness and sensory were investigated. The regression models that explained the significant effects of different percentages of ingredients level on all the response variables were determined. The developed regression model of all the responses had a coefficient of determination (R2) and adjusted R2 value of higher than 0.9245 and 0.8490, respectively. The optimum conditions for maximum acceptability of NFS food having 70% barnyard millet, 28% quality protein maize and 2% flaxseed.

**Key words**: Barnyard millet, Flaxseed, Nutritional snack food, Quality protein maize, RSM

Health and nutrition are the most important contributory factors for human resource development particularly in rural people. Protein energy malnutrition (PEM), micronutrient deficiencies and vitamin B-complex deficiencies are the frequently encountered nutrition problems among the rural poor and urban slum communities (Anonymous 2011). The nutritional profile of millets comprising of high dietary fibre, micronutrients, non-gluten proteins and phytochemicals deserves special attention in bakery products applications (Angioloni and Collar 2012, Patil *et al.* 2016).Minor millets are claimed to be the future foods for better health and nutrition security. Among the minor millets, barnyard millet is a fastest growing grain even under adverse agro climatic conditions. In India, barnyard millet is the second important smallmillet after finger millet having production and productivity 0.87 MT and 857 kg/ha, respectively (Padulosi *et al.* 2009, Sood *et al.* 2015) and cultivated in Uttarakhand and Tamil Nadu of India. Barnyard millet grains had superior nutritional quality (amino acid) than other crop (Kim *et al.* 2011) and due to antioxidant effects,this is called as functional food crop (Sood *et al.* 2015).The demand of the crop has increased due to its highly nutritious and providing both food and nutritional security in hills. Barnyard millet is highly suitable for commercial foods for diabetics, infants and pregnant women because of high iron content. Due to the non-availability of ready-to-use processed products has limited the usage. Hence, need to develop barnyard millet-based food products, viz. ready-to-use and functional foods to meet the demands of the present-day consumers. Value addition to barnyard millet may help increase the area under cultivation.(Sood *et al*. 2015).

Quality protein maize (QPM) had similar test weight, density and kernel size with 45% more lysine and 38% more tryptophan compared to food grade maize (FGM) (Ana *et al.* 2003).Maize has high carbohydrate content, but very low protein content and low levels of lysine and tryptophan. These deficiencies can be rectified by the use of QPM and extruded snack food made from QPM will be more nutritional than other snack type products available in the market (Ruiz-Ruiz *et al.* 2008).Flax seed added as functional ingredient in the blend formulation and are most suitable for human consumption as a rich source of α-linolenic acid, lignin content, phenolic compounds and also bioactive peptides. It gives health benefits for chronic diseases such as cardiovascular disorders, obesity and hormone-dependent cancers (Giacomino *et al.*2013).

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Among the available technologies for preparing snack food, extrusion cooking is a multi-step, multi-functional and thermal cum mechanical process, high temperature cum short time process (Arun Kumar *et al.* 2015,Sharma *et al.* 2015, Kaur *et al.* 2015). Response surface methodology (RSM) is a collection of statistical and mathematical techniques for developing,improving and optimizing product/processes. This techniques had been applied in different products such as sweet potato based pasta (Singh *et al.* 2004), soyfortified instant upma mix (Yadav and Sharma 2008) baking parameters of chapatti (Yadav *et al.* 2008), natural polymeric enteral feed formula (Vijayakumar and Deepa 2010) and weaning mix based on malted and extruded pearl millet and barley (Balasubramanian *et al.* 2014). Two component lattice simplex mixture design based on RSM was applied to determine the optimum combination of nixtamalized extruded maize flour and extruded chickpea flour for producing a weaning food. The component proportions were expressed as fractions of the mixture and the sum (X1 + X2) of the component proportions equaled 100% and experimental design generated 11 assays (Carrillo *et al.* 2007).

Even though Barnyard millet and flax seeds are having high nutritive value, they are not used as ingredient for snack food in the commercial market. In view of the above facts, the present study was carried out to rectify the nutrition problems such as Protein Energy Malnutrition (PEM), micronutrient deficiencies and vitamin B-complex deficiencies to make nutri-functional snack food (using barnyard millet, quality protein maize, flax seed flour and skimmed milk powder). The primary aim of the study was to optimize the ingredients level for production of best nutritive and acceptable barnyard millet based Nutri-Functional Snack (NFS) food.

