

Quality assessment of probiotic weaning mix from fermented cereal-legume blends

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ABSTRACT

Weaning mix was developed from underutilized crops of Uttarakhand (finger millet, barnyard millet, black soybean, amaranth grain). Malting and fermentation were adopted as ways of improving cereal protein quality and decreasing anti-nutritional property and probiotic weaning mix was produced using probiotic strain *Lactobacillus plantarum*. Box-benken design of response surface methodology was used to design the experiments design for three variables with three levels [blend ratio (cereals : legume:: 50:50, 60:40, 70:30), fermentation time (12 h, 24 h, 36 h), inoculums concentration (1%, 3%, 5%)] and four responses (protein, tannin, water absorption capacity, water absorption index). The probiotic fermentation resulted in favourable changes in nutritional profile of weaning mix resulting 48.2% increase in protein and 92.3% decrease in tannin content. The optimized set of independent variables (cereals (FM+BM): legume: 70:30 fermented for 25 h with 2.9% inoculum concentration) obtained after statistical analysis, was used to develop final probiotic weaning mix.

Highlights

- Weaning mix was developed from underutilized crops of Uttarakhand (finger millet, barnyard millet, black soybean, amaranth grain).
- Weaning food was produced from probiotic fermentation technology by using probiotic strain *Lactobacillus plantarum*.

Keywords: Underutilized crops, weaning mix, fermentation and LAB

Early childhood malnutrition is detrimental to the mental and physical development of the child and has a negative impact on the growth and productivity of a nation. In India, around 63% of the children under five years of age are malnourished (Thathola and Srivastava 2002). This accounts for 75 million malnourished children and is one of the worst levels in the world. Food and nutrition plays a major role to build up their immunity. Proper nutrition is required for their proper physical growth. Weaning, the substitution of the mother's milk with other foods, is a gradual process of introducing an infant to adult diet. It is very crucial period of infant's life and also

known as "food accustomed" period, which covers 4-6 months to 3 years.

In developing countries, complementary foods are based on local staple foods, usually cereals that are processed into porridges. Apart from their bulkiness, cereal based gruels are of poor nutritional value. Traditional weaning foods are improved by the incorporation of locally available protein-rich materials such as grain legumes and proteinized seeds (Egounlety 2001). Legumes contain high lysine but low content of sulphur amino acids. Cereals, on the other hand, are high in sulphur amino acids



but deficient in lysine. A mutual complementation of amino acids and consequent improvement in protein quality is, therefore, achieved when legumes are blended with cereals in the right proportions (Ghavidel and Prakash 2010. Some traditional technologies (fermentation and malting) may be preferred as they are economic, improve nutritional quality and easy to perform. It has been also suggested that weaning foods with improved nutritive value can be prepared by using cereal-legume blends, fermented and germinated cereals and legumes (Sajilata *et al.*, 2002).

The potential of fermented foods for reducing or alleviating food related factors of malnutrition, particularly among weaning age children is important considering the beneficial properties inherent in these types of food (Lorri 1993). Fermentation is mainly by a complex micro flora, which develops spontaneously with lactic acid bacteria generally overcoming the other flora. Spontaneous fermentation is difficult to control, sometimes not safe since they are liable to contamination by pathogen. There is therefore the need to replace the present uncontrolled fermentation process existing in the developing countries with a pure culture fermentation so as to obtain a consistent product quality. Lactic acid bacteria are gram positive, anaerobic, non-sporulating and acid tolerant bacteria that are generally regarded as safe (GRAS) for use in food and food products. They contribute to rapid acidification of food products and also to flavor, texture and nutrition. Fermentation with the help of Lactic acid bacteria (LAB) improves digestibility and reduces diarrhea illness in infants. In view of the above information, this research therefore aimed at to study the effect of blend ratios, fermentation time and inoculums concentration on nutritional and functional property of probiotic weaning mix and also to get the optimized set of independent variables using RSM to develop underutilized crops based nutritionally enriched weaning mix.

