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Development of Various Millet Based Probiotic Beverages Using Different Strains of *Lactobacillus*

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Abstract: ***In present times, there has been an increased interest to adapt healthy diets, which help in preventing diseases, and as a consequence, the study and development of new functional foods has gained much importance. The allergy to dairy products affects negatively some persons. Lactose intolerance and the cholesterol content are two major drawbacks related to the fermented dairy products. Traditions and economic reasons that limit the use of dairy fermented products in some developing countries promote the idea of reduction of milk components as vehicles for the probiotic agents. Most of the Probiotics are Lactic acid bacteria and traditionally dairy products have been the vehicle for the delivery of Probiotics. Due to the consumer demand recently, has converted to non-dairy based probiotic products such as fruits and vegetables and cereal based products. Millets are unique among the cereals because of the richness of both Micronutrients and Macronutrients. Fermentation of the Millets have the positive influence due to the change of increase in amino acids, breakdown of proteins etc... In this describes the application of probiotic cultures in non-dairy food products.***

Keywords: Millet, Probiotics, Non- Dairy, Fermentation

1. Introduction

1.1 Millets

Millets are one of the cereals apart from the major wheat, rice, and maize. Millets are highly variable small-seeded grasses, widely thriving around the world as cereal crops or grains for grub and human food. Millets are important crop in the semiarid tropics of Asia and Africa (predominantly in India, Mali, Nigeria and Niger), with 97% of millet production in developing countries. The crop is recommended due to its productivity and dwindle season under dry, increased temperature conditions. Millets are endemic to many parts of the world. The most widely grown millet is pearl millet (kambu), which is a supreme crop in India and many parts of Africa. Finger millet, foxtail millet, Kodu millet are also vital crop species. Millets have been taken by humans from 7,000 years ago, especially by those who live in hot, dry areas of the world. Probably millets are small-grained, annual, warm weather cereals belonging to the grass family. They are highly tolerant of drought and other extreme weather conditions and other extreme weather conditions and have a similar nutrient content to other major cereals. They are cultivated chiefly in peripheral areas under agricultural conditions in which major cereals fail to give considerable yields (Adekunle, 2012).Millets are classified with maize, sorghum, coix (job`s tears) in the grass sub-family *Panicoideae* (Yang et al., 2012).Millets are important foods in many underdeveloped countries because of their ability to grow under unfavorable weather conditions like finite rainfall. Millets is the major source of energy and protein for millions of people. It has been reported that millet has many nutritious and medical functions (Obilana and Manyasa, 2002; Yang et al., 2012). It is a famine resistant crop and can be preserved for a long time without any nematode damage (Adekunle, 2012); hence, it can be important during drought.

1.2 Types of Millets

Millet is relevant to sorghum and belongs to the *Poaceae* (predominantly known as *Gramineae*) flowering plant family. In 2007, Global millet production reached about 32 million tons with the top producing countries. It is very hardy crop which is drought tolerant and grown on marginal soils where other crops cannot grow, and can supply 400-900 kg per hectare Shakshi et al., 2015).

S.No	Types of Millets	Common Name	Scientific Name
1	Finger millet	Ragi	*Eleusine coracana*
2	Kodu millet	Varagu	*Paspalum scrobiculatum*
3	Barnyard millet	Kuthiraivali	*Echinochloa* spp
4	Foxtail millet	Thinai	*Setaria italica*
5	Proso millet	Pani varagu	*Panicum miliaceum*
6	Little millet	Samai	*Panicum sumatrense*
7	Sorgham	Cholam	*Sorghum* spp

