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Studies on Process Standardization and Organoleptic Evaluation of Cereal based Probiotic Beverage

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ABSTRACT

The present investigation focuses on standardizing the process for preparation of cereal based probiotic beverage. A combination of barley slurry, sorghum slurry, garden cress seed powder and pumpkin seed powder was used for the preparation of probiotic beverage. The lactic acid fermentation with LAB starter culture containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* was carried out. The most acceptable probiotic beverage having 70 ml barley slurry, 30 ml sorghum slurry, 4 gm garden cress seed, 4 gm pumpkin seed powder and 3 percent starter culture with fermentation period of 4 hours was found to be most desirable in terms of sensorial quality profile. It has total energy value of 75.127 Kcal and the shelf life of beverage was 9 days under refrigerator storage (4ºC). The process of preparation of cereal based probiotic beverage being a techno-economically feasible, justified the suitability of cereals in probiotic based functional food for commercial exploitation.

Introduction

"Probiotic" is used to refer to cultures of live microorganisms which, when administered to humans or animals, improve properties of indigenous microbiota. In the food industry, the term is described as "live microbial food ingredients that are beneficial to health" (Clancy, 2003). The term probiotic was technically defined by an Expert Committee as ''live microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition''. Species of *Lactobacillus* and *Bifidobacterium* are most commonly used probiotics, but the *Saccharomyces cerevisiae* and *Bacillus* species are also used as a probiotics (Tomisik and Tomasik, 2003).

Recently, probiotics have been more widely defined as bacteria that work to maintain the host's health (Holzapfel, 2002; Saito, 2004; Grajek *et al.*, 2005). Probiotics have some health benefits like they offers increased resistance to establishment of infection by potentially pathogenic organisms in the intestine, decreased duration of diarrhoea (antibiotic associated, travellers, infective), used in lactose intolerance (promotion of intestinal lactose digestion). increased nutritional value (better digestibility, increased absorption of vitamins and minerals), regulation of gut motility (constipation, irritable bowel syndrome), maintenance of mucosal integrity of the intestine, reduction in serum cholesterol concentration, reduction in allergy, prevention of colon cancer, reduction in carcinogen production etc. (Orrhage and Nord, 2000). Associated with probiotics are prebiotics. Prebiotics are non-digestible food ingredients that have a beneficial effect on the host by selectively stimulating growth of health-promoting bacteria (Desai *et al.,* 2004). They are assimilated by beneficial bacteria such as *Bifidobacteria* hence improving their growth activity leading to an enhanced intestinal balance. For a food to qualify as a prebiotic, it: has to be non-digestible by human enzymes; has to undergo selective fermentation by potentially beneficial bacteria in the colon; should cause an alteration in the composition of the colonic microbiota towards a healthier composition and all these changes should lead to a beneficial health to the host (Pariyaporn *et al.,* 2003). Some researches with prebiotics have reported reduction in putative risk factors for colon cancer and control of serum triglycerides as well as cholesterol (Roberfroid, 2001).

Most probiotic foods available today are milk based, but consumer preference today lie more with botanical dietary supplements, which are either free from or have minimal cholesterol content. Also, the market for functional foods is in its infancy in many countries; however, product innovation throughout a number of sectors, such as drinks, bakery and probiotics, is evident, with trends generally following those of the U.S. and U.K. (Luckow and Delahunty, 2004). With an increase in the consumer vegetarianism throughout the developed countries, there is also a demand for the vegetarian probiotic products. Furthermore, lactose intolerance and the cholesterol content are two major drawbacks related to the fermented dairy products.

There are a wide variety of traditional non-dairy fermented beverages produced around the world. Much of them are non-alcoholic beverages manufactured with cereals as principal raw material. Studies may be classified based on the source of raw material for the production of the non dairy probiotic beverage (Heenan *et al.,* 2004). The cereal products often ferment spontaneously, resulting in improved shelf life and better nutritional properties compares with raw materials. Fermentation procedures have been used to develop new foods with enhanced health properties (Blandino *et al.,* 2003).

