

Development of Corn Milk Based Fermented Milk Inoculated with *Lactiplantibacillus plantarum*

ABSTRACT

This fermented drink is based on the corn milk and the bovine milk which got enormous nutritional and health benefits. In the mixture of pasteurized corn milk and bovine milk, mother culture containing the 2% of pure probiotic strain of *Lactiplantibacillus plantarum* was inoculated aseptically and allowed to ferment at 37°C for 6–8 hrs. Fermented mixture was then sweetened with honey and flavoured with vanilla essence for its final tuning of the flavour. Sensory evaluation was carried out to study the consumer acceptance of the product. Results for microbiological parameters were found in compliance with the microbial safety standard of food products. Product had 15.47% protein, 3.83% total sugar and 1.47% total lipids. The initial values for the pH were recorded at 4.9 ± 0.02 and titratable acidity at 0.319 % lactic acid. Shelf- life study of the product was conducted by examining the variation of Lactobacillus counts on MRS agar plates. Lactic acid produced from the Lactobacillus bacteria metabolism causes variation in titratable acidity and pH. These variations also indicated shelf life of the product.

Keywords: Probiotics, corn milk, Lactiplantibacillus plantarum, fermented milk and Lactic acid bacteria.

1. INTRODUCTION

According to FAO/WHO report, probiotics are “live microorganisms which when administered in adequate amounts conferring a health benefit on the host” (2002). As per FSS Regulations, amended since 2021, the viable amount of incorporated probiotic organism in food shall be $\geq 10^8$ colony forming unit (CFU) in the daily recommended serving portion (Food Safety and Standards Regulations, 2016).

Probiotics are beneficial bacteria that helps in the balancing of the gut microbiota (X. Wang *et al.*, 2021), inhibit the harmful bacterial growth (Ahmad and Khalid, 2018; Kopp-Hoolihan, 2001) aid in digestion (Cammarota *et al.*, 2009; O’Sullivan *et al.*, 1992), enhance immune function (Adel *et al.*, 2017; Cammarota *et al.*, 2009; Maldonado Galdeano *et al.*, 2019; Mazziotta *et al.*, 2023; Umair *et al.*, 2022; Vanderpool *et al.*, 2008; Yan and Polk, 2020) and improve resistance against infection (Yan and Polk, 2011). Consumption of probiotic products around 100 g/day can meet the required daily intake 10^8 – 10^9 CFU/g of probiotics which could survived the upper digestion and confers the physiological benefits (Terpouet *et al.*, 2019). According to the Standard for Fermented Milks CXS 243–2003, flavoured fermented milks are composite milk as defined in General Standard for the Use of Dairy Terms CXS 206–1999. They are fermented milk– based drinks which are obtained by combining fermented milk and potable water, optionally incorporating the other additional non–dairy ingredients and flavourings. Non– dairy ingredients encompasses both nutritive and non–nutritive sweeteners, fruits, vegetables, fruit juices, purees, pulps, preparations and preserves derived from them. Cereals, honey, chocolate, nuts, coffee, spices and other natural flavouring are also non–diary ingredients. By mass (m/m), drinks based on fermented milk contain a minimum 40% of fermented milk while the flavoured fermented milks contain a

maximum of 50%. The non-dairy ingredients can be added either before or after the fermentation process. Maize (*Zea mays*), cereal crop, which is originated from the Southern Mexico before 1000 years ago. Corn that has attained the productive stage R6 was used for corn milk production. According to ICAR-Indian Institute of Maize Research (IIMR) and (Ritchie and Wei, 2015), corn of R6 reproductive stage shall contain 30–35% of kernel moisture and attains its maximum dry matter by approximately 45–50 days. As milk is rich in lactose sugar, it serves as one of ingredients in the growth medium of the probiotic culture i.e *Lactiplantibacillus plantarum*. It also enhances the consistency and flavour of the product. Honey contains oligosaccharides and low molecular weight polysaccharides that possess prebiotic properties. Oligosaccharides in honey remains undigested in the upper part of gastrointestinal tract human and animals but are fermented by beneficial microorganisms in the large intestine. Fresh honey also contains probiotics (Gaifullina *et al.*, 2016). Thus, honey would increase the probiotic activities while serving as a prebiotic. Sensory evaluation for consumer acceptance was carried out using 5-point hedonic scale. The microbial safety of the product was established through enumerating the microbiological counts. Microbial parameters analyzed were Total Plate Count (TPC), coliforms and Yeasts and Molds counts using pour plating method. Physico-chemical parameters analyzed were pH, titrable acidity, protein, sugar and total Lipid content. Shelf-life study of the product was conducted by examining the variation of Lactobacillus count on MRS agar plates, pH and titratable acidity. Even it helped in indicating the viability and growth of the probiotic strain.

