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Optimization of Rabadi-like Sorghum-based Fermented Milk Beverage

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ABSTRACT

Rabadi, prepared by fermenting sorghum (Sorghum bicolor variety PC-9) flour with buttermilk, is a traditional popular beverage of North-Western states of India. A process for sorghum-based *Rabadi*-like fermented milk beverage was attempted. Skim milk and flour of 24 h germinated sorghum grains (FGG-24 h) were used as sources of solids. FGG-24 h was mixed in skim milk before fermentation and the level of flour and water were determined using Response Surface Methodology (RSM) with central composite rotatable design (CCRD). The product developed using 4.7% flour and 69% water based on curd gave the most acceptable product. For further stabilization during storage, pectin and/or carboxymethyl cellulose were tried at different levels and a level of 0.6% pectin was selected. The standardized product was packaged in glass bottles and stored under refrigeration (5–7 °C). The shelf life of the product was 7 days.

KEYWORDS

Rabadi, Sorghum, Fermented milk beverage

INTRODUCTION

Fermentation is one of the oldest applied biotechnologies used in food preservation for over 6,000 years. Traditional fermented foods are made under primitive conditions, which result in low yield and poor quality. They can be "reinvented" by applying modern process technologies for mass production to meet the consumer's new demand in response to changing lifestyles. The fermented milk-cereal flour products are extremely popular in most African countries and the Indian sub-continent. The process of manufacture of these products differs from place to place and even within the same country. For the manufacture of these products generally, buttermilk or whey or curd and cereals like sorghum (*Sorghum bicolor* L.), wheat (*Triticum aestivum* L.) and pearl millet (*Pennisetum typhoideum* L.) are used. Besides, spices may be added along with salt with various combinations and levels. In the present study, sorghum was selected for the preparation of *Rabadi* because of its unique functional bland flavor, light colour and nutritional quality (High protein; 10.4% with good biological value and digestibility).

Rabadi is an indigenous natural cereal-based lactic fermented milk beverage popular in North-Western semi-arid regions of India. Traditionally *Rabadi* is prepared by admixing cereal flour with sour buttermilk in different proportions. The mixture is further fermented by placing in sunlight for 3–4 hrs, followed by boiling, salting and cooling. Rabadi is a nutritionally superior, microbiologically safe and organoleptically acceptable natural beverage. So far, the technology of producing Rabadi remains a household art, which results in wide variation and reduced shelf-life.

Moreover, the traditional method is not suitable for large-scale production. Excellent prospects exist in India for value-addition and improving health benefits of milk and milk by-products by combining with traditional cereals and applying advanced technologies for their processing and preservation. No work of technological or scientific significance has been reported in the literature on either standardization of method or large-scale production of Rabadi with standard composition. It was, therefore, planned to develop processing technology for preparing *Rabadi*-like sorghum-based fermented milk beverage.

MATERIALS AND METHODS

The essential ingredients like sorghum (Sorghum bicolor variety PC-9), salt, cumin (*Cuminum cyminum*) and black pepper (*Piper nigrum*) were procured from the local market. Fresh mixed skim milk was taken from the Experimental Dairy, NDRI, Karnal. The fat and total solids (TS) content of skim milk ranged from 0.05% to 0.2% and 8.8% to 9.2%, respectively. The titratable acidity of skim milk was between 0.14 and 0.16 percent. Mesophilic mixed strain Dahi culture NCDC-167 was supplied by the National Collection of Dairy Cultures (NCDC), Dairy Microbiology Division, NDRI, Karnal. Churning Dahi prepared sour buttermilk in hand churn. The buttermilk had 1.2% fat, 7.78% TS and 0.93% acidity as determined by Indian Standard Part XI (1981) and AOAC (1995) methods. All chemicals used were of AR grade.