Materials and Methods

Sample Collection

The raw grains i.e. Finger millet (*Eleusinecoracana*), Barnyard millet (*Echinochloa frumentacea*), Black soybean (*Glycine max*) and Amaranth (*Amaranthus*

spp.) of local varieties were purchased from the local market of Bheemtal and Haldwani. Grains were cleaned, dried and kept in moisture proof plastic containers for the purpose of study.

Pretreatments to the grains

Preliminary trials were performed for identifying the range of independent parameters effecting the pretreatments and fermentation. Soaking time was set as 24h for finger millet and barnyard millet as there was no spoilage and mould contamination observed. Germination time was decided on the basis of vegetative growth (shoots and roots) as observed for seeds. It was found that appropriate germination time for finger millet was 24 h and 48h for barnyard millet. The malted grains were dried at 55°C for 5-7h. Appropriate roasting temperature and time for black soybean and amaranth grain was determined on the basis of aroma and appearance. Roasting temperature for Amaranth grain was decided as 100-120°C for 3 min. and for Black Soybean, it was decided as 100-120°C for 10 min. The grains after pre-treatment method were grinded at a constant speed of using mixer and grinder, and sieved using mesh size 0.05 mm.

Starter Development

The starter used *Lactobacillus plantarum* MTCC 6160 in this work were collected from MTCC and Gene bank, Institute of Microbial Technology, Chandigarh. *L. plantarum* was propagated in MRS broth.

Experimental Design

Statistical software Design-Expert 8.0.7.1 was used to design the experiment. Box-Behnken for the three independent variables was performed. The independent variables considered were blend ratio, fermentation time and inoculums concentration. Dependent variables were tannin, protein, water absorption capacity (WAC) and water absorption index (WAI). Response surface methodology was used to investigate the effect of fermentation condition on the product responses. The independent



variable levels like blend ratio (50:50, 60:40, 70:30), fermentation time (12h, 24h, 36h) and inoculums concentration (1%, 3%, 5%) considered for study were selected on the basis of the results of earlier researches (Table 1).

Table 1. Independent variables considered (probiotic fermentation)

Independent variables	Coded values	Coded levels
		(-1) (0) (+1)
Blend ratio [cereals (FM + BM) : legume (BS)]	X1	50:50 60:40 70:30
Fermentation time	X2	12h 24h 36h
Inoculum concentration	X3	1% 3% 5%

*FM= Finger millet, BM= Barnyard millet, BS= Black soybean

Sample preparation

Grinded powders of grains were used to prepare composite blend ratio of weaning mix. Three levels were kept of blend ratio i.e. 50:50, 60:40 and 70:30. Amaranth grain was mixed in fixed amount 50g. Total 150g sample was prepared with varying cereals and legume ratio.

Fermentation of blend

150g of prepared sample with varying blend ratio was dissolved in distilled water up to 500ml volume. Slurry concentration was fixed at 30 % (w/v) referred from previous work done (Wakil and Oriola 2012). Probiotic strain

Table 2. Box-Behnken matrix for quality parameters of probiotic weaning mix

Expt. No.	Coded Variables			Responses			
	Blend ratio	Fermentation time(h)	Inoculum concentration(%)	Tannin %	Protein %	WAC ml/100g	WAI g/g
1	70:30	24	1	0.0007	16.4	2.1	1.05
2	60:40	24	3	0.0008	18.9	2.4	0.65
3	60:40	12	5	0.0008	17.56	2.2	1.42
4	50:50	24	1	0.001	20.1	2.6	0.58
5	50:50	12	3	0.0008	19.3	2.4	1.28
6	60:40	36	5	0.0006	18.7	2.7	1.06
7	60:40	24	3	0.0008	18.8	2.3	0.6
8	60:40	24	3	0.0008	18.8	2.4	0.64
9	60:40	12	1	0.001	17.2	2.2	1.43
10	60:40	36	1	0.0007	18.5	2.6	1.04
11	50:50	24	5	0.0007	20.3	2.5	0.6
12	60:40	24	3	0.0007	18.7	2.5	0.65
13	70:30	12	3	0.0006	15.5	2.0	1.34
14	60:40	24	3	0.0007	18.6	2.5	0.62
15	50:50	36	3	0.0006	15.5	2.8	0.95
16	70:30	24	5	0.0006	16.96	2.2	1.05
17	70:30	36	3	0.0003	16.7	2.4	1.25



Lactobacillus plantarum was inoculated in slurry with 3 varied levels of concentration i.e. 1%, 3% and 5%. Sample was kept in incubator for 12h, 24h, and 36h at 37°C. After these respective time periods, fermented slurry was transferred to aluminum trays and then kept in tray dryer at 55- 65°C for 13h. After drying, the dried sample was grounded to powder form.

