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Storage studies of probiotic rice milk during refrigerated conditions

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Abstract
The rice milk is plant based milk alternate, which is rich in carbohydrate and low in fat gives nutritional benefit to the consumer who opted for milk substitutes. The broken rice were 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 at ambient condition. 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 cfu/ml at the beginning of the storage and decreased to 9.24, 8.01 and 6.78 log cfu/ml after 28 days in T1P1, T1P2 and T1P3 ; 9.12, 7.54 and 6.5 log cfu/ml)after 28 days in T1P1, T1P2 and T1P3, (8.12, 7.01 and 6.53 log cfu/ml) after 28 days in T1P1, T1P2 and T1P3, (4.23, 8.45 and 9.02 log cfu/ml) after 28 days in T1P1, T1P2 and T1P3 and (9.24, 8.01 and 6.78 log cfu/ml) after 21 days in T1P1, T1P2 and T1P3. It was observed that the viable counts decreased with increase in storage time. However, in the treatment T1P1 the viable count of the all probiotic bacteria was more when compared to the other treatments and followed by T1P2 and T1P3.

Keywords: Rice milk, probiotic rice milk, storage studies, viable count, packaging materials

Introduction
Rice is cereal crop which grows annually and belongs to the grass species *Oryza sativa* (Asian Rice) or *Oryza glaberrima* (African Rice) widely cultivated for its seed. Rice, a monocot, is the most staple food for a large part of the world's human population (Juliano, 1993). According to (FAOSTAT, 2012) data, rice is the agricultural produce with the third highest worldwide production after sugarcane and maize. One seed of rice yields more than 3,000 grains; it is the highest yielding cereal grain and can grow almost anywhere. As reported by (Ricepedia, 2016), rice provides 19% of global human per capita energy, and 13% per capita protein Micronutrients are essential vitamins and minerals needed in very small amounts and must be supplied by a variety of foods in the diet (Agbon, 2009) [1] to stimulate cellular growth and metabolism (Kennedy *et al*. 2003).
Most of the probiotic beverages in the market are milk based and very few attempts were done for the development of probiotic products by utilizing cereals as alternative fermentation substrates. Their important nutritive value and large distribution have focused the attention on their use as raw materials for new fermented functional foods (Angelov *et al*. 2006) [2]. The development of non-dairy probiotic products is increasing due to consumer interest for exotic and different tastes, an emerging number of vegan consumers and also because some consumers are lactose intolerant or have milk protein allergies (Prado *et al*. 2008) [17]. Cereal products often ferment automatically, resulting in extended shelf-life and better nutritional properties compared with the raw material. The combination of cereals are 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 procedures have been used to develop new foods with enhanced health properties (Blandino *et al*. 2003) [4]. 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.

Methodology

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*) was used for preparation of probiotic rice milk. The prepared probiotic milk was stored under refrigerated condition at 5±1°C. The sample was filled in the bottles (Glass, HDPE and LDPE). The bottles stored at refrigerated condition were labelled as Glass bottles (T1P1), HDPE (T1P2) and LDPE (T1P3). 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 refrigerated and ambient storage condition.

Heat treatment (90 °C/ 20 min)

↓

Cooling (37 °C)

↓

5% honey

↓

Inculcation with ABT-5 starter culture

↓

Incubation (37 °C / 16 hr)

↓

Probioc rice milk

↓

Filled in the sterilised bottles

↓

Refrigerated storage (5 °C) and ambient storage (25 °C)

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; Vinderola & Reinheimer, 1999; Tharmaraj & Shah, 2003) [19].