Forms of sorghum

Solids sorghum was used in the form of flour of raw grains, germinated grains and in the form of a slurry of cooked grains in water with 15% TS. Types of milk solids

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were used in the form of skim milk and sour buttermilk. The sorghum solids and milk solids were blended at two different stages namely before & after fermentation for the formulation of the *Raabdi*-like beverage. Before fermentation Skim milk was heated to 40°C and sorghum solids (flour, slurry and germinated flour) at 5% on milk basis were added. This blend was then further heated to 90°C for 5 min and cooled to 37°C. It was then inoculated with starter culture at 3% and incubated at 37°C for 12 h. The set curd was then appropriately mixed and blended with water to have a viscosity of 45–65 cp. The mixture was blended with salt (0.80%), cumin (0.25%) and black pepper (0.05%) in an electric mixer (Sumeet Machines Pvt. Ltd., Nasik, India) (EM). The levels of these ingredients were decided on the basis of preliminary trials. The final product was cooled to 5°C and stored in a refrigerator (5–7°C).

After fermentation

Skim milk was heated to 90°C for 5 min and cooled to 37°C. It was then inoculated with starter culture at 2% and incubated at 37°C for 12 h. The set curd was then mixed properly and blended with sorghum solids (flour, slurry and germinated flour) at 5% on milk basis and water to have viscosity of 45–65 cp. The mixture was blended with salt (0.80%), cumin (0.25%) and black pepper (0.05%) in an EM. The final product was cooled to 5°C and stored in refrigerator (5–7°C). Beverage prepared by both methods was evaluated for its sensory characteristics by a panel of 8 judges from the faculty of Dairy Technology Division; using 9-point Hedonic scale (Stone *et al.*, 1974). The combination of germinated flour addition before fermentation had maximum average overall acceptability. Thus, it was selected for further investigation.

Formulation of beverage

The beverage was prepared using the before fermentation method as described earlier with varying levels of 24 h germinated flour @ 3 to 8% and curd is set. The set curd was then mixed and added with water at varying levels of 50 to 100% by weight of the curd formed and blended, added with spices and stored as described under before fermentation method.

Selection of Stabilizer

Two stabilizers that are effective at acidic pH, namely, pectin and carboxy methylcellulose (CMC) were selected to determine their levels in the final product. During the manufacture of beverage, the water to be mixed was heated to 60C and varying levels of stabilizers (not in combination) were added to it as follows: CMC @ 0.05 to 0.15%; pectin @ 0.4 to 0.6%.

Storage study of the optimized sample

The selected final product was filled in sanitized 200 ml glass bottles. Filled glass bottles were crown corked with the help of a manually operated crown-corking machine in the UG/PG lab, DT Division, NDRI, Karnal. Then these bottles were kept at refrigerated temperature (5–7°C) and analyzed daily for changes in acidity, viscosity, sedimentation and sensory attributes.

Analysis of sedimentation value and wheying-off

Ten milliliters of the beverage was taken in a 15 ml graduated capped tube and kept undisturbed in the refrigerator for 24 h. The amount of cereal flour settled in ml was noted as sediment and the amount of whey separated was noted and expressed as ml sediment and percentage wheying-off, respectively.

Viscosity

The viscosity of the samples was determined at 25±0.1°C using a 1-1 system of coaxial cylinder viscometer (Contraves Rheomat 108 ER, Switzerland). The viscosity was measured at a shear rate of 100 s^{-1}. The experiment was conducted using a statistically thermo-controlled water bath. The result obtained in Pa.s. was then converted into cP using the following formula:

$$\text{Viscosity (cP)} = \text{Viscosity (Pa.s.)} \times 100$$

Statistical analysis

Statistical analysis of the present investigation was designed using response surface methodology (RSM) by employing central composite rotatable design (CCRD). The experiment was designed using software Design-Expert version 6.0.10. In these two process variables, namely, FGG-24 h and water (by weight of the curd) were taken. The matrix designed is illustrated in Table 1. All physicochemical determinations were an average of three replicates, whereas sensory data were average of eight replicates.

