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## Study to select the sugar concentration and compatible flavour for preparation of finger millet enriched probiotic fermented milk product

**Aijaz Shaikh and Sreeja V**

### Abstract

In the current study, finger millet enriched probiotic fermented milk products were prepared using, caramel @ 0.8%, chocolate syrup @ 1% and strawberry flavour @ 0.4% and sugar as sweetening agent @10%. These products were prepared using culture C1 (*S. thermophilus* and *Lb. helveticus*). Products were analysed for physico-chemical, sensory and microbiological characteristics. Product made using 0.4 % strawberry flavour had significantly ( $P < 0.05$ ) greater sensory scores. Hence it was taken and its rate of incorporation was further optimized. Out of the three rates (0.3%, 0.4% and 0.5%) tried, strawberry flavour @0.4% was found best. Product made using 0.4 per cent strawberry flavour had significantly ( $P < 0.05$ ) greater scores for flavour ( $42.68 \pm 0.55$ ), body & texture ( $27.80 \pm 0.62$ ), acidity ( $8.39 \pm 0.17$ ), colour & appearance ( $12.80 \pm 0.16$ ) and overall acceptability ( $91.26 \pm 1.30$ ) compared to product made using 0.3 and 0.5 per cent strawberry flavour. Both starter and probiotic counts in the product was  $>10$  log cfu/g.

**Keywords:** Finger millet, Strawberry flavour, Probiotic, Fermented milk, quality attributes

### 1. Introduction

Milk products are considered excellent media to generate an array of products that fit to current consumer demand for health benefitting foods (Shortt *et al.*, 2004) [20]. Fermented dairy products enriched with probiotic bacteria have developed into one of the most successful category of functional foods and the market of such probiotic dairy foods is increasing annually. Even though milk is considered as an almost complete food, it is deficient in dietary fibre, micronutrients such as iron and vitamin C.

Finger millet or Ragi (*Eleusine coracana*) is one of the common millets in several regions of India and is a good source of dietary fibre (Desai *et al.*, 2010, Devi *et al.*, 2014) [7, 8]. This millet is exceptionally rich in calcium (344 mg/100g) compared to all other cereals and millets and contains 283 mg % phosphorus, 3.9 mg % iron (Gopalan *et al.*, 2009) [10], and many other trace elements and vitamins. Potassium content of finger millet is also high (408 mg %) compared to other cereals and millet. The nutritive value of finger millet is better than other cereals (Manay and Shadaksharaswamy, 2001) [14]. It contains important amino acids such as isoleucine (4.4 g), leucine (9.5 g), methionine (3.1 g) and phenyl alanine (5.2 g) which are deficient in other starchy meals. Millets also contains B vitamins, especially niacin, B6 and folic acid. Additionally it is being studied for its health benefitting properties such as antimicrobial (Chethan and Malleshi, 2007; Isingoma *et al.*, 2015) [4, 11], antioxidant (Chethan and Malleshi, 2007; Chandrasekara and Shahidi, 2010) [4, 1], cholesterol lowering (Mathanghi and Sudha, 2012; Dayakar rao *et al.*, 2016) [16, 6], blood glucose lowering effect (Rachie and Peters, 2002; Rajasekaran *et al.*, 2004; Glew *et al.*, 2008; Mathanghi and Sudha, 2012) [18, 19, 9, 16], nephroprotective (Dayakar rao *et al.*, 2016; Mathanghi and Sudha, 2012) [6, 16], anti-cataractogenic (Mathanghi and Sudha, 2012) [16], anti-inflammatory (Taylor *et al.*, 2006; Taylor and Emmambux, 2008; Chandrasekara and Shahidi, 2011b and 2011c) [23, 22, 2, 3], antiviral (Mathanghi and Sudha, 2012) [16], anticancer (Thompson, 1993), and antidiabetic effect (Mathanghi and Sudha, 2012) [16]. Finger millet as potential prebiotics contains good amount of healthy components (Manisseri and Gudipati, 2012). As the millets are gluten free, it has considerable potential in foods and beverages which can meet the growing demand for gluten free foods.

Very few research reports are available related to combining the nutritional aspects of milk, finger millet and fermentation.

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Finger millet (*Eleusine Coracana*) enriched fermented milk product prepared using an indigenous probiotic bacteria could be a novel concept of a functional food. Being a rich source of calcium and iron, and the fact that the bioavailability can be improved by simple processing such as germination and fermentation, the resultant product can be a good supplement for improving bone health and hemoglobin. Hence, supplementing milk with finger millet and fermentation of the same using starter and probiotic strains can lead to a novel high nutrition food product with health benefitting effects. The present study was planned and executed to develop a finger millet enriched probiotic fermented milk product.

