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Interaction Effect of Starter Cultures, Forms and Rates of Finger Millet flour on Quality Parameters of Finger Millet Enriched Probiotic Fermented Milk

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Abstract

In the present study, three starter cultures *viz*., C1 (*Streptococcus thermophilus* MTCC 5460 + *Lactobacillus helveticus* MTCC 5463), C2 (*Streptococcus thermophilus* MTCC 5460 + *Lactobacillus rhamnosus* MTCC 5945) and C3 (*Streptococcus thermophilus* MTCC 5460 + *Lactobacillus rhamnosus* MTCC 5946), two forms of finger millet flour *viz*., malted and unmalted were used to prepare finger millet enriched probiotic fermented milk. Both forms of finger millet flour were incorporated in toned milk at 15% and 20% rates. Products were analysed for titratable acidity, sensory and microbiological parameters. Study results revealed a significant interaction effect between type of cultures, forms and rates of incorporation of finger millet especially with respect to titratable acidity and culture count in the products. Malted finger millet supported better growth of all starter cultures which was evident from the higher titratable acidity and culture counts in the malted products. Sensorily also these products were superior compared to unmalted products. Among the cultures, the products made using culture C1 was found to be superior sensorily in comparison to cultures C2 and C3. Rate of incorporation of finger millet @20% supported a better growth of cultures. Average count of all the strains in the products were more than10 log cfu/g.

Keywords: Starter cultures, Finger millet, Fermented milk, Probiotic, Quality attributes

Lactic acid bacteria (LAB) are widely used as starter cultures for fermentation in the dairy industry. The most common use of starter cultures in the dairy industry is for the production of lactic acid from lactose, which assists in the coagulation of milk protein. Certain starter organisms are added specifically for their ability to produce flavor compounds such as diacetyl and acetaldehyde. Starter organisms also influence flavor and texture of cultured products through the breakdown of proteins, fats and other milk constituents. In addition to nutrition and flavor, these cultures can also improve the safety and shelf-life of the fermented products. Starter / probiotic cultures of lactic acid bacteria are also being increasingly finding application as functional starters because of their ability to produce antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, vitamins, or useful enzymes, or provide a health benefit (Mudgal, 2015).

Cereals and its ingredients are well known for its functional properties as it provides dietary fibre, proteins, energy, minerals, vitamins and antioxidants required for human health. A number of research studies have tried to blend cereals and milk to compensate for deficiency of each other (Mugocha *et al.* 2000; Modha and Pal, 2011; Addis *et al*.; Pardhi *et* *al*. 2014; Shivakumar *et al*. 2014; Isingoma *et al*. 2015; Chilakwar and Pawar, 2015). Finger millet, popularly called *Ragi,* has excellent nutritional value and health benefits. It is very rich in calcium and phosphorus and contains iron (Gopalan *et al*. 2009). The nutritive value of finger millet is better than other cereals with respect to minerals, dietary fibre and essential amino acids. The interest in finger millet is growing due to its health benefits such as antimicrobial and antioxidant activities of its polyphenols (Chethan and Malleshi, 2007; Isingoma *et al*. 2015), cholesterol lowering, blood glucose lowering effect, nephro protective, anti-cataractogenic, antiviral, antidiabetic effect (Mathanghi and Sudha, 2012; Rao *et al*. 2016), anti-inflammatory (Taylor and Emmambux, 2008) and anticancer (Thompson, 1993) properties. Finger millet starch is a relatively bland functional ingredient that has the potential to be utilized in lower fat foods. Because of its good thickening and water binding properties the incorporation of finger millet in fermented milk products as a functional ingredient can improve the consistency of final product. It also possess potential prebiotic properties (Manisseri and Gudipati, 2012).

