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Development of novel indigenous pearl millet based fermented Skim milk product

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Abstract

The present study was aimed to develop pearl millet based fermented skim milk product. The strain, *L. rhamnosus* RSI3 isolated from indigenous cereal based fermented milk product Rabaadi was screened and finally selected for starter formulation on the basis of appreciable phytate degrading activity and significant antibacterial and antifungal activity against pathogens. However, this culture didn't happen to be a good acid producer and hence was paired with a prolific acid producing compatible *S. thermophilus* ST20, a native strain isolated from dahi. With these strains as starter cultures, method of development of cereal based fermented milk product was standardized using skim milk and ungerminated pearl millet (*Pennisetum glaucum*) flour as raw material and cumin (*Cuminum cyminum*) , black pepper (*Piper nigrum*), curry patta (*Murraya koenigii*) and salt as flavouring agents. The product so developed was analyzed for fat, protein, moisture, ash, acidity content and HCl extractability of minerals (as an index of bioavailability of minerals).

Keywords: *L. rhamnosus* RSI3, *S. thermophilus* ST20, Rabaadi, Phytate, pearl millet, HCl extractability of minerals

The earliest production of fermented foods was based on spontaneous fermentation which was optimised through backslopping, i.e., inoculation of the raw material with a small quantity of a previously performed successful fermentation. But the main difficulty of backslopping is lack of consistency in the product. So the direct addition of selected starter cultures to raw materials has been a breakthrough in the processing of fermented foods, resulting in a high degree of control over the fermentation process and standardization of the end product. Strains with the proper physiological and metabolic features were isolated from natural habitats or from successfully fermented products (Oberman and Libudzisz, 1998).

Pure cultures isolated from complex ecosystems of traditionally fermented foods exhibit a diversity of metabolic activities that diverge strongly from the ones of comparable strains used as industrial bulk starters (Klijn *et al.*, 1995). These include differences in growth rate and competitive growth behaviour in mixed cultures, adaptation to a particular substrate or raw material, antimicrobial properties, flavour, aroma and quality attributes. There is a growing trend in the isolation of wild-type strains from traditional products to be used as starter cultures in food fermentation (Beukes *et al.*, 2001).

Fermentation has special advantage in context of cereals as is known to reduce the level of phytic acid and improve the digestibility of protein as well as starch of various food grains (Gupta *et al.,* 1992). Possible breakdown of starch to oligosaccharides may partially be responsible for the improvement in starch digestibility during fermentation (Cronk *et al.*, 1977). There are various cereals and pulses based fermented foods which have been studied by different researchers e.g. *idli, dhokla, nan, papadam, jalebi, raabadi* etc. (Dhankher and Chauhan, 1987; Soni and Arora, 2000).

Cereals are limited in essential amino acids such as threonine, lysine, and tryptophan, thus making their protein quality poorer compared with animals and milk. This can be compensated by making food products with combination of milk and cereals. There are some traditional cereal based fermented milk products prevalent in some regional areas around the globe. Among them *Tarhana* and *Kishk* hail from Middle East while in India, it is *raabadi* which is popular in Western region of Rajasthan, Punjab, Haryana and Uttar Pradesh. It is prepared by mixing and fermenting the flour of pearl millet, barley, wheat, sorghum or maize with homemade butter milk in earthen and metallic vessel in hot summer days in open sun or at room temperature (35-45^{o}C), kept for 4-6 h, followed by boiling, salting to taste, cooling and consuming. But till date it is not commercially available in market. LAB fermentation of different cereals has been found to effectively reduce the amount of phytic acid and tannins, as well as improve protein availability. Increased amounts of riboflavin, thiamine, niacin, and lysine, due to the action of LAB in fermented blends of cereals, were also reported (Charalampopoulos *et al*., 2002).

