



E-ISSN: 2278-4136

P-ISSN: 2349-8234

JPP 2019; 8(5): 2230-2234

Received: 07-07-2019

Accepted: 09-08-2019

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Viability of *Lactobacillus* in non-dairy matrices and its effect on quality characteristic of product

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Abstract

This work investigated the effect of LAB starter cultures on the titratable acidity with respective incubation time and its viability during refrigeration storage in a fermented gruel from wheat flour and green gram malt mixture. The microorganisms used were *Lactobacillus acidophilus* and *Lactobacillus plantarum*. There were significant increases in TA of non dairy probiotic drink with respective time of incubation. It was observing that the decrease in viscosity of drink as storage time and viable count of LAB were increased at refrigeration temperature. It observed that in first four days of storage, there was constant increase in viable count of bacteria tested on MRS media while the percentage of growth is slightly decrease showed by six day of storage. The results of this work showed that LAB were good candidates for grow on the non dairy substrate content wheat flour and green gram malt based gruel with inhibiting the survival of other undesirable bacteria.

Keywords: *L. acidophilus*, *L. plantarum*, viability titratable acidity, non dairy probiotic drink

Introduction

Most probiotic foods at the markets worldwide are milk based and very few attempts are made for development of probiotic foods using other fermentation substrates such as cereals. Their large distribution and important nutritive value have focused the attention on their use as raw materials for the development of new fermented functional foods (Angelov, A. *et al.* 2006) [2]. Fermentation procedures have been used to develop new foods with enhanced health properties (Blandino, A. *et al.* 2003) [4]. Historically, *Lactobacilli* had been found to be associated with food. The food containing *Lactobacillus* are thought to be safe (Adams M.R., 1995) [1]. *Lactobacillus* is an important organism recognized for their fermentative ability as well as their health and nutritional benefits (Gilliand S.E. *et al.* 1999) [9].

