

## Sensory, Chemical and Microbial Quality of Fermented Probiotic Cereal Based Health Drink

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**Abstract:** Fermentation of the foods destroys undesirable components, enhance the nutritive value and appearance of the food and make a safer product. Probiotic organisms confer a health benefit on the host by improving the properties of the indigenous micro flora and they can be used as starter culture for fermentation of the food. The cereals offer another alternative for the production of functional foods. The three cereal flours viz. sorghum, finger millet and sorghum+finger millet were used to prepare the fermented probiotic cereal based health drink. The *Lb. acidophilus* strains as Strain 1, Strain 2 and Strain 3 were used as culture. On the basis of overall acceptability and maximum counts of *Lb. acidophilus*, the 10% rate of addition of each cereal flour; addition of 2% jaggery and 10% rate of addition of culture of each strains were selected in main experiment. The 16 hours was found the best incubation period. Experimental trials were conducted using Factorial Completely Randomized Design by replicating three times. The fresh and refrigerated stored samples of health drinks were evaluated for chemical, microbial and sensory quality. It was observed that for sensory, chemical and microbial quality of fermented probiotic cereal based health drink treatment T<sub>2</sub> (10% culture of strain 2 + 10% sorghum flour) shown maximum scores. It was concluded that, the best quality of fermented probiotic cereal based health drink can be prepared by using 10% sorghum flour; 10% culture of *Lb. acidophilus* strain 2, and 2% jaggery, incubating at 37°C/16 hours.

**Keywords:** Probiotics, fermentation, health drink, *Lb. acidophilus*.

### INTRODUCTION

Since the beginning of human civilization there has been an intimate companionship between the human being, his fare and the fermentative activities of microorganisms. These fermentative activities have been utilized in the production of fermented foods and beverages, which are defined as those products which have been subordinated to the effect of microorganisms or enzymes to cause desirable biochemical changes. The microorganisms responsible for the fermentation may be the microbiota indigenously present on the substrate, or they may be added as starter cultures (Harlender, 1992).

Since the dawn of civilization, methods for the fermentation of milks, meats, vegetables and cereals have been described. The industrial revolution resulted in the concentration of large masses of people in towns and cities. As a consequence, food

had to be prepared in large quantities, requiring the industrialization of the manufacturing process. The blossoming of Microbiology as a science in the 1850's formed the biological basis of fermentation, and the process was understood for the first time (Caplice and Fitzgerald, 1999).

The WHO food safety unit has given high priority to the research area of fermentation as a technique for preparation/storage of food. A food item prepared from water contaminated with pathogenic microorganisms will successively be contaminated, and a health risk. Lactic acid fermentation of food has been found to reduce the risk of having pathogenic microorganisms grown in the food (Peter, 1999).

The word probiotic is derived from Greek and means "for life". It was Metchnikoff at the beginning of 20<sup>th</sup> century that first acknowledged the health benefits related to the regular consumption of

fermented milks (Metchnikoff, 1908). Naidu *et al.* (1999), defined probiotic as “a microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract”.

Among lactic acid bacteria; *Lactobacilli* are most important group and are gaining increasing attention in food fermentation industry because of their biotechnological interest. These organisms produce antimicrobial substances such as organic acids, hydrogen peroxide and bacteriocins which prevent the growth of pathogens (Arokiyamary and Sivakumar, 2011).

Cereals are globally number one as food crops as well as substrates for fermentation. Cereal based foods are a major source of inexpensive dietary energy and nutrients in developing countries.

Consumer trends with respect to food choice are changing due to the increasing awareness of the link between diet and health. Properly formulated probiotic-containing foods having the potential to promote health benefits offer consumers a low risk and low cost dietary component. By considering the consumer awareness and demand, the present investigation is planned for preparation of fermented probiotic cereal based health drink with the objectives: To assess the chemical quality of cereal based health drink, To assess the sensory quality of cereal based health drink, To assess the microbial quality of cereal based health drink.

## MATERIAL AND METHODS

The present investigation entitled “Sensory, Chemical and Microbial quality of fermented probiotic cereal based health drink” was carried out at the Department of Animal Science and Dairy science, PGI, MPKV, Rahuri. Following materials were used in laboratory for analytical purpose during the entire research work.