Analysis

Nutritional quality of weaning mix was analyzed according to the method prescribed by the Association of Official Analytical Chemists (AOAC 1984). Tannin was determined using La Motte Tannin Kit. Water absorption capacity (WAC) and water absorption index (WAI) was determined using procedure (Anderson *et al.*, 1969).

Functional properties

Water absorption capacity

Water absorption capacity (WAC) of the flour was determined using procedure (Anderson *et al.*, 1969). A 1g sample was taken in a beaker and 10 ml of distilled water was added to it. Suspension was stirred for 5 min with magnetic stirrer and centrifuged at 3555 rpm for 30 min. volume of supernatant was measured. Difference between the volume added to the sample and volume of supernatant gave water absorption capacity.

$$\text{WAC (ml/g)} = \frac{\text{Initial volume of water} - \text{Volume of supernatant}}{\text{Wt. of flour sample}}$$

Water absorption index

The water absorption index (WAI) of the flour was determined using procedure (Anderson *et al.*, 1969). A 2.5 g sample was taken in centrifuge tubes, and 30 ml of distilled water was added to it. The tubes were then heated in a water bath at 95°C for 30 min and stirred with a glass rod after every 5 minutes. Heated samples were then centrifuged at 3000 rpm for 15 min. The supernatant was decanted completely and gel weight was taken for estimation.

$$\text{WAI (g/g)} = \frac{\text{Wt. of gel}}{\text{Wt. of dry sample}}$$

Statistical Analysis

The obtained experimental data was subjected to suitable statistical analysis using Design Expert software 8.0.7.1 version. Box Behnken design was applied to determine the significant effect of three independent variables (blend ratio, fermentation time, inoculums concentration) on dependent variables (tannin, protein, and water absorption capacity and water absorption index). A complete second order model was fitted to the data and adequacy of the model was tested considering R² (the coefficient of determination) and Fisher's F-test. The models were then used to interpret the effect of various parameters on the response.

Optimization of process parameters was carried out and then optimized set of independent variables were used to develop the final weaning mix and then other nutritional contents were evaluated. The model was considered adequate when

- The calculated F ratio was more than that of table value and the value R² was more than 80%.

A second order response function for three independent variables has the following general form:

$$Y = \hat{\alpha}_0 + \sum_{i=1}^3 \hat{\alpha}_i X_i + \sum_{i=1}^2 \sum_{j=i+1}^3 \hat{\alpha}_{ij} X_i X_j + \sum_{i=1}^3 \hat{\alpha}_i X_i^2$$

Where,

β_0 , β_{ii} , β_{ij} are constants and X_i , X_j are variables (coded)

The experimental data were then analyzed employing regression analysis to develop response functions and variable parameters optimized for best outputs. Finally verification experiment was conducted to validate the optimal results as given by Design Expert software under specific set (as selected) of experiment conditions. The experimental samples



were prepared using prescribed composition and the predicted and actual scores of attributes were compared in order to determine the validity of model. A non-significant difference between predicted and actual score of prescribed attributes revealed the accuracy of suggested optimal point

Results and Discussion

The effect of three independent variables (blend ratio, fermentation time, inoculum concentration) having

three variables with three levels [blend ratio(cereals : legume:: 50:50, 60:40, 70:30), fermentation time (12h, 24h, 36h), inoculum concentration (1%, 3%, 5%)] and four responses (protein, tannin, water absorption capacity, water absorption index) which gave 17 experiments (Table 2). A quadratic model was fitted into each response. The adequacy of the model was tested using coefficient of determination (R²) and Fisher's F-test. The model was used to analyze the effect of independent variables on responses. If the