Table 1: Process variables and their coded and uncoded levels

	-1.414	-1	0	1	1.414
FGG24 h, % of skim milk, X_1	3	3.73	5.5	7.27	8
Water, % of curd, X_2	50	57.32	75	92.68	100

RESULTS AND DISCUSSION

Effect of milk solid source

Two sources of milk solids, namely, sour buttermilk and skim milk, were tried. The beverage made from sour buttermilk and skim milk were acceptable in terms of sensory quality (scores 7.4 - 7.6). The viscosity of beverage prepared from buttermilk (62 cp) was slightly higher than that of skim milk (55 cp) based beverage. The beverages made from both the bases were found to be more or less similar in both sensory and physicochemical aspects. The preparation of sour buttermilk is a time consuming and tedious process in addition to losses of fat in it. Taking into consideration the industrial production of beverage, skim milk was selected as the base for the preparation of *Rabadi*-like beverage. Further experiments were therefore carried out using skim milk as source of milk solids.

Effect of form and stage of the addition of sorghum solids to skim milk

Out of total seven combinations tried (Raw sorghum flour, FGG-24 h and flour of 48 h germinated sorghum grains (FGG-48 h) in 2 stage before and after fermentation and re-fermentation of raw flour), flour of 24 h germinated grains when added to milk solids before fermentation was found better than other forms and stage of addition with average overall acceptability score of 7.37 on 9-point Hedonic scale. Also Dhankher and Chauhan (1987a); Dhankher and Chauhan (1987b) had reported that germination followed by fermentation proved to be more effective in increasing the protein digestibility of sorghum. Raw sorghum flour and wet grounded slurry in all forms provided astringent flavour to the beverage, thus the scores were low. FGG-48 h caused excessive settling, thus increasing sedimentation in both stages of addition, i.e. before and after fermentation. Thus, in all subsequent trials beverage was prepared by adding sorghum flour obtained by grinding 24 h germinated grains (FGG-24 h) to skim milk before fermentation.

Effect of variables on beverage properties

Sensory scores of *Rabadi*-like beverage for colour and appearance varied from 6.75 to 7.75, consistency 6.5 to 7.5, flavour 6.45 to 7.31 and overall acceptability 6.64 to 7.27. The scores of physicochemical responses varied from 0.49 to 0.65% (lactic acid), 0 to 1.5 ml/10 ml and 37 to 140 cp for acidity, sedimentation and viscosity, respectively. A second-order polynomial was fitted into the data for all responses and the results are reported in Table 2.

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{12} AB$$

Where, Y is dependent variable; β_0, β_1, β_2, β_{11}, β_{22} & β_{12} are regression coefficients; A & B are independent variables.

R^2>0.80 for sensory attributes is statistically adequate for developing a model or equation (Henika, 1982). Presently R^2 was >0.80 for all the sensory responses (Table 2). The probability value (p) showed the adequacy of the models so used to describe the effect of variables on different responses. The effect of flour (FGG-24 h) and water on the responses are shown in Table 2. The sign and magnitude of coefficients indicate the effect of the variable on the responses.

Effect of variables on sensory responses

Table 2 shows that flour (FGG-24 h) affected most of the sensory responses except overall acceptability at the linear level and water decreased consistency at a linear level. The interaction between the variables also had an effect on the sensory responses except for consistency.