Combining milk with finger millet and fermenting this composite substrate using suitable probiotic starter culture can be a natural and novel way to improve the nutritional as well as functional properties of such composite foods. Most of the traditional finger millet foods are prepared through natural fermentation process. LAB are reported to be associated with such fermentation. During fermentation, finger millet will undergo major biochemical reactions like starch hydrolysis, sugar transformation and softening. So the conventional process of fermentation is believed to increase the nutritive value of grains. The fermentative action of specific LAB strains can result in removal of toxic or antinutritive factors such as phytic acid and tannins from finger millet to increase mineral bioavailability (Coulibaly *et al*. 2011).The good growth of LAB in finger millet substrate suggests that this cereal alone or in combination with milk can be a good delivery vehicle for probiotics. Finger millet being a potential prebiotic source, the milk-millet fermented product can possibly deliver health-promoting properties by combining the probiotic and prebiotic concept. In the development of such composite foods, it becomes essential to study about the growth and activity of different starter cultures in the presence of different forms and rates of incorporation of finger millet in milk and its effect on the overall fermentation of milk-cereal mix and the quality parameters of the resulting product. Also, very few reported research studies are available regarding the development of milk-finger millet fermented probiotic dairy products. Hence, the present study focused on understanding the growth and activity of starter/probiotic cultures in the presence of different forms and rates of finger millet in milk and selecting the most suitable starter on the basis of chemical, sensory and microbiological parameters of the resulting product.

MATERIALS AND METHODS

Toned milk (3.0% milk fat, 8.5% MSNF) for the preparation of finger millet enriched probiotic fermented milk was obtained from AMUL Shoppe, Anand, Gujarat, India. Finger millet variety PRM 9802 (dark colored) of AGMARK (Grade I) was procured from local supermarket of Anand, Gujarat, India.

Starter cultures and growth conditions

Starter cultures used in the study comprised of *Streptococcus thermophilus* MTCC 5460, *Lactobacillus helveticus* MTCC 5463, *Lactobacillus rhamnosus* MTCC 5945, *Lactobacillus rhamnosus* MTCC 5946. All the cultures were obtained from the culture collection of SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India. The cultures were propagated in sterilized reconstituted skim milk (10% T.S.) medium by incubation at 37±1ºC for 8-12 h and was stored at 5±2ºC. Three successive transfers of cultures were given in the same medium prior to their use to ensure activity of cultures during the course of the study. All the cultures have been studied for their potential probiotic attributes. *Lactobacillus helveticus* MTCC 5463 has been proven for its potential as probiotic (Prajapati *et al*. 2011; Senan *et al.* 2015).

Preparation of Finger Millet Enriched Probiotic Fermented Milk Product

Malted finger millet flour was prepared as per the procedure of Shaikh *et al.* (2017). Three different starter cultures *viz*., C1 (*Streptococcus thermophilus* MTCC 5460 + *Lactobacillus helveticus* MTCC 5463), C2 (*Streptococcus thermophilus* MTCC 5460 + *Lactobacillus rhamnosus* MTCC 5945) and C3 (*Streptococcus thermophilus* MTCC 5460 + *Lactobacillus rhamnosus* MTCC 5946) were used in the study. Toned milk (3.0% Fat, 8.5% SNF) was pre-heated to 40°C and added with malted and unmalted finger millet flours (preheated at 70°C) at two different concentrations *viz*., 15% and 20% on milk basis. It was mixed to ensure uniform mass. The mixture was then heated to 80°C for no hold. It was cooled to 40°C and inoculated with starter culture @2%. Incubation was done at 37 ± 2°C till titratable acidity reached to about 0.7% LA. The products were then cooled (5 ± 2°C) and the curd was broken to obtain a uniform viscous product. Products were filled in HDPE bottles and stored at refrigeration temperature (7 ± 2°C).

Estimation of titratable acidity

Titratable acidity of the product was determined by the method described in the Indian Standards (1960).

Sensory evaluation

Sensory evaluation of the products for flavour, body, acidity, colour and appearance and overall acceptability was carried out by expert panel of judges using 9 point hedonic scale.

Microbiological analysis

Probiotic and streptococcal counts were analysed according to methods described in Indian Standards ISI Handbook of food analysis (BIS, 1989).

Statistical analysis

Study data were analyzed by Factorial Completely Randomized Design as per the methods described by Steel and Torrie (1980). The values for microbial counts were log transformed before analysis.