Table 3. Results of regression analysis for various responses of weaning mix

Source	Tannin		Protein		WAC		WAI	
	Coeff.	P (%)	Coeff.	P(%)	Coeff.	P(%)	Coeff.	P (%)
Model	0.0008	0.02***	18.76	<0.01***	2.42	0.05***	0.63	0.02***
X1	-0.0001	0.02***	-1.77	<0.01***	-0.2	<0.01***	0.16	0.14***
X2	-0.0001	0.01***	0.54	<0.01***	0.21	<0.01***	-0.15	0.23***
X3	-0.00009	0.1***	0.17	0.5***	0.013	60.88	0.0038	90.79
X1 X2	-0.00003	30.83	0.12	6.78*	0.0	100	0.06	21.68
X1 X3	0.00005	6.40*	0.090	16.41	0.05	17.36	-0.005	91.31
X2 X3	0.00003	38.83	-0.04	51.20	0.025	47.35	0.0075	87.01
X12	-0.0001	0.21***	-0.22	0.03***	-0.047	18.33	0.078	11.41
X22	-0.00008	0.87***	-0.67	<0.01***	0.028	42.09	0.50	<0.01***
X32	+0.00001	0.36***	-0.1	11.22	-0.023	50.68	0.11	11.22
R ²	0.97		0.996		0.96		0.97	
Adj R ²	0.92		0.992		0.91		0.92	
F-value	21.9***		332***		20***		24.3***	
LOF	NS		NS		NS		NS	

three levels each (blend ratio: 50:50, 60:40, 70:30, fermentation time: 12h, 24h, 36h, inoculum concentration: 1%, 3%, 5%) on nutritional (protein), antinutritional(tannin)andphysical(waterabsorption index and water absorption capacity) was studied. The experiments were planned using Response Surface Methodology with Box Behnken Design for

model was found significant, the best fit equations were developed in order to draw contour plots for graphical presentation of influence of independent variables on responses and for selecting the best range of variables for developing an acceptable product. Optimization of various processes conditions was done using software Design-Expert 8.0.7.1. The data

from all the seventeen experiments were analyzed and the response functions were developed using multiple regression. ANOVA was used to analyze the models.

The statistical analysis indicated that the models were adequate because they have satisfactory values of the R² for all the responses and model significance. Results showed that the lack-of-fit test was non-significant for all responses.

Development of control weaning mix

Weaning mix formulated from untreated and unfermented grains (finger millet, barnyard millet, black soybean, and amaranth) was considered as control. Three levels [cereals (FM+BM): legume:: 50:50, 60:40, 70:30] of blend ratio same as used in experimental design (Full Factorial and Box Behnken) was decided to develop control weaning mix and similar responses (tannin, protein, water absorption capacity and water absorption index) as taken in experimental design was evaluated so that on the basis of its results, effect of pretreatments (malting and roasting) and fermentation on quality of weaning mix could be observed. The data obtained for control weaning mix is presented in Table 4.

Table 4. Experimental data for control weaning mix

Blend Ratio	Tannin (%)	Protein (%)	WAC (ml/g)	WAI (g/g)
50:50	0.91	12.63	0.35	3.4
60:40	0.9	12.1	0.32	3.7
70:30	0.87	11.7	0.28	4.3

Response surface analysis for tannin

Tannin is considered as an anti-nutritional factor as it has the capability to form insoluble complexes with proteins thereby reducing digestibility of food proteins (Ikpe-Emmanuel *et al.*, 2012). It can be clearly observed that probiotic fermented weaning mix had very low tannin content in comparison to control weaning mix. Fermentation and pretreatments given to grains reduced the tannin level. The reduction can be attributed to the result of enzymatic activity of the organism while hydrolyzing

ability was enhanced by the fermentation. The results were also found to be in accordance with those found by (Chavan and Kadam 1989) who reported decreased tannin content in cereals on fermentation. Germination has been shown to reduce tannin levels in high tannin sorghum to non-detectable levels (Mukuru *et al.*, 1992).