## Materials and Methods

### Materials

Toned milk (3.0% milk fat, 8.5 % MSNF) for the preparation of finger millet enriched probiotic fermented milk product was obtained from Vidya shoppe Anand, Gujarat, India. Finger millet variety PRM 9802 (dark colored) of AGMARK (Grade I) was procured from local supermarket of Anand, Gujarat, India. High quality sugar, free from impurities was purchased from local supermarket of Anand. Chocolate syrup was prepared in the laboratory using cocoa powder purchased from the local market. Caramel essence and strawberry essence No. 1, was obtained from Vijay industries Eagle brand Vadodara Gujarat (India). Indigenous starter culture used in the preparation of product comprised of *Streptococcus thermophilus* MTCC 5460 and probiotic strain *Lactobacillus helveticus* MTCC 5463. Both cultures were obtained from the culture collection of SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India. *Lactobacillus helveticus* has been proven for its potential as probiotic. All the cultures were propagated in sterilized reconstituted skim milk (10% T.S.) medium by incubation at  $37\pm 1^\circ\text{C}$  for 8-12 h and stored at  $5\pm 2^\circ\text{C}$ . 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.

### Methods

#### Preparation of malted finger millet flour

Malting of finger millet was carried out according to the procedure suggested by Chilakawar *et al.* (2015) [5] with necessary modifications. Finger millet grains were weighed, dust, dirt were removed and washed well. The grains were kept for steeping in water for 12 hours at room temperature. The grains: water ratio was kept at 1:3. During steeping, the water was changed after every 4 hours. It was then hanged overnight at room temperature to remove excess water. Steeped grains were spread on perforated trays lined with muslin cloth and germinated at  $25\pm 2^\circ\text{C}$  for 24 h in an incubator. The seeds were then spread uniformly on a stainless steel tray and dried in a vacuum tray dryer maintained at  $40\text{--}45^\circ\text{C}$  for 5 to 6 h. The malted, dried finger millet seeds were ground and made into flour using commercial flour mill (Milcent flour mill, compression-cum-shearing type (roller) mills, Ahmadabad) adjusted to a fine setting. Subsequently, the flours were sieved and stored in air-tight containers at  $7\pm 2^\circ\text{C}$ . The malted finger millet flour was given a heat treatment of  $70^\circ\text{C}$  prior to its incorporation in the milk during product preparation.

#### Preparation of finger millet enriched probiotic fermented milk product

Toned milk (3.0% Fat, 8.5% SNF) was pre heated to  $40^\circ\text{C}$  and

added with malted finger millet flour at 20% on milk basis. It was mixed to ensure uniform mass. Sugar was added at 10% on milk basis. The mixture was then heated to  $80^\circ\text{C}$  for no hold. It was cooled to  $40^\circ\text{C}$  and inoculated with probiotic and streptococcal culture @2%. It was incubated at ( $37\pm 2^\circ\text{C}$ ) till titratable acidity reached to about 0.7% LA. The product was then cooled ( $5\pm 2^\circ\text{C}$ ) and curd was broken to obtain a uniform viscous product. Caramel flavour at 0.8%, chocolate syrup at 1% and strawberry essence was added at 0.4% on curd basis. It was filled in HDPE bottles and stored at refrigeration temperature ( $7\pm 2^\circ\text{C}$ ).

#### Titrateable acidity

The titrateable acidity of the product was determined by the method described in the Indian Standards (IS: 1479-Part I, 1960).

#### Sensory evaluation

Sensory evaluation of the products for flavour, body and texture, acidity, colour and appearance and total score was carried out by expert panel of judges.

#### Microbiological analysis

Probiotic and streptococcal count were analysed according to methods described in Indian Standards (IS: 1479, Part III, 1962).

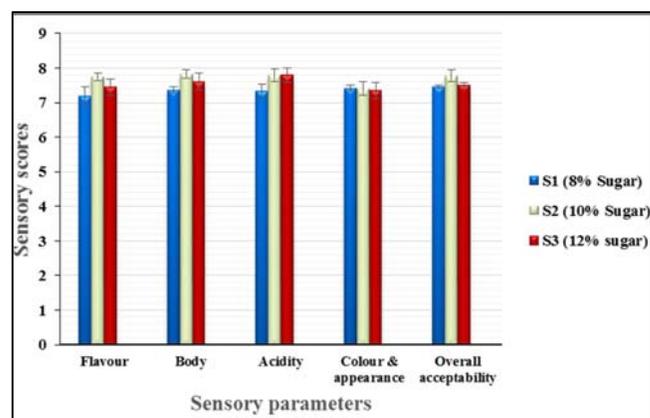
#### Statistical analysis

All the parameters under study were analyzed by statistical methods. Study data were analyzed by Completely Randomized Design as per the methods described by Steel and Torrie (1980) [21]. The values for microbial counts were log transformed before analysis.