## MATERIALS AND METHODS

Barnyard millet (var. CO 2) having 10% ± 1.5 (w.b.) moisture content were cleaned by a combination of manual and mechanical means to remove all foreign matter, broken and immature grains. De-hulling of barnyard millet was done using a double chamber centrifugal de-huller. Quality Protein Maize (QPM) variety $f_2$ was procured from the ICAR-Indian Institute of Maize Research, New Delhi. The procured material was subjected for cleaning, grading followed by drying and stored in cloth bags. Flax seed and Skim Milk Powder (SMP) was purchased from local market of Delhi. As per requirement of the experiment, clean and dry grain was milled in a laboratory scale multipurpose grain mill (capacity 40 kg/h) and cleaned and graded by power operated cleaner cum grader equipped with 60-mesh IS sieve.

The ingredients of blend were barnyard millet (1), Quality Protein Maize (QPM) (2) and flax seed (3) with constant level of skim milk powder (SMP) 5%. The ingredients levels of each variable were established on the preliminary trials. The layout of experimental design with the actual level is presented in Table 1. Dependent variables were bulk density (BD), Colour, Expansion ratio (ER), Water Absorption Index (WAI), Water Solubility Index (WSI), Crispiness and Overall Acceptability (OAA).The effects of extrusion conditions on the product responses were investigated through RSM. The ingredients level (independent variable) varied as; barnyard millet (50–70%), quality protein maize (20–50%) and flax seed (0–20%) with constant level of skim milk powder (5%). The selection was based on preliminary trials. To determine the ingredients level for the production of nutri functional snack food D-optimal mixture design was used. The design contains 11 different combinations of ingredients as experimental run in design expert 7.0.0 software.

The extrusion studies were conducted at Department of Food Science and Technology, Punjab Agricultural University, Ludhiana. It was performed on a co-rotating and intermeshing twin-screw extruder Model BC 21. The barrel diameter and its length to diameter ratio (L/D) were 2.5 mm and 16:1, respectively. The extruder had four

Table 1 Effect of extrusion conditions on dependent variables

| Actual values | | | Product responses (Mean±SE) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| BM | QPM | FS | BD | colour | ER | WAI | WSI | Crispiness | OAA |
| 70 | 20 | 10 | 0.1016±0.003 | 53.24±0.929 | 2.4470±0.040 | 3.9574±0.039 | 50.35±0.620 | 10.91±0.160 | 7.40±0.142 |
| 60 | 30 | 10 | 0.1430±0.014 | 55.32±0.958 | 2.4721±0.058 | 4.2758±0.062 | 49.98±0.622 | 17.43±0.833 | 7.53±0.117 |
| 50 | 50 | 0 | 0.0644±0.021 | 57.58±0.428 | 2.7644±0.058 | 4.3599±0.081 | 58.71±0.460 | 13.62±0.609 | 7.55±0.142 |
| 60 | 40 | 0 | 0.0894±0.006 | 58.96±0.801 | 2.5155±0.058 | 4.5807±0.089 | 57.51±0.688 | 18.82±0.178 | 7.82±0.128 |
| 70 | 30 | 0 | 0.0571±0.004 | 61.19±0.913 | 2.8225±0.058 | 4.7889±0.096 | 56.94±0.770 | 12.86±0.470 | 7.80±0.136 |
| 50 | 30 | 20 | 0.1946±0.005 | 52.41±0.487 | 1.4778±0.064 | 4.7586±0.079 | 45.13±0.104 | 18.08±0.565 | 7.47±0.159 |
| 55 | 25 | 20 | 0.1720±0.002 | 52.68±0.441 | 1.6990±0.064 | 4.4322±0.068 | 43.36±0.893 | 19.18±0.695 | 7.22±0.147 |
| 50 | 30 | 20 | 0.2060±0.005 | 53.82±0.549 | 1.246±0.058 | 4.7488±0.037 | 45.49±0.630 | 18.03±0.681 | 7.32±0.136 |
| 50 | 50 | 0 | 0.0650±0.006 | 58.57±0.285 | 2.7381±0.059 | 4.1175±0.022 | 57.09±0.768 | 13.25±0.054 | 7.56±0.146 |
| 50 | 40 | 10 | 0.1120±0.004 | 56.78±0.282 | 2.0460±0.048 | 4.1373±0.056 | 50.88±0.866 | 15.94±0.051 | 7.56±0.154 |
| 60 | 20 | 20 | 0.1488±0.005 | 50.42±0.242 | 1.7856±0.025 | 4.3631±0.057 | 45.48±0.836 | 18.51±0.600 | 7.27±0.169 |

