The Pharma Innovation Journal 2021; 10(11): 902-906

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(11): 902-906

www.thepharmajournal.com
Received: 03-08-2021
Accepted: 11-10-2021

# Development of probiotic rice milk and its storage studies

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**Abstract**
The rice milk is a plant-based milk alternative, which is rich in carbohydrate and low in fat giving nutritional benefit to the consumers opting for milk substitutes. The broken rice which is main bi-product of rice industry can be utilized for the preparation of value-added products which in turn gives profit to the producers. The broken rice was used to prepare rice milk with the optimised process parameters and added with probiotic culture. The storage studies were analysed by filling the probiotic rice milk in Glass, HDPE and LDPE under ambient conditions (Specify temperature) here. The viable count of the *L. case, B. longum, L. bulgaricus, S. thermophilus, L. acidophilus* during the ambient storage at a temperature of 25±5 °C in three types of packaging materials for the storage period of 4 days. The viable count of the *L. casei, B. longum, L. bulgaricus S. thermophilus and L. acidophilus* were 9.66, 9.75, 8.77, 7.71 and 9.77 log cfuml$^{-1}$ at the beginning of the storage and decreased to (7.57, 3.21 and 2.34 log cfuml$^{-1}$), (8.5, 6.3 and 2.67 log cfuml$^{-1}$), (6.99, 6.12 and 2.12 log cfuml$^{-1}$), (5.23, 4.32 and 2.01 log cfuml$^{-1}$) and (2.63, 8.78 and 8.89 log cfuml$^{-1}$) at the last day of the storage in glass bottles, HDPE and LDPE.

**Keywords:** Probiotic rice milk, storage studies, viable count

## Introduction
Probiotic beverages available are milk based and now-a-days research was focused on the development of probiotic beverages using cereals as alternative fermentation substrates. The probiotic beverages are rich in nutritive value and large distribution has focused the attention to use as raw materials for new probiotic functional foods (Angelov *et al*., 2006). A combination supplements of prebiotics and probiotics in a single product is called synbiotic (Bielecka *et al*., 2002) [3]. A probiotic microorganism is one of the polysaccharide compounds and it can simulate the growth of one or more species of colonic bacteria (Gibson and Roberfroid, 1995) [10]. The most important fundamental property of the probiotic beverage is acid and bile tolerance which indicate the tolerance of probiotic bacteria in the gastrointestinal tract. The probiotic bacteria should able to tolerate the acidic conditions in the stomach and also bile conditions on the entry of the small intestines (Prasad *et al*., 1998) [19]. The success of new probiotic formulations does rely only on the ability to provide enough probiotic cells that may survive the human gastrointestinal tract. The organoleptic properties of these products must also be acceptable for consumers. An appropriate selection of substrate composition and strains is necessary to efficiently control the distribution of the metabolic end-products (De Vuyst, 2000) [7]. Several factors have been suggested to influence probiotic survival during processing and storage.

The development of cereal based probiotic products is increasing due to consumer interest for nutritional beverages with different tastes, an increasing number of vegetarian consumers opt for the non-dairy beverages and also because some consumers are lactose intolerant or have milk protein allergies (Prado *et al*., 2008). Cereal products often ferment automatically, resulting in extended shelf-life and better nutritional properties compared with the raw material. The combination of cereals is used as a substrate for the development of fermented beverages, the final product may vary with the type of raw material used as a substrate, fermentation conditions may affect the microbial population. Fermentation process was used to develop new foods with beneficial health properties (Blandino *et al*., 2003) [4]. Rice is a cereal grain and which is used for preparation of traditional fermented beverages and foods (Blandino *et al*., 2003) [4]. Rice having high levels of dietary fibers which are soluble in nature such as β-glucans and zinc, selenium and antioxidant (Johansson *et al*., 2004), barley and oat were used for making yogurt like beverages (Gee *et al*., 2007) [9].

Based on properties like rheological and nutritional emmer flour was used recently for production of fermented beverages (Coda *et al*., 2012) [6].

After polishing of rice, it is graded based on size, a rice kernel that does not meet the required size is considered as broken (Van Dalen, 2004). Broken rice is generally sold without prior separation or it can be ground into flour (Mukhopadhyay and Siebenmorgen, 2017) [16]. Currently, the USDA has reported that 10% of the total rice consumed in the US is used in both pet food and brewery industry, most of which is broken rice (USDA, 2016). The cost of the broken rice is less when compared to head rice, which makes to utilize the rice by-product. Large and medium rice mills generate 10 to 15% broken rice, 25% in small mills and depends on immature concerns, moisture absorption, chalkiness, relative humidity, insect infection and other factors (Siebenmorgen *et al*., 1998, Muthayya *et al*., 2014, Bruce and Atungulu, 2018) [22, 17, 5]. The cost of the broken rice is 60–80% cost of the head rice, which can impact profits (Mukhopadhyay and Siebenmorgen, 2017) [16]. Utilization of rice brokens can lead to production of low-cost value-added products with nutritional and functional quality. It is necessary to develop new industrialized food products and to evaluate some important properties, such as keeping quality, shelf life and viability of probiotics.