## Results and Discussion

### Optimization of sugar concentration for the preparation of finger millet enriched probiotic fermented milk product

To select the optimized sugar concentration for preparation of finger millet enriched probiotic fermented milk product, three different sugar concentrations *viz.*, 8%, 10% and 12% on milk basis were tried. These levels were selected based on preliminary trials. Level of sugar concentration was optimized on the basis of sensory score.



**Fig 1:** Optimization of sugar concentration for the preparation of finger millet enriched probiotic fermented milk product.

The data furnished in Figure 1 indicates that use of different sugar concentrations had a significant ( $p<0.05$ ) effect on

flavour, body and texture, acidity and overall acceptability of the product. The finger millet enriched probiotic fermented milk made using 10 per cent level of sugar had significantly ( $P < 0.05$ ) greater score for all the above mentioned attributes compared to all other samples.

Lisak *et al.* (2012) [13], prepared a strawberry (@ 0.2%) flavoured yoghurt by addition of three different type of sweeteners, viz., sucrose, stevia and equal portion of sucrose and stevia. He found that, the best sensory evaluation had the yoghurts sweetened with addition of 4.5 g/100 g equal portions of sucrose and stevia.

**Optimization of compatible flavour for the preparation of product**

To select the compatible flavour for preparation of finger

millet enriched probiotic fermented milk product, three flavours viz., caramel, chocolate and strawberry were incorporated on curd basis. All the three flavours were selected on the basis of preliminary trials taken in the department. Flavours were optimized on the basis of sensory score.

The data furnished in Table 1 indicates that use of different flavours had a significant ( $p < 0.05$ ) effect on flavour, body and texture, acidity, colour and appearance and total score of the product. Strawberry essence had a significant ( $P < 0.05$ ) influence on all of the sensory attributes of finger millet enriched probiotic fermented milk. Highest sensory scores were obtained for strawberry flavoured product. No significant ( $p > 0.05$ ) effect was observed between control, caramel and chocolate flavoured product.

**Table 1:** Optimization of compatible flavour for the preparation of product.

Types of flavour	Sensory attributes				
	Flavour (out of 45)	Body & Texture (out of 30)	Acidity (out of 10)	Colour & Appearance (out of 15)	Total score (out of 100)
Control	36.68 ± 1.17	24.16 ± 0.94	7.46 ± 0.19	10.04 ± 0.18	78.72 ± 0.83
A	37.64 ± 0.78	22.28 ± 0.80	7.60 ± 0.35	10.80 ± 0.50	78.82 ± 1.09
B	37.54 ± 0.72	21.80 ± 0.22	7.78 ± 0.17	10.70 ± 0.15	78.24 ± 0.82
C	41.14 ± 1.60	26.72 ± 0.89	8.31 ± 0.24	11.90 ± 0.80	88.52 ± 2.97
SEm	0.505	0.345	0.113	0.150	0.755
CD <sub>(0.05)</sub>	1.513	1.034	0.338	0.449	2.265

Each observation is a mean ± SD of five replicate experiments (n=5)

[Where, Control = Product without flavour and sugar addition; A=0.8% Caramel flavour+ 10% Sugar; B= 1% Chocolate syrup + 10% Sugar; C= 0.4% Strawberry essence + 10% Sugar]

Patel *et al.* (2015) [17] in their study used mango, caramel, chocolate and vanilla for flavouring ragi ice-cream and reported that among different flavours, chocolate flavoured ragi ice-cream was preferred most followed by mango, caramel and vanilla.

Kalpak (2015) [12] developed a oat based probiotic smoothie using mango pulp@ 5, 10, 12 and 15% and reported that among different concentration, 12% mango pulp preferred most.

**Quality attributes of finger millet enriched probiotic fermented milk products as affected by level of strawberry flavour**

In order to further optimize the level of incorporation of strawberry flavours, finger millet enriched probiotic

fermented milks were prepared using strawberry essence at three different rates viz., 0.3%, 0.4% and 0.5% on curd basis. Sugar at the rate of 10.0 percent of milk was kept constant. The results of the study are given and discussed here under.