BM - Barnyard millet (%), QPM-Quality Protein Maize (%), FS – Flaxseed (%), BD – Bulk Density (g/cc), ER- Expansion Ratio, WAI – Water Absorption Index (g/g), WSI – Water Solubility Index (%), OAA- out of 9.

barrel zones. Temperature of the first, second and third zone were maintained at 40, 70 and 100ºC, respectively, throughout the experiments, while the temperature at last zone (compression and die section) was varied according to the experimental design (150ºC). The diameter of die opening was 3 mm. The extruder was thoroughly calibrated with respect to the combinations of feed rate and screw speed to be used.

Raw materials were equilibrated to room temperature and blended as per the experimental design by mixing desired proportion of feed formulation. The moisture content of the feed, screw speed and barrel temperature of the extruder were maintained at 16% (wb), 400 rpm and 150ºC respectively. When the extruder reached a steady state, as indicated by nearly constant values for extruder torque and product temperature, samples were collected in steel trays and kept at ambient temperature for cooling. The extruded samples were sealed in polythene (HD) bags and stored at room temperature (25±4°C) till analysis.

The product response as variables for the optimization of ingredients level were bulk density, colour, expansion ratio, WAI, WSI, Crispiness and sensory evaluation.

BD (g/cc) of extrudates was determined by a volumetric displacement procedure as described by Patil *et al.* (2007). The volume of the expanded sample was measured using a 100-mL graduated cylinder by rapeseed displacement. The volume of 20 g randomized samples was measured for each test. The ratio of sample weight and the replaced volume in the cylinder was calculated as BD (w/v).

The colour values of ground extrudates samples in terms of L, $a^*$ and $b^*$ were measured using Hunter Lab Lab Scan XE (NR-3000; 10°/D65). Color values were recorded as L= lightness (0=black, 100=white), a (–a=greenness, +a = redness), and b (–b=blueness, +b = yellowness). Total colour change was calculated using Eq 1.

$$\text{Total colour change} = \sqrt{(L-L_0)^2 + (b-b_0)^2 + (a-a_0)^2} \quad (1)$$

where, the subscript '0' indicates initial colour values of the raw material.

Expansion ratio was measured as the ratio of cross sectional area of the dried cylindrical extrudate to that of the die (Chakraborty *et al.* 2009). The diameter of the extrudate was the average of ten random measurements. This index describes the degree of puffing undergone by the sample as it exits the extruder.

Water Solubility Index and Water Absorption Index were determined according to the method developed for cereals (Yagci and Gogus 2008, Balasubramanian *et al.* 2014). The ground extrudates were suspended in water at room temperature for 30 min, gently stirred during this period, and then centrifuged at 3000× g for 15 min. The supernatants were decanted into an evaporating dish of known weight. The WAI was the weight of gel obtained after removal of the supernatant per unit weight of original dry solids. The WSI was the weight of dry solids in the supernatant expressed as a percentage of the original weight of sample.

$$\text{Water absorption index} = \frac{\text{weight gain of gel (g)}}{\text{sample weight (g)}} \quad (2)$$

$$\text{Water solubility index (per cent)} = \frac{\text{weight of dry solids in supernatant (g)}}{\text{Sample weight (g)}} \times 100 \quad (3)$$

Crispness of the extrudates were measured using texture analyzer supplied with "Texture Expert" software, fitted with 5 kg load cell. Force-deformation data were obtained using 2 mm diameter test probe. Tests were conducted using compression mode and the probe was allowed to penetrate the product for a distance of 3 mm. The test settings included test speed of 0.5 mm/s, and strain of 90%. Force time curve was recorded and analyzed by the inbuilt software program which indicated the peak force and also counts the number of major peaks obtained during the test and expressed in terms of crispiness (Subir *et al.* 2011). Ten randomly collected samples were measured for each extrusion condition and mean of the observations was recorded for reporting.

A nine point hedonic scale was used for Sensory evaluation (Xiong *et al.* 2002). A semi-trained panelist consisting of 50 members selected from the CIPHET, Ludhiana and IARI, New Delhi of different age group (21-55 years).Appearance and color, texture, odor, flavour and taste as well as overall acceptability were evaluated for the barnyard millet based nutrifunctional snack food.