Glassware, Incubator, Electronic Balance, Autoclave, Hot Air Oven, Chemicals as analytical (AR) or guaranteed reagent (GR), Media Ingredients, Colony Counter, Laminar Air Flow, Milk, Cereals as sorghum and finger millet, Jaggery, Water etc.

### (A) Starter Culture, its Maintenance and Propagation

The freeze dried pure cultures of *Lactobacillus acidophilus* were obtained from the NDRI, Karnal, Haryana and SMC College GAU, Anand. The cultures were maintained separately in sterilized reconstituted skim milk test tubes.

The sterilized skim milk test tubes were separately inoculated with these cultures and incubated at 37°C for 8 hr and there after stored at refrigerated temperature. In order to keep these cultures active, they were propagated once in a week (Shankar 1975).

### (B) Preparation of fermented probiotic cereal based health drink

The flour required for ambali preparation was obtained by following the procedure described by Krishne Gowda *et al.* (2006), with slight modifications. The ambali was prepared by the procedure described by Ramkrishnan (1979) with slight modifications. 10% of flour each Sorghum, finger millet and sorghum+finger millet, 10% culture of each strain - strain 1, strain 2 and strain 3 and 2% jaggery were used to prepare fermented probiotic cereal based health drink. It was stored at 37°C for 16 hrs and the prepared health drink was analyzed for sensory, chemical and microbial quality. The fermented probiotic cereal based health drink was prepared by following method.

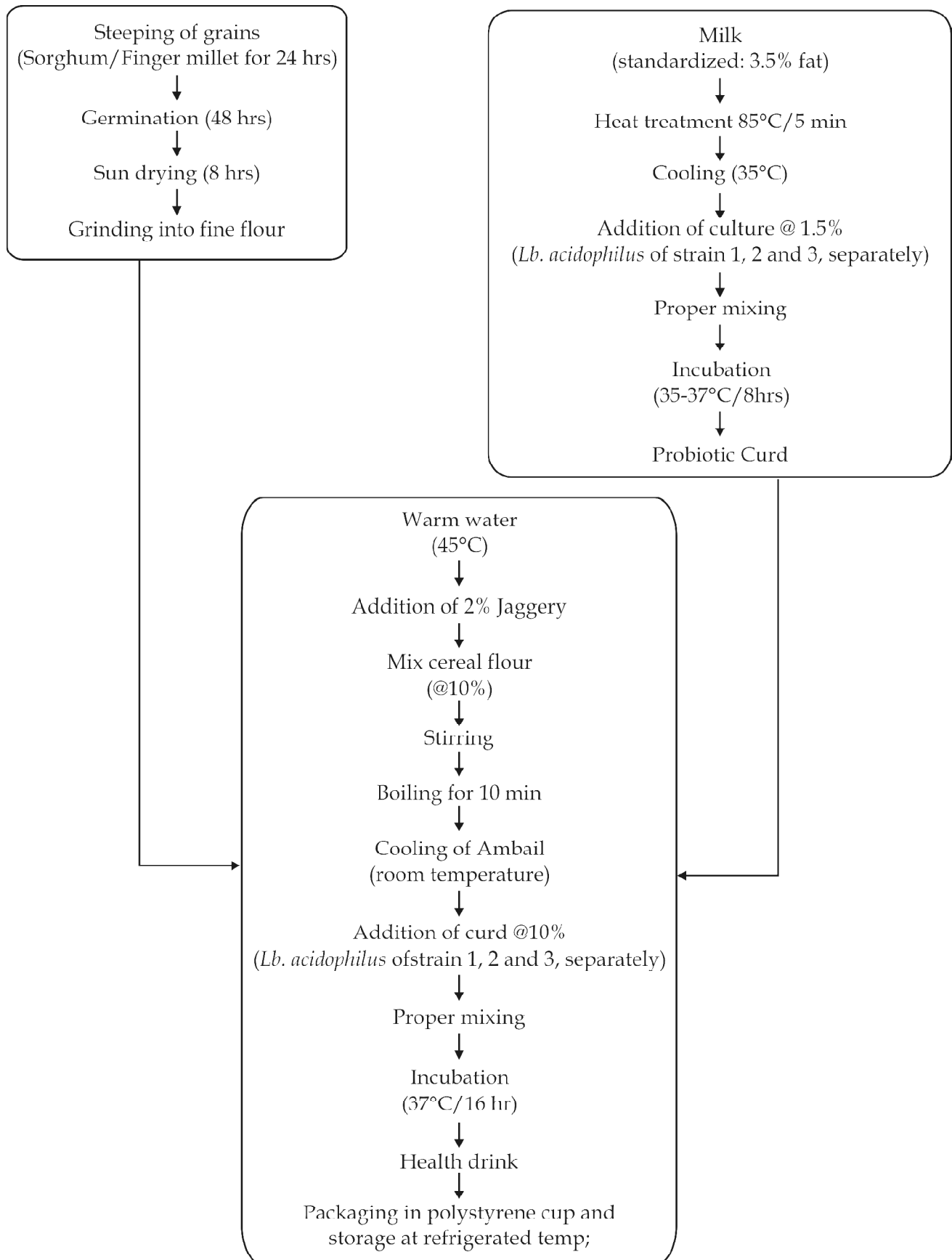
### Analysis of Fermented Probiotic Cereal Based Health Drinks