In the Bergey's manual of systematic bacteriology, *Lactiplantibacillus plantarum* belongs to group 19 and Lactobacillus genus. It is a regular, non-sporing and gram-positive rods. These bacteria are facultative anaerobes. They are also aerotolerant and sometime exhibits microaerophilic behaviour. The cells have rod shaped and usually measuring $0.5\text{--}1.2 \times 1.0\text{--}10.0\mu\text{m}$. They are usually elongated rods, sometimes almost coccoid and commonly appearing in short chains. Cells are rarely motile which is achieved through peritrichous flagella. Utilizes carbohydrates anaerobically. The major fermentation products are mainly lactate and may produce some acetate, ethanol and CO_2 . They are widely distributed in fermentable materials of animal and vegetable food products (Seddik *et al.*, 2017). Additionally, they can also be found in gastrointestinal tracts of birds, mammals and vaginal tracts of some mammals. These bacteria exhibit enhanced growth in the presence of 5% CO_2 and have an optimum growth temperature between 30–40°C (Bergey and Holt, 1994).

The *L. plantarum* strains have larger genome size as compared to other LAB that ranges between 3.0–3.6 Mbps (Siezenet *et al.*, 2010) and 3–3.3Mbps (Goel *et al.*, 2020; Li *et al.*, 2015). The *L. plantarum* is considered as Generally Recognized As Safe (GRAS) and Qualified Presumption of Safety (QPS) (EFSA Panel on Biological Hazards (BIOHAZ) *et al.*, 2016; Kandasamy *et al.*, 2022; Ray and Joshi, 2014). These statuses were given by United States Food and Drug Administration (US FDA) and European Food Safety Authorities (EFSA) respectively (Echegaray *et al.*, 2023). Numerous beneficial and functional probiotic properties of *L. plantarum* are reported in many literatures such as antioxidant (Jeong *et al.*, 2021; Tang *et al.*, 2018; Tian *et al.*, 2022), antimicrobial (Liu D, -Met *et al.*, 2022; Liu Y *et al.*, 2022; Riaz Rajoka *et al.*, 2020; Surve *et al.*, 2022), acidic pH tolerance (Nath *et al.*, 2020; Tang *et al.*, 2018), GI resistance (Liu D, -M *et al.*, 2022; Liu Y *et al.*, 2022; Riaz Rajoka *et al.*, 2020; Surve *et al.*, 2022), and adhesion capacity to intestinal mucosa (Liu D, -M *et al.*, 2022; Liu Y *et al.*, 2022; Metrouhet *et al.*, 2022; Surve *et al.*, 2022). The several strains of *L. plantarum* are reported to have potential in compositional modification of the gut microbiota (Echegaray *et al.*, 2023).

Many *L. plantarum* strains are capable of producing bacteriocins normally referred as plantaricin belonging to both class I (lantibiotic) and class II (non-lantibiotic) bacteriocins, while most of them belongs to class II (Choi *et al.*, 2023). These bacteriocins increases

shelf-life of food products and protects against unwanted colonization in the hosts (Yilmaz *et al.*, 2022). Bacteriocins of certain *L. plantarum* strains have antimicrobial activities toward both pathogenic and food spoilage bacteria (Wang *et al.*, 2018; Wang *et al.*, 2018; Wang *et al.*, 2018; Zeng *et al.*, 2014). It also produces several other antimicrobial compounds including organic acids such as lactic, citric, isobutyric, and acetic acids, ethanol, diacetyl, and H₂O₂ (Arena *et al.*, 2016; Liu D.-M. *et al.*, 2022; Liu Y *et al.*, 2022; Markkinen *et al.*, 2022). Furthermore, it can produce natural antifungal exopolysaccharides (Riaz Rajoka *et al.*, 2020). Eubiosis is well balanced intestinal microbiome which is crucial for the prevention of both infectious as well as non-infectious diseases. The distortion in this microbiome equilibrium is referred as dysbiosis (Al-Rashidi, 2022). Probiotics possess the ability to adjust, modify the gut microbiota composition and rectify the abnormal immune responses (Zhang *et al.*, 2019). Bacteriocins of *L. plantarum* are considered as safe to utilize as bio-preservatives in the food industries. No genes related to antibiotic resistance and pathogenic characteristics were identified in *L. plantarum* genomic sequence (Yilmaz *et al.*, 2022).

2. MATERIAL AND METHODS

2.1 MATERIAL

Corn, Pasteurized milk, Probiotic culture – *L. plantarum*, natural honey and vanilla essence were used for the preparation of the product.