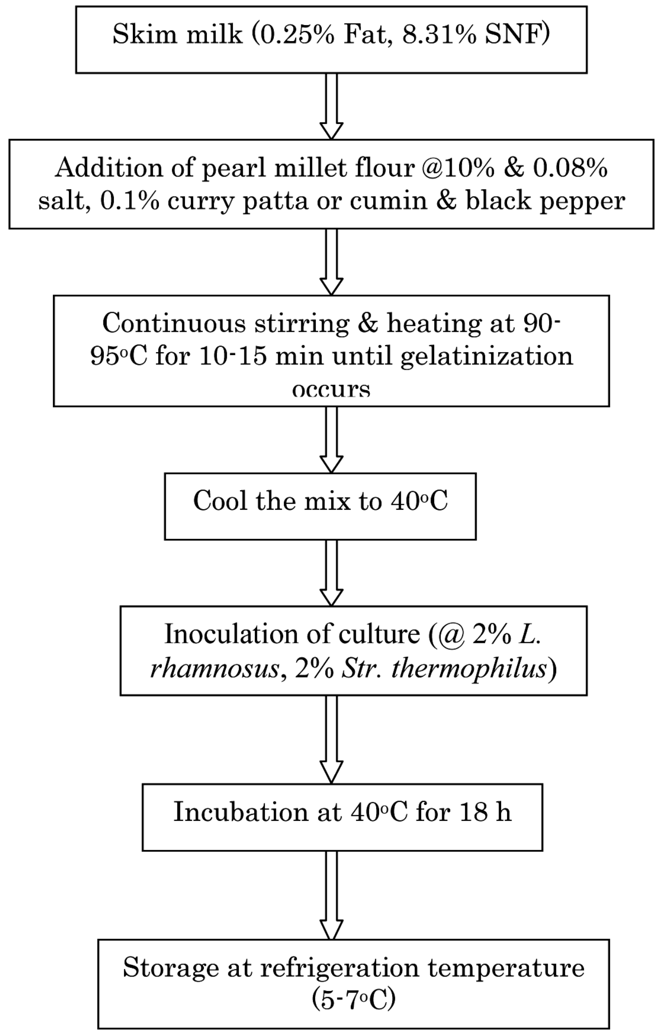
Materials and Methods

Preparation of Cereal-Based Fermented Skim Milk Product

Skim milk (0.25% fat, 8.31% SNF) was collected from NDRI Experimental Dairy. Pearl millet (bajra) was selected for the preparation of cereal-based fermented milk product. The pearl millet grains of Nandi 67 variety was collected from Karnal local grain market and were processed (soaking, drying, grinding, sieving) properly before use. The primary culture RSI3 was isolated from indigenous cereal based fermented milk product *raabadi* and secondary one ST20 (dahi isolate) was procured from another study going on in our laboratory.

Study on Relation between Two Starter Cultures Tentatively Selected for Preparation of Cereal-Based Fermented Milk Product

RSI3 (*L. rhamnosus*) was primarily selected as a starter culture on the basis of its good phytase activity and promising antimicrobial activity.



Flow Chart for Preparation of Pearl Millet-based Fermented Skim Milk Product

But at the time of product development RSI3 was not singly able to produce organoleptically good sour taste within the fermentation period (12 – 14 h) (judged by panel of experts). Then it was decided to add another compatible culture with RSI3 for the production of the same. Indigenous *Streptococcus thermophilus* culture ST20 isolated form dahi was opted for this purpose as *Streptococcus thermophilus* and *Lactobacillus bulgaricus* combination was already successfully used for preparation of yoghurt. The growth temperatures of both are also compatible with each other (37 – 42°C). But before using the culture combination of *L. rhamnosus* RSI3 and *Str. Thermophilus*, we intended to see how far compatible they were with each other by following agar-well-assay method.

As far as procedure was concerned here only RSI3 was inoculated in soft agar. The wells were bored in the middle of the plates and 100 µl of RSI3 culture supernatant was inoculated in well surrounded by ST20 culture and vice-versa.