Cereal grains are an important source of protein, carbohydrates, vitamins, minerals and fiber for people all over the world, and can be used as sources of non-digestible carbohydrates that besides promoting several beneficial physiological effects can also selectively stimulate the growth of *Lactobacilli* and *Bifidobacteria* present in the colon, thereby acting as prebiotics (Manthey *et al.,* 1999, Wood and Beer, 1998).

Cereals contain water-soluble fiber (such as β-glucan and arabinoxylan), oligosaccharides (such as galacto and fructo oligosaccharides) and resistant starch, and thus have been suggested to fulfil the prebiotic concept (Andersson *et al.,* 2001). The whole barley grain consists of about 65-68% starch, 10-17% protein, 4-9%, β-glucan, 2-3% free lipids, 1.5-2.5% minerals, 11-34% total dietary fibers and 3 to 20% soluble dietary fiber (Quinde *et al.,* 2004). According to USDA, (2010) sorghum is an excellent source of energy, containing about 75% complex carbohydrates (fibers and starches) which are usually digested slowly and therefore provide satiety and delayed hunger.

The pumpkin seeds were found to be rich in oil 50%, 38% protein and 3g/100g α-tocopherols (Younis *et al.,* 2000). The garden cress seed contains 34.15% protein, 1.86% crude oil, 9.85%crude fiber, 5.89% ash and 48.25% nitrogen free extract (NFE), on a dry weight basis (Gaafar *et al*., 2013).

The beneficial effects of food with added live microbes (probiotics) on human health are being increasingly promoted by health professionals. Probiotic products available in the markets today, are usually in the form of fermented milks and yoghurts; however, with an increase in the consumer vegetarianism throughout the developed countries, there is also a demand for the vegetarian probiotic products.

Materials and Methods

Sorghum, barley, pumpkin seeds and garden cress seeds, sugar were collected from local market

Preparation of starter culture

The starter culture was prepared with the help of the method described by Ghadge *et al*., (2008) with some modifications.

Composition of MRS medium

All the ingredients were suspended in distilled water and heated to dissolve the medium completely. The medium was sterilized in autoclave at 15 lbs pressure for 15 minutes (De Mann *et al*., 1960) (Table 1).

Isolation of lactic acid bacteria from the commercial yoghurt sample

The sample of yoghurt was used for isolation of culture on MRS agar. The serially diluted sample was inoculated on MRS agar and incubated at 37^0c for 24 hours. Then selected colonies were again inoculated in to MRS broth for 24-48 hours. After vigorous growth of culture then again inoculated and incubated on MRS agar to get pure culture. This culture was grown on nutrient agar by standard procedure.

Purity of the cultures

The staining of the obtained pure cultures of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* was carried out by using Gram positive staining technique for their identification (Harley and Prescott, 2002) (Table 2).

Sub-culturing of pure culture

The pure cultures i.e. *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were sub-cultured on slants prepared from MRS media in laminar air flow. This was incubated at 37º C for 24 hours in incubator. It is having microbial count nearly about 32 x 10^7cfu/ml.

Preparation of Probiotic cereal beverage

The production of probiotic cereal beverage was carried out by using *Lactobacillus acidophilus* and *Bifidobacterium bifidum* starter culture. The recipe used for preparation of cereal based probiotic beverage are mentioned below in Table 4 and the three standard compositions of composite flour are represented in Table 3 and pure LAB cultures used in recipe in different concentration is mentioned in Table 5.

