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Development of calcium fortified rice milk and its storage studies

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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 calcium carbonate. The chemical compositions of plain and fortified rice beverages were analysed and the storage studies were analysed by filling the rice milk and fortified rice milk in Glass, HDPE and LDPE at ambient and refrigerated condition. After the fortification the protein content was decreased from 1.12 to 1.05 and ash content, TSS, pH were increased from 0.1 to 0.4, 10.2 to 12, and 6.21 to 6.53. It was noted that the total plate count generally increased on storage for all the treatments but high total plate count values were observed in the T2P3, and followed by T2P2 and T2P1, and similar trend was observed for the control milk in this study during the 15 days storage period under refrigerated condition and 2 days under ambient condition. It was noted that the total plate count generally increased on storage for all the treatments but high total plate count values were observed in the T2P3, and followed by T2P2 and T2P1, and similar trend was observed for the control milk in this study.

Keywords: Rice milk, calcium fortified rice milk, storage studies, 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. 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) [6].
Rice milk is lactose free making them prefect alternative for patients suffering from lactose intolerance (Lomer *et al*. 2008) [8]. Further, rice milk can also act as an alternative in the case of patients with the increasing allergy issues caused by soybean and almonds (Gizzarelli *et al*. 2006., Roux *et al*. 2003) [5, 15]. Research has also showed that consumption of rice milk as an alternative to cow's milk without proper care can result in malnutrition, especially in the case of infants because of the varied difference in the nutrient profile (Katz *et al*. 2005., Massa *et al*. 2001) [7, 9]. Kwashiorkor, a form of protein-energy malnutrition was observed in infants that were on a rice based vegan diet. Rice milks that are unfortified, especially that are homemade also lack in minerals and vitamins like calcium and B-12 unless fortified as in the case of most commercial milks (Craig, 2009., Messina and Reed Mangels, 2001) [3, 10]. Low intake or absorption of minerals like calcium, iron and zinc might generate deficiencies which in turn are related to many human health problems including stunted growth in children, weak bones, and immune system disorders. Food fortification could play a key role to overcome this problem. In this regard a beverage developed with enrich in calcium to meet the requirements provided by the raw milk.

Methodology
The broken rice was purchased from the local market Sangareddy, India. The broken rice was stored in an air tight container and used or further studies. The rice milk was prepared with the optimised process conditions was taken for preparation of the calcium fortified rice milk. The process of preparation of calcium fortified rice milk was presented in the Figure 1.

The fortified rice milk was filled in the sterilized bottles i.e., Glass, HDPE and LDPE and stored under refrigerated condition at 5±1 °C, ambient conditions at 25±5 °C. The microbial analysis of calcium fortified rice milk was done for every 5 days of the storage period under refrigerated condition and every one day for ambient condition.