Table 2: Coefficient of second order polynomial regression models

Coefficient	C&A#	Consistency	Flavour	OA$	Acidity	Sediment	Viscosity
β_0	7.256	7.334	7.256	7.208	0.558	0	64.6
β_1	-0.186*	0.121*	-0.154*	-0.045	0.047*	0.515*	23.38*
β_2	-0.121	-0.228*	-0.099*	-0.095*	0.040*	0.338*	23.54*
β_{11}	-0.121	-0.170*	-0.289*	-0.186*		0.437*	11.32
β_{22}	-0.113	-0.132*	-0.154*	-0.151*		0.187	2.075
β_{12}	0.175	0.080	0.225*	0.195*		0.250	2.250
R^2	0.89	0.87	0.97	0.94	0.86	0.93	0.89
F	5.31	9.37	49.31	25.42	30.40	19.77	11.27

* $p \leq 0.05$, # Colour & Appearance, $ Overall Acceptability

Colour & appearance, as well as Consistency of the product, were significantly influenced by levels of both parameters. The negative sign of the partial coefficient indicates the negative effect of flour on colour and appearance score. Higher levels of water and flour (FGG-24 h) have an inverse effect on the consistency score of a product. The water had a significant negative effect while flour (FGG-24 h) had a positive effect at the linear level. The flavour of the product was also significantly influenced by levels of both parameters, with the increase in flour (FGG-24 h) above a certain level; a significant decrease in flavour score was observed.

The regression equation of overall acceptability reveals that both flour and water have negative coefficients at both linear and quadratic levels. The significance of level of flour at quadratic level indicates that a decrease in overall acceptability after attaining a particular value. But the level of water has a significant negative effect at both linear and quadratic level this indicate as the level of water increases the overall acceptability score decreases significantly. The response surface for overall acceptability scores in relation to flour (FGG-24 h) and water is shown in Fig 1. The maximum score of overall acceptability was observed when 5.5% flour (FGG-24 h) and 75% water was used in the formulation.

Effect of variables on physicochemical responses

All the physicochemical responses were affected significantly by both the parameters (Table 2). The acidity of the beverage was significantly influenced by the level of both flour (FGG-24 h) and water but the flour had a more significant effect. There was a decrease in acidity as the amount of water and flour (FGG-24 h) increased. Sedimentation and viscosity were also affected by both flour (FGG-24 h) and water, both variables tend to favour sedimentation in the beverage, while flour (FGG-24 h) increased and water decreased the viscosity.

Optimization of independent variables

The optimum level obtained for flour (FGG-24 h) was 4.7% by weight of skim milk, and for water 69% by weight of set curd. The predicted and actual response values (obtained after making the product using the optimum level of ingredients) have been presented in Table 3 from which it can be observed that both the values were almost similar, hence, the above levels of ingredients were recommended. Optimized *Rabadi*-like beverage contained 0.23% fat, 8.87% TS, 2.47% protein and 1.32% ash. The acidity of beverage was 0.55% lactic acid. The standard plate count was 9.5×10^6 cfu/ml, the yeast and mold count was to be 25 cfu/ml and no coliforms were found in the beverage.

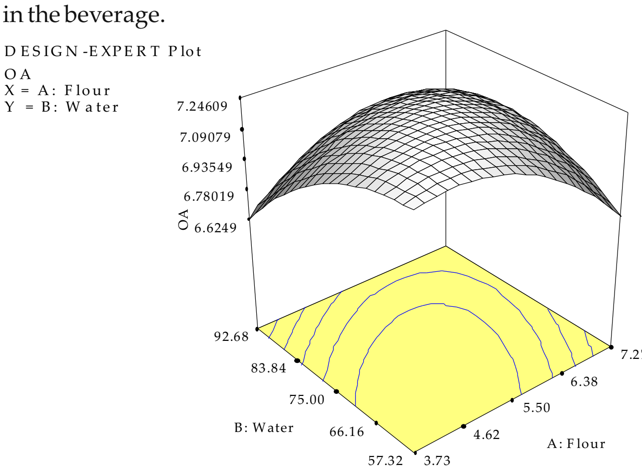

Fig 1: Response surface of Overall Acceptability (OA) as influenced by level of flour (FGG-24 h) and water

Table 3: Comparison of Predicted v/s Actual values of responses (Student's t-test)