Results and Discussion

Chemical Analysis of Pearl Millet Based Fermented Skim Milk Product

After fermentation the product was analyzed for acidity, pH, moisture, fat, protein, ash and HCl extractability of minerals (Zn, Fe, Ca, Mn, Cu).

Determination of Acidity and pH in pearl millet Based Fermented Skim Milk Product

Ten g of well mixed sample was taken in a beaker. Then 25 ml of distilled water was added to it and mixed well. One ml of phenopthalein indicator was added to it and it was titrated against N/10 NaOH solution until it developed pinkish colour.

Calculation

Volume of N/10 NaOH soln. required to titrate × 0.09 = % Lactic Acid in sample

The pH of the product was determined by pH meter (Thermo Electron Corporation).

Estimation of Fat in pearl millet Based Fermented Milk Product by Soxhlet Method

Fat was determined in the same way as it was done in case of pearl millet flour. About 4 g of well mixed sample was taken for determination of fat.

Estimation of Crude Protein in pearl millet Based Fermented Milk Product by Kjeldahl's Method

Protein was determined in the same way as it was done in case of skim milk. About 2 g of well mixed sample was taken for determination of crude protein.

Determination of Moisture and Total Solids in pearl millet Based Fermented Skim Milk Product

About 3 g of well mixed sample was weighed in a previously dried aluminum dish. Then it was dried in a oven at 98 – 100°C for 7-8 h to constant weight. Then dish was transferred to desiccator and weighed soon after reaching room temperature. Sample residue was reported as total solids and loss in weight was considered as moisture. It was done in triplicate.

Estimation of Ash in pearl millet Based Fermented Skim Milk Product

Ash was determined in the same way as it was done in case of pearl millet flour. Near about 2 g of well mixed sample was taken for determination of ash.

Estimation of HCl Extractability of Minerals in Cereal Based Fermented Skim Milk Product

Pearl millet flour was added @10% to skim milk and heated to 90-95°C for 10 to 15 min to prepare cereal based fermented skim milk product base. One sample was fermented with defined starter (2% *L. rhamnosus* and 2% *S. thermophilus*) for 18 h and another sample was left without fermentation. The fermented and unfermented samples were oven-dried at 60°C for 48 h to constant weight and finely grounded by using mortar.

Extraction method For Total Mineral: Twenty 5 ml of diacid mixture (HNO_3:$HClO_4$::5:1 v/v) was added to 1 g of moisture-free sample and mixture was kept overnight. The next day it was digested by heating until clear white precipitates settled at the bottom. The crystals were dissolved by diluting in milliQ water and the contents were filtered through Whatman No. 42 filter paper. The filtrate was made upto 50 ml with millQ water and used for the determination of total iron, calcium, zinc and manganese by atomic absorption spectrophotometry according to the method of Lindsey and Norwell (1969).

Extraction Method for HCl-Extractable Mineral: Minerals were extracted from the samples in 0.03 N

HCl by incubating at 37ºC in a shaker with water bath for 3 h (Peterson *et al*., 1943). After filtration through Whatman No. 42 filter paper, the clear filtrate was oven-dried at 100ºC and then wet digested with diacid mixture and the amounts of the above-mentioned minerals were determined.

$$\text{Mineral extractability (\%)} = \frac{\text{Mineral extractable in 0.03 N HCl}}{\text{Total mineral}} \times 100$$

$FeSO_4$, $7H_2O$, $CaCl_2$, $2H_2O$, $ZnSO_4$, $7H_2O$, $MnSO_4$, H_2O and $CuSO_4$, $5H_2O$ salts were used for preparation of standard solution. For Ca estimation 1 ml of 2% SCl2 was added to 1 ml of digested sample to eliminate interfering elements (such as Al, Be, P, Si, Ti) and the volume was made to 10 ml with milliQ water. The instrument used for mineral estimation was Polarized Zeeman Atomic Absorption Spectrophotometer – Double Layer (Hitachi, Japan).