Regression analysis (Table 3) reveals that the coefficient of determination (R²) for the regression model for tannin was quite high 0.97, which implies that the model could account for 0.97 data. R²-adj (0.92) value was observed to be relatively close to the R² (0.97) value. It ensures a relatively satisfactory adjustment of the quadratic model to the experimental data. Model was found to be highly significant (p<0.02). The F value (21.9) was greater than F table value (6.72) at 1% level of significance.

Table 3 expresses the partial coefficients of the regression model of tannin score in terms of linear order, second order and interaction terms. Blend ratio had highly significant (p<0.02 at 1% level of significance) negative effect on tannin content in both linear and quadratic terms. The negative coefficient of linear term of blend ratio indicated that tannin content decreased with increase in level of blend ratio due to reduction in the amount of black soybean which possesses higher anti-nutritional property in comparison to finger millet and barnyard millet. Fermentation time had highly significant (p<0.01 at 1% level of significance) negative effect in linear and quadratic terms. It appears that tannin percentage decreased with increase in level of fermentation time as fermentation helps in reducing anti-nutritional property (tannin) of grains. The reduction in tannin content might be as a result of enzymatic activity of the organisms whose hydrolyzing ability was enhanced by fermentation. The similar results were found (Lopez *et al.*, 1993), who reported that lactic acid fermentation decreased the tannin content in maize. Inoculum concentration had also negative significant effect on tannin content (p<0.1 at 1% significance level). It indicates that percentage of tannin decreased with increase in level of inoculum concentration because of probiotic strain which helps in reducing the anti-nutrients in grains.



Interactive effect of blend ratio and fermentation time had negative but non-significant effect on tannin content whereas interactive effect of blend ratio and inoculum concentration had positive significant effect at 10% level of significance. The interaction effect of fermentation time and inoculum concentration on tannin profile was insignificant.

In quadratic terms blend ratio and fermentation time had highly significant ($p < 0.2$ and 0.8 at 1% significance level) negative effect on tannin profile whereas inoculum concentration had significant positive effect.

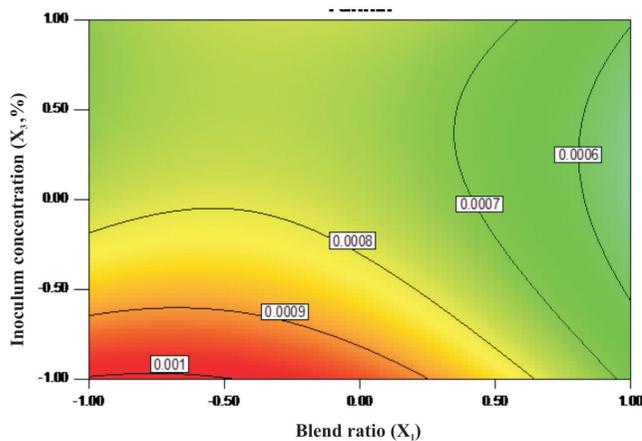


Figure 1. Contour plot between blend ratio and inoculum concentration for tannin

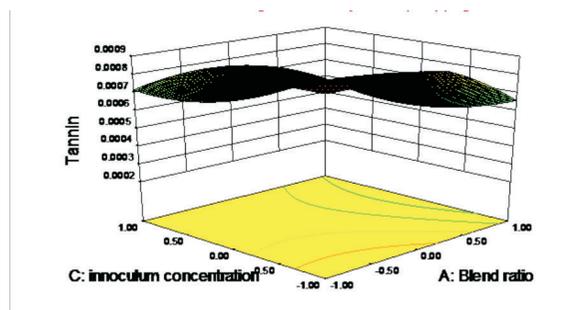


Figure 2. 3D plot showing the effect of blend ratio and inoculum concentration on tannin

Response surface analysis for protein

Protein is an important nutritional parameter to be considered to develop protein rich weaning food for infants to prevent protein energy malnutrition.

Protein in probiotic fermentation was increased due to increased microbial proliferation utilizing carbohydrate to produce protein. The response surface model was developed with values of coefficient of determination R^2 of 0.996 for protein which implies that the model could account for 99.69% data. R^2 -adj (0.992) value was observed to be relatively close to the R^2 (0.996) value. It insures a relatively satisfactory adjustment of the quadratic model to the experimental data. Model was found highly significant ($p < 0.01$). The F value (332) was greater than FTab value (6.72) at 1% level of significance.