**Titrateable acidity and pH**

The average values presented in Table 2 shows that the rate of addition of strawberry essence and sugar in the preparation of finger millet enriched probiotic fermented milk had a significant ( $p < 0.05$ ) influence on pH and acidity of the products. Acidity of the products increased with increased level of incorporation of essence. Since there is an inverse relation between acidity and pH, the pH value of product containing 0.2% strawberry essence was higher than its counterparts containing 0.4 and 0.5% levels of essence.

**Table 2:** Changes in the acidity and pH of finger millet enriched probiotic fermented milk prepared with different concentrations of strawberry essence.

Levels of flavour incorporation	pH	Titrateable acidity (% LA)
Control (0%)	4.89 ± 0.015	0.73 ± 0.02
S1 (0.3%)	4.86 ± 0.018	0.74 ± 0.04
S2 (0.4%)	4.84 ± 0.012	0.76 ± 0.01
S3(0.5%)	4.76 ± 0.012	0.78 ± 0.02
SEm	0.018	0.006
CD <sub>(0.05)</sub>	0.055	0.017

Each observation is a mean ± SD of five replicate experiments (n=5)

**Probiotic and Streptococcal counts**

Levels of flavour incorporation had no significant ( $p > 0.05$ )

effect on streptococcal and probiotic count (log cfu/g) of products (Table 3).

**Table 3:** Microbial counts of finger millet enriched probiotic fermented milk as affected by the level of strawberry essence.

Levels of flavour incorporation	Probiotic counts (log cfu/g)	Streptococcal counts (log cfu/g)
Control (0%)	10.94 ± 0.28	10.92 ± 0.20
S1 (0.3%)	10.87 ± 0.24	10.82 ± 0.24
S2 (0.4%)	10.86 ± 0.22	10.83 ± 0.20

<b>S3 (0.5%)</b>	10.85 ± 0.21	10.84 ± 0.22
SEm	0.101	0.073
CD(0.05)	NS	NS

The average probiotic counts of products prepared using strawberry flavour S1, S2 and S3 were found to be  $10.87 \pm 0.24$ ,  $10.86 \pm 0.22$ ,  $10.85 \pm 0.21$  log cfu/g respectively. The average streptococcal counts of products made using different rates of strawberry flavour S1, S2 and S3 were found to be  $10.82 \pm 0.24$ ,  $10.83 \pm 0.20$ ,  $10.84 \pm 0.22$  log cfu/g respectively.

### Sensory attributes

The data furnished in Table 4 indicates that use of strawberry essence at varying level had a significant ( $P < 0.05$ ) influence on all of the sensory score of finger millet enriched probiotic fermented milk.

**Table 4:** Sensory properties of finger millet enriched probiotic fermented milks prepared with different concentrations of strawberry essence.

Levels of flavour incorporation	Sensory attributes				
	Flavour (out of 45)	Body & Texture (out of 30)	Acidity (out of 10)	Colour & Appearance (out of 15)	Total score (out of 100)
Control (0%)	36.32 ± 1.83	24.72 ± 1.74	7.72 ± 0.08	10.18 ± 0.13	78.14 ± 1.44
S1 (0.3%)	39.18 ± 0.57	25.58 ± 0.63	7.70 ± 0.40	11.60 ± 0.42	84.58 ± 0.77
S2 (0.4%)	42.68 ± 0.55	27.80 ± 0.62	8.39 ± 0.17	12.80 ± 0.16	91.26 ± 1.30
S3 (0.5%)	40.40 ± 0.95	26.46 ± 0.66	7.99 ± 0.32	12.02 ± 0.47	87.59 ± 1.40
SEm	0.495	0.463	0.123	0.219	0.565
CD (0.05)	1.483	1.389	0.370	0.658	1.693

The finger millet enriched probiotic fermented milk made using 0.4 per cent level of strawberry essence had significantly ( $P < 0.05$ ) greater scores for all sensory attributes when compared to the other levels (Table 4). All the three samples of finger millet enriched probiotic fermented milk had uniform pink colour throughout the mass.

Since finger millet enriched probiotic fermented milk made using 0.4 per cent strawberry essence had significantly ( $P < 0.05$ ) greater scores for flavour, body & texture, acidity, colour & appearance and its total sensory score was highest compared to the products containing 0.3 and 0.5 per cent strawberry essence.

There was no scientific published data available to see the effect of strawberry flavour addition on sensory properties of composite fermented milk products. Hence a comparative discussion was not possible.

In conclusion, the results of physico-chemical, sensory and microbiological analysis of finger millet enriched probiotic fermented milk products using different levels of strawberry essence incorporation revealed that the products prepared using strawberry flavour @0.4% was found to be superior compared to other products.

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