Attributes	Predicted results*	Actual Results[@]	t-value[#]
C&A	7.28	7.25	0.15
Consistency	7.35	7.33	0.12
Flavour	7.28	7.25	0.37
OA	7.24	7.21	0.49
Acidity	0.55	0.56	-0.38
Sedimentation	0.50	0.55	-0.36
Viscosity	65.0	64.6	0.03

* Predicted values of Design Expert 6.0.10 package
[@] Actual values (average of 3 trials) of optimized product
[#] t-values found non-significant at 5% level of significance (Table value of t = 4.303)

Selection of type and level of stabilizer for Rabadi-like beverage

Though in the beverage prepared by using solution suggested by response surface methodology, the sedimentation of cereal solids was zero on day 1, it gradually increased on storage. Whey separation in acidic milk beverages made without sufficient stabilizers could be due to the formation of large casein aggregates or an unstable weak gel, which undergoes further rearrangements of the strands and clusters during storage resulting in a reduction in its water-holding ability. This phenomenon in cultured milk could be eliminated or reduced by increasing viscosity through the introduction of hydrocolloids in the system (Glahn 1982; Basset 1983; Towler 1984). So, to have stability in terms of cereal sedimentation and wheying-off during storage, carboxy methyl cellulose (CMC) and pectin (not in combination) were used to attain stability in the beverage. Levels of CMC were 0.05, 0.10 and 0.15% and that of pectin were 0.4, 0.5 and 0.6% suggested by Sathish (2004) and the most suitable one was selected.

Effect on sedimentation and wheying-off

Sedimentation and wheying-off of all samples were measured and results are presented in Table 4. It was obsereved from the result that the sedimentation was minimum (significantly, $p<0.05$) for the sample which was made using pectin at all levels as compared to CMC and wheying-off was minimum for the sample which was made using pectin at 0.5% and 0.6% as compared to its level at 0.4%. So, pectin as a stabilizer is selected at two levels (0.5% and 0.6%) and sensory attributes

Table 4: Effect of stabilizers on sedimentation and wheying-off

Stabilizer	Concentration	Statistical average*	
		Sedimentation	Wheying -off
Control	-	5.62 ^{d}	10.31 ^{c}
CMC	0.05%	4.38 ^{cd}	7.50 ^{c}
CMC	0.10%	3.12 ^{bc}	8.12 ^{c}
CMC	0.15%	1.25 ^{ab}	6.88 ^{c}
Pectin	0.40%	0 ^{a}	5.94 ^{bc}
Pectin	0.50%	0 ^{a}	1.25 ^{ab}
Pectin	0.60%	0 ^{a}	0.62 ^{a}

* at 5% level of significance

were checked by a panel of judges. The sensory score of beverage was higher (7.47) for the product made using pectin at 0.6% level as compared to 0.5% level (7.18). So, pectin at 0.6% level was selected for beverage preparation. Sathish (2004) also reported that the addition of pectin in *lassi*-like beverage decreases the sedimentation.

Changes in sensory and physicochemical parameters during storage of beverage

The average scores of colour and appearance, sedimentation, consistency, flavour and overall acceptability of beverage on 7th day decreased from 7.6 to 7.2, 7.7 to 7.1, 7.5 to 7.0, 7.5 to 6.9 and 7.5 to 6.85, respectively (Fig. 2).

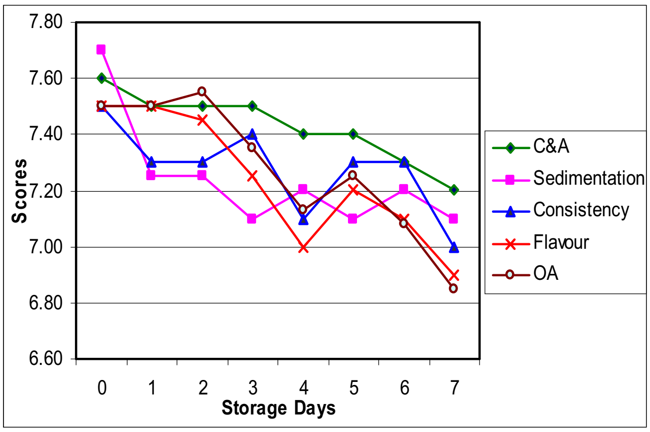

Fig. 2: Changes in sensory attributes (C&A, Sedimentation, Consistency, Flavour and OA) during storage