Sensory Evaluation of the Product

Sensory evaluation of cereal based fermented skim milk product prepared by using pearl millet flour and skim milk as main ingredients, spices (cumin, black pepper, curry patta) and salt as flavour and taste enhancers and *L. rhamnosus* RSI3 and *S. thermophilus* ST20 (each @ 2% level) as starter culture, was carried out after setting and storing of the curd at 5-7ºC for 18 h.

Fig. 1: Pearl Millet Based Fermented Skim Milk Product

The flavour, body and texture, colour and appearance, acidity and overall acceptability were used as sensory attributes. All the samples were evaluated for the above sensory characteristics and overall acceptability by a panel of 5 judges keeping at least two judges ready every time for the sensory analysis, on the basis of 9-point Hedonic Scale as described by Larmond (1977).

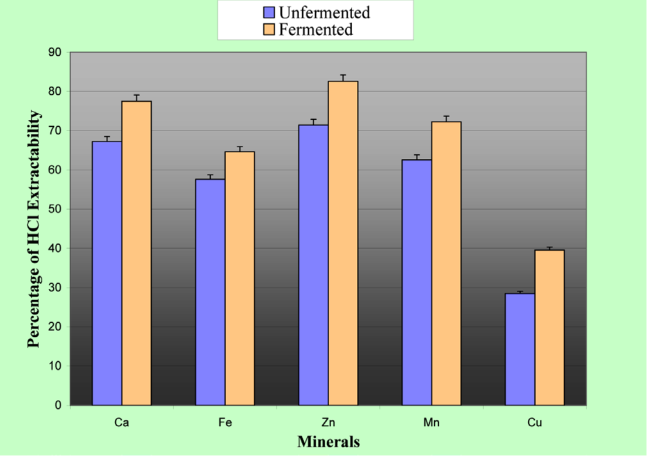


Fig. 2. Effect of Fermentation on HCl Extractability of Minerals

Chemical analysis of skim milk, pearl millet flour and Pearl millet based fermented skim milk product

Before preparing pearl millet based fermented milk product, the main ingredients skim milk (collected from Experimental Dairy, NDRI, Karnal) and pearl millet flour (grain collected from Karnal local market) were chemically analyzed. The chemical composition of skim milk was as follows: Fat → 0.25%, Protein→ 3.6%, SNF→ 8.31%, Total Solids→ 8.56%, Carbohydrate→ 4.81% (Table 1). The chemical composition of pearl millet flour was as follows: Moisture→ 7.5%, Ash→ 1.6%, Fat→ 5.6%, Crude Protein→ 14.5%, Crude Fiber→ 1.63% (Table 2).

Table 1 : Chemical Composition of Skim Milk

Fat%	0.25
Protein%	3.6
SNF%	8.31
TS%	8.56
Carbohydrate%	4.81

*Data are average of three observations

Table 2: Chemical Composition of Pearl Millet Flour

Moisture%	7.50
Ash%	1.60
Fat%	5.60
Crude Protein%	14.50
Crude Fiber%	1.63

* Data are average of three observations

The chemical composition, nutritionally valuable minerals and functional properties of pearl millet (*Pennisetum typhoides*) were studied by Oshodi *et al.* (1999). In this study the chemical composition of pearl millet flour was as follows: Moisture→ 10.2%, Crude protein→ 11.4%, Fat→ 7.6%, Ash→ 1.8%, Crude fiber→ 3.1%, Carbohydrate→ 56.9% (Table 3).

The final developed product was also chemically and microbiologically analyzed. The chemical composition of final product was as follows: Total Solids→ 17.82%, Fat→ 0.73%, Protein→ 5.01%, Ash→ 0.82%, Acidity → 0.82% L.A., Ca- 651.73 mg/100 g, Fe- 8350 μg/100 g, Zn- 3830 μg/100 g, Mn- 990 μg/100 g, Cu- 190 μg/100 g and the microbiological quality of final product was as follows: LAB count on MRS agar at 37°C→ 8.95 log10 cfu/g, Coliform count→ <10 cfu/g, Yeast and mould count→ <10 cfu/g.

