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Development of Fermented Millet Sprout Milk Beverage Based on Physicochemical Property Studies and Consumer Acceptability Data

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Millets play an important role in the food and nutritional security of the poor in arid and semiarid regions of the world. The present study focused on the use of underexploited millets for production of fermented millet based milk beverage. The beverage was prepared using skimmed milk along with three different millets (finger millet, pearl millet and sorghum). The techniques used for processing millets are soaking, sprouting and extraction of milk from millets. The ratio of millet milk blending with skimmed milk was optimized using Mixture Design based on physicochemical properties viz., sediment, viscosity, wheying off, acidity and sensory responses. The overall acceptability of the optimized sample was 7.1, which is very close to the predicted value. The nutritional analysis was done for the sample prepared using optimized combination of millet milk.

Keywords: fermentation, millet sprouts, beverage, mixture design, sensory response

Introduction

Fermentation is a natural way to augment the nutritive value and appearance of the food, to reduce the energy required for cooking and to make a safer product[1]. Fermented foods, which contribute to about one-third of the diet worldwide, are produced using various manufacturing techniques, raw materials and microorganisms[2]. The fermented milk-cereal flour products like Boza, Boshera, Mahewu, Pozol, Rabbadi and Togwa are extremely popular in most of the African countries and Indian sub-continent[3]. For the preparation of these products, generally, buttermilk or whey or curd and cereals like pearl millet (*Pennisetum typhoideum* L.), wheat (*Triticum aestivum*), rye (*Secale cereale*) and sorghum (*Sorghum vulgare*) are used. Processing and utilization of millets are largely confined to home scale that renders many of the valuable nutrients unavailable to human beings on wider scale. Millets are nutritionally, especially in micronutrient content superior to the commonly consumed cereals[4]. Luteolin, a flavone present in sorghum and millets is reported to have antioxidant, anti-inflammatory, cancer preventive and anti-arrhythmic properties. Value-addition and improving health benefits of millets by combining with traditional cereals and milk and by applying advanced technologies for their processing and preservation opens new avenues for the product diversification. The aim of the present study is to formulate and analyse the fermented millet milk beverage using sorghum (*Sorghum vulgare*), pearl millet (*Pennisetum typhoideum*) and finger millet (*Eleusine coracana*).

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Materials and methods

Skimmed milk was purchased from Aavin milk dairy located in Erode, India and heated to 90˚C for 5 min before use so as to suppress the microbial activity. Millets and spices like cumin (*Cuminum cyminum*) and black pepper (*Piper nigrum*) were purchased from the local market. The total solids content (TS) and fat of skimmed milk was in the range of 8.4% to 9% and 0.1% to 0.4% respectively. The titratable acidity of skimmed milk was between 0.12 and 0.15%. Fresh curd was used as a starter culture. All chemicals used were of AR grade.

Preparation of millet milk

From soaked millets

Millets were washed well with water to remove dirt and soaked in water separately for about 3 hours. The millets were ground well using mixer- grinder for milk extraction. Millets and water were taken in the ratio of 1: 2 for grinding. The milk obtained was filtered using muslin cloth for clarification.

From millet sprouts

Millets were washed well with water to remove dirt and soaked in water separately for about 12 hours and allowed to germinate for 48 hours. Millet milk was extracted as the same from soaked millets.

Formulation of beverage

Skimmed milk was combined with millet milk from soaked millets and sprouts separately in three different proportions (25:75,50:50, 75:25) and inoculated with starter culture on the basis of 2 % for the whole milk and incubated at 37º C for 12 hours. It was kept undisturbed for 12 hours to enhance efficient curdling of milk. After the formation of curd, it was blended well in an electric mixer (Preethi, Chennai, India). Salt and spices were added during the blending process. Beverage prepared by different combinations of skimmed milk and millet milk was evaluated for its sensory characteristics by a panel of 8 judges from the faculty of Food Technology Department; using 9-point Hedonic scale[5]. The combination of skimmed milk and millet milk from sprouts in the ratio of 25:75 had maximum average overall acceptability. Thus, it was selected for further investigation.