Responses obtained as a result of the proposed experimental design were subjected to mixture design analysis in order to assess the effects of barnyard millet, quality protein maize and flax seed with constant proportion of skim milk powder. Second-order quadratic regression models were established for the dependent variables to fit experimental data for each response using statistical software Design-Expert 7.0.0 (Stat-Ease Inc., Minneapolis, MN).

$$y = \sum_{i=0}^{3} b_i x_i + \sum_{i=0}^{3}\sum_{j=0}^{3} b_{ij} x_i x_j$$

where $x_i$ (i = 1, 2, 3) are independent variables (barnyard millet, quality protein maize and flax seed) respectively, and bi and bij are coefficient for linear and interactive effects, respectively. Data was analyzed by multiple regression analysis and statistical, significance of the terms was examined by analysis of variance (ANOVA) for each response. The adequacy of regression model was checked by correlation coefficients. The lack of fit test was used to judge the adequacy of model fit. To aid visualization of variation in responses with respect to processing variables, series of three-dimensional response surfaces plots were drawn.

## RESULTS AND DISCUSSION

Different composition of barnyard millet, quality protein maize and flax seed with constant proportion of skim milk powder affected the BD, colour, ER, WAI, WSI, crispiness and overall acceptability of barnyard millet based Nutri-Functional Snack food (Table 1). The independent and dependent variables were fitted to the second-order model and examined for the goodness of fit. The analyses of variance were performed to determine the lack of fit and the significance of the linear, and interaction effects of the independent variables on the dependent variables. Suggested quadratic models were chosen for all the factors with highly significant effects ($P < 0.001$) on response variables. Coefficient of determination or $R^2$ is the proportion of variation in the response attributed to the model rather than to random error and was suggested that for good fit model, $R^2$ should be at least 80% (Gan *et al.* 2007). The results showed that the models for all the response variables were highly adequate because they had satisfactory levels of $R^2$ of more than 90% and that there was no significant lack of fit in all the response variables (Table 2).

Hence, it can be concluded that the proposed models approximate the response surfaces and could be used suitably for prediction at any values of the parameters within experimental range. The fitted model for each of the response variables could be derived from the predicted values of each response variable (Table 3).

*Effect of product parameters on bulk density*

The bulk density of the nutri-functional snack (NFS) food ranged between 0.0571 and 0.2060 g/cm$^3$ (Table 1). For predicting the bulk density the multiple regression model gave 94.88% variations in the observations (Table 2). It indicated that the bulk density was significantly affected by the quantity of barnyard millet, QPM and flax seed (linear terms, $P<0.01$, Table 3). Flax seed had more significant positive effect ($P < 0.01$) on bulk density than barnyard millet and QPM. When flaxseed content in the blend increased, its bulk density increased due to less expansion of the extrudates (Fig 1).This may be due to the increase in oil content, which might have given more rigid extrudate with higher bulk density. Also Wu *et al.* (2015) reported that the physicochemical properties were affected significantly by the level of flax seed. The interaction of barnyard millet- QPM, barnyard millet-flaxseed and QPM-flaxseed showed that no significant effect on bulk density.

*Effect of product parameters on colour*

The colour value of the NFS food ranged between 50.42 to 61.19 (Table 1). The regression model for predicting the colour value gave 96.48% variations in observation (Table 2). The colour value was found to be significantly affected by the quantity of barnyard millet, QPM and flax seed (linear terms, $P<0.01$, Table 3.).Flax seed had more significant positive effect ($P < 0.01$) on colour than barnyard millet and QPM (Fig 2). This may be due to the Maillard reaction in extrusion process. As the flax seed content increased in blend, the extrudates became darker resulting in decrease in colour value. The interaction of barnyard millet-QPM, barnyard millet-flaxseed and QPM- flaxseed shows that no significant effect on colour.