#### *Sensory Quality of Health Drink*

The samples of health drink were evaluated for sensory qualities by using 9 point hedonic scale for flavour, colour and appearance, body and texture and overall acceptability.

#### *Chemical Analysis of Health Drink*

The prepared fermented probiotic cereal based health drink was chemically analyzed for Fat, Protein, Reducing sugar, Total sugar, Ash, Total Solids, Acidity (% lactic acid) and pH content.



## Microbiological Analysis

### Phosphate Buffer (stock solution)

The potassium dihydrogen orthophosphate ( $\text{KH}_2\text{PO}_4$ ) 3.4 g was dissolved in 100 ml of distilled water. The pH was adjusted to 7 with 1 N NaOH solution so that after sterilization it would be 7.2. Then total volume was made to 100 ml with distilled water.

### Phosphate Buffer (dilution blanks)

To prepare dilution blanks, 1.25 ml of stock phosphate buffer solution was added to 1 lit; of distilled water and transferred in 9.3 ml quantities into glass test tubes (18×150 mm) and sterilized at 121°C/15 lb/15 min.

## Microbial Analysis of Health Drink

The samples of health drink were examined for probiotic counts, coliform counts and yeast and mould counts as per the standard methods of analysis of milk and milk products.

## Storage Studies

The samples of health drink were stored at refrigerated temperature for 7 days and analyzed for their sensory, chemical and microbiological quality over a period of 7 days.

## Statistical Analysis

The data generated was analyzed in respect of Factorial Completely Randomized Block Design (Panse and Sukhatme, 1985).

## Experimental Details

### Treatment Combinations

- $T_1$  :  $S_1F_1$  (10% culture of strain 1 + 10% sorghum flour)  
 $T_2$  :  $S_2F_1$  (10% culture of strain 2 + 10% sorghum flour)  
 $T_3$  :  $S_3F_1$  (10% culture of strain 3 + 10% sorghum flour)  
 $T_4$  :  $S_1F_2$  (10% culture of strain 1 + 10% finger millet flour)

$T_5$  :  $S_2F_2$  (10% culture of strain 2 + 10% finger millet flour)

$T_6$  :  $S_3F_2$  (10% culture of strain 3 + 10% finger millet flour)

$T_7$  :  $S_1F_3$  (10% culture of strain 1 + 10% sorghum + finger millet flour)

$T_8$  :  $S_2F_3$  (10% culture of strain 1 + 10% sorghum + finger millet flour)

$T_9$  :  $S_3F_3$  (10% culture of strain 3 + 10% sorghum + finger millet flour)

Where,

T - Treatment  $F_1$  - Sorghum flour

$S_1$  - Strain 1  $F_2$  - Finger millet flour

$S_2$  - Strain 2  $F_3$  - Sorghum + Finger millet flour

$S_3$  - Strain 3

## RESULTS AND DISCUSSION

The fermented probiotic cereal based health drink was prepared by using 10 per cent culture of *Lb. acidophilus* of each strain, i.e. Strain 1, Strain 2 and Strain 3, and 10 per cent of each cereal flour, i.e. Sorghum, Finger millet and Sorghum + Finger millet, 2 per cent jaggery and incubated at 37°C/16hrs in main experiment. The products were evaluated for sensory qualities, Chemical qualities and microbial qualities.