Experimental Design

Mixture experiment is a special type of response surface experiment in which factors are the proportions of components in a mixture and their levels are not independent of each other[6]. Mixture experimental designs are used for the optimization of ingredients of products that involve more than one ingredient or ingredients that possess an alternative substance. The design provides a predictive mathematical representation of the relationship between mixture factor and responses to observe the effects of the ingredients on foods, and ingredient interactions[7]. Mixture Simplex Lattice Design was used to design the experiment using Design Expert® software version 8.0.7.1. Therefore, the aim of this methodology was to verify how the properties of interest were affected by the variation in millet milk mixture keeping their total composition constant. The proportional levels of the millet milk were set at 15–30%. Fourteen combinations of the millet milk are given in Table 1. The following quadratic equation was fitted to data obtained from the experimental point. The following polynomial equation was used: $Y= \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_{12} + \beta_{13} X_{13} + \beta_{23} X_{23}$ where Y is the predicted response (sediment, viscosity, wheying off, acidity and overall acceptability); β_1, β_2, β_3, β_{12}, β_{13} and β_{23} are the linear and non-linear constants for each term and X is the millet milk concentration used in the fermented millet milk beverage (X_1 finger millet; X_2 pearl millet and X_3 sorghum)

Physico-Chemical Analysis

Sediment and wheying off (%)

10 ml of fermented millet milk beverage was taken in a graduated measuring tube and kept undisturbed in the refrigerator for 24 hours. The amount of whey separated at the top and sediment settled was measured and expressed in percentage and millilitre respectively.

Viscosity

The viscosity of the fermented millet beverage was analysed at 25±0.1° C using 1-1 coaxial cylinder Brooke field viscometer. When the instrumental set up was made ready, the torque was set to zero and the viscosity (cP) was measured at a minimum rpm of 50.

Overall acceptability

The overall acceptability of the fermented millet beverage was analyzed using 9 point hedonic scale based on the taste.

Nutritional Analysis

The carbohydrate, crude protein, fat and iron were determined by AACC and AOAC methods[8,9].

Statistical analysis

The experiment was designed and responses were analyzed using software Design Expert version 8.0.7.1.

Table 1—Millet milk composition of fermented millet milk beverage in a simplex lattice mixture design

Sample code	Finger millet (FM) milk (%) X_1	Pearl millet (PM) milk (%) X_2	Sorghum (SM) milk (%) X_3
S1	25	25	25
S2	22.5	30	22.5
S3	30	15	30
S4	30	22.5	22.5
S5	15	30	30
S6	30	30	15
S7	22.5	22.5	30
S8	20	27.5	27.5
S9	30	15	30
S10	15	30	30
S11	30	30	15
S12	22.5	22.5	30
S13	27.5	27.5	20
S14	27.5	20	27.5

All physico-chemical determinations were done with an average of three replicates, whereas sensory analysis was carried out with an average of eight replicates.

Results and discussion

Effect of germination

The fermented beverage prepared using millet milk extracted from soaked millets and millet sprouts were subjected to sensory analysis. The beverage made from milk of millet sprouts were acceptable in terms of sensory property (scores 7.1–7.3). On account of better acceptability by the sensory panellists, milk from millet sprouts were selected for preparation of fermented millet milk beverage for further experiments. The beverage prepared from milk of soaked millets was unacceptable, which may be due to high sedimentation value and wheying-off percentage. Also the beverage prepared from the sprouts have higher protein digestibility since germination is followed by fermentation[10].

Physico-chemical Properties of fermented millet sprouts milk beverage

The sedimentation value of the studied samples varied between 0.4 – 0.85 ml/10ml, being the highest for the sample S7 and lowest for the S2 sample (Table 2). The viscosity of the samples was in the range of 149.1 and 166.3 cP. The maximum viscosity was observed for the S6 and S7 samples. The wheying off of the samples varied from 0.014 – 0.020%. The highest was observed in S7, S10 and S12 samples and lowest in S4 and S13 samples. The acidity of the samples were in the range of 0.608 – 0.987% lactic acid. The samples S7 and S12 showed highest acidity and lowest was found in S2 sample. Table 2 Sediment, viscosity, wheying off, acidity and overall acceptability of the model fermented millet milk beverage

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Sensory responses of fermented millet sprout milk beverage

Sensory score for fermented millet milk beverage varied for 6.4 – 7.1 for overall acceptability. The predicted model for the overall acceptability is reported in Table 3. R^2>0.80 for sensory attributes is statistically adequate for developing a model or equation for biological materials[11]. Table 3 Predicted models for the experimental data of the fermented millet milk beverages

Effect of the interaction between the millet milk on the physico-chemical and sensory responses