*Effect of product parameters on expansion ratio*

Extrusion process incorporate air cells in the extrudates that gives expanded porous structure to the snack food. The

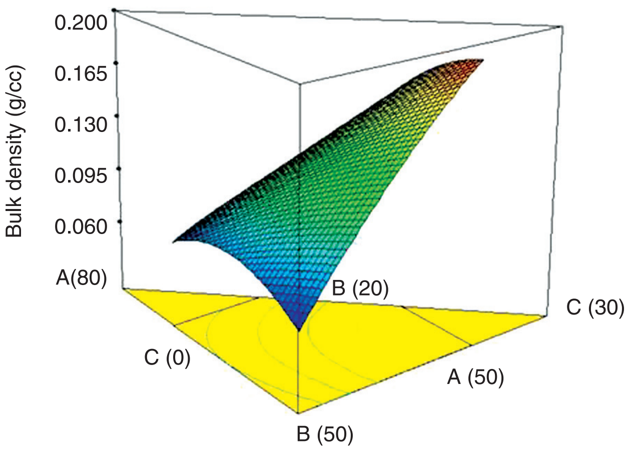


Fig 1 Effect of barnyard millet (A), QPM (B), flax seed (C) on bulk density of nutri-functional snack food.

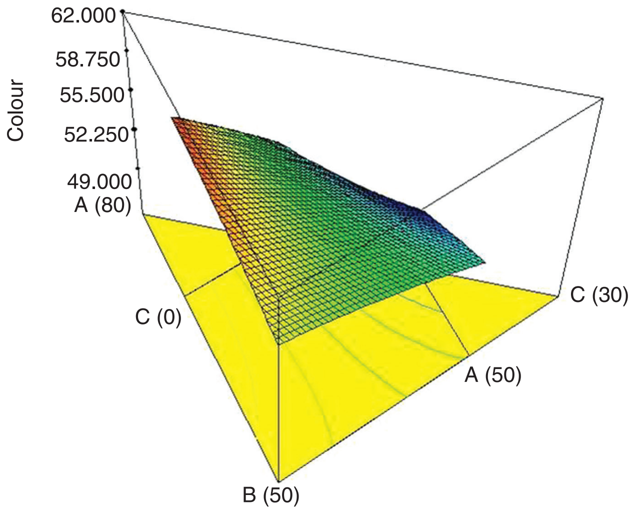


Fig 2 Effect of barnyard millet (A), QPM (B), flax seed (C) on colour of nutri-functional snack food.

Table 2. Analysis of variance for the fit of experimental data to response surface model

| Regression | Sum of squares | | | | | | |
|---|---|---|---|---|---|---|---|
| | BD | Colour | ER | WAI | WSI | Crispiness | OAA |
| Adequate precision | 10.167 | 15.852 | 11.349 | 10.044 | 22.390 | 24.704 | 10.789 |
| R square | 0.9488 | 0.9648 | 0.9598 | 0.9261 | 0.9898 | 0.9864 | 0.9245 |
| Adjusted R square | 0.8976 | 0.9296 | 0.9196 | 0.8522 | 0.9796 | 0.9728 | 0.8490 |
| CV% | 13.84 | 1.59 | 7.25 | 2.46 | 1.62 | 2.97 | 1.01 |
| Lack of fit | 0.066ns | 0.512ns | 0.308ns | 0.646ns | 0.216ns | 0.093ns | 0.560ns |

Table 3 Regression coefficients for fitted Models

| Parameters | Regression coefficients | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | BD | Colour | ER | WAI | WSI | Protein | Crispiness | OAA |
| A. BM | -0.189** | 59.0** | 2.82** | 4.00** | 63.13** | 38.2** | -61.1** | 6.8** |
| B. QPM | -0.577** | 39.3** | 2.87** | -0.95** | 80.55** | 69.3** | -156.6** | 3.747** |
| C. FS | 0.621** | 108.1** | -18.84** | 42.77** | 53.93** | -31.4** | -4.5** | 7.394** |
| AB | 1.782 | 35.4 | -0.53 | 10.81 | -53.90 | -164.7** | 489.9** | 9.175 |
| AC | 0.151 | -171.6 | 26.29 | -55.34** | -88.95 | 19.2 | 245.4** | -3.720 |
| BC | 0.856 | 41.2 | 5.74 | -27.33* | -74.68 | 19.6 | -25.7 | 6.139 |

* Significant at P <0.05. ** Significant at P <0.01. BM - barnyard millet; QPM - Quality protein maize; FS- Flax seed; BD - Bulk Density; ER -Expansion ratio;