1.3 Probiotics

Probiotics are defined as live microbes that are beneficially affect the host by improving its intestinal microbial flora when administrated in adequate amounts (Fuller, 1989). It aids the existing flora or help to repopulate the colon when bacteria levels are reduced by antibiotics, Chemotherapy or diseases. Most of the probiotic foods provides fatty acids, Vitamins and other vital nutrients that improve the body`s resistance against pathogenic microorganisms (FAO/WHO, 2001).The abundant probiotics in fermented foods also improve digestion and the production of nutrients, like vitamins, probiotic microbes inhibit the growth of food spoilers and can both prevent and treat diarrhea. They inhibit tooth decay, can help to manage some types of diabetes and some have been proved to reduce “bad” cholesterol. Many researches proved that addition of Probiotics to food leads to several health benefits including the reduction of level of serum cholesterol, the improvement of gastrointestinal function, the enhancement of the immune system, the suppression of diarrhea in young children and the lowering of the risk of colon cancer (Berner and Donnel, 1998). Most of the Probiotics are Lactic acid bacteria and traditionally

dairy products have been the vehicle for the delivery of Probiotics. The usage of probiotics foods which may helps in not getting the Irritable bowel syndrome but due to the consumer demand recently, has converted to non-dairy based probiotic products such as fruits and vegetables and cereal based products.

1.4 Nutritional Composition of Millet Grains

Millets are unique among the cereals because of their richness in calcium, dietary fibre, polyphenols and proteins (Devi et al., 2011). Millets is nutritionally an excellent source of minerals, especially calcium, potassium, phosphorus and magnesium that provides to a large part of the recommended dietary allowance value. Millets primarily contain significant amount of essential amino acids (methionine and cysteine). They are also higher in fat content than maize, rice and sorghum (Obilana and Manyasa, 2002). In general, cereal proteins including millets are limited in lysine and tryptophan content. Plant nutrition are largely used in the food industry, and cereal grains constitute a major source of dietary nutrients worldwide (Amadou *et al*.,2011a; Izadiet et al.,2012).Modification of a protein is usually realized by physical, chemical, biological such as fermentation or an enzymatic treatment, which changes its structure and consequently its physicochemical and functional properties (Lestienne et al.,2007;Amadou et al.,2011b). Table 2 represents the nutritional content of different millets such as pearl millet, finger millet, foxtail millet, kodu millet, kuthiraivali and little millet.

Jowar (*Sorghum vulgare*), Pearl millet (*Pennesetum glaucum*), Finger millet (*Eleusine coracana*), Thinai(*Setaria italic*) are the multibeneficial grains which are consumed by large population of India due to their high availability and low cost. Jowar contains nutrients like iron, calcium, potassium, phosphorus, B- vitamins and photochemical which are usefulness in reducing obesity. Pearl millet is high in protein as compared to other cereals and contains all essential amino acid and is rich in folate, potassium, magnesium, copper, zinc, vitamins E, B-complex, calcium and iron. It helps in maintaining the cardiovascular health and reduces acidity problems, control cholesterol, prevents diabetes and minimizes the risk of cancer. On other hand Ragi is a power house of health benefiting nutrients that helps in reducing weight and also it used as treatment for multiple diseases like brittle bones, osteoporosis, anemia and diabetes. It is a natural relaxant that helps in relieving stress and anxiety (Michaelraj and shanmugam 2013).These millets are constitutionally similar to wheat(Koehler and Wieser 2013), they are expected to contain probiotic molecule like Inulin besides arabinoxylan found in latter (Samanta koruri et al.,2016). Millets are recognized nutritionally for being a good source of minerals, magnesium, manganese and phosphorus and also rich in phytochemicals, including phytic acid (Shakshi et al. 2007), which is believed to lower cholesterol and phytate, which is associated with reduced cancer risk. Therefore there is an enormous scope growing in this crop to explore the technological possibilities of its utilization of its food industry for the preparation of various food products.

1.5 Fermentation

Traditional fermented foods are receiving extensive scientific attention globally and many traditional preparations have been analyzed for their microbiological, enzymological and biochemical changes (Omemu et al, 2007).The various millet based traditional preparations are available through the worldwide and the fermentation of the millets are more common. Fermentation of millets has a positive influence.