Table 3: Chemical Composition and Microbiological Analysis of Pearl Millet Based Fermented Skim Milk Product

Moisture%	82.18
Fat%	0.73
Crude Protein%	5.01
Ash%	0.82
TS%	17.82
Acidity %L.A.	0.82
LAB count on MRS agar at 37°C (log10 cfu/g)	8.95
Coliform count (cfu/g)	<10
Yeast and Mould count (cfu/g)	<10

*Except coliform count and yeast and mould count data are average of three observations

Effect of Fermentation on HCL-Extractability of Minerals

The HCl extractability of minerals was tested on mainly 5 minerals namely calcium, iron, zinc, manganese and copper. Unfermented product was taken as control sample. In case of unfermented product HCl extractability of Ca, Fe, Zn, Mn and Cu were 67.20%, 57.61%, 71.45%, 62.45% and 28.49% respectively (Table 4) whereas in case of fermented product these were 77.54%, 64.62%, 82.57%, 72.27% and 39.56% respectively (Table 5). So there was an increase in HCl extractability in case of all of the 5 minerals. This can be concluded from these data that fermentation with phytate degrading starter RSI3 and ST20 will definitely increase the HCl extractability of minerals in pearl millet based fermented skim milk product.

Gupta and Khetarpaul (1993) studied the HCl extractability of minerals (an index of their bioavailability to the human system) from *raabadi* (a wheat flour fermented food). *Raabadi* was prepared from a precooked wheat flour and buttermilk mixture. The mixture was fermented at different temperatures, i.e., 30, 35 and 40°C for varying periods, viz. 6, 12, 18, 24 and 48 h. A significant improvement occurred in the HCl extractability of dietary essential minerals including calcium, copper, iron, zinc, manganese and phosphorus in all of the samples of *raabadi* fermented at various temperature and time period combinations. The longer the period and the higher the temperature of fermentation, the greater was the increase in HCl extractability of the minerals.

Sensory evaluation of pearl millet based fermented skim Milk product

Two types of product of different flavour combinations were developed using RSI3 and ST20 as defined culture. One was developed with cumin and black pepper (@ 0.1% w/v) and common salt (@ 0,08% w/v) and another was developed with curry patta powder (@0.1% w/v) and common salt (@0.08%). Both types of products were moderately accepted by panel of 5 judges (Table 6 & 7). Although first one was slightly more liked by panel of judges over second one as far

Table 4: HCl Extractability of Unfermented Product

	Total Mineral of Unfermented Product (mg/100 gm)			HCl Extractable Mineral of Unfermented Product (mg/100 gm)			HCl Extractability of Unfermented Product (%)			
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**Mean**
Ca	632.7	632.9	633.1	424.83	424.98	426.12	67.15	67.15	67.31	67.20±0.09
Fe	7.96	8.11	8.14	4.49	4.72	4.74	56.41	58.20	58.23	57.61±1.04
Zn	3.55	3.73	3.79	2.53	2.63	2.75	71.27	70.51	72.56	71.45±1.04
Mn	0.956	0.959	0.971	0.597	0.603	0.606	62.45	62.88	62.41	62.58±0.26
Cu	0.163	0.172	0.178	0.048	0.049	0.049	29.45	28.49	27.53	28.49±0.96

Table 5: HCl Extractability of Fermented Product

	Total Mineral of Fermented Product (mg/100 gm)			HCl Extractable Mineral of Fermented Product (mg/100 gm)			HCl Extractability of Fermented Product (%)			
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**Mean**
Ca	651.5	651.5	652.2	504.74	505.76	505.25	77.47	77.63	77.52	77.54±0.06
Fe	8.27	8.37	8.41	5.33	5.35	5.47	64.45	63.92	65.51	64.62±0.66
Zn	3.71	3.87	3.91	3.08	3.17	3.17	83.02	81.91	82.77	82.57±0.47
Mn	0.983	0.986	0.991	0.711	0.713	0.712	72.33	72.31	72.16	72.27±0.07
Cu	0.178	0.194	0.195	0.07	0.078	0.074	39.32	40.21	39.15	39.56±0.46