## Materials and Methods

### Preparation of probiotic Rice milk

The rice milk which was prepared from optimised conditions was taken for production of probiotic rice milk. The preparation of probiotic rice milk is shown in Figure 1. ABT-5 starter culture consisting of *L. Casei*, *B. Longum, L. bulgaricus*, *S. thermophilus*, *L. acidophilu*) were used for preparation of probiotic rice milk. The prepared probiotic milk was stored under ambient condition at 25±5ºC. The sample was filled in the bottles (Glass, HDPE and LDPE). The bottles stored at ambient condition were labelled as (T2P1), HDPE (T2P2) and LDPE (T2P3). The viability of the probiotic strains *i.e.*, ABT-5 starter culture (*L. Casei*, *B. Longum, L. bulgaricus, S. thermophilus, L. acidophilus*) was studied at ambient storage condition25±5ºC.

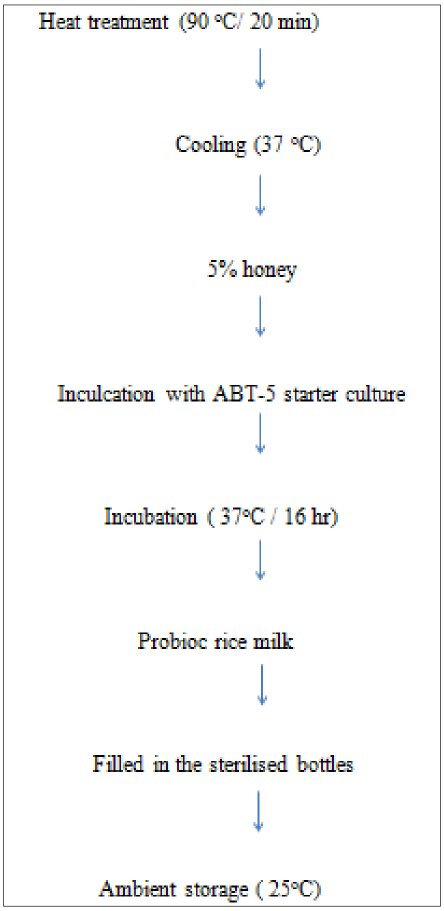


**Fig 1:** Process flow chart for preparation of probiotic rice milk

### Determination of viable count of probiotic bacteria

The viable count of the probiotic bacteria ABT-5(*Bifidobacterium longum counts, Lactobacillus bulgaricus, Streptococcus thermophiles, L. Casei* counts, *L. Acidophilus* counts**)** during storage was measured according to method described by (Lapierre, Undeland& Cox, 1992; Tharmaraj Shah, 2003) [15, 25].

## Results and Discussion

**Changes in the physical and chemical composition after fermentation:** The chemical composition of plain and probiotic rice beverages is presented in the Table 1. The protein content, fat content, ash content and total phenolic content of the rice milk beverage in comparison to probiotic rice milk increased from 1.12 to 2.2g/100g, 0.1 to 0.4%, 0.08 to 0.25% and 0.01 to 0.03%, but the carbohydrate content decreased from 7.76 to 5.93g 100g$^{-1}$. Lactic acid bacteria (LAB) are a group of bacteria that produce lactic acid as their major end products from carbohydrates through fermentation which results in decrease in the carbohydrates during fermentation (Martensson *et al*. 2000). Ash content and phenolic content of the probiotic rice milk increased due to the fermentation process. These results were in agreement with the work of Tseng *et al*. (2006) in fermented rice beverage.

**Table 1:** Composition of plain rice milk with probiotic rice milk

| Control Rice milk | Probiotic Rice Milk | composition |
|---|---|---|
| 6.21 | 4.94 | pH |
| 0.34 | 0.54 | Titrable acidity |
| 4.27 | 6.55 | Dry Matter |
| 0.94 | 1.64 | Protein |
| 0.082 | 0.086 | Ash |
| 0.1 | 0.1 | Fat Content |
| 7.74 | 7.61 | Total carbohydrates |
| 0.01 | 0.01 | Total Phenols |

**Changes in pH and titratable acidity:** As shown in Fig. 2.

The pH values of the probiotic rice milk beverage decreased with increasing fermentation time from 6.21 to 4.95. The titrable acidity values of the probiotic rice milk increased from 0.08 to 0.54% up to the fermentation time of 16 hours. This could be due to the fact that microorganisms consume the nutrients and produce organic acids, which are released into the medium, which in turn causes decrease in pH values. The decrease in pH and increase in titrable acidity values during fermentation process were in accordance with the results presented by Tangular and Erten, (2012) [23], Ramos *et al*. (2011) [20], and Wongkhalaung *et al*. (2000) [27].