Millets is the primary source of energy and protein and also have nutritious and medical functions (Obilana and Manyasa, 2002). Millets grains are easily digestible and have a long shelf life and do not contain gluten, hence advised for celiac (Gluten sensitive enteropathy) patients (Chandrasekhar and Shahidi, 2010). Additionally a major development in functional foods also pertains to foods having probiotics which impart immense health benefits to human system.

Functional foods with probiotics are used universal wide at fast scale and these have become ultra popular level among the consumers (Saarela et al .2000). Therefore, health food containing probiotics/synbiotics constitute present and future prospects in evolution of the food developing process.

Enhancement of biological value (BV), net protein utilization (NPU), thiamin, riboflavin and niacin contents are increased in fermented millets (Aliya and Geervani, 1981). Changes that are take place during fermentation include increase in amino nitrogen, the breakdown of proteins and destruction of any inhibitors that may be present (Davidek et al.,1990).

2. Review of Literature

Millets is a primary category for several species of small grained cereal crops and is a staple food in parts of India, Africa, China and everywhere. The millet is employed for several related genera, some used to produce grain, or forage or both. Millet has been cultivated since prehistoric times in regions of North Africa and Central Asia, though its origin is ambiguous. Mostly millet is produced in Asia and Africa. In Europe and USA millets bare grown as forage crops for live stock and bird feed. Millets are the family of Grasses which are known as “Little gaint”.

Mainly millets are classified into two types i.e major millets and minor millets. The *serobiculatum*), Finger millet (*Eleusine coracana*),Kodu millet (*Paspalum scrobiculatum*) and major millets which includes maize (Zea maize), Pearl millet (*Pennisetum glaucum*).Minor millets includes grain crops like little millet Samai (*Panicum sumatrense*), Proso millet (*Panicum miliaceum*) Fox tail millet (*Paspalum serobiculatum*), Finger millet (*Eleusine coracana*), Kodu millet (*Paspalum scrobiculatum*).

Millet contains an average of 10-12% protein. While its protein is capital to that of wheat or corn in use of content of essential amino acids, it nonetheless contains less than half the amount of the essential amino acid lysine that is found in high quality protein sources such as meat.

Millet lack gluten, the wheat protein that makes dough prepared from wheat flour elastic; hence millet flour generally is used in making flat cakes and breads. The whole grain is used in soups or in a cooked cereal. Millet is also popped, roasted or sprouted (Robert Ronzio,2004).

Functionality of probiotics

The beneficial effects of food with added live microbes (probiotics) on human health and particularly on children and other high- risk populations are being increasingly promoted by health professionals. It has been reported that probiotics can play a significant in immunological, digestive and respiratory functions and have a important effect in alleviating infectious diseases in children. However, some health benefits, for example immune modulation, may be achieved even with dead bacteria (Kalliomaki et al., 2001).

Prevention of diarrhea

Several probiotic strain especially *Lactobacillus rhamnosus* have been shown to prevent or alleviate infantile diarrhea, which is caused by Rota virus. It is also well- defined that some probiotic strains can both prevent and shorten antibiotic- associated disorders. (Fuller et al., 2008).

Stimulation of the immune system

Many human studies performed to investigate the effects of probiotic cultures on the immune system reveal that the probiotic bacteria are able to enhance the both innate and acquired immunity by increasing the natural killer cell activity and phagocytosis, making change in cytokine profiles and increasing the levels of immunoglobulins. The strains of *Lactobacillus* have been demonstrated in several studies to enhance natural immune system in healthy peoples (Fuller et al., 2008).

Inflammatory bowel syndrome

There is growing evidence that probiotics have a potential therapeutic benefit for patients suffering from inflammatory bowel disease (IBS).