RESULTS AND DISCUSSION

Effect of starter cultures, forms and rates of finger millet flour on the quality attributes of finger millet enriched probiotic fermented milks

The demand for health benefitting foods are encouraging the food industry to orient its research towards newer developments in the area of functional foods. Dairy foods containing finger millet and probiotic strains can offer multiple beneficial effects. Additionally, the presence of millets in such composite food can serve as sources of non-digestible carbohydrates that besides promoting several beneficial physiological effects can also selectively stimulate the growth of beneficial organisms present in the colon and act as prebiotics. In addition to milk, millets are used as a fermentable substrate for the growth of probiotic microorganisms (Ganguly and Sabikhi, 2012; Kocková *et al*. 2013; Fasreen *et al*. 2017). In such cases, the important parameters to be considered are the processing of the grain, growth capability and viability of the cultures, stability of the strains during storage, organoleptic properties and nutritional value of the resulting products.

Titratable acidity of the products

Starter cultures differed significantly ($p<0.05$) from each other with respect to titratable acidity of the products. Highest acidity was seen in product made using culture C1 followed by cultures C3 and C2 (Table 1). This may be because of the inherent difference in the fermenting ability of the cultures. It is a well-known fact that use of different starter strains tend to produce fermented dairy products having varying acidity; even change in the proportion of component starters can affect the final acidity of product. Acidity production was more in case of products made using malted finger millet (A1) compared to unmalted (A2). This may be because the malted millet is said to be nutritionally superior to unmalted one as it is a good source of amylases and during germination the amylases partially hydrolyze the starch to lower molecular weight carbohydrates such as oligo- and disaccharides (Ganguly and

Table 1: Effect of starter cultures, forms and rates of finger millet flour on titratable acidity (% LA) of fermented milk products

Type of Culture (C)	Malted Finger millet (A1)		Unmalted Finger millet (A2)	
	15% (B1)	20% (B2)	15% (B1)	20% (B2)
C1	0.710± 0.01	0.760±0.01	0.703±0.005	0.730±0.01
C2	0.663± 0.005	0.683±0.005	0.650±0.00	0.683±0.005
C3	0.687±0.005	0.697±0.005	0.650±0.02	0.683±0.005
*CD (0.05) C=0.008, A=0.007; B=0.007,A*B=NS; A*C=NS; B*C=NS A*B*C = 0.016;*				

NS = Non significant at 5% level of significance; C1= Streptococcus thermophilus MTCC 5460 + Lactobacillus helveticus MTCC 5463; C2= Streptococcus thermophilus MTCC 5460 +Lactobacillus rhamnosus MTCC 5945; C3= Streptococcus thermophilus MTCC 5460 + Lactobacillus rhamnosus MTCC 5946; Each observation is mean ± SD of three replicate experiments (n=3).

Sabikhi, 2012) which may have supported a better activity of lactic cultures (Shaikh *et al*. 2017). With respect to rates of addition of finger millet, both 15% and 20% differed significantly (p<0.05). Rate of addition @20% gave higher acidity. According to Mugocha*et al*. (2000), Starter culture YC380 produced a pH of 4.5 and less when the proportions of finger millet gruel in the product varied between 0 and 90%. The interaction effect of cultures, forms and rates was also significant. Both malted forms and 20% rate of addition significantly favoured the growth of starter cultures and the effect was evident in all the products irrespective of the type of cultures used. In one of our research articles (Shaikh *et al*. 2017), we reported that rates of incorporation of finger millet had a significant (P<0.05) effect on acidity of the finger millet enriched products in comparison to product without fingermillet. Finger millet incorporated products showed a higher titratable acidity and it increased with increase in the rate of finger millet concentrations. Similar observation was reported by Pardhi *et al*. (2014) who added finger millet flour at different concentrations of 2%, 3% and 4% during the preparation of finger millet lassi. They reported an increase in the acidity of finger millet lassi with an increased level of finger millet flour. Not much scientific published data about the research works carried out on effect of cultures, forms and rates of finger millet flour addition on titratable acidity of composite fermented milk products are available.