This change in scores of sensory attributes during the storage of beverage was however non-significant and may be due to a slight increase in acidity and wheying-off. Decrease in viscosity of the beverage during storage should be obvious for decrease in consistency score. The decrease in flavour scores may be mainly attributed to an increase in acidity of beverage during storage period. As overall acceptability (OA) is a combined perception of other attributes like colour & appearance (C&A), consistency, and flavour. Thus, if the scores of other attributes decreased, the scores of OA also decreased.

The acidity of beverage increased from an initial value of 0.595 to 0.75% (as lactic acid) at the end of 7th day (Fig. 3). The increase in acidity is attributed to the degradation of carbohydrates, mainly lactose by the microorganisms added during the inoculation of culture. The viscosity of fresh beverage increased from 69 cP to 73 cP on second day of storage but subsequently showed a decreasing trend, on 7th-day viscosity found to be 58 cP (Fig.3). Initially, the slight increase in viscosity may be due to gelatinization of starch and interaction of protein with pectin having a negative charge (Herbesteith and Fox, 1999).

The possible reason for decrease in viscosity could be the degradation of starch by microorganisms during storage of the beverage. Microorganism breakdown starch to smaller molecules (Mono, di or oligosaccharide), which are soluble and unable to hold water (Cronk *et al.,* 1977). Thus, water bonded to starch becomes free and helps in decreasing the viscosity. No wheying-off was observed up to 4 days of storage in the beverage (Fig. 3). After four days, a significant increase in wheying-off was noticed. This may be due to the breakdown of starch (Cronk *et al.,* 1977) during storage of cereal-based products. The extent of breakdown increases with the rate of fermentation and so the wheying-off. Another reason behind increase in wheying-off could be the decrease in viscosity during storage of beverage. The study was terminated at the end of the 7th day because on 8th-day product became unacceptable because of increased acidity and wheying-off.

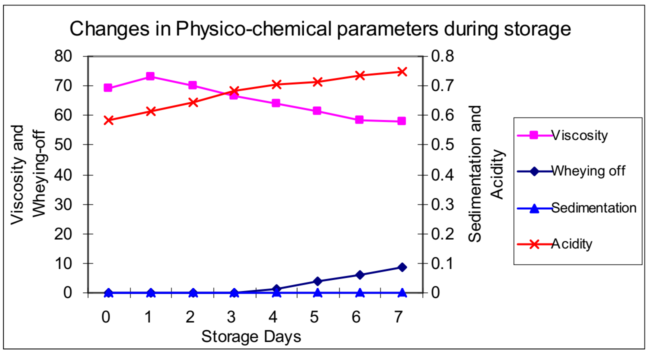

Fig. 3: Changes in physicochemical parameters (Acidity, viscosity, Sedimentation and Wheying-off) during storage

CONCLUSION

The sorghum-based *Rabadi*-like fermented milk beverage developed by the optimized process was found to contain 0.3% fat, 8.87% TS, 2.47% protein, and 1.32% ash. The average overall acceptability score of the final beverage was 7.5. The current study resulted in the development of a fermented sorghum based milk beverage of sensorily acceptable quality having shelf-life of 7 days at refrigerated storage (5–7°C) without any preservative when packed in glass bottles. The technology developed for the manufacture of *Rabadi*-like traditional fermented milk beverage appears to have considerable potential to facilitate its commercial production and marketing.

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