### I. Sensory Quality of Probiotic Cereal Based Health Drink

#### (a) Sensory Evaluation of Fresh Probiotic Cereal Based Health Drink

The Sensory Evaluation of fresh cereal based fermented probiotic cereal based health drink has been presented in Table 1.

From Table 1, it was observed that, the colour and appearance have shown non significant variation among different treatments. The treatments  $T_1$ ,  $T_2$  and  $T_3$  have shown the maximum scores 8.72 each for colour and appearance. The treatments  $T_1$ ,  $T_2$  and  $T_3$  have shown the maximum score 8.55 each for body and texture.

The flavour score has shown the significant ( $P < 0.05$ ) variation among the treatments. Treatment

**Table 1**  
Sensory Evaluation of Fresh Probiotic Cereal Based Health Drink

Fresh				
Sensory Attributes				
Treatments	Colour and Appearance	Body and Texture	Flavour	Overall Acceptability
T <sub>1</sub>	8.72	8.55	8.33 <sup>d</sup>	8.33 <sup>de</sup>
T <sub>2</sub>	8.72	8.55	8.66 <sup>f</sup>	8.66 <sup>g</sup>
T <sub>3</sub>	8.72	8.55	8.50 <sup>e</sup>	8.50 <sup>f</sup>
T <sub>4</sub>	8.55	8.00	7.83 <sup>a</sup>	7.66 <sup>a</sup>
T <sub>5</sub>	8.61	8.05	8.33 <sup>d</sup>	8.27 <sup>cd</sup>
T <sub>6</sub>	8.61	8.05	8.00 <sup>b</sup>	7.94 <sup>b</sup>
T <sub>7</sub>	8.55	8.22	8.22 <sup>c</sup>	8.16 <sup>c</sup>
T <sub>8</sub>	8.55	8.22	8.50 <sup>e</sup>	8.44 <sup>ef</sup>
T <sub>9</sub>	8.55	8.22	8.33 <sup>d</sup>	8.27 <sup>cd</sup>
SE(m) ±	0.059	0.056	0.020	0.041
CD at 5%	NS	NS	0.062	0.124

T<sub>2</sub> (10 per cent culture of strain 2 and 10 per cent sorghum flour) found superior (8.66 ± 0.062). Treatment T<sub>2</sub> (10 per cent culture of strain 2 and 10 per cent sorghum flour) found significantly differed for score for overall acceptability, yielding maximum score (8.66) than other treatments.

#### (b) Sensory Evaluation of Stored Probiotic Cereal Based Health Drink

The Sensory Evaluation of stored cereal based fermented probiotic cereal based health drink has been presented in Table 2.

From Table 2, it was observed that, the colour and appearance and body and texture score has shown non significant variation among different treatments. Treatments T<sub>1</sub> and T<sub>2</sub> have shown the maximum score 8.66 for colour and appearance and Treatments T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> have shown the maximum score 8.55 for body and texture.

Flavour score has shown the significant ( $P < 0.05$ ) variation among the treatments. Treatment T<sub>2</sub> (10 per cent culture of strain 2 and 10 per cent sorghum flour) found superior (8.61 ± 0.040).

Treatment T<sub>2</sub> (10 per cent culture of strain 2 and 10 per cent sorghum flour) found significantly differed for score for overall acceptability, yielding maximum score, 8.61, than other treatments.