Starter and Probiotic culture count in the products

Type of cultures, forms of finger millet and rates of incorporation of finger millet had a significant (P<0.05) effect the starter and probiotic counts of the products (Table 2). Product made using culture C1 and containing 20% malted finger millet flour had highest streptococcal and probiotic counts. All the interaction effects were found to be significant (p<0.05). The culture counts were higher in products made using 20% finger millet. Average count of the strains in the products were more than10 log cfu/g. The growth of all the strains were found to be better in malted form than unmalted form of finger millet. Malted millet is said to be nutritionally superior to unmalted one. The complete process of malting mainly include four stages *viz*., soaking, germination, roasting, and milling, where the most desired physico–chemical changes occur during the soaking and germination stages (Swami *et al*. 2013). During germination the amylases partially hydrolyze the starch to lower molecular weight carbohydrates such as oligo- and disaccharides (Shobana *et al*. 2013). A 23% reduction in the starch content and an increase in the amount of glucose, fructose, maltose, and sucrose in malted finger millet has been reported (Malleshi, 2002; Omary *et al*. 2012) which the lactic acid bacterial strains can utilize for their growth. There was no scientific published data on similar kind of work available to compare the effect of finger millet flour addition on streptococcal and probiotic counts of fermented milk products.

Sensory attributes

Flavour is probably the most important criteria for measuring the quality of fermented milk products

Table 2: Culture count (log cfu/g) in the finger millet enriched fermented milk products

Type of culture (C)	Malted Finger millet (A1)		Unmalted Finger millet (A2)	
	15% (B1)	20% (B2)	15% (B1)	20% (B2)
Streptococcal count (log cfu/g)				
C1	10.66±0.02	10.75±0.01	10.58±0.04	10.66±0.01
C2	10.62±0.02	10.74±0.01	10.62±0.01	10.57±0.01
C3	10.68±0.02	10.61±0.02	10.52±0.01	10.62±0.02
*CD (0.05) C=0.016, A=0.013; B=0.013, A*B=0.018; A*C=0.022; B*C=0.022 A*B*C=0.032*				
Probiotic count (log cfu/g)				
C1	10.71± 0.01	10.85±0.01	10.45±0.01	10.69±0.01
C2	10.60±0.01	10.80±0.01	10.56± 0.01	10.70±0.02
C3	10.51±0.01	10.66±0.01	10.59±0.00	10.58±0.01
*CD (0.05) C=0.011, A=0.009; B=0.009, A*B=0.013; A*C=0.016; B*C=0.016 A*B*C=0.022*				

NS = Non significant at 5% level of significance; C1= Streptococcus thermophilus MTCC 5460 + Lactobacillus helveticus MTCC 5463; C2= Streptococcus thermophilus MTCC 5460 + Lactobacillus rhamnosus MTCC 5945; C3= Streptococcus thermophilus MTCC 5460 + Lactobacillus rhamnosus MTCC 5946; Each observation is a mean ± SD of three replicate experiments (n=3).

which in turn determine its acceptability. The effect of type of cultures, forms and rates of incorporation of finger millet on sensory attributes of the fermented milk products is shown in Table 3. Type of cultures had a significant (P<0.05) effect on the sensory attributes of the fermented milk products. Among all products, product made using culture C1 and 20% malted finger millet flour had maximum flavour score. Malted finger millet products scored better for flavour than unmalted products and in both cases products made using 20% rate of addition was preferred over finger millet incorporation @15%.

Other than the cultures none of the other parameters had a significant effect on the body of the products. Body explain the physical nature of the product with respect to smooth or coarse or uniform or settled. The body of the product helps in its marketing value. It also shows product's inner makeup. Body scores were found to be higher for products prepared using culture C1. This may be because the strain MTCC 5463 used in the study has reported to have noticeable amount of EPS production ability which was also confirmed by presence of EPS producing gene in that particular strain (Prajapati *et al*. 2011; Vishwanath *et al*. 2012) and this might have contributed to a smooth uniform body as well as a better flavour to the product.

Forms of finger millet and type of cultures had a significant (P<0.05) effect on acidity score of the products as perceived by judges during sensory evaluation of the products. The product made using culture C1 and containing 20% malted finger millet flour had maximum acidity scores. This is because the starter growth and acid production was comparatively higher in the product made using the said combination. All the interaction effects were found to be non-significant (p>0.05) except the rates of incorporation and type of cultures. Pardhi *et al.* (2014) prepared a finger millet based lassi containing 2, 3 and 4% finger millet flour and reported the acidity scores as 7.24, 7.33 and 7.19 % LA respectively.

Forms of finger millet and type of cultures had a significant (P<0.05) effect on colour and appearance score of the products. Among all products, products made using culture C1 had maximum colour and appearance score. In the current study, rate of incorporation of finger millet had not shown any significant effect on colour and appearance score of the products.