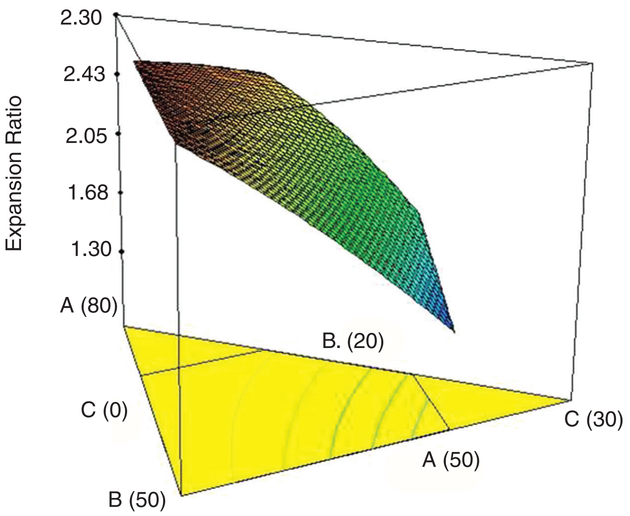


Fig 3 Effect of barnyard millet (A), QPM (B), flax seed (C) on expansion ratio of Nutri-Functional Snack food.

expansion ratio of the nutri-functional snack (NFS) food ranges between 1.246 and 2.822 (Table 1). For predicting the expansion ratio the multiple regression model gave 95.98% variations in the observations (Table 2). It indicated that the expansion ratio was significantly affected by the quantity of barnyard millet, QPM and flax seed (linear terms, P<0.01, Table 3).The coefficient estimates of expansion ratio model showed that the levels of flax seed had highly significant (P < 0.01) negative effects in linear terms (Fig 3). There is an increase in the expansion ratio of NFS food when the level of barnyard millet in the blend was increased. Similar trend were also found in the level of QPM. However, flaxseed showed opposite trend.

*Effect of product parameters on water absorption index (WAI)*

The values of WAI for the NFS food varied between 3.9574 and 4.7889g/g (Table 1). The developed regression model of WAI had a coefficient of determination ($R^2$) and adjusted $R^2$ value of 0.9261 and 0.8522 respectively and CV value of 2.46% (Table 2).The level of barnyard millet and flaxseed increased than significant increases in WAI. The negative coefficients of the linear term of QPM (Table 3) indicated that WAI decreases with increase of QPM level in the blend preparation (Fig 4).

*Effect of product parameters on water solubility index (WSI)*

The values of WSI for the NFS food varied between 43.36 and 58.71% (Table 1). The developed regression model of WAI had a coefficient of determination ($R^2$) and adjusted $R^2$ value of 0.9898 and 0.9796 respectively and CV value of 1.62% (Table 2).The WSI was significantly affected by the quantity of barnyard millet, QPM and flax seed (linear terms, P<0.01, Table 3).Flax seed had more significant positive effect (P < 0.01) on WSI than barnyard millet and QPM.

*Effect of product parameters on crispiness*

The crispiness value of NFS food ranged between 10.91 and 19.18 (Table 1). For predicting the bulk density he multiple regression model gives 98.64% variations in the observations (Table 2).The crispiness was significantly affected by the quantity of barnyard millet, QPM and flax seed (linear terms) and interaction of barnyard millet–QPM,

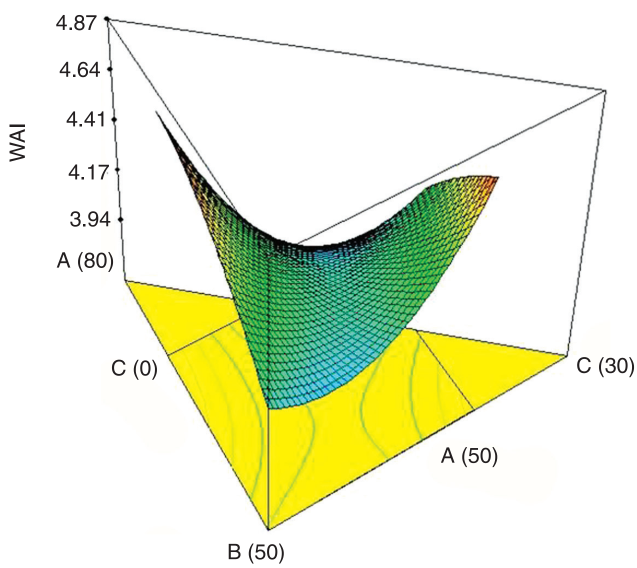


Fig 4 Effect of barnyard millet (A), QPM (B), flax seed (C) on WAI of nutri-functional snack food.