Table 6: Sensory Score Card for Pearl Millet Based Fermented Skim Milk Product

	Product 1					**Product 2**				
	J1	J2	J3	J4	J5	J1	J2	J3	J4	J5
Flavour	7	6	7	7	7	6	6.5	7	7	7
Mouthful	7	6	7	8	7	6.5	7	8	7	7
Body	6.5	7	8	7	7	7	7	7	8	7
Texture	7	6	8	7	7	6	6.5	7	6.5	7
Overall Acceptability	7	6.5	7	7	7	6	6.5	7	6.5	7

Table 7: Sensory Score Card for Mean Value

	Product 1	**Product 2**
Flavour	6.8±0.45	6.7±0.40
Mouthful	7.0±0.71	7.1±0.49
Body	7.1±0.55	7.2±0.40
Texture	7.0±0.71	6.6±0.37
Overall Acceptability	6.9±0.22	6.6±0.37

*Product 1→ Flavour combination of cumin, black pepper and salt

Product 2→ Flavour combination of curry patta and salt as sensory quality is concerned, yet the difference in sensory quality of the both products is statistically insignificant ($p<0.05$). The product was judged on the basis of 9-point hedonic scale. This product was tried to develop in two different types of flavour combinations to make it popular in Northern region as well as Southern region of our country as curry patta flavour is liked by South Indian people.

Cumin, black pepper and curry patta were chosen as flavouring agents for development of pearl millet based fermented skim milk product. They not only mask the off-flavour of the particular product but also impart some appealing flavour characterists. They do have some beneficial medicinal values also.

Cumin (*Cuminum cyminum*) is the second most popular spice in the world after black pepper. Cumin seeds are used as a spice for their distinctive aroma, popular in Nepalese, Indian, Pakistani, North African, Middle Eastern, Sri Lankan, Cuban, northern Mexican cuisines. Some scientific evidence suggests cumin may aid digestion by stimulating enzymes to break down foods. Cumin seeds contain a relatively large percentage of iron.

Black pepper (*Piper nigrum*) is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning. Black peppers are native to India and are extensively cultivated there and elsewhere in tropical regions. Black Pepper was believed to cure illness such as constipation, diarrhea, earache, gangrene, heart disease, hernia, hoarseness, indigestion, insect bites, insomnia, joint pain, liver problems, lung disease, oral abscesses, sunburn, tooth decay, and toothaches. Black pepper either powdered or its decoction is widely used in traditional Indian medicine and as a home remedy for relief from sore throat, throat congestion, cough etc.

The curry pattas (*Murraya koenigii*) are highly valued as seasoning in southern and west-coast Indian cooking, and Sri Lankan cooking, much like bay leaves, and especially in curries. They are also used as a herb in Ayurvedic medicine. Their properties include much value as an anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, anti-hypercholesterolemic etc. Curry leaves are also known to be good for hair, for keeping it healthy and long.

Conclusion

In this project, we are trying to develop pearl millet based fermented skim milk product by using defined starter as a combination of *L. rhamnosus* RSI3 and *S. thermophilus* ST20. This fermented product is almost similar to *"Raabadi"*, a traditional household-fermented food of India, especially in Western region of Rajasthan, Punjab, Haryana and Uttar Pradesh. This product has good nutritional value of higher level of HCl extractability of several minerals. Further, we are trying to improve the commercial acceptability of the product by adding some spicy flavouring agents like cumin, black pepper, curry patta and salt. The sensory evaluation of the product was done by panel of ten expert judges. They appreciated the product moderately and they have also suggested further study to improve its sensory quality.

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