**Table 2**  
Sensory Evaluation of Stored Probiotic Cereal Based Health Drink

Sensory Attributes				
Treatments	Colour and Appearance	Body and Texture	Flavour	Overall Acceptability
T <sub>1</sub>	8.66	8.55	8.27 <sup>de</sup>	8.27 <sup>d</sup>
T <sub>2</sub>	8.66	8.55	8.61 <sup>h</sup>	8.61 <sup>f</sup>
T <sub>3</sub>	8.61	8.55	8.50 <sup>gh</sup>	8.44 <sup>e</sup>
T <sub>4</sub>	8.55	8.11	7.66 <sup>a</sup>	7.66 <sup>a</sup>
T <sub>5</sub>	8.55	8.00	8.27 <sup>de</sup>	8.27 <sup>d</sup>
T <sub>6</sub>	8.55	8.00	7.83 <sup>b</sup>	7.83 <sup>b</sup>
T <sub>7</sub>	8.50	8.22	8.16 <sup>cd</sup>	8.11 <sup>c</sup>
T <sub>8</sub>	8.50	8.11	8.44 <sup>fg</sup>	8.39 <sup>de</sup>
T <sub>9</sub>	8.50	8.22	8.33 <sup>ef</sup>	8.33 <sup>de</sup>
SE(m) ±	0.038	0.052	0.040	0.049
CD at 5%	NS	NS	0.122	0.149

## II. Chemical Quality of Probiotic Cereal Based Health Drink

The perusal of data from Table 3 it is revealed that the treatments had non significant effect on moisture content for fresh products. for stored product all treatments have shown the reduction in moisture content as compare to fresh samples.

From Table 3 it is observed that the treatment T<sub>2</sub> (2.11 per cent) have the maximum protein content as compare to other treatments. The protein content of refrigerated stored samples has shown no significant change as compare to fresh samples.

The treatments T<sub>4</sub> (0.56 per cent) and T<sub>5</sub> (0.56 per cent) have shown the minimum fat content as compare to other treatments for fresh samples.

The reducing sugar has shown the non significant variation among the treatments. The treatments T<sub>4</sub> (6.13 per cent), T<sub>5</sub> (6.15 per cent) and T<sub>6</sub> (6.14 per cent) have shown comparatively maximum reducing for fresh samples while reducing sugar has been increased in the refrigerated stored samples. The treatments T<sub>1</sub> (6.48 per cent), T<sub>2</sub> (6.51 per cent) and T<sub>3</sub> (6.49 per cent) have shown the comparative increase in the reducing sugar of the stored product.

The treatment T<sub>5</sub> (7.45 per cent) have shown comparatively maximum reducing sugar. It showed