2.2 METHODOLOGY

2.2.1. Preparation of Stock Culture

10mL milk is taken and pasteurized at 63°C for 30 minutes. Two loops of culture were inoculated from stock cultures and incubated at 37°C for 6–8h. It was refrigerated immediately at 4°C.

2.2.2. Preparation of Mother Culture

It was prepared by aseptically transferring 1mL of stock culture into 10mL pasteurized milk. Incubation was carried out at 37°C for 6–8hrs. It was refrigerated immediately at 4°C.

2.2.3. Preparation of Corn Milk

Husks and silks were removed from harvested corn cobs. They are then washed with clean water. The kernels were then separated manually from the cleaned cobs by hands. The washed kernels were boiled at 100 ± 5 °C for 5 minutes. The kernels were grinded with water 1:3 (Yasni and Maulidya, 2014). Slurry was then filtered using a fine mesh strainer to produce a corn milk. The corn milk was finally heated treated at 63 °C for 30 minutes and stored at 4°C until use.

2.3. Standardization of Fermented milk

Corn milk was blended with milk at various percentage of proportions. The proportions of corn milk increases while milk proportion decreases by 6% (v/v). The proportion of the starter culture and honey were kept constant as 2% and 16% (v/v) respectively. The different proportions were given in the form of different treatments of T1, T2, T3 and T4 as shown Table 1.

Table 1. Various proportions of bovine milk and sweet corn milk

Treatment	Corn milk (%)	Pasteurized Milk(%)	Honey(%)	Probiotic culture (%)
T1	38	44	16	2
T2	44	38	16	2
T3	50	32	16	2
T4	56	26	16	2

2.3.1. Product preparation

Husk and silk were removed and the cob was washed with filtered water. The corn kernels were separated from the cleaned cob. These washed kernels were boiled at 100 ± 5 °C for 5 minutes. The kernels were ground with water (1:3) and the slurry was then filtered to produce a milk solution. Mix heat-treated milk (44%) and corn milk (38%) and transfer into sterilized glass bottles. The mixed milk was pasteurized at 63 °C for 30 minutes. The lactic culture *Lactiplantibacillus plantarum* was inoculated into cooled milk at 2 % rate and incubated at 37°C for 6-8 h. Honey was added at rate of 16 % and 0.15 ml of vanilla essence and stored at 4 °C.

2.4 Sensory Analysis

The sensory evaluation was carried for all the different treatments of corn milk probiotic drink. Quality attributes like colour, consistency, taste, aroma/odour and overall acceptability considered as sensory characteristics for the panelists. Sensory scores were given based on 5–point hedonic scale as 5 for highly acceptable and 1 for highly unacceptable. Sensory scores were further interpreted through mean score ranking.

2.5 Chemical Analysis

Since treatment T1 got highest mean score in overall acceptability during sensory analysis, T1 was analyzed for the different physico– chemical parameters. TSS was measured using Erma Hand Refractometer with range of 0–32% and A–Contrast 11–520–0. pH variation during the storage period was recorded using pH meter (Model: LMPH–10, Make: LABMAN, Bangalore, India). Even the Total titratable acidity values during storage period were measured as per the FSSAI manual of method of analysis of foods– Milk and Milk products, 2005. The 5 readings of pH were taken at the 3 days intervals from 0th day when the product completed its formulation procedure followed by the days at the refrigerated condition. Total sugar content was determined through Anthrone Method, Protein content through Pyne's method and Total lipid through Bligh and Dyer's method.

2.6 Microbial Analysis

Microbial enumeration was done as per the procedure prescribed in IS 5402: 2012 for establishing the microbial safety of the product. Microbial analysis in terms of Total plate count, Coliform and yeast and molds were enumerated using pour plate method.

2.7 Shelf-life study

The pH values were taken as one of the parameters for studying the shelf life of the product. There will be the production of the lactic acid, acetic acid (in some cases), ethanol and

CO₂ due to lactic acid bacteria metabolism. Thus, the quantity of titratable acids and metabolism rate of probiotic bacteria is directly correlated with the variation of pH values of the product. Acidity of the product is related to the palatability of the product thus, giving the clues about the shelf life of the product. Since *L. plantarum* was used as the probiotic strain in product, taking their microbial cell count through plating allows us to check its viability, stability and metabolic activities of the bacteria. Thus, determining the variation in counts of *L. plantarum* growth on the MRS agar plates serve as the prime indicator for the shelf-life study.

3. RESULTS AND DISCUSSION

3.1 Sensory Analysis

Table 2. Mean scores of Sensory evaluation

Sensory characteristic	T1	T2	T3	T4
Colour/Appearance	4.22	4.22	4.22	4.22
Taste	4.11	3.88	3.77	3.61
Flavour	4.33	3.55	3.44	3.44
Consistency	4.00	3.88	3.77	4.00
Overall acceptability	4.22	3.66	3.77	3.61

The overall acceptability mean scores of the T1 > T3 > T2 > T4. Thus, treatment 1 (T1) formulation was considered appropriate for further analysis.