Preparation of starter culture

Stock bacterial cultures

(*Lactobacillus acidophilus* and *Bifidobacterium bifidum*)

↓

Activation of bacterial strains in MRS broth separately at 37º C for 48 hr

↓

Starter culture Containing *L. acidophilus* and *B. bifidum* in equal proportions

↓

Centrifugation at 4000 rpm for 7 min

↓

Starter culture

Preparation of Barley Slurry

Barley

↓

Soaking
(12 hr)

↓

Draining

↓

Wet milling

↓

Addition of water (1:1)

↓

Filtration

↓

Settling and sedimenting

↓

Removing the top layer

↓

Adding water to make Barley Slurry

↓

Stored till use

Preparation of Sorghum Slurry

Sorghum
↓
Soaking (12hr)
↓
Draining
↓
Cooking (in boiling water for 2-3 min)
↓
Blending with water (1:1)
↓
Sorghum Slurry
↓
Stored till use

Preparation of Cereal Based Probiotic Beverages

Barley and Sorghum slurry (as per selected composition)
↓
Heat treatment (65 °C for 5 min)
↓
Cooling (22 °C)
↓
Addition of 5% sugar (w/w)
↓
Mixing for 10 min
↓
Filtration
↓
Unfermented cereal beverage
↓
Inoculation with 3% starter culture (*L. acidophilus* and *Bifidobacterium bifidum*)
(containing equal amount of LAB culture, having initial conc. of cells 32 x 10^7cfu/ml)
↓
Fermentation (37°C for 4hr)
↓
(Pumpkin seed powder 2%) → Plain beverage ← (Garden Cress seed powder 2%)
↓
Probiotic cereal beverage
↓
Packing in PET bottle and storage (4°C)

Results and Discussion

Proximate composition of raw material

The proximate composition of raw material is depicted in Table 6.

The data presented in Table 6 revealed that highest moisture content was observed in barley (12.3%) followed by sorghum (10.41%), pumpkin seed (7.43%) whereas lowest moisture content was recorded in garden cress seed (5.15%). Highest protein content was recorded in pumpkin seed (29.12%) whereas lowest protein was observed in barley (10.6%). It could be observed that highest fat content was recorded in pumpkin seeds (27.83%) whereas lowest value was recorded for barley (2.6%). Highest ash content was recorded for garden cress seeds (4.65%) whereas lowest ash was observed in sorghum (1.18%). Highest value of crude fibre was recorded in pumpkin seeds (12.3%) whereas lowest value was recorded in sorghum (1.3%). It could be observed that cereal grains had higher carbohydrate content than the pumpkin seeds and garden cress seeds. Highest value was recorded in sorghum (65.72%) whereas lowest was recorded in pumpkin seeds (22.21%). Similar results were obtained by Belitz *et al.*, (2009); Doke and Guha, (2014).

Sensory evaluation of cereal based probiotic beverage

The obtained mean score values for sensorial characteristics of beverage are summarized in Table 7.

It is evident from the Table 7 that among various sensory characteristics color, flavor and taste were significantly affected by the various levels of addition of starter culture i.e. *Lactobacillus acidophilus* and *Bifidobacterium bifidum ranging from 3 to 4 per cent* and its incubation time period 4 to 5 hr. The results presented in Table 7 showed that the color of beverage was found to get lighter with increase in concentration of LAB starter culture and acceptable color was observed in Sample A and containing 3 per cent of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* starter culture. The maximum score for color of beverage in treated sample was obtained by Sample A (i.e. 9.0). The scores assigned to flavor of beverage ranged from 8.4 to 9.0 among the beverage with highest score for Sample A. The taste (sourness) was found to increase with increase in concentration of culture. Maximum score for 'taste' was noted in the Sample A followed by C, while the B Sample scored the lowest value. Textural properties of all Samples were found to be increase with addition of starter culture. The beverage Samples added with starter culture are more viscous than control. Maximum textural score (i.e. 8.6) was secured by Sample A. The maximum score (i.e. 8.8) for overall acceptability was observed in Sample A having 70 per cent barley and 30 per cent sorghum and 3% *Lactobacillus acidophilus* and *Bifidobacterium bifidum* starter culture while the minimum score (i.e. 8.3) was observed in control Sample B.

Physicochemical analysis of cereal based probiotic beverage

As sample A containing 65 per cent barley in composite flour and 27 per cent sorghum flour and 3 per cent of L*actobacillus acidophilus* and *Bifidobacterium bifidum* starter culture had shown maximum consumer acceptability as observed through sensory evaluation of all Samples. Sample A was selected for further studies. The obtained results for various parameters are presented in Table 8.