Lactose intolerance

Bacterial cultures, yogurt starter cultures as well as some probiotic cultures are known to improve the lactose digestion in lactose maldigestors. Subjects suffering from lactose intolerance have a very low concentration of the lactose- cleaving enzyme β- galactosidase, and bacteria in fermented or unfermented food products release their β-galactosidase in the small intestine, where it supports the lactose digestion (Li et al., 2012).

Allergies

Pelto et al. found that *Lactobacillus rhamnosus* confers an immunostimulatory effect in healthy adults. Probiotics have also been used successfully in the management of atopic eczema in infants. Furthermore, *Lactobacillus rhamnosus* proved to be effective in the prevention of early atopic disease in children at high risk. The *Lactobacillus rhamnosus* product when given prenatally to mothers and postnatally for 6 months to the mothers or to their infants directly, reduced the frequency of atopic eczema in the probiotic group to half that of the placebo group at the age of 2 years. The preventive effect was reconfirmed at the age of 4 years (Delcenserie et al., 2010).

High cholesterol

Many human studies have evaluated the effects of culture-containing dairy products or probiotic bacteria on cholesterol levels with equivocal results. A fermented milk containing *Enterococcus faecium* and *Streptococcus thermophilus* was reported to produce a small but significant decrease in total and LDL-cholesterol in patients with primary hypercholesterolemia. (Pereira et al., 2002)

3. Materials and Methods

3.1 Collection of Cultures

The different strain of *Lactobacillus* was collected from ICAR-NDRI- National Collection of Dairy Cultures, Karnal, Haryana. The freeze- dried cultures in ampoule, strains of *Lactobacillus* which was bought such as

S.No	Strain Name	NCDC Accession No
1.	*Lactobacillus casei spp. casei*	17
2.	*Lactobacillus plantarum,*	020
3.	*Lactobacillus acidophilus,*	14
4.	*Lactobacillus rhamnosus*	19
5.	*Lactobacillus fermentum*	604

Activation of Cultures

The lyophilized cultures which was activated by using skimmed milk medium which was mentioned in the NCDC catalogue.

Composition of skim milk media

#Skim milk powder : 28 g
#Tryptone : 5 g
#Yeast extract : 2.5 g
#Dextrose : 1 g
#Agar : 15 g
pH :7.0 ± 0.2

Then the cultures which was incubated at 37-40º for 48 hours but when the turbidity does not appeared in 48 hours incubate for another 24 hours because the lyophilized culture may take high incubation time rather then other liquid form cultures.

3.1 Collection of Samples

The millet grains were bought from commercially available market from town hall, Coimbatore. The millet such as Kodu millet, Thinai, Saamai, Kambu, Kudhiraivaali, Ragi, Sorghum are the various types of millet which was bought and used. All samples were segregated, cleaned and stored in air tight containers till further use.

3.1.1 Washing the Impurities

Millets which was used was soaked in water and the impurity which was appeared on the top of the water was discarded.

3.1.2 Roasting

Roasting and grinding processes render the grain digestible, without the loss of nutritious components. The puffing and roasting are almost similar process but the volume expansion in puffing is higher.

Grinding
A part of millet seeds was grinded in a mixer and was sieved using 1.0 mm sieve and then it was stored in the air tight container for further studies.

Formulation of Millet Beverage
The 25 grams of ground and sieved seven different types of flour was cooked with 500 ml of distilled water in an sterile container. When the mixture of solution was boiled at 78ºC and it was retained for a further 10 minutes. Then the mixture was cooled to 40ºC, then the activated probiotic *Lactobacillus* strains which was inoculated in the cooked millet mixture.

Then millet containing probiotic culture was incubated at 37ºC for 4 hours in the metabolic shaker. Subsequently 150 ml of milk was pasteurized (78ºC for 1 minute) and 46g/L of sugar/ cane sugar, 7.9 g/L of cocoa powder were added to the pasteurized milk and stir well.

Then this mixture was added to the previously incubated fermented millet solution and it was stored in the air tight sterilized containers under 4ºC for weeks and the shelf life of the beverage was determined.