Table 3 expresses the partial coefficients of the regression model of protein score in terms of linear order, second order and interaction terms. Blend ratio had highly significant ($p < 0.01$ at 1% level of significance) negative effect on protein content in linear term. The positive coefficient of linear term of blend ratio indicated that percentage of protein decreased with increase in the level of blend ratio. This can be attributed to the fact that black soybean contains high amount of protein. Therefore, when blend ratio was increased i.e. percentage of black soybean decreased, protein percentage got decreased. Fermentation time had highly significant ($p < 0.01$ at 1% level of significance) positive effect in linear term. It was clearly observed (Table 2) that percentage of protein increased with increase in fermentation time upto 24h but decreased after further increase in fermentation time upto 36h. This can be attributed to the fact that till 24h, due to high activity of microorganism consuming carbohydrate to produce protein and energy for their proper growth, protein percentage was increased but after that activity of microorganisms got reduced as they entered stationary phase. Inoculum concentration had also positive significant effect on protein content ($p < 0.5$ at 1% significance level). It was observed that a slight increment in protein percentage was found with increase in inoculum concentration due to the increased growth and microbial proliferation in the form of single cell protein of the starter culture (Oboh 2006). It may also be due to the structural proteins

that are an integral part of the microbial cell (Tortora et al 2002). The similar result was found (Onilude et al 2004), who reported that fermentation of blends with mixture of *L.plantarum* and *S.cerevisiae* increased the protein value of weaning food.

Interactive effect of blend ratio and fermentation time had positive significant effect ($p < 6.78$ at 10% significance level) on protein content whereas interactive effect of blend ratio and inoculum concentration had positive but non-significant effect. The interaction effect of fermentation time and inoculum concentration on protein profile was also insignificant.

In quadratic terms blend ratio and fermentation time had highly significant ($p < 0.01$ and 0.01 at 1% significance level) negative effect on protein profile

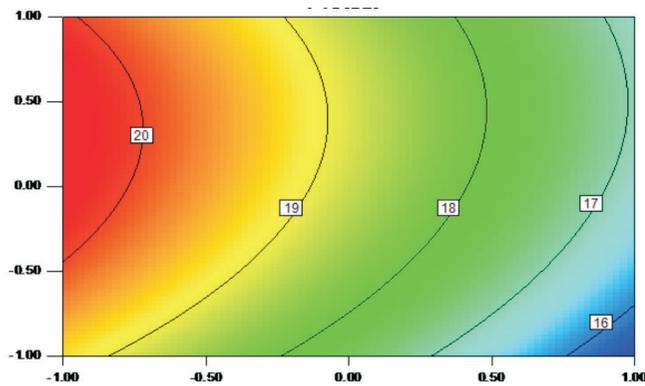


Figure 3. Contour plot between blend ratio and fermentation time

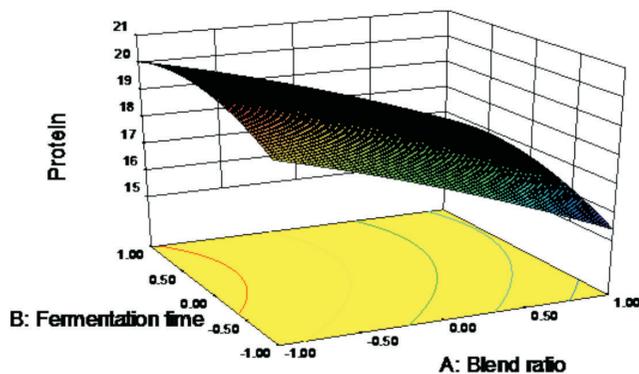


Figure 4. 3D plot showing the effect of blend ratio and fermentation time on protein

whereas inoculum concentration had negative but insignificant effect.