From the results presented in Table 8, it has been observed that the pH, Titratable acidity, total solids and viscosity of accepted beverage

Sample were found to be 4.01, 0.44 per cent, 14.67 per cent and 35 centipoises respectively. Decrease in pH might be due to the less acidic nature of pumpkin seeds and garden cress seeds. These results are also corroborated with findings of Salwa and Diekmann, (2000).

Proximate composition of cereal based probiotic beverage

The proximate composition of cereal based probiotic beverage is depicted in Table 9.

Data presented in Table 9 revealed that the beverage Sample contained 81.28 per cent moisture, 3.43 per cent crude protein. Higher protein content in probiotic beverage might be might be due to the protein content presented in pumpkin seed powder and garden cress seeds. It was observed that ash content of probiotic Sample was 1.270 percent. Ash content increased significantly due to fermentation process and fortification with pumpkin and garden cress seed. It was observed that fat content of probiotic beverage was 1.823 per cent. It was increased due to the fortification of pumpkin seed powder and garden cress seeds. It was observed that the carbohydrate content of prepared probiotic beverage was 11.25 per cent.

Effect of storage period on physicochemical properties of cereal based probiotic beverage

The prepared probiotic beverage sample was further analyzed for physicochemical properties during storage up to 9 days. The data regarding the physicochemical properties of prepared probiotic beverage sample are presented in Table 10.

The data represented in Table 10 revealed that the pH of accepted beverage Sample was found to be 4.08, 3.98, 3.83, and 3.80 on the day of preparation, third, sixth, and ninth days after production respectively. It could be observed that the Titratable acidity of Sample increased during storage period. During storage period, the acidity values of the beverage Sample significantly increased from 0.30 per cent to 0.47 per cent from the day of preparation until the nine days of storage. It could be observed that the viscosity of the prepared probiotic beverage Sample increased with the storage. The viscosity of fresh beverage Sample was 36 cP at 25^0C whereas it was increased to 43 cP at 25^0C on 9^{th} day of storage. It could be observed that the Titratable acidity of the Sample was 0.30 per cent on the day of preparation, 0.39 on third day of storage, 0.42 per cent on sixth day and 0.47 per cent on 9^{th} day of storage. Data indicated that the storage time significantly affected the acidity level in the probiotic cereal beverage; Titratable acidity increased, while the pH was decreased. The positive change in Titratable acidity and negative change in pH level was due to the ongoing multiplication of LAB during storage period. These results are in agreement with those reported by Tangular and Erten (2012).

Measurement of textural (consistency) properties of cereal based probiotic beverage

The viscosity of different samples of cereal based probiotic beverage was calculated on Brookfield Viscometer DVE at constant speed 100rpm with a spindle number S-63 at different temperatures i.e. at 30, 32, 34 and 36^0C and expressed in terms of centipoises (cP). The results obtained regarding the consistency of probiotic beverage are presented in Table 11.

The data presented in Table 11 revealed that the viscosity of the beverage decreased with increase in temperature.

Table.1 Composition of MRS media for *LAB culture*

Ingredients (g)	*L. acidophilus*	*B. bifidum*
Proteose peptone	10	10
Yeast extract	5.0	5.0
Beef extract	10	10
Dextrose	20	20
Tween-80	1.0	1.0
Ammonium citrate	2.0	2.0
Sodium acetate	5.0	5.0
Magnesium sulphate	0.1	0.1
Manganese sulphate	0.5	0.5
Di-potassium phosphate	2.0	2.0
Distilled water (lit)	1.0	1.0
L.cysteine	-	0.5

Table.2 Confirmation tests for *Lactobacillus acidophilus* and *B. bifidum*

Biochemical test	*Lactobacillus acidophilus*	*B. bifidum*
Morphology	Rod, pair, chain	Y shape
Gram staining	+ ve	- ve
Catalase test	- ve	- ve