3.2 Chemical Analysis

Table 3. Results for Chemical analysis

Sl. No.	Chemical Parameters	Observation
1	TSS	21.78 ⁰ ± 0.03 brix (10.89 ± 0.25%)
2	Protein	15.47 ± 0.15%
3	Total lipids	1.47 ± 0.20%
4	Total sugar	3.83 ± 0.35%

3.3 Shelf-life study



Figure 1. pH variation of the product during refrigerated storage.

During the initial day of storage, pH value was recorded at 4.9 ± 0.02 which further shift towards the acidity range at 3.16 ± 0.02 upon 12th day of storage period. The decreasing pH and increasing acidity level in corn milk yoghurt was showed by (Yasni and Maulidya, 2014). As the product storage period increase, the pH gradually decreases due to increase in the lactic acid production as the result of probiotic bacteria metabolism.

During the initial day, titratable acidity value was recorded as 0.319 which further increases to 0.58. As per the book published by Codex Alimentarius on "Milk and Milk products", second edition, 2011, titratable acidity, expressed as % lactic acid (% m/m) for fermented milk must contain minimum of 0.3%. While yogurt, Alternative culture yogurt and Acidophilus milk and kefir (min. 0.6%) and koumys must contain (min. 0.5). The storage period increase, the titratable acid produced by the probiotic bacteria metabolism also increases.

In microbial analysis the TPC was $2.778 \pm .65 \log_{10}$ CFU/ml, no coliforms were detected and yeast and mold count was less than 10 CFU/ml.

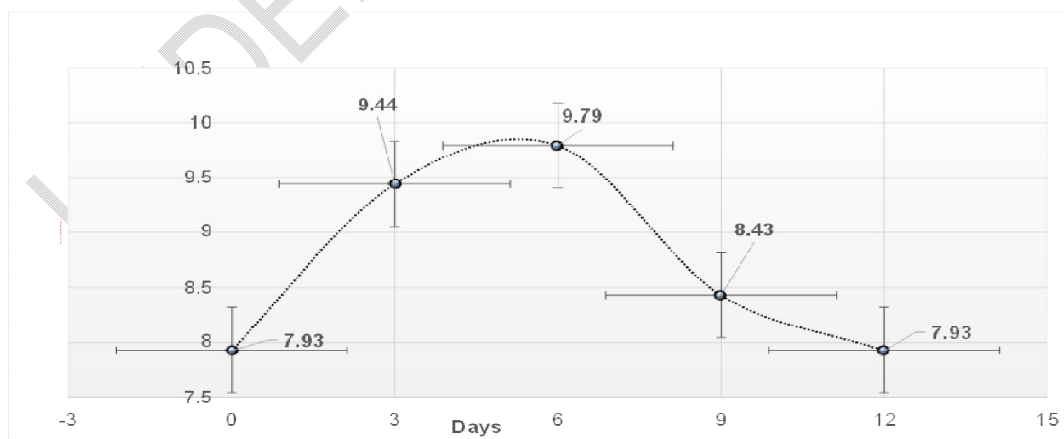


Figure 2. Logarithmic curve of the *Lactiplantibacillus plantarum* refrigerated condition

The probiotic drink must to contain at least $\geq 10^6$ CFU/ml of total lactic acid bacteria (A.Y. Tamime and R K Robinson, 2007). Lactobacillus counts for the product are above the required values, thus it qualifies as the probiotic product. Initial reading was 8.6×10^7 cfu/ml which gradually increase and gradually decreases by 12th day of storage. Highest value for count was on 6th day i.e. 6.17×10^9 CFU/ml. Thus, the shelf life for the product was determined as less than two weeks (12 days) at refrigeration storage condition. All the microbial parameters are found within its acceptance level which assures microbial safety of the product.

4. CONCLUSION

Many research shows that probiotic products have enormous health benefits where it improves our immunity, gut micro-flora which is further link with reducing numerous gastro-intestinal diseases. Many people don't consume probiotic products due to its acid/sour /vinegary taste so developing such sweetened probiotic product will improve the consumer acceptance. The probiotic product being developed utilizing a single bacterial strain of *L. plantarum*, could increase product varieties of corn, milk and honey. Physico-chemical and the microbiological parameters analyzed for the probiotic drink was in accordance with the standards. Shelf life for the product was established at 2 weeks. This product having good taste, color, consistency and aroma, sensory acceptance is also high.

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