**Table 3**  
**Chemical Analysis of Probiotic Cereal Based Health Drink**

Fresh									
Chemical Parameters									
Treatments	Moisture (%)	Protein (%)	Fat (%)	Reducing Sugar (%)	Total Sugar (%)	Ash (%)	Total Solids (%)	Acidity (%)	pH (%)
T <sub>1</sub>	89.57	2.08	0.70	6.09	7.21	0.44	10.43	0.80 <sup>f</sup>	4.64
T <sub>2</sub>	89.53	2.11	0.70	6.11	7.22	0.44	10.47	0.86 <sup>h</sup>	4.52
T <sub>3</sub>	89.57	2.10	0.69	6.08	7.20	0.44	10.43	0.84 <sup>g</sup>	4.60
T <sub>4</sub>	89.93	1.39	0.56	6.13	7.44	0.68	10.07	0.66 <sup>a</sup>	5.15
T <sub>5</sub>	89.92	1.39	0.56	6.15	7.45	0.68	10.08	0.69 <sup>c</sup>	5.00
T <sub>6</sub>	89.92	1.41	0.55	6.14	7.44	0.68	10.08	0.68 <sup>b</sup>	5.08
T <sub>7</sub>	89.73	1.78	0.63	6.03	7.30	0.56	10.27	0.72 <sup>d</sup>	4.90
T <sub>8</sub>	89.71	1.79	0.63	6.05	7.31	0.57	10.29	0.74 <sup>e</sup>	4.78
T <sub>9</sub>	89.72	1.79	0.62	6.04	7.30	0.57	10.28	0.74 <sup>e</sup>	4.84
SE(m)±	0.114	0.111	0.011	0.095	0.083	0.013	0.114	0.035	0.094
CD at 5%	NS	NS	NS	NS	NS	NS	NS	0.010	NS
T <sub>1</sub>	89.51	2.10	0.70	6.48	7.24	0.44	10.49	0.85 <sup>f</sup>	4.40
T <sub>2</sub>	89.48	2.13	0.71	6.51	7.23	0.45	10.52	0.92 <sup>h</sup>	4.30
T <sub>3</sub>	89.51	2.12	0.70	6.49	7.22	0.45	10.49	0.90 <sup>g</sup>	4.38
T <sub>4</sub>	89.89	1.40	0.56	6.39	7.44	0.69	10.11	0.70 <sup>a</sup>	5.00
T <sub>5</sub>	89.88	1.41	0.56	6.43	7.46	0.69	10.12	0.74 <sup>c</sup>	4.85
T <sub>6</sub>	89.87	1.43	0.55	6.41	7.46	0.69	10.13	0.72 <sup>b</sup>	4.91
T <sub>7</sub>	89.69	1.79	0.63	6.32	7.32	0.57	10.31	0.77 <sup>d</sup>	4.62
T <sub>8</sub>	89.65	1.81	0.63	6.36	7.33	0.57	10.35	0.79 <sup>e</sup>	4.52
T <sub>9</sub>	89.67	1.81	0.63	6.34	7.33	0.57	10.33	0.78 <sup>de</sup>	4.59
SE(m)±	0.114	0.111	0.012	0.129	0.083	0.014	0.114	0.006	0.113
CD at 5%	NS	NS	NS	NS	NS	NS	NS	0.019	NS

finger millet based health drink has comparatively maximum total sugar.

The finger millet based health drinks, *i.e.* treatments T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub>, shown the maximum ash content *i.e.* 0.68 per cent for each, as compare to that one prepared from sorghum and sorghum+finger millet.

Treatment T<sub>2</sub> (10.47 per cent) shown the comparatively maximum total solids content for fresh samples. The total solid content of the stored product has been increased as compare to fresh samples.

From Table 3, it was observed that the treatments were significantly varied among each other. Treatments T<sub>1</sub> (6.48 per cent), T<sub>2</sub> (6.51 per cent)

and T<sub>3</sub> (6.49 per cent) have shown the maximum acidity. During storage the acidity of all samples were increased as compare to fresh.

pH of the product is related with the acidity. As the acidity of the product increases pH of the product decreases. From Table 3 it was observed that pH of treatments T<sub>1</sub> (4.64 per cent), T<sub>2</sub> (4.52 per cent) and T<sub>3</sub> (4.60 per cent) have comparatively minimum pH.

### III. Microbial Quality of Probiotic Cereal Based Health Drink

The *Lb. acidophilus* counts of fresh and stored samples fermented probiotic cereal based health drinks are presented in Table 4.

**Table 4**  
***Lb. acidophilus* Counts of Fresh and Stored Samples of Probiotic Cereal Based Health Drinks**

Treatments	<i>Lb. acidophilus</i> count ( $10^6$ cfu/gm)	
	Fresh	Stored
T <sub>1</sub>	78.33 <sup>d</sup>	63.33 <sup>c</sup>
T <sub>2</sub>	150.67 <sup>i</sup>	113.00 <sup>g</sup>
T <sub>3</sub>	113.67 <sup>b</sup>	92.00 <sup>f</sup>
T <sub>4</sub>	45.67 <sup>a</sup>	36.67 <sup>a</sup>
T <sub>5</sub>	98.00 <sup>f</sup>	80.00 <sup>e</sup>
T <sub>6</sub>	72.33 <sup>c</sup>	62.33 <sup>c</sup>
T <sub>7</sub>	63.33 <sup>b</sup>	50.67 <sup>b</sup>
T <sub>8</sub>	109.67 <sup>g</sup>	92.00 <sup>f</sup>
T <sub>9</sub>	93.33 <sup>e</sup>	69.67 <sup>d</sup>
SE(m)±	1.310	0.958
CD at 5%	3.951	2.888

From Table 4 it treatment T<sub>2</sub> (10 per cent culture of strain 2 and 10 per cent sorghum flour) was highly superior to all other treatments. The *Lb. acidophilus* counts for fresh T<sub>2</sub> products was  $150.67 \times 10^6$  cfu/g. There was reduction in the *Lb. acidophilus* counts in the refrigerated stored samples. In refrigerated stored samples also, the treatment T<sub>2</sub> has shown the maximum counts. The counts of Treatment T<sub>2</sub> reduced from  $150.67 \times 10^6$  cfu/g in fresh samples to  $113.00 \times 10^6$  cfu/g in refrigerated stored samples.