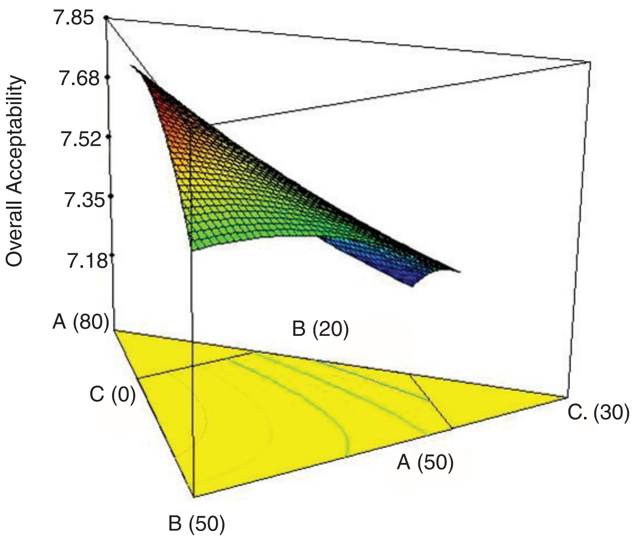


Fig 5 Effect of barnyard millet (A), QPM (B), flax seed (C) on OAA of nutri-functional snack.

barnyard millet–flaxseed (P<0.01, Table 3).The negative coefficients of the linear terms of barnyard millet, QPM and interaction of barnyard millet –flaxseed level indicated that crispiness decreased with increase of these variables, while positive coefficients of the interaction of barnyard millet –QPM and barnyard millet –flaxseed indicated that crispiness increases with increase in their level.

*Effect of product parameters on sensory evaluation*

Sensory evaluation conducted based on nine point hedonic scale for developed NFS food in terms of overall acceptability (OAA). OAA score for the NFS food varied between 7.22 and 7.82 (Table 1).The developed regression model of OAA had a coefficient of determination ($R^2$) and adjusted $R^2$ value of 0.9245 and 0.8490 respectively and CV value of 1.01% (Table 2).The OAA was found to be significantly affected by the quantity of barnyard millet, QPM and flax seed (linear terms, P<0.01, Table 3). The interaction of barnyard millet – QPM, barnyard millet –flaxseed and QPM- flaxseed shows that no significant effect on OAA (Fig 5).

*Optimisation of ingredients level*

Numerical optimisation of ingredients level in blend preparation was carried out for the production of NFS food. Desired goals were assigned for all the parameters for obtaining the numerical optimum values for the responses. All the levels of barnyard millet, QPM and flaxseed were kept in ranges of maximization, in range and minimization. The response parameters like bulk density was kept minimum, and all other responses kept maximum respectively. The optimum values obtained for ingredients levels, viz. barnyard millet, QPM and flaxseed were 70%, 28% and 2%, respectively. The corresponding optimum values of BD, colour, ER, WAI, WSI, Crispiness and OAA were 0.0944 g/cm$^3$, 58.11, 3.02,112.08 Wh/kg, 4.57g/g, 24.15% , 12.62 and 7.72 respectively.

The significant effect of all three ingredients, viz. barnyard millet, QPM and flaxseed through Response Surface Methodology was observed on physical properties of barnyard millet based NFS food. Within the minimization range, flaxseed was the most important factor affecting the physical properties of the NFS food. After complete evaluation of all the product responses, viz. bulk density, colour, expansion ratio,water absorption index, water solubility index, crispiness and overall acceptability, it was found that there was strong relationship between all the responses and ingredients.

Barnyard millet can be the alternate source of commercially available raw material, viz rice, maize for production of extruded snack food. The developed nutri-functional snack (NFS) food is rich in protein, micro nutrients and fibre. The optimum conditions for maximum acceptability of NFS food were found to be 70.0% barnyard millet, 28% quality protein maize (QPM) and 2% flaxseed with constant level of 5% skim milk powder (SMP). Hence, the developed NFS food may reduce the problems of protein energy malnutrition (PEM), micronutrient deficiencies and vitamin B-complex deficiencies. Barnyard millet is cheap compared to commercially available raw material in extruded snack industry. This may reduce the cost of production of extruded snack food with tremendous nutritional advantages.

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