Table.3 Standardization of composite flour

Grain Flour	Sample A (g)	Sample B (g)	Sample C (g)	Control (g)
Barley	65	55	46	100
Sorghum	27	37	46	0
Pumpkin	4.0	4.0	4.0	0
Garden cress	4.0	4.0	4.0	0

Table.4 Standard recipe for preparation of cereal based probiotic beverage

Ingredient	Quantity (g)
Composite flour	100
Sugar	5
Garden cress seed	4
Pumpkin seed powder	4
Water (liter)	1

Table.5 Various levels of addition of starter culture at different Incubation period

Sample	Starter Culture Concentration (%)		Time (hr)	
	L. acidophilus	*B. bifidum*		
Control	3	4	3	4
A	3	4	3	4
B	3	4	3	4
C	3	4	3	4

Table.6 Proximate composition of raw material

Cereal Grain	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Ash (%)	Crude Fiber (%)	Total Carbohydrates (%)
Barley	12.3	10.6	2.6	2.1	9.5	62.01
Sorghum	10.41	11.1	3.1	1.18	1.3	65.72
Pumpkin Seeds	7.43	29.12	27.83	4.1	12.3	22.21
Garden Cress Seeds	5.15	21.47	2.78	4.65	6.8	34.24
SE	0.06954	0.03716	0.03653	0.00192	0.00319	0.00167
CD at 5%	0.20663	0.11148	0.9478	0.00572	0.00948	0.00495

Table.7 Sensory evaluation of cereal based probiotic beverage using hedonic scale

Sample	Color	Flavor	Taste	Texture	Overall acceptability
Control	8.8	8.4	8.6	8.2	8.5
A	9.0	9.0	8.8	8.6	8.8
B	8.2	8.4	8.5	8.3	8.3
C	8.6	8.5	8.6	8.4	8.5
SE	0.05963	0.09129	0.05963	0.05963	0.06393
CD at 5%	0.17889	0.27124	0.17717	0.17717	0.18996

Table.8 Physicochemical characteristics of cereal based probiotic beverage

Parameter	Values (%)
pH	4.01
T.S.S (^{0}Bx)	14.67
Viscosity (cP)	35
Titrable Acidity (%)	0.4

Table.9 Proximate analysis of cereal based probiotic beverage

Parameter	Values (%)
Moisture	81.28
Crude fat	1.823
Crude protein	3.43
Ash	1.270
Total Carbohydrate	11.25

Table.10 Physicochemical properties of probiotic beverage during storage

Storage (Days)	pH	Titrable Acidity (%)	Consistency at 25^0C (cP)
0	4.08	0.30	36
3	3.98	0.39	37
6	3.83	0.42	40
9	3.80	0.47	43
SE ±	0.012	0.006	0.06393
CD at 5%	0.036	0.019	0.18996

Table.11 Textural (consistency) properties of cereal based probiotic beverage

Temperature (^{0}C)	Consistency (cP)			
	Control	A	B	C
30	33	35	40	43
32	31	34	38	39
34	28	32	35	37
36	25	29	33	35