Sedimentation of fermented millet sprout milk beverage

The mixture design was applied to determine the significance of the interaction between the three different millet sprout milk. Table 3 shows the predicted models, significance of regression coefficients and R^2 values for sediment value of the fermented millet sprout milk samples. It is observed that R^2 value of the model was higher than 0.75, indicating that the equation can be used for the prediction of the parameters[12]. All the physico-chemical responses were significantly influenced by the milk from the sprouts of pearl millet, finger millet and sorghum. Sedimentation refers to settling of solids in the beverage. Though the beverage was

Table 2—Sediment, viscosity, wheying off, acidity and overall acceptability of the model fermented millet milk beverage

Sample code	Sediment (ml)	Viscosity (cP)	Wheying off (%)	Acidity (% LA)	Overall acceptability
S1	0.55	153.2	0.016	0.643	6.9
S2	0.4	149.1	0.015	0.608	6.4
S3	0.9	159.5	0.016	0.913	6.5
S4	0.7	156.5	0.014	0.63	7.1
S5	0.7	160.7	0.02	0.912	5.8
S6	0.7	165.3	0.015	0.612	6.8
S7	0.85	166.3	0.019	0.987	6.7
S8	0.55	154.8	0.018	0.792	6.7
S9	0.8	158.3	0.016	0.814	6.5
10	0.75	158.3	0.019	0.863	5.9
S11	0.7	164.7	0.015	0.612	6.8
S12	0.8	165.2	0.019	0.987	7
S13	0.55	153.6	0.014	0.612	6.9
S14	0.71	159.3	0.016	0.805	6.8

Table 3—Predicted models for the experimental data of the fermented millet milk beverages

Parameter	Predicted models	R^2
Sediment	$7.04142X_1^* - 3.25382X_2^* + 11.87418X_3^* + 2.02020\ X_1X_2 - 33.99219\ X_1X_3^* - 9.84933\ X_2X_3^{**}$	0.8066
Viscosity	$272.50230X_1 - 53.23580X_2 + 739.53079X_3 + 602.09363\ X_1X_2^* - 1452.41586\ X_1X_3^* - 634.55872\ X_2X_3^*$	0.8408
Wheying off	$-1.04552E\text{-}003X_1^* - 8.90266E\text{-}003X_2^* + 0.13583X_3^* + 0.13246\ X_1X_2^* - 0.21621\ X_1X_3^* - 0.15192\ X_2X_3^*$	0.9507
Acidity	$-0.13658X_1^* - 1.50349X_2^* + 10.16607X_3^* + 9.73875\ X_1X_2^* - 16.32916\ X_1X_3^* - 12.63630\ X_2X_3^*$	0.8663
Overall Acceptability	$-3.45189X_1^* - 18.45189X_2^* + 2.47629X_3^* + 67.74537\ X_1X_2^* + 11.40995\ X_1X_3 + 41.40995\ X_2X_3^*$	0.8447

**p*<0.01; ***p*<0.05

prepared by using solution suggested by Mixture design, the sedimentation of millet solids was zero after 10 hours, it gradually increased on standing. Because of low pH, acidic milk products suffer from protein sedimentation, which leads to whey separation on storage[13]. Interpreting from the regression coefficients of the linear terms of the predicted model equation, FM milk and SM milk tend to increase sedimentation value of the beverages. As seen in the Table 3, all of the linear terms were significant (p<0.01), and the interaction between FM/SM and PM/SM milk combinations were also found to be significant. Both of them exerted an antagonistic effect on the sedimentation value of the samples. The sample S7 had the highest sedimentation value of 8.5ml. The variation in the sedimentation value may have resulted from the solid content of the millet milk.

Viscosity of fermented millet sprout milk beverage

Table 3 shows the predicted models, significance of regression coefficients and R^2 values for viscosity value of the fermented millet sprout milk samples. The p values were used to identify the significance of the coefficients. The higher the magnitude of p, the less significant the corresponding coefficient is. If the p values are less than 0.05 or 0.01(based on significance level), the model terms are accepted as significant[7]. There were no significant linear terms for the prediction of viscosity while all the non-linear terms were significant. The combination of FM/PM milk had synergistic effect on viscosity. The sample S6 and S7 had highest viscosity values of 166.3 cP.