**(a) Yeast and Mould Counts of Fresh and Stored Probiotic Cereal Based Health Drinks**

The Yeast and mould counts of fresh and stored samples fermented probiotic cereal based health drinks are presented in Table 5.

From Table 5 it is observed that the for yeast and mould count for all the treatments were non significant with each other for fresh and store samples. Treatment T<sub>2</sub> has shown the minimum yeast and mould ( $1.67 \times 10^6$  cfu/g) as compare to other treatments. The yeast and mould count increased in the refrigerated stored samples as compare to fresh samples.

**(b) Coliform Counts of Fresh and Stored Probiotic Cereal Based Health Drinks**

The coliform counts of fresh and stored samples fermented probiotic cereal based health drinks are presented in Table 6. The fresh samples have shown

**Table 5**  
**Yeast and Mould Counts of Fresh and Stored Samples of Probiotic Cereal Based Health Drinks**

Treatments	Yeast and mould count ( $10^1$ cfu/gm)	
	Fresh	Stored
T <sub>1</sub>	2.33	5.67
T <sub>2</sub>	1.67	3.67
T <sub>3</sub>	2.00	5.00
T <sub>4</sub>	3.33	7.67
T <sub>5</sub>	2.67	5.00
T <sub>6</sub>	2.67	6.33
T <sub>7</sub>	3.00	6.67
T <sub>8</sub>	2.33	4.67
T <sub>9</sub>	3.33	6.00
SE(m)+	0.530	0.966
CD at 5%	NS	NS

the presence of some coliform count in the range of  $0.33$  to  $1.67 \times 10^1$  cfu/g. From Table 6 it was observed that the coliform counts were decreased in the refrigerated stored samples as compare to fresh samples.

**Table 6**  
**Coliform Counts of Fresh and Stored Samples of Probiotic Cereal Based Health Drinks**

Treatments	Coli form count ( $10^1$ cfu/g)	
	Fresh	Stored
T <sub>1</sub>	1.00	0.33
T <sub>2</sub>	0.33	0.00
T <sub>3</sub>	1.33	0.00
T <sub>4</sub>	1.67	0.67
T <sub>5</sub>	0.33	0.00
T <sub>6</sub>	0.67	0.33
T <sub>7</sub>	1.33	0.33
T <sub>8</sub>	0.33	0.00
T <sub>9</sub>	0.33	0.00
SE(m)+	0.455	0.243
CD at 5%	NS	NS

**CONCLUSIONS**

1. The best quality of fermented probiotic cereal based health drink can be prepared by using 10 per cent culture of *Lb. acidophilus* strain 2, 10

per cent sorghum flour and 2 per cent jaggery incubating at 37°C/16 hours on the basis of sensory quality

2. The chemical composition of fresh and stored sorghum based health drink was 89.53, 2.11, 0.70, 6.11, 7.22, 0.44, 10.47, 0.86 and 4.64 per cent and 89.48, 2.13, 0.71, 6.51, 7.23, 0.45, 10.52, 0.92 and 4.30 per cent for moisture, protein, fat, reducing sugar, total sugar, ash, total solids, acidity and pH, respectively.
3. The *Lb. acidophilus* count of fresh fermented probiotic cereal based health drink of treatment T<sub>2</sub> (10 per cent culture of strain 2 + 10 per cent sorghum flour) was superior over other treatments. There was reduction in the *Lb. acidophilus* count of stored samples of fermented probiotic cereal based health drink. But however these counts are fulfilling recommended dose of probiotics in products, which will possess the therapeutic value.

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