Evaluation of Microbial Profile
The activated probiotic strains of *Lactobacillus* were further inoculated into Man, Rogosa, Sharpe (MRS) medium and microbial evaluation was done.

Composition of Man, Rogosa, Sharpe (MRS) medium (Aneja, 2003)

- Peptone : 10g
- Beef extract : 10g
- Yeast extract : 5g
- Dextrose : 20g
- Ammonium citrate : 2g
- Distilled water : 1000 ml
- pH : 6.5

Then the MRS agar plates were prepared by adding 20 grams/1L of agar and then the organism was streaked and then isolates were primarily examined according to their colony morphology, catalase reaction and gram reaction.

Gram Staining (Gram, 1984)
Cultures were grown in appropriate medium at 37ºC for 24 hours under anaerobic conditions. Cells from fresh cultures were used for gram staining. After incubation, cultures were transferred aseptically into 1.5 ml eppendrof tubes and centrifuged for 5 minutes at 6000 rpm. Then, supernatant was removed and cells were resuspended in sterile water. Gram staining procedure was followed which was formulated by Gram in 1984. Then the gram stained slides were observed under microscope and then observed for the rods.

Catalase test (Aneja, 2003)
Catalase test was performed for cultures in order to observe for catalase reaction. Overnight cultures were grown on selective medium at suitable conditions. After 24 hours 3% hydrogen peroxide was randomly dropped onto the culture which was placed in the slide. Also fresh liquid cultures were also used for catalase test by dropping 3% hydrogen peroxide solution onto 1 ml of overnight cultures. Therefore, the strains which were used did not give gas bubbles (Catalase negative).

Biochemical Tests
The following biochemical tests were performed with the strains of Lactobacillus viz. MRVP and Casein hydrolysis.

Methyl Red and Voges- Proskauer (MRVP) test (Aneja, 2003)
Tubes of MRVP broth (pH 6.9) were inoculated with the strains separately followed by the incubation at 35ºC for 48 hours. Then the tubes were examined for change in the color of methyl red for MR test and crimson red to pink for VP test.

Composition of MRVP broth (Aneja, 2003)

Peptone	:	7.0 g
Dextrose	:	5.0 g
Potassium phosphate	:	5.0 g
Distilled water	:	1000 ml
pH	:	6.9

Casein hydrolysis (Aneja, 2003)
Skimmed milk agar medium was autoclaved at 15 lb pressure for 15 minutes. The medium was then poured into sterile petri plates and the medium was allowed to solidify. Then the strains of *Lactobacillus* was streaked onto the plates containing medium and then it was incubated at 37º C for 24 hours in an inverted position. The result which was interpreted according to the presence and absence of zone of clearance around the line of growth was examined.

Tritrable acidity and pH
The titratable acidity of the product was determined by method described in the Indian standards (IS: 1479-Part I, 1960) and A 25 g sample was titrated with 0.1N of NaOH according to the method described. The acidity based on lactic acids (acid- base titration method) (Sharma, 2006)

The pH was measured by a pH meter against a standard buffer of 4.0 pH.

Sensorial Evaluation
Nine point hedonic scale method as given by Amerine et al.(1965) was followed for conducting the sensorial evaluation of probiotic food products. The panel of 10 judges was selected to evaluate the probiotic millet beverage. Sensory evaluation of the beverage for flavor, texture, acidity, color and appearance and total score was carried out by panel of judges.

4. Results and Discussion

Collection of cultures
The different strain of *Lactobacillus* was collected from ICAR-NDRI- National Collection of Dairy Cultures, Karnal, Haryana. The freeze- dried cultures in ampoule, strains of *Lactobacillus* was used. The Lactobacillus strains which carried the accession number for each different strain which includes,

1.	*Lactobacillus casei spp. casei*	17
2.	*Lactobacillus plantarum,*	020
3.	*Lactobacillus acidophilus,*	14
4.	*Lactobacillus rhamnosus* and	19
5.	*Lactobacillus fermentum*	604

Collection of samples
The millet grains were bought from commercially available market from town hall, Coimbatore. The millet such as Kodu millet, Thinai, Saamai, Kambu, Kudhiraivaali, Ragi, Sorghum are the various types of millet which was bought and were cleaned and then stored in sterilized air tight container.