Overall acceptability is an indicative parameter of sensory quality of products in totality and consists of flavour, colour and appearance, body characteristics which represent the total performance of the product

Table 3: Interactive effect of cultures, forms and rates of finger millet flour addition on sensory attributes of finger millet enriched fermented milk products

Type of culture (C)	Malted Finger millet (A1)		Unmalted Finger millet (A2)	
	15% (B1)	20% (B2)	15% (B1)	20% (B2)
Flavour score (out of 9)				
C1	7.670± 0.22	8.393±0.39	7.533±0.28	8.180±0.19
C2	7.510± 0.32	7.717±0.20	7.290±0.03	7.403±0.12
C3	7.497±0.14	7.683±0.16	7.217±0.20	7.430±0.13
*CD (0.05) C=0.188, A=0.153, B=0.153, A*B=NS, A*C=NS, B*C=0.188, A*B*C=NS*				
Body score (out of 9)				
C1	7.900± 0.02	8.087±0.06	7.537±0.09	7.780±0.30
C2	7.680± 0.25	7.493±0.24	7.550±0.22	7.293±0.31
C3	7.790±0.11	7.440±0.25	7.607±0.24	7.257±0.22
*CD (0.05) C=0.169, A=NS, B=NS, A*B=NS, A*C=NS, B*C=0.239, A*B*C=NS*				
Acidity score (out of 9)				
C1	7.757± 0.39	8.293±0.30	7.530±0.35	8.143±0.27
C2	7.513± 0.38	7.530±0.21	7.310±0.31	7.237±0.17
C3	7.587±0.27	7.420±0.28	7.240±0.32	7.197±0.22
*CD (0.05) C=0.254, A=0.207, B=NS, A*B=NS, A*C=NS; B*C=0.254, A*B*C=NS*				
Colour and appearance score (out of 9)				
C1	7.860±0.30	7.790±0.21	7.647±0.11	8.020±0.16
C2	7.607±0.20	7.770±0.11	7.553±0.10	7.367±0.14
C3	7.530±0.31	7.793±0.12	7.553±0.20	7.347±0.27
*CD (0.05) C=0.153, A=0.125, B=NS, A*B=NS; A*C=NS; B*C=NS, A*B*C=0.306*				
Overall acceptability score (out of 9)				
C1	7.850± 0.01	8.040±0.40	7.707±0.13	7.840±0.31
C2	7.410±0.25	7.587± 0.25	7.337±0.29	7.420±0.11
C3	7.383±0.11	7.643±0.11	7.117±0.10	7.477±0.06
*CD (0.05) C=0.154, A=NS, B=NS, A*B=NS; A*C=NS, B*C=NS, A*B*C=0.308*				

NS = Non significant at 5% level of significance; C1= Streptococcus thermophilus MTCC 5460 + Lactobacillus helveticus MTCC 5463; C2= Streptococcus thermophilus MTCC 5460 + Lactobacillus rhamnosus MTCC 5945; C3= Streptococcus thermophilus MTCC 5460 + Lactobacillus rhamnosus MTCC 5946; Each observation is a mean ± SD of three replicate experiments (n=3).

in the mind of judges while evaluating the product. Type of cultures and the interaction effects had a significant (P>0.05) effect on overall acceptability score of the products. Product made using culture C1 and containing 20% malted finger millet flour showed highest overall acceptability score. The same products had the highest flavour and body scores also.

CONCLUSION

Current study results revealed that the forms and rates of incorporation of finger millet has a significant influence on the growth and activity of starter cultures. Growth of all the culture strains were found to be better in malted form than unmalted form of finger millet. All the culture counts were higher in products made using 20% finger millet. At the same time, type of culture and its inherent properties such as acid production, exopolysaccharide production has a significant influence on sensory attributes, titratable acidity and culture counts of the finger millet enriched probiotic fermented milk products. Use of exopolysaccharide producing strain improved the product characteristics such as flavour and body. The product made using malted finger millet @20% and fermented with culture comprising of *Streptococcus thermophilus* MTCC 5460 and *Lactobacillus helveticus* MTCC 5463 was found to be superior in terms of sensory attributes and culture count.

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