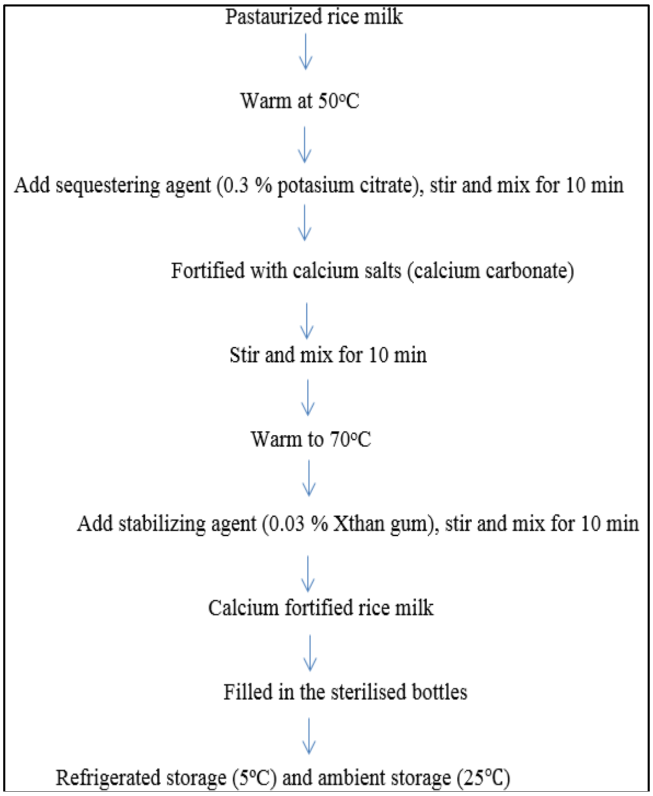

Fig 1: Process Flow Chart for Preparation of Calcium Fortified Rice Milk

Sensory Evaluation

The standardized products were subjected to sensory evaluation as specified by (Meilgaard *et al.* 1999) [11] using a nine-point hedonic scale (0 = Dislike extremely to 9 = Like extremely). Thirty semi-trained panel members from college of Agricultural Engineering, Sangareddy of Professor Jayashankar Telangana State Agricultural University, Hyderabad, India, evaluated the products for acceptability based on its colour, flavour, texture, taste and overall acceptability using standardized protocol. The panelists were provided with water to cleanse their palate between samples. Four samples were coded by three digit numbers and subjected to evaluation during each session. The sensory evaluations were carried out under normal daylight conditions at ambient temperature.

Nutrient Composition

The protein and ash content of the rice milk and calcium fortified rice milk were measured according to the (AOAC, 2012) [2].

TSS: Total soluble solids of the rice milk was determined using calibrated Hand Held pocket Refractometer (Make: Atago, Model: PAL-1, Range :0-53 %Brix), Accuracy: ±0.2° Brix, with automatic temperature compensation).

TSS (°Brix) = Measurement value …………1

pH: pH of the sample was measured with calibrated digital pH meter in triplicates. The probe which was attached to the pH meter was inserted into the sample. The reading which was displayed on the pH was recorded as the pH value.

pH = Measurement value ………..……….2

Microbiological analysis

Microbiological properties of rice milk and value added products during storage were determined by total plate count, mould count according to method described by AOAC (2012) [2].

Results and Discussion

Changes in the physical and chemical composition after fortification

The chemical composition of rice milk and fortified rice milk is shown in the Figure 2. The decrease in the protein content was observed from 1.12g/100g to 1.05g/100g and increase in the ash content, TSS, pH were observed from 0.1% to 0.4%, 10.2% to 12%, 6.21 to 6.53 of the rice milk with comparison to fortified rice milk. The results were in accordance with the results predicted by Yazici *et al.* (1977) [16] for the soy milk fortified with the calcium fortified soy milk.

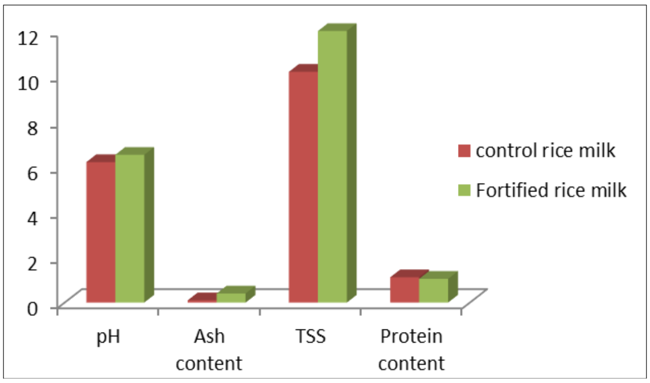

Fig 2: Changes in physico-chemical composition of fortified rice milk

Microbial Analysis of Stored Rice Milk

Changes in total plate count of rice milk during storage

The plate count of rice milk during 15days of refrigerated storage study is shown in Figure 3. It was noted that the TPC increased on storage for all the treatments, but high TPC values were observed in T2P3 (0.78×10^6 cfu/ 10 ml), followed by T2P2 (0.55×10^6 cfu/ 10 ml) and T2P1 (0.33×10^6 cfu/ 10 ml). Similar trend was noticed for control milk in this study, and the values were 0.66×10^6 cfu/ 10 ml, 0.33×10^6 and 0.33×10^6 cfu/ 10 ml. The TPC values were within the permissible limit as per (FSSAI, 2011) and similar results were reported by Akalin *et al.* (2004), Pescuma *et al.* (2010) [13], Pereira *et al.* (2011) [12], Wang *et al.* (2002) [15] for the probiotic rice milk. Statistical analysis with ANOVA was done for data obtained from the storage studies and presented in Appendix-C. F-values indicated that there was a significant effect with packaging on the TPC of rice milk. Interaction terms had also affected the TPC of rice milk ($p<0.05$).