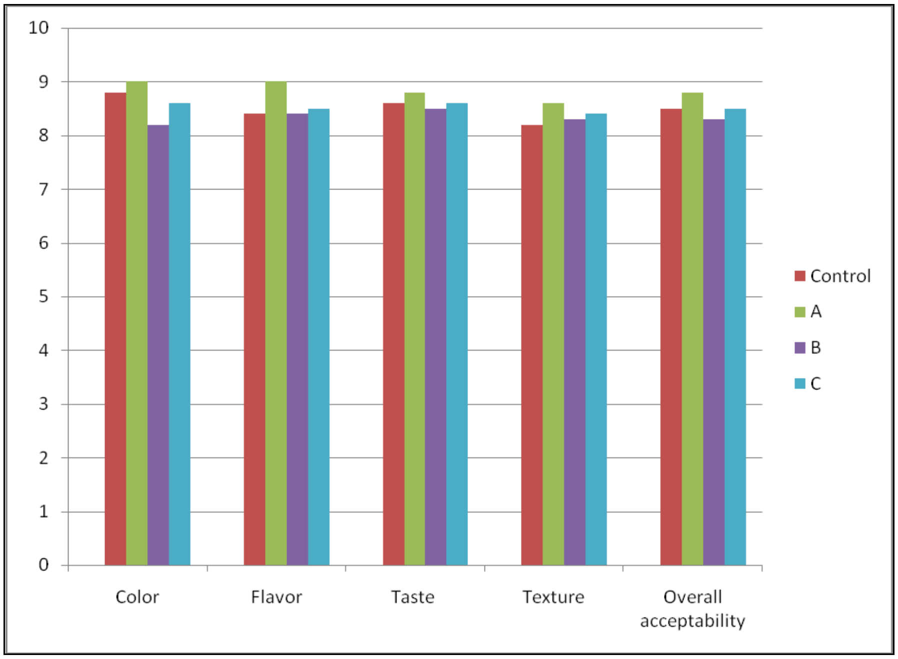
Table.12 Microbial analysis of probiotic beverage during storage

Storage (Days)	Total Plate Count (cfu/gm) x 10^6	Yeast and Mold Count (cfu/gm) x 10^6	Coliform Count
0	19	17	Absent
3	23	11	Absent
6	29	9	Absent
9	33	4	Absent

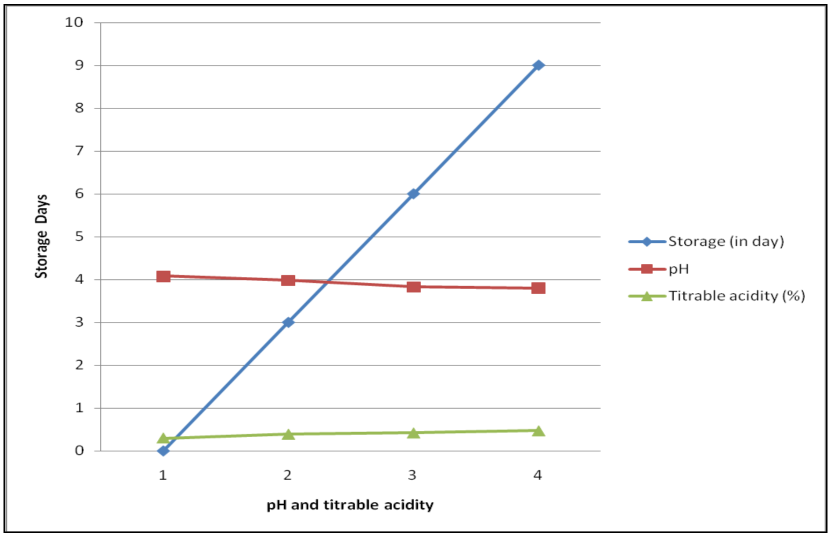
Table.13 Theoretical energy value of probiotic beverage

Parameter	Energy Value (Kcal)
Carbohydrate	45.00
Protein	13.72
Crude Fat	16.407
Total Energy	75.127

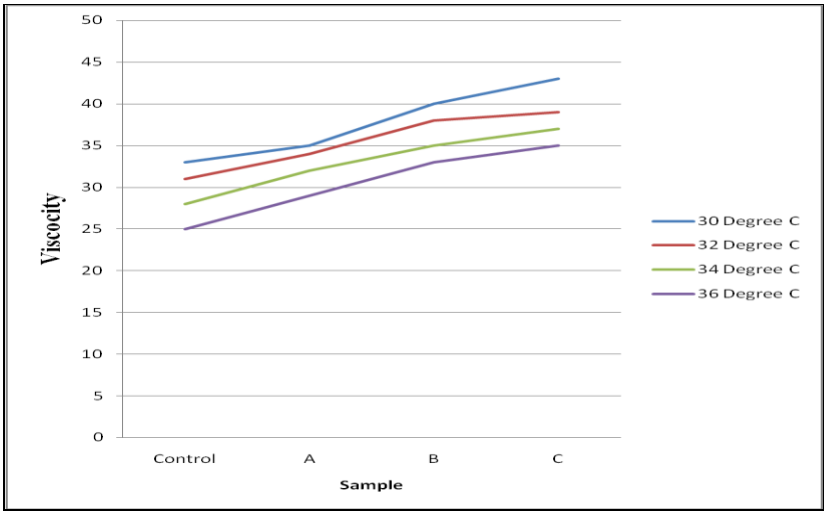
Graph.1 Sensory evaluation of cereal based probiotic beverage