Activation of Cultures
The cultures were activated by using the Skimmed milk medium and it was incubated at 37ºC for 48 hours and observed for turbidity, if the turbidity of the cultures is not seen then it was further incubated for another 24 hours.

3.1.1 Washing the Impurities
Millets which was used was soaked in water and the impurities which was appeared on the top of the water was discarded and then it was shade dried.

Formulation of Millet Beverage
The seven different types of millet flour were cooked to 78º C with 500 ml sterile water and it was retained for 10 minutes and then the mixture allowed to cool to 40ºC and probiotic strain of *Lactobacillus* was inoculated.

The five different strains of *Lactobacillus* strains were used for the fermentation of the millets. Hence, the millet flour was cooked for inoculating the five different cultures. For another, inoculation of the *Lactobacillus casei* only the ragi flour was cooked in 500 ml sterile water and it was also retained for 10 minutes in 78º C and cooled down to 40ºC and the strain was inoculated in it.

The millet mixture was incubated at 37º-40ºC for 4 hours.

Subsequently 150 ml of milk was pasteurized (78ºC for 1 minute) and 46g/L of sugar/ cane sugar, 7.9 g/L of cocoa powder were added to the pasteurized milk and stirred well.

Then the mix was added to the previously incubated millet beverage and mixed.

Later it was stored in a sterile air tight container in 4ºC for weeks.

Evaluation of Microbial Profile
The activated probiotic strains of *Lactobacillus*were further inoculated in MRS broth.

Gram Staining (Gram, 1984)
The gram staining was performed to the strains which was isolated from MRS medium.
Cultures were grown in appropriate temperature at 37ºC for 24 hours under anaerobic conditions.
The stained slides were observed under the microscopes at different magnifications.
The Lactobacillus is a gram positive, bacillus which absorbs the primary stain, and appears rod shaped.

Catalase test (Aneja, 2003)
Catalase test was performed for cultures in order to observe for catalase reaction. After 24 hours 3% hydrogen peroxide was randomly dropped onto the culture which was placed in the slide. The catalase test which was done for strains of the *Lactobacillus* and the result was observed to be **negative** to the catalase test.

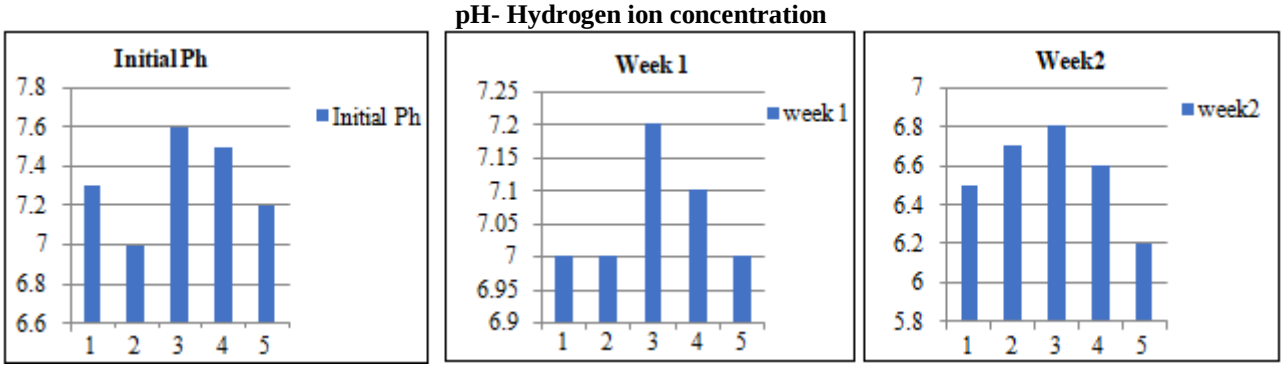
Casein hydrolysis (Aneja, 2003)
The result which was interpreted according to the presence and absence of zone of clearance around the line of growth.