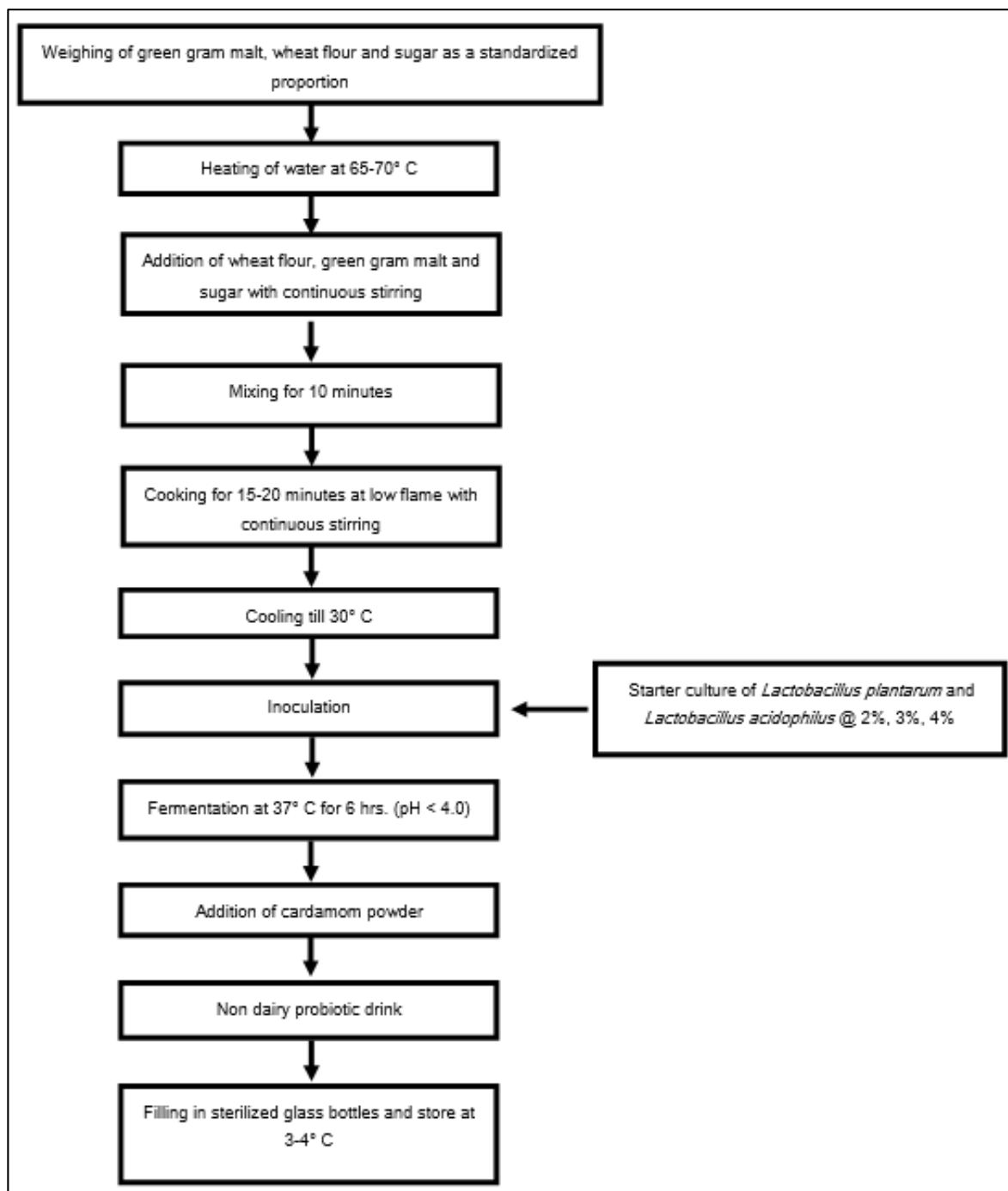
Lactic acid bacteria (LAB) have been used successfully, with few adverse effects, to prevent antibiotic associated diarrhea, to treat acute infantile diarrhea and recurrent *Clostridium difficile* disease and to treat various diarrheal illnesses (Siitonen S. *et al.* 1990, Saavedra J.M. *et al.* 1994, Biller J.A. *et al.* 1995) [23, 19, 3]. The antagonistic property is attributed to the lowered pH, the undissociated acids and production of other primary and secondary antimicrobial metabolites produced by LAB. That result in growth inhibition of food spoilage or poisoning bacteria and detoxification of noxious compounds of plant origin (Schillinger U., 1989 and Salminen, S., 1996) [22, 20].

Lactic acid bacteria (LAB) are important in much fermentation and the antagonistic effects of LAB are attributed to some of their features. They can utilize carbohydrates and produce organic acid as lactic acid. The majority of food borne contaminants, either pathogenic or non-pathogenic sensitive to these acid and the resulting low pH. They also produce antibacterial substances such as bacteriocines, hydrogen peroxide, diacetyl and CO₂ which may also play part in the antagonism of LAB on other microorganisms (maganusson and schnurer, 2001) [16]. LAB have shown a major potential for use in bio-preservation because of safety for human consumption (GRAS status) and the prevalent microflora during storage in many foods (Vignolo *et al.*, 2008) [25]. The health benefits offered by LAB can be nutritional or therapeutic including production of vitamins, immunomodulation, reduction in the risk of diarrhea, and mutagenic activity and a decrease in serum cholesterol (Briand *et al.*, 2006; Myllyluoma *et al.*, 2007; Linsalata *et al.*, 2010) [6, 18, 15]. Exploitation of LAB as a preservative agent is advantageous not only in improving the microbial safety of food but also as a probiotic in animals and humans to improve the balance of microflora and to inhibit pathogenic bacteria in intestinal tract (Soomro *et al.*, 2002) [24]. The Lactic acid bacteria (LAB), generally considered as food grade organisms, show special promise for selection and implementation as protective cultures. There are many potential applications of protective cultures in various food systems (Holzapfel *et al.*, 1995) [12].