Response surface analysis for WAC

Water absorption capacity is a functional property. It indicates the extent to which water can be added or gives an indication of the amount of water available for gelatinization. Lower absorption capacity is desirable for making thinner gruels (Ghavidel and Prakash 2010). WAC was higher in case of probiotic fermentation due to increased number of microorganisms as starter culture was inoculated in blend. Thus increased number of active microorganisms having high proteolytic activity caused rapid denaturation of legume protein which in result increased the availability of protein functional group in flour. The response surface model was developed with values of coefficient of determination R^2 of 0.96 for WAC which implies that the model could account for 0.96 data. R^2 -adj (0.91) value was observed to be relatively close to the R^2 (0.96) value. It ensures a relatively satisfactory adjustment of the quadratic model to the experimental data. Model was found highly significant ($p < 0.05$). The F value (20) was greater than F table value (6.72) at 1% level of significance.

Table 3 expresses the partial coefficients of the regression model of WAC score in terms of linear order, second order and interaction terms. Blend ratio had highly significant ($p < 0.01$ at 1% level of significance) negative effect on WAC in linear term. WAC was found to be increased for the samples which had high amount of soybean in blend ratios. It was due to hydrophilic property of protein present in black soybean. Fermentation time had highly significant ($p < 0.01$ at 1% level of significance) positive effect in linear term. Water absorption capacity increased with increase in level of fermentation time. It was due to proteolytic activity which takes place during fermentation and helped in increasing the availability of polar groups in protein and thus, increased the hydrophilicity of flour protein. Inoculum concentration had also positive but insignificant effect on WAC.



Interactive effect of blend ratio and fermentation time had highly non-significant effect on WAC whereas interactive effect of blend ratio and inoculum concentration had positive but non-significant effect. The interaction effect of fermentation time and inoculum concentration on tannin profile was also positive but insignificant.

In quadratic terms blend ratio and inoculum concentration had negative but non-significant effect on WAC profile whereas fermentation time had non-significant positive effect.

Response surface analysis for WAI

Water absorption index or swelling capacity is a functional property of flour and it depends on the availability of starch content in weaning mix. It measures the volume occupied by the starch after swelling in excess water (Mason and Hosene 1986) which corresponds to the volume of the gel formed. WAI depends on the availability of hydrophilic groups and on the capacity of gel formation of the macromolecules (Gomez and Aguilera 1984). It can be clearly expressed that in the presence of starter culture, water absorption index decreased rapidly as water absorption index for probiotic weaning mix was less than that for control weaning mix. This result could be correlated with the fact that due to inoculation of starter culture, increased microbial population could account for higher degradation of starch molecules and resulted in less starch which was available to be gelatinized. This statement can be supported by the work of (Ejiofor and Okafor 1981) who confirmed the reduction of carbohydrate by the activities of amylase for the breakdown of starch to simple sugars. Thus, reduction in volume occupied by starch after swelling in excess water i.e. volume of gel formed resulted in decreased water absorption index during fermentation.

The coefficient of determination (R^2) for the regression model for WAI was 0.97 which implies that the model could account for 0.97 data. R^2 -adj (0.92) value was observed to be relatively close to the R^2 (0.97) value. It ensures a relatively satisfactory adjustment of the quadratic model to the experimental data. Model

was found highly significant ($p < 0.02$). The F value (24.3) was greater than F_{tab} value (6.72) at 1% level of significance.

Table 3 expresses the partial coefficients of the regression model of WAI score in terms of linear order, second order and interaction terms. Blend ratio had significant ($p < 0.14$ at 1% level of significance) positive effect on WAI in linear term. Water absorption index increased with increase in level of blend ratio. This can be attributed due to the high amount of carbohydrate (starch) present in blend containing the highest amount of cereals (70:30) as water absorption index depends upon the starch content. Fermentation time had significant ($p < 0.23$ at 1% level of significance) negative effect in linear term. It is clearly evident (Table 2) that water absorption index decreased with increase in fermentation time upto 24 h whereas an increment in value of WAI was observed with further increase in fermentation time up to 36h. This could be due to the high consumption of starch during enhancement activities of microorganisms till 24h but after that gradually decreased activity of microorganisms increased the availability of macromolecules (carbohydrate), thus increased WAI. Inoculum concentration had positive but non-significant effect on WAI.