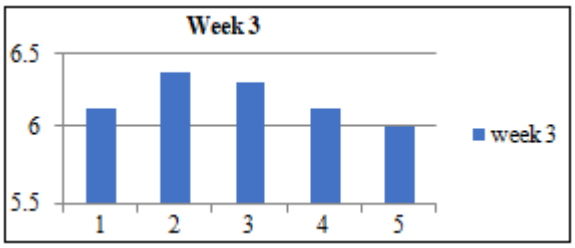
Biochemical Tests
The following biochemical tests were performed with the strains of Lactobacillus viz. MRVP and Casein hydrolysis.

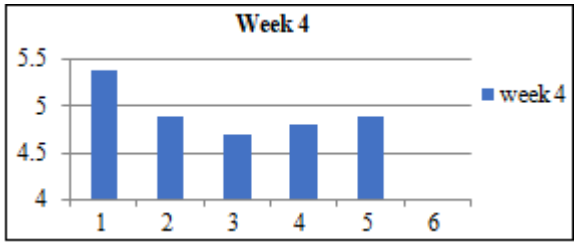
Methyl Red and Voges- Proskauer (MRVP) test (Aneja, 2003)

S.No	*Organisms*	*MR*	*VP*
1	*Lactobacillus plantarum*	+	+
2	*Lactobacillus casei*	+	+
3	*Lactobacillus acidophilus*	+	_
4	*Lactobacillus rhamnosus*	+	+
5	*Lactobacillus fermentum*	+	+

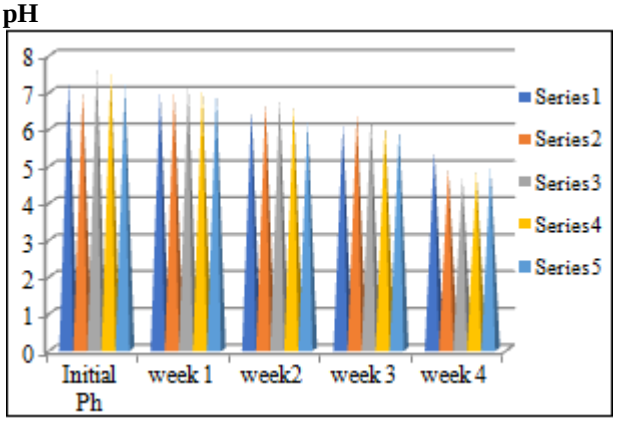
pH- Hydrogen ion concentration







Comparison of initial pH with storage period of 4 weeks pH



Acidity of the fermented millet beverage

The titration was by using burette containing 0.1N sodium hydroxide.

The 10 ml of fermented millet was taken and then diluted with 10 ml distilled water.

Then 3-5 drops of phenolphthalein solution were added to the mixture.

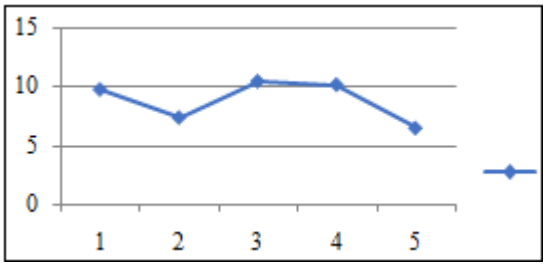
Mixed and observed for the first persistent pink color with 0.1N NaOH.

By using the formula, find the percentage of the volatile acidity and lactic acid acidity.