Cereal-based products ability to support the growth of probiotics is mainly due to their high concentration of fibres such as xylooligosaccharides, xylan and arabinoxylan, which may act as a growth substrate for probiotics. Strains of *Lactobacillus* have been recognised as complex microorganisms that require fermentable carbohydrates, amino acids, vitamin B, nucleic acids and minerals to grow (Gomes AMP and malcata, 1999) ^[10]. Cereal grains readily support the growth of microorganisms, including lactic acid bacteria, provided the presence of water and hydrolytic

enzymes (Salovaara & Geanzle, 2011) ^[21] Lactic acid fermentation usually improves nutritional value and digestibility of cereals (Charalampopoulos *et al.*, 2002) ^[7]. Hence the present study investigated that to prove as the cereal are ideal substrate for growth of LAB based on the viability of LAB in the non dairy matrices during storage period and the changes in TA by incubation period.

Materials and methods



Flowchart for preparation of non-dairy probiotic drink

Materials

Processing and analytical equipments

The processing and analytical equipments included hot air oven, incubator, glass wares, laminar air flow inoculation chamber, and an electronic balance with the accuracy of 0.0001g for weight measurements were obtained from

Department of Food and Industrial Microbiology, College of Food Technology, V.N.M.K.V., Parbhani.

Pure cultures of LAB

Pure cultures of *Lactobacillus acidophilus* and *Lactobacillus plantarum* procured from NCL pune.

Methodology

Viable cell counts

Bacterial cell numbers were determined using the surface plating method. To enumerate viable cell, 1 mL of each samples was homogenized aseptically with 9 mL of sterile physiological saline (0.85%, w/v) and suitable dilutions were plated on appropriate media. *Lactobacillus* spp. was enumerated on MRS agar (Chengcheng Li, 2014) [8].

Titrateable acidity

The acidity of sample was calculated by standard A.O.A.C. method (2000). The weighed sample (5g) was ground gently in mortar and pestle by using distilled water (for beverage 5ml of sample was taken). The final volume was made to 100ml and it was then filtered through Whatman no.4 filter paper. A measured aliquot (10ml) was then titrated against 0.1N NaOH using phenolphthalein as indicator to a light pink colour. It was expressed as per cent acidity.

$$\% \text{Acidity} = \frac{\text{Titre} \times \text{Normality of NaOH} \times \text{Volume made up} \times \text{equivalent wt. of acid} \times 100}{\text{volume of sample taken for estimation} \times \text{weight or volume of sample taken} \times 1000}$$

Organoleptic evaluation of non dairy probiotic drink

Freshly prepared non dairy probiotic drink was evaluated for sensory characteristics like color, taste, texture (consistency), flavor and overall acceptability at room temperature in sensory evaluation laboratory by a panel of semi trained judges, comprised of postgraduate students and academic staff members of College of Food Technology, V.N.M.K.V., Parbhani. Samples were scored based on a nine point hedonic scale. Hedonic scale was in the following sequence: like extremely- 9, like very much - 8, like moderately -7, like slightly-6, neither like nor dislike -5, dislike slightly- 4,

dislike moderately-3, dislike very much-2, dislike extremely-1 (BIS, 1971) [5].

Results and Discussion

In present investigation sincere efforts were made to standardize the addition levels of LAB starter culture viz. *Lactobacillus acidophilus* and *Lactobacillus plantarum* and incubation period by standardize the TA of cereal and legume based non-dairy probiotic drink.

Viable counts (LAB) present in non dairy probiotic drink during storage

The total viable counts of drink observed at different storage period on MRS agar media. It is evident that viable counts are observed at dilutions rate of 10^{-6} , 10^{-7} and 10^{-8} on the storage day of 0, 2, 4 and 6.