Graph.2 pH and titratable acidity of accepted probiotic beverage during storage



Graph.3 Effect of temperature on viscosity of accepted probiotic beverage



Viscosity of the Sample C is highest i.e. 43 cP at temperature 30^0C and it decreased as temperature increased and Control Sample showed least viscosity at 36^0C i.e. 25 cP. Control Sample had lowest viscosity among all the other Samples i.e. 33 cP at temperature 30^0C which decreased with increased in temperature and recorded 25 cP viscosity at 36^0C temperature. It can be observed that addition of sorghum resulted in increase in the viscosity of the probiotic beverage. The decreased in acceptability of B and C as compared to control and A could be attributed to increased viscosity contributing to poor mouth feel of the probiotic beverage. Similar results were observed by Hasan *et al.*,(2012).

Microbial analysis of cereal based probiotic beverage during storage

The accepted beverage Sample was subjected to microbial studies for total plate count, yeast and mold count and *Coliform* growth during the storage period as per method adopted by Cappuccino and Sherman, (1996). The results recorded during the present investigation are presented in Table 12.

It is evident that the total plate count was the highest (33×10^6) in beverage after 9 days of storage period. However, the total plate count was lowest (19×10^6) in beverage Sample on production day. Total plate Counts (TPC) were high ranging from 19×10^6 cfu/g at zero time and reached 33×10^6 cfu/g at the end of storage period. Data presented in Table 11 indicated that the total plate counts of accepted beverage Sample is 19×10^6, 23×10^6, 29×10^6 and 33×10^6 cfu/g for the Sample on day of production, third, sixth and ninth days after production respectively. Maximum numbers of yeast and mold colonies were recorded in Sample on the day of production (17×10^6). However, the fungal count was lowest (4×10^6) in Sample after 9 days of storage period. From the Table 11 it is also observed that the yeast and mold counts of accepted beverage Sample is 17×10^6, 11×10^6, $9\times10^6$and 4×10^6cfu/ml for the Sample on day of production, third, sixth and ninth days after production respectively. The results showed that addition of lactic acid starter culture in beverage was effective in enhancing the shelf life of beverage by inhibiting mold growth. The results from Table also shows that, the beverage Sample was free from *Coliform* and *E. coli* when the Sample was fresh and throughout the storage period (of 9 days) at refrigerator temperature of 4ºC as result of good hygienic and sanitary conditions, during the preparation of cereal based probiotic beverage.

A minimum of 10^6–10^7plate microorganisms per gram or milliliter should be present in food product in order to meet the requirements of a probiotic food, as by the Japanese Fermented Milk and Lactic Acid Bacteria Beverages Association (Ishibashi and Shimanura, 1993).

Theoretical energy value of prepared cereal based probiotic beverage

The energy value of prepared probiotic beverage Sample is presented in Table 13.

It is evident from the Table 13 that the total energy value of the probiotic cereal beverage is 75.127 Kcal as calculated by adding energy value obtained from crude protein, crude fat and total carbohydrates.

Hence concluded, thus in light of scientific data of the present investigation, it may be concluded that the most acceptable probiotic beverage i.e. sample "A" having 70 ml barley slurry, 30 ml sorghum slurry, 4 g garden cress seed, 4 g pumpkin seed powder and 3 per cent starter culture with fermentation period of 4 hours was found to be most desirable in terms of sensorial quality profile of health food. The shelf life of beverage is calculated (9 days) under refrigerator storage (4ºC).

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