Interactive effect of blend ratio and fermentation time had positive but non-significant effect on WAI, whereas interactive effect of blend ratio and inoculum concentration had negative insignificant effect. The interaction effect of fermentation time and inoculum concentration on WAI was positively insignificant.

In quadratic terms blend ratio and inoculum concentration had positive but non-significant effect on WAI profile whereas fermentation time had highly significant ($p < 0.01$ at 1% significance level) positive effect on WAI.

Optimization of independent variables

Optimization is a process of making compromises between responses, to achieve a common target. Numerical optimization was carried out using

Design-Expert 8.0.7.1 software. During optimization, 13 solutions were obtained out of which the one that suited the criteriamost was selected. The optimum level of independent variables in coded and actual form is given in the Table 5. The values of corresponding responses were 0.0005% for tannin, 16.8% for protein, 2.18 ml/g for WAC and 0.87 g/g for WAI.

The optimized set of independent variables (blend ratio 70:30, fermentation time 25h, inoculum concentration 2.9%) obtained from Design-Expert

Table 5. Optimum values of independent variables

Value	Blend ratio (X1)	Fermentation time(h) (X2)	Inoculum concentration (%) (X3)
Coded	+1	0.08	-0.04
Actual	70:30	25	2.9

(tannin, protein, water absorption capacity and water absorption index) were compared with the values given by software after optimization to verify the results. Verification of the optimized formulations was done by preparing the selected sample three times and evaluating all attributes. The results revealed that the actual values of all attributes are close to predicted values (as shown in Table 6) and ANOVA analysis shows that variation between predicted score and actual score is highly non-significant ($F_{tab} > F_{cal}$). Thus, the formulation of weaning mix containing 23.3% finger millet, 23.3% barnyard millet, 20% black soybean, 33.3% amaranth grain with 2.9% inoculums concentration of probiotic strain *Lactobacillus plantarum* and 25h fermentation time could be considered as optimum.

Conclusion

The present work focused on nutritional enhancement of weaning food made of underutilized crops of

Table 6. Comparison of actual and predicted values of responses

Responses (Quality Attributes)	Predicted Score* (Per 100g)	Actual Score# (Per 100g)	PFA Standards (per 100g)	F-Value
Carbohydrate	-	70.99g	≥55.0g	
Protein	16.8g	16.3g	≥15g	4.2
Fat	-	2.5g	≥9g	
Iron	-	12.6mg	≥5mg	
Calcium	-	386.4 mg	≥230mg	
Total Calorific Value	-	406 kcal	-	
Tannin	0.0005mg	0.0006mg	-	1.45
Water Absorption Capacity	218ml	212ml	-	20.2
Water Absorption Index	87g	85g	-	2.14
Microbial Count	-	4.2×106 CFU	5×106 CFU	

*Predicted values obtained from Design Expert.

#Actual (Average of three trials) of optimized weaning mix.

F_{tab}= 21.19 (1% significance level)

Verification of optimized results

software, was used to develop optimized weaning mix. Skimmed milk powder (Sagar Skimmed Milk Powder of Amul Brand) was added 20% (w/w). Proximate composition was evaluated and compared with PFA standards. The values of four responses

Uttarakhand viz. Finger millet, Barnyard millet, Amaranth grain and Black soybean. Weaning food was produced from probiotic fermentation technology by using probiotic strain *Lactobacillus plantarum*. Experimental studies and data analysis revealed that probiotic Fermentation was quite



effective for increase in protein content and decrease in tannin. Slight reduction in water absorption index was observed, while water absorption capacity was slightly increased. The end product (weaning mix) contained 23.3% finger millet, 23.3% barnyard millet, 20% black soybean, 33.3% amaranth grain. The probiotic fermentation resulted in favourable changes in nutritional profile of weaning mix resulting 48.2% increase in protein and 92.3% decrease in tannin content. From the present study, nutritional benefits of probiotic weaning mix could be observed. Hence, it can be recommended that the probiotic fermentation technology possess a good potential to develop nutritionally enriched weaning mix by utilizing the underutilized crops of Uttarakhand.

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