Table 1: Viable counts (LAB) of non-dairy probiotic drink during storage at 4°C

Storage in (days)	Viable counts		
	(cfu/gm)x10 ⁶	(cfu/gm)x10 ⁷	(cfu/gm)x10 ⁸
0	3.3	2.1	1.5
2	4.1	2.6	1.9
4	4.6	2.9	2.1
6	4.9	3.0	2.1

The result showed that the viability of LAB is increased with storage period. It proved that the ability of non dairy substrate to survive the LAB. A minimum range of 10^6 – 10^7 plate 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 Drinks Association (Ishibashi and Shimanura, 1993) [13].

Table 2: Effect of starter culture added by various concentrations on the TA of non dairy probiotic drink

Sample code	Starter culture (%)	Incubation period (hrs)	% Titrateable acidity (in terms of lactic acid)
A ₁	2	5	0.180
A ₂	3	5	0.315
A ₃	4	5	0.342
B ₁	2	6	0.225
B ₂	3	6	0.360
B ₃	4	6	0.405
C ₁	2	7	0.270
C ₂	3	7	0.387
C ₃	4	7	0.450

Final non-dairy probiotic drink was prepared from 8.5 g of composite flour (Control, A, B and C), 100ml tap water, 7 per cent (w/w) sugar and LAB starter culture (2, 3 and 4 %) containing equal proportions of *Lactobacillus acidophilus* and *Lactobacillus plantarum*. Which have 0.360% TA (in terms of lactic acid) at 3.98 pH. The prepared drink evaluate by standardized it TA in the manner of lactic acid against starter

culture was added and the incubation period. Results showed that increase the concentration of starter culture leads to reducing pH with increasing TA that show the improvement in sensory quality of food. Also the same results cited by Molin N. (1991) [17] in the production of lactic acid fermented oatmeal.

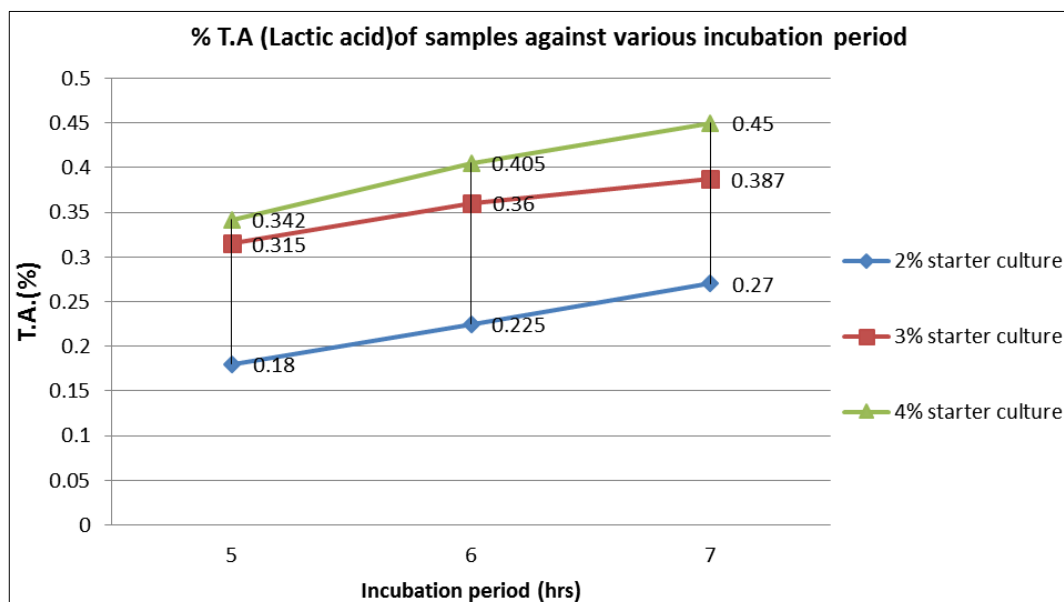


Fig 1: Effect of concentration of starter culture with different incubation period on titratable acidity (in terms of lactic acid) of non dairy probiotic drink

Effect on textural properties of prepared probiotic drink

The viscosity of different samples of cereal and legume based non dairy non-dairy probiotic drink 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°C and expressed in terms of centipoises (cP).