Results and Discussion

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 the storage in refrigerated conditions

Figure. 2 shows the viable cell count of *L. casei B. longum, L. bulgaricus, S. thermophilus, L. acidophilus* during the refrigerated storage at a temperature of 5±1 ℃ in three types of packaging materials for 21 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 cfu/ml at the beginning of the storage and decreased to 9.24, 8.01 and 6.78 log cfu/ml after 28 days in T1P1, T1P2 and T1P3 ; 9.12, 7.54 and 6.5 log cfu/ml)after 28 days in T1P1, T1P2 and T1P3, (8.12, 7.01 and 6.53 log cfu/ml) after 28 days in T1P1, T1P2 and T1P3, (4.23, 8.45 and 9.02 log cfu/ml) after 28 days in T1P1, T1P2 and T1P3 and (9.24, 8.01 and 6.78 log cfu/ml) after 21 days in T1P1, T1P2 and T1P3. It was observed that the viable counts decreased with increase in storage time. However, in the treatment T1P1 the viable count of the all probiotic bacteria was more when compared to the other treatments and followed by T1P2 and T1P3.

The viable cell count of probiotic strains decreased with increase in the storage period, and but remained at sufficient levels (> 6 log cfu/ml) up to the end of the storage period in all three types of packaging materials. It was observed that the survival rate of the all probiotic strains were higher in the glass bottles, except *S. thermophilus*, which decreased in glass bottles and increased in HDPE and LDPE. *S. thermophilus*, depends heavily on the availability of oxygen for its metabolic activities, by consumption of the dissolved oxygen present in the yoghurt. Due to low oxygen permeability in glass bottles, *S. thermophilus* viable cells decreased in glass bottles and increased in HDPE and LDPE bottles. The quality of the probiotic bacteria is dependent on the type of packaging materials and the storage conditions (Mattila-Sandholm *et al*. 2002). The viable count of the probiotic strains was reduced to about 1-2 log cycles, during the refrigerated storage. Speck, (1976) reported that 10^8 to 10^9 viable cells of *L. acidophilus* should be ingested daily to fulfil the health benefits to the consumers.

The viable count of *L. casei, B. longum, L. acidophilus and L. bulgaricus* content in the probiotic rice beverage during storage did not change much from 0th day to 7th day in glass containers, EVOH and LDPE. However there was a decrease in the viable count of *L. casei, B. longum, L. acidophilus and L. bulgaricus* in EVOH and LDPE during 14th day and 21st day of storage indicating that, glass containers were better packaging material to retain the *L. casei, B. longum, L. Acidophilus and L. bulgaricus* content in the probiotic rice milk. *S. thermophilus* on the other hand showed different results as compared to other strains like *L. casei, B. longum, L. Acidophilus and L. bulgaricus.* There was a consistent decrease in the viable count of *S. thermophilus* from 0th day to 7th day to 14th day to 21st day stored in glass bottles, as compared to EVOH and LDPE packaging, indicating that *S. thermophilus* is more stable in EVOH and LDPE containers.

The results were in accordance with those described by Olson and Aranya, (2008). More over the viability of *L. casei, B. longum, L. bulgaricus S. thermophilus* and *L. acidophilus* survives well in the yogurt throughout the shelf life and these results were in agreement with the findings of Akalin *et al*. (2004)., Pescuma *et al*. (2010) [16]., Pereira *et al*. (2011) [15]. and Wang *et al*. (2002) [21]. The viability losses may be due to the decrease in pH values, post acid production (Wang *et al*. 2011), sensitivity to oxygen by (Frank and Marth, 1988), and metabolisms such as hydrogen peroxide and ethanol and bacteriosins produced by lactic acid bacteria and this can be help to reduce exposure of the oxygen to Bifidobacteria. The viable counts and survival of probiotic strains stored in glass bottles were more than those stored in plastic cups due to low oxygen permeability (Dave and Shah, 1997).