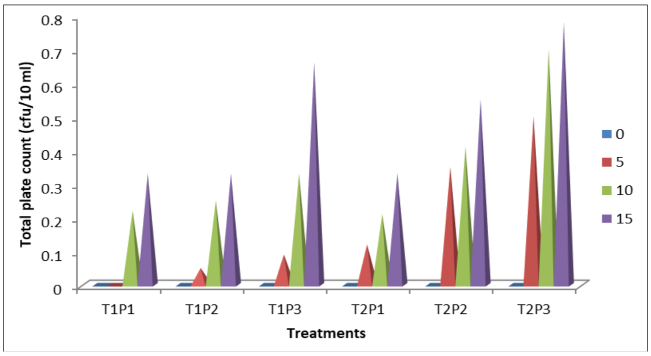

Fig: 3 Total plate count of different treatments of rice milk stored at refrigerated conditions

The results of total plate count of rice milk stored for 2 days at ambient conditions i.e., 25±5 °C is shown in Figure 4. The results pertaining TPC are given in Appendix. From the Figure 4, it was observed that TPC values increased on storage in all the treatments, but high TPC values were observed in T2P3 (1.77×10^6 cfu/ 10 ml), followed by T2P2 (1.55×10^6 cfu/ 10 ml) and T2P1 (1.33×10^6 cfu/ 10 ml). Same trend was observed in control milk in this study and the maximum values obtained were in T1P3 i.e., 1.33×10^6 cfu/ 10 ml, followed by T1P2 (1×10^6 cfu/ 10 ml) and T1P1 (1×10^6 cfu/ 10 ml). However, the count was within the permissible limit as per (FSSAI, 2011). The results obtained by Pereira *et al*. (2011) [12], Wang *et al*., (2015) for the probiotic rice milk were similar and Papageorgiou *et al*. (1997) reported a shelf life of 24 h to 48 h for the rice milk stored at ambient conditions. Statistical analysis with ANOVA was done for data obtained from the storage studies and presented in Appendix-C. F-values indicate that there is a significant effect of packaging on the TPC of rice milk. Interaction terms had also affected the TPC of rice milk ($p<0.05$).

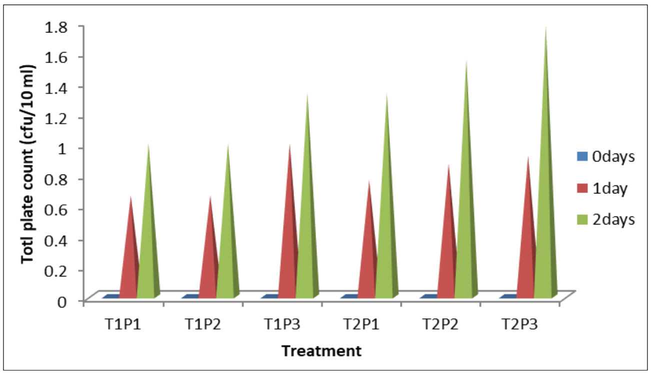

Fig 4: Total plate count of different treatments of rice milk stored at ambient condition

Changes in mould count of rice milk during storage

The mould count of rice milk during 15days of refrigerated storage at 5±1 °C is shown in Figure 5. The results pertaining to mould count are given in Appendix. From the results obtained (Figure 5), it was observed that the mould count increased on storage in all the treatments. High mould count was noted in the T2P3 i.e 0.6×10^4 cfu/ 10 ml, followed by T2P2 (0.33×10^4 cfu/ 10 ml) and T2P1 (0.33×10^4 cfu/ 10 ml). Similar trend was observed in the control milk also (T1P3 - 0.33×10^4 cfu/ 10 ml, followed by T1P2 - 0.33×10^4 cfu/ 10 ml and T1P1 - 0.1×10^4 cfu/ 10 ml). Also the count was within the permissible limit as per (FSSAI, 2011). The results are in agreement with results observed by Barooah *et al*. (2018), Pereira *et al*. (2011) [12] in rice milk. Statistical analysis with ANOVA was done for data obtained from the storage studies and presented in Appendix-C. F-values indicate that there is a significant effect of packaging on the mould count of rice milk. Interaction terms had also affected the mould count of rice milk ($p<0.05$).