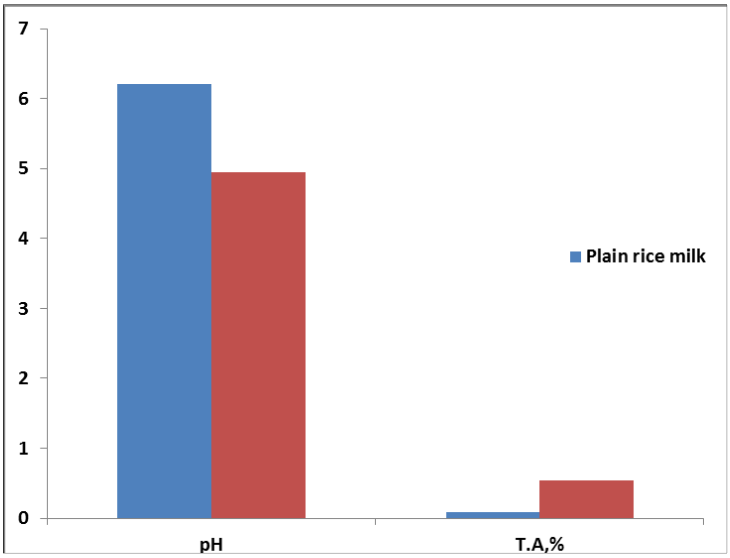


**Fig 2:** Changes in pH and titrable acidity during fermentation

**Assessment of the probiotic rice milk during storage**

In this study, rice milk was used as the primary substrate for the starter culture ABT-5 and its effect on the survival and acidifying activities of probiotic strains *L. Casei, B. Longum, L. bulgaricus, S. thermophilus, L. acidophilus*stored in a different packaging material i.e., glass, HDPE and LDPE was studied. Storage studies were conducted at refrigerated and ambient conditions

**Survival of probiotic strains during storage at ambient conditions**

Figure 3 shows the survival of probiotic strains i.e., *L. casei B. longum, L. bulgaricus, S. thermophilus, L. acidophilus* during storage at ambient temperature of 25±5°C in three types of packaging materials. The storage period for probiotic rice milk at ambient conditions was estimated as 4 days. The viable cell count of *L. casei, B. longum, l. bulgaricus S. Thermopiles* and *L. acidophilus* were 9.66, 9.75, 8.77, 7.71 and 9.77 log $cfuml^{-1}$ at the beginning of the storage and decreased to (7.57, 3.21 and 2.34 log $cfuml^{-1}$), (8.5, 6.3 and 2.67 log $cfuml^{-1}$), (6.99, 6.12 and 2.12 log $cfuml^{-1}$), (5.23, 4.32 and 2.01 log $cfuml^{-1}$) and (2.63, 8.78 and 8.89 log $cfuml^{-1}$) at the last day of the storage for the treatments T2P1, T2P2 and T2P3. It was noticed that with increase in the storage time the viable count of the probiotic strains decreased and survival of probiotic bacteria was more in T2P1 when compared to the other treatments and followed by T2P2 and T2P3.

The survival of the probiotic strains at ambient conditions was less when compared to storage at refrigerated conditions. A storage time of 4 days was noticed in the glass bottles and HDPE bottles, but the storage life reduced to 2 days in LDPE bottles. The decrease in storage life with different packaging material was in accordance with findings of Joshita *et al*. (2017) [12]. Shelf life of 4 days was noticed by Jayamanne and Adams, (2004) for buffalo milk stored in plastic and glass cups. The survival of probiotic strain decreased with increase in the permeability of the oxygen in the packaging material. The acidity of probiotic rice milk increased after astorage period of 4 days, rendering it to be unsuitable for consumption. Chilled storage slows post-fermentation acidification which prolongs viability, while packaging materials which presents a greater barrier to oxygen, had a similar effect at ambient temperature. The viability of bacteria is dependent on the type of packaging material used for storage, and it was proved that the level of dissolved oxygen increased significantly in the LDPE bottles, whereas HDPE bottles and the glass bottles maintained the recommended viability, as the oxygen levels remained low. These results were in accordance with those explained by Tripathi and Giri (2014) [25] and Weinbreck *et al*. (2010) [28].