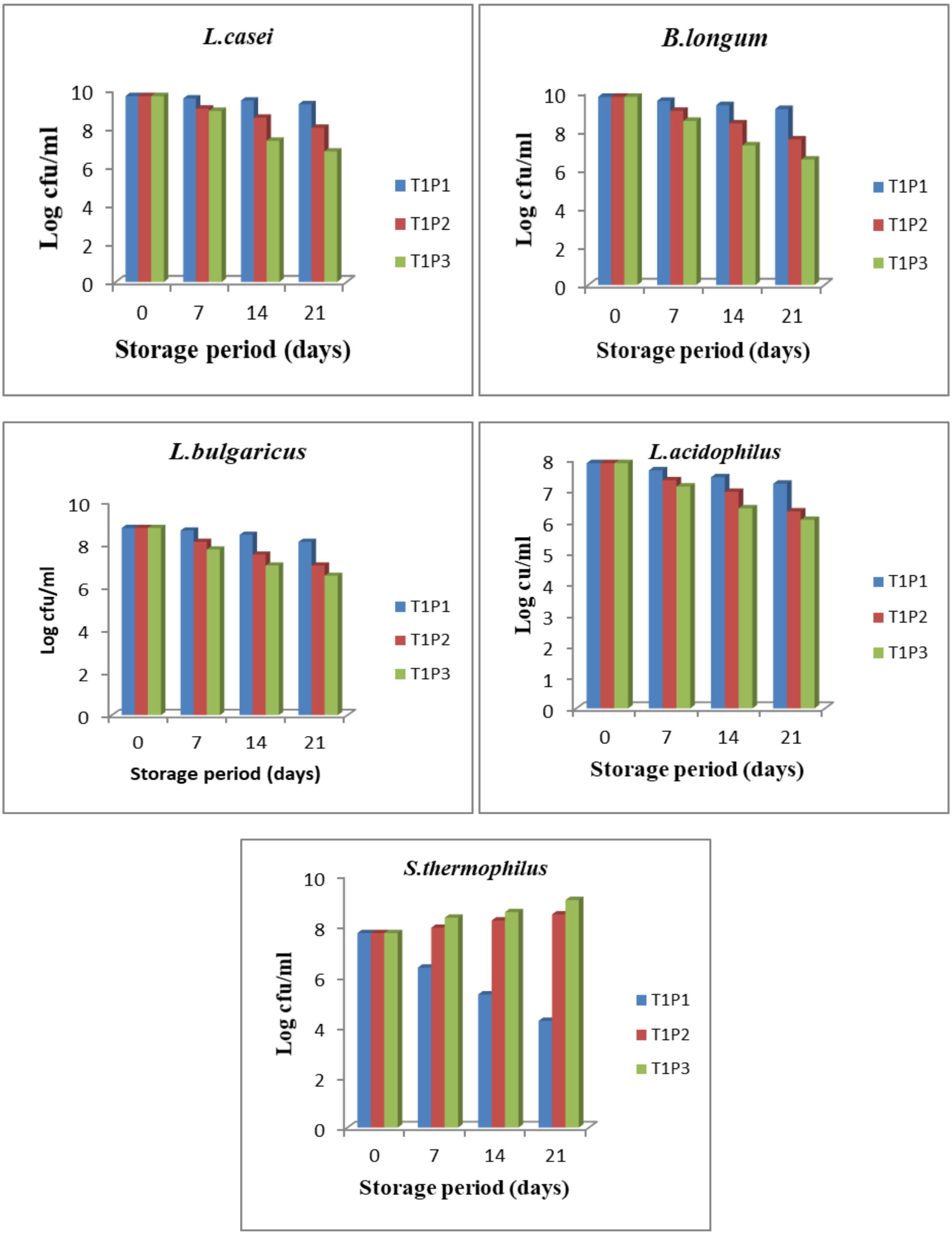

Fig 2: Viability of the probiotic strain during storage at refrigerated conditions

Conclusions

This kind of study can facilitate the development of new, non-dairy, nutritionally well-balanced food products with unique physical properties. Shelf-life study revealed that during 21 days storage at 4 °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 a new possibility to make an acceptable fermented product based mainly on rice brokens which are suitable substrates that's can support high cell viability during cold storage for 21 days for different probiotic strains. In addition these produced beverages increases nutritional qualities and help to get knowledge on probiotic characteristics.

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