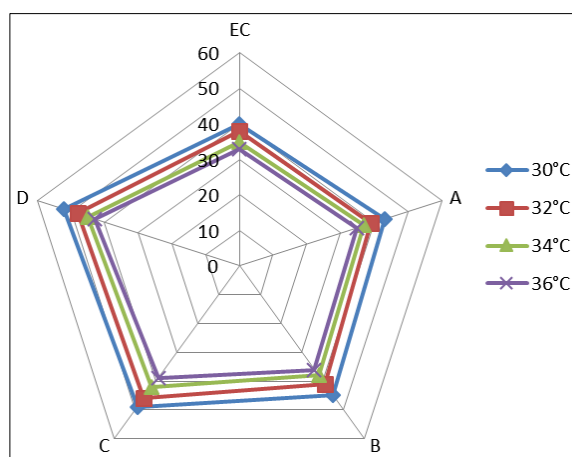


Fig 2: Effect of temprature on textural (consistency) properties of non dairy probiotic drink

It can be observed that addition of green gram malt (1.5%, 2.5%, 3.5%, and 4.5%) resulted in increase in the viscosity of the non-dairy probiotic drink. Also the incubation period and concentration of starter culture affect on the viscosity of non-dairy probiotic drink. The acceptability of C (containing 5% wheat flour and 3.5% green gram malt) is more as compared to A, B and D could be attributed to contributing to poor mouth feel of the non-dairy probiotic drink. Similar results were observed by Hasan *et al.*, (2012)

Effect of starter culture on sensory quality of non dairy probiotic drink

The sensorial quality characteristics of cereal based non-dairy probiotic drink play a vital role in attracting consumers to purchase the product. Consumer judges drink quality on the basis of its sensory parameters such as color, flavor, taste, texture etc. Sensorial evaluation was done using hedonic scale.

Table 2: Organoleptic evaluation of prepared probiotic drink

Sample code	Color	Flavor	Taste	Mouth feel	Overall acceptability
A1	7.8	7.2	7.3	7	7.3
A2	7.8	7.3	7.4	7.3	7.4
A3	7.9	7.3	7.8	7.4	7.6
B1	7.9	7.5	7.8	7.7	7.7
B2	8	7.8	8	8.2	8
B3	7.8	7.3	7.4	7.8	7.5
C1	7.8	7.3	7.3	7.7	7.5
C2	7.7	7.1	7.3	7.4	7.3
C3	7.7	7.1	7.3	7	7.2

The result showed that the sensorial parameter such as color, flavor, taste and mouthfeel were improved till 6 hrs incubation period and 3% starter culture with increasing titratable acidity. The starter culture added by the quantity of 3% for 6 hrs incubation period showed the highest hedonic score at the pH 3.8. (i.e. 8) in overall sensory parameters. Apart that the increased incubation period and starter culture concentration constantly reduces the consumer acceptability. It is observed that the sourness produces due to increased in TA mask the aroma developed by LAB results in increasing consumer acceptability with respective incubation period. Incubation period with high viable microbial counts and when added to certain foods, it accelerates fermentation leading to a final product with a desirable alteration in the aroma, texture and flavor profile (Holzapfel, 2002) ^[11]. The same results were found by Kingamkono RR (1997) ^[14] as the pH of non dairy probiotic drink (*Togwa*) is 4.0-3.2 for better taste of drink.

Conclusion

Results from this research work shows that LAB plays an important role in reducing the pH of food by producing lactic acid and the majority of food borne contaminants, pathogenic or non- pathogenic sensitive to these acids and the resulting low pH. It improves the overall acceptability and palatability of the final product by imparting a unique taste. Cereals and legumes can be an ideal substrate for the culture of probiotics, since they already contain beneficial nutrients like carbohydrates, proteins, minerals, vitamins and dietary fibers.

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