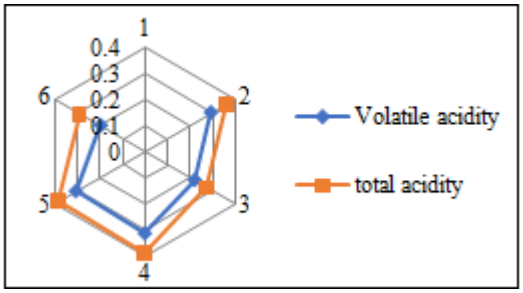
$$\textbf{Total acidity of lactic acid} = \frac{\text{ml of alkali} \times \text{Normality of alkali} \times 7.5}{\text{Weight of sample in grams}}$$

$$\textbf{Volatile acidity} = \frac{\text{ml of alkali} \times \text{Normality of alkali} \times 6.0}{\text{Weight of samples in grams}}$$

S.No	ml if alkali	Volatile acidity	total acidity
1	9.8	0.294	0.367
2	7.4	0.222	0.277
3	10.5	0.315	0.393
4	10.2	0.306	0.382
5	6.5	0.195	0.288



ml of alkali



Total and volatile acidity

Sensorial Evaluation of the Millet Beverage

Sensory Evaluation Sheet

Evaluation for sensorial quality of fermented millet beverage

Name of the Analyst:

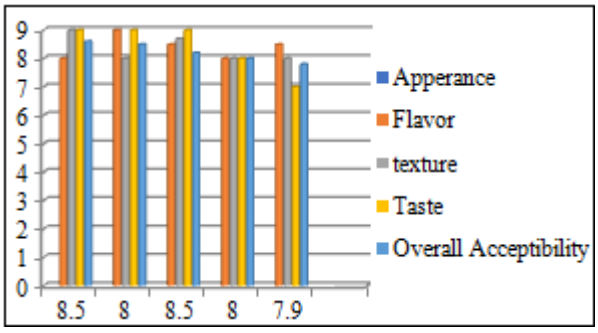
Product Name : Heptads millet beverage

Lactose Intolerant : Yes / No

Kindly evaluate the given sample on Hedonic scale (1 to 9) according to attributes mentioned below:

S.no	Sample	Sensory Parameters				
		Appearance/ Color	Flavor	texture	Taste	Overall Acceptibility
1	*L. plantarum*	8.5	8	9	9	8.6
2	*L. rhamnosus*	8	9	8	9	8.5
3	*L. casei*	8.5	8.5	8.7	9	8.2
4	*L.acidophilus*	8	8	8	8	8
5	*L. fermentum*	7.9	8.5	8	7	7.8

The fermented millet beverage samples were assessed by 10 panalist using a 9 point sensory hedonic scale for some sensory parameters (viz. appearance/color, flavor, texture, taste and overall acceptability), as described by Amerine *etal*. (1965). In sensory evaluation the millet beverage *L.plantarum* and *L. rhamnosus.*



Shelf life evaluation

Titration acidity was increased and pH was reduced at the end of the storage period of 4 weeks. But it remained in total acidity and pH level to the viable of probiotic bacteria.

There no no change in sensory attribution upto IV week. Therefore, this beverage could serve as a ready to drink functional beverage could serve as a ready to drink functional beverage under refrigerated (5ºC ±1) storage upto 4 weeks.

Consequently 4 hours fermentation was enough to maintain an overall sensory quality and functional level of the beverage which was proven by the results obtained by sensory.

5. Summary

Based according to the obtained results of the study, it can be concluded there was better survivability of the probiotic strains of *Lactobacillus* in the fermented millet beverage.Thecount of probiotic organisms was within the level of standards (108CFU/ mL) until theend of 4 weeks. Finally, this research has revealed that sensory acceptable various millet based fermented probiotic beverage can be developed using *Lactobacillus* byinoculating 1ml/Linoculum of activated *Lactobacillus* and could serve asa ready to drink functional beverage in refrigerated (5 ± 1 °C) storage up to 4 weeks.

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