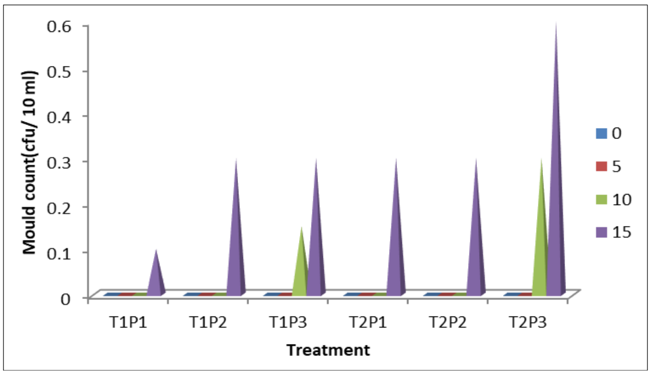

Fig 5: Mould count of different treatments of rice milk stored at refrigerated condition

The results of mould count in rice milk stored for 2 days at ambient conditions i.e., 25±5 °C is shown in Figure 6. It was noted that the mould count increased on storage in all the treatments. High yeast values were observed in the T2P3 i.e., 1.67×10^4 cfu/ 10 ml, followed by T2P2 and T2P1 i.e., 1.54×10^4 and 1.33×10^4 cfu/ 10 ml respectively. Similar trend was observed in the control milk i.e., 1.33×10^4 cfu/ 10 ml, followed by T1P2 and T1P1 (0.67×10^4 and 0.66×10^4 cfu/ 10 ml). Also the count was within the permissible limit as per (FSSAI, 2011). The results obtained are in agreement with observations made by Pescuma *et al*. (2010) [13], Pereira *et al*. (2011) [12] in probiotic rice milk. Papageorgiou *et al*. (1997) reported a shelf life of 24 h to 48 h for the rice milk when stored at ambient conditions. Statistical analysis with ANOVA was done for data obtained from the storage studies and presented in Appendix-C. F-values indicated that there is a significant effect between packaging and mould count of rice milk. Interaction terms had also affected the mould count of rice milk ($p<0.05$).

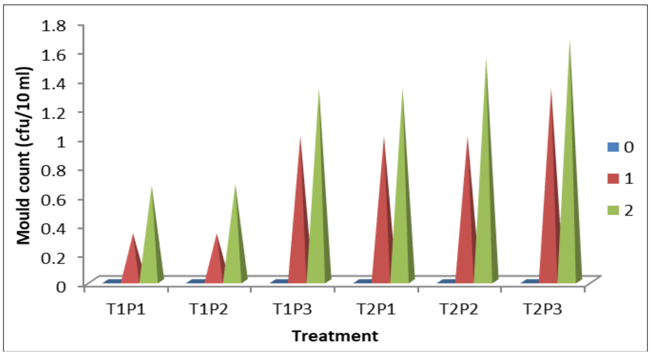

Fig 5: Mould count of different treatments of rice milk stored at ambient conditions

Sensory Evaluation

The results of sensory evaluation of value added products developed with rice milk and spray dried rice milk powder are shown in Table.1.However, a score of 7.00 and above on a 9.0 point hedonic score card indicates good acceptability of the products. The results of our study indicate good acceptance of all the products tested. Probiotic rice milk was the best among all the products, indicating a new probiotic product which can be further validated and tested, for commercialization.

Table 1: Sensory evaluation of value added rice milk products

S. No.	Name of the product	Colour	Texture	Taste	Flavor	Overall acceptability
1	CRM	7.95 ± 0.16^{ab}	7.50 ± 0.25 ^{c}	7.80 ±0.20^{bc}	7.30 ± 0.25^{bcd}	7.95 ± 0.11^{bc}
3	CFRM	7.45 ± 0.17 ^{ab}	7.70 ± 0.37^{abc}	7.85 ± 0.31^{abc}	7.35 ±0.24$^{a b}$	7.45 ± 0.17^{ab}

Note: All the values are expressed as Mean ± SD. Values with similar superscripts within rows are statistically similar at 0.05% level. Control Rice Milk (CRM), Calcium Fortified Rice Milk (CFRM).

Conclusions

This type of study can help in the development of the non-dairy milk, and also for development of the fortified products which are nutritionally well balanced with unique physical properties. The non-dairy milk and calcium fortified milk developed in this report have more benefits, for the people suffering from lactose intolerant since they won't be suffering from maldigestion of lactose as in case of dairy products.

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