Wheying off and acidity of fermented millet sprout milk beverage

Wheying off refers to the separation of water from the beverage on standing. The R^2 value of the predicted model for the wheying off was close to unity, indicating this equation may be used for the prediction of the wheying off values of the samples based on different millet sprout milk combinations. These values are important to determine the significance of equations or models developed from the design[11]. Interpreting from the regression coefficients of the linear terms of the predicted model equations, FM milk and PM milk caused to decrease wheying off of the samples and SM milk tend to increase. As seen in the Table 3, all the linear terms and non-linear terms were significant (p<0.01). The samples S4 and S13 showed lowest wheying off value. Acidity of the beverage was significantly influenced by all the linear terms and non-linear terms. The highest acidity of 0.987% lactic acid was found in samples S7 and S12 and lowest of 0.608% was found in S2 sample.

Sensory responses of fermented millet sprout milk beverage

Table 3 shows those linear and non-linear terms significantly influence the overall acceptability of the beverage. The model demonstrates the interaction between FM/PM milk, FM/SM milk and PM/SM milk has synergistic effect on overall acceptability. Fig. 1 Effect of pearl millet, finger millet and sorghum millet on overall acceptability

Optimization of millet sprouts milk combination based on sensory responses

Our aim was to optimize the millet milk combination for the preparation of beverage based on the overall acceptability of the product. The optimum level obtained for FM milk, PM milk and SM milk was 30%, 23.9% and 21.1% respectively. The predicted and actual response values for overall acceptability of the fermented millet sprouts milk beverage (obtained after making the product using the optimum level of millet milk) was 7.09 and 7.1

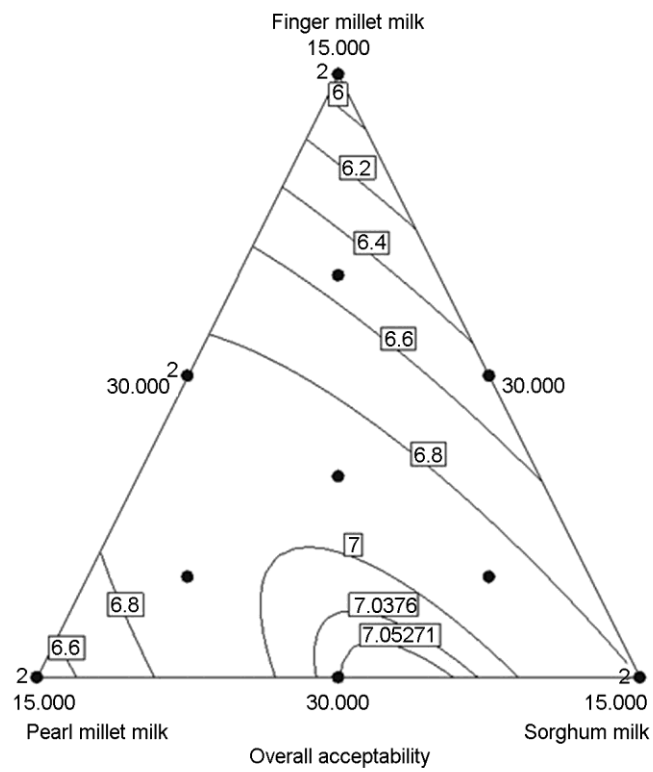

Fig. 1—Effect of pearl millet, finger millet and sorghum millet on overall acceptability

respectively, which can be observed that both the values were almost similar, hence, the above levels of millet milk were recommended for the preparation of the beverage.

Nutritional analysis

The variation in nutrient content is important because of the effects it can bring in nutritional requirements. Even though RSM methodology was used to optimize the combination of ingredients for the product development, the nutritional value of the final product plays a vital role in enhancing the consumer acceptance of the product. Henceforth the nutritional value of the optimized combination of millet milk was analyzed for the nutrients like fat, iron, protein and carbohydrates and found to be 2.1%, 340 mg, 450 mg and 14.57 mg respectively, in 100ml of fermented millet sprout milk beverage.

Conclusion

Optimized fermented millet sprout milk beverage contained 0.5% protein, 1.3% fat, 7.1% TS and 0.23% iron. The acidity of beverage was 0.587% lactic acid. The average overall acceptability score of final beverage was 7.1. The current study revealed the feasibility for the development of fermented millet sprout milk beverage based on sensory response and physicochemical properties. The method standardized for production of fermented millet sprout milk beverage is very simple and can be applied for industrial production. As a result of this study, it may be concluded that these types of models may be used in industry to optimize the formulation of products, to minimize product costs and to ensure consumer acceptability of the product.

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