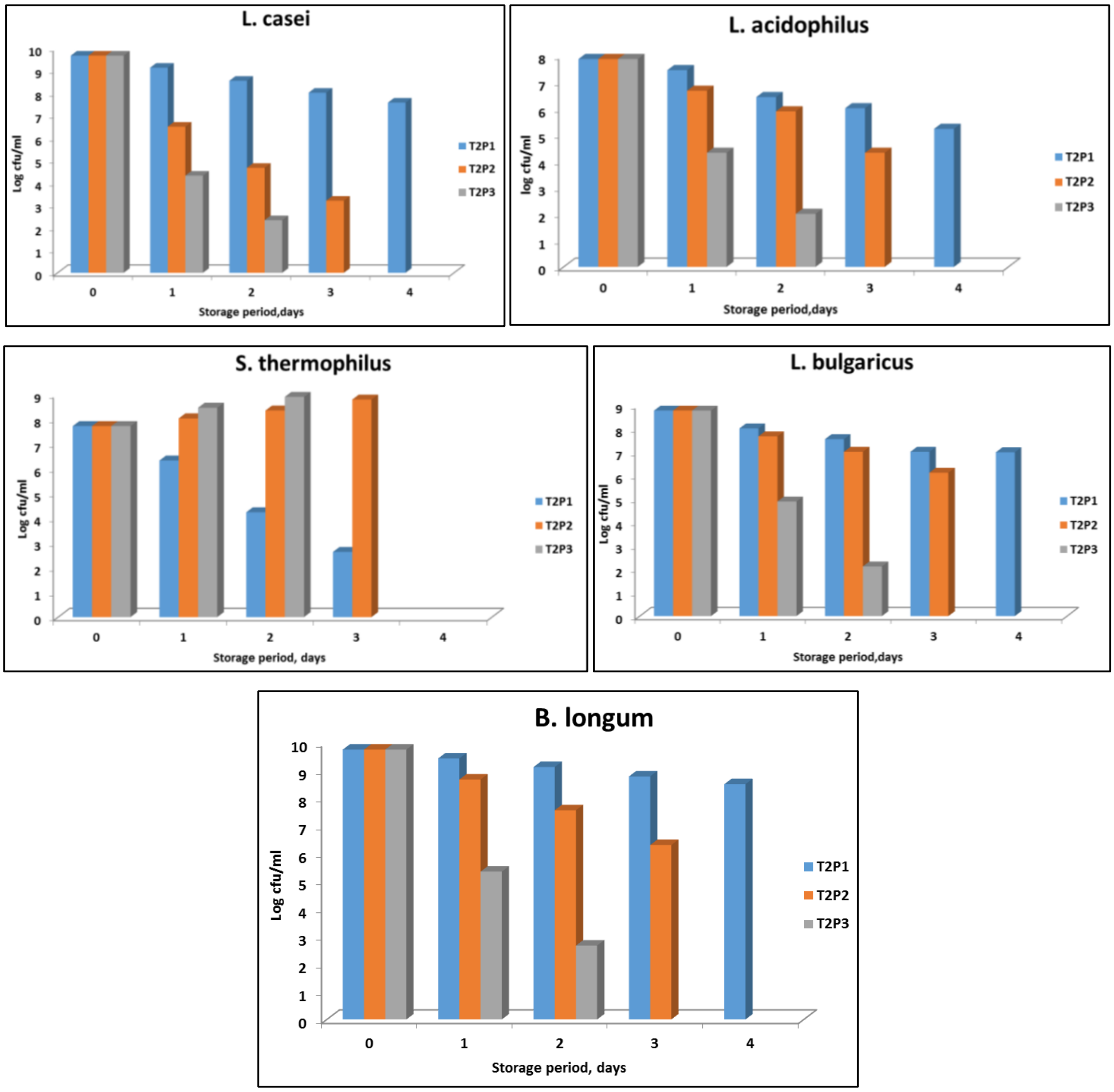


**Fig 3:** Viability of the probiotic strain during storage at ambient conditions

## Conclusions

Shelf-life study revealed that during 4 days storage at 25±5°C, pH and acidity of rice beverage remained above 4 and lower than 1%, respectively, while viable count of *L. casei*, *B. longum*, *L. bulgaricus, S. thermophilus* and *L. acidophilus* remained above 5 log cfu ml$^{-1}$. This study shows that the viability count were more in the glass bottles followed by HDPE and LDPE for *L. casei*, *B. longum*, *L. bulgaricus, and L. acidophilus*. Negetives effect was shown for the the viability of *S. thermophilus*.

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