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ORIGINAL ARTICLE

Development of rice based probiotic yogurt enriched with some fruit pulps and its quality analysis

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Abstract Yogurt is one of the popular dairy products produced by the bacterial fermentation of milk. Researches proved that fruit enriched Rice-based yogurt is good alternative for the traditional yogurt. Hence, four different combinations of rice-based yogurt were prepared by adding rice slurry. Rice-based yogurt containing 25% milk and 75% rice slurry scored best in microbial analysis. Count of both *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* were higher in this combination but organoleptic quality was poor. The one with 75% milk and 25% slurry scored best in organoleptic analysis as it scored high in all the characters under study. Hence the combination with 75% milk and 25% slurry was selected and was enriched with the fruit pulps of Annona, Papaya and Guava. The one with 20% Annona fruit pulp and one with 5% Papaya fruit pulp were selected as the best combinations as they got the highest overall acceptability score and the count of both the bacteria were higher in these combinations, proved that fruit enrichment doesn't affect bacteria in yogurt. Cost analysis proved that collection of fruits from wild will help to reduce the price of the yogurt. Thus, Rice-based Probiotic Yogurt with 75% milk and 25% rice slurry with 20% Annona fruit pulp and one with 5% Papaya fruit pulp were developed as best combinations and can replace normal yogurts currently available in the market with low cost.

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Abbreviations

ssp.	subspecies
T_0–T_9	Treatments 0–9

Introduction

Yogurt is one of the most popular fermented milk products worldwide and has gained widespread consumer acceptance as a healthy food (Mckinley 2005). It is a dairy product, created by bacterial fermentation of cow's milk. It is produced using a culture of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*.

Fermented milk products have probiotic effect because their consumption leads to high amount of live bacterial content that exert benefits in the balanced intestinal microbiota and health, beyond basic nutrition (Mckinley 2005; Adolfsson et al. 2004). The probiotic food plays a major role in ensuring the nutritional security and hence it gets a momentum in the present market.

Effective utilization of milk obtained from the local market itself, by production and popularization of probiotic preparations like yogurt will be a great advantage to the rural people. Also, it is a nutrient-dense food which provides an array of nutrients in significant amounts and the bone density problems especially for women can be controlled up to a certain extent by including calcium rich food in their daily diet (Anonymous 2015). Researches proved that addition of fruits to milk products like yogurt will enrich the vitamin and mineral content (Barnes et al. 1991; Con et al. 1996). In this backdrop, development of rice-

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based yogurt enriched with medicinally important fruits as probiotic food was attempted.

A rice-based yogurt is the one which is made by adding rice flour in milk during the preparation of yogurt. It is a good alternative for the conventional yogurt as it can be made in low cost and also the rice-based yogurt has a promising health benefit when compared to the normal milk yogurt. It contains low amount of fat than the other (Sarabhai 2012).

Annona muricata is a species of the genus *Annona* of the custard apple family, Annonaceae, known mostly for its edible fruit. The traditional use of *A. muricata* is recorded worldwide in folk medicine systems. In traditional Indian medicine it is widely used for the treatment of kidney troubles, fever, ulcers, nervousness and wounds (Ana et al. 2016; Mootoosamy and Mahomoodally 2014).

Carica papaya, one of the 22 accepted species in the genus *Carica* of the family Caricaceae. The health benefits of consuming Papaya include a reduced risk of heart disease, diabetes, cancer, aiding in digestion, improving blood glucose control in people with diabetes, lowering blood pressure, and improving wound healing. The milky juice is extracted, dried and used as a chewing gum for digestive problems, toothpaste and meat tenderizers (Nivaasini 2017).

Psidium guajava (common guava, lemon guava) is a small tree in the family *Myrtaceae*. The traditional uses of *P. guajava* are for oral/dental infections, skin infections, cancer, women problems, diabetes, hypertension, wounds, pain relief and reducing fever (Poonam et al. 2017).

Combining the various beneficial properties of rice-based yogurt with *A. muricata*, *C. papaya* and *P. guajava* may yield a better combination of low cost consuming healthy food. Such a combination is hitherto not formulated and no reports are available in the publications.

In this context, the aim of the present work was to develop rice-based yogurt, which can replace the traditional yogurts, enriched with fruit pulps of selected fruits as probiotic food.

Materials and methods

Materials

The pure culture of *L. delbrueckii* ssp. *bulgaricus* and *S. salivarius* ssp. *thermophilus* were collected from the College of Dairy Science and Technology, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, pure cow milk from a dairy farm, and raw rice, *C. papaya*, *P. guajava* and *A. muricata* fruits were collected from the local market.

Pretreatments

The oven dried raw rice was powdered and sterilized by autoclaving at 121 °C for 15 min. Rice slurry was prepared by mixing 10,000 mg of rice flour in 100 ml sterile water.

Preparation of plain yogurt and rice based yogurt

Plain yogurt was prepared by using *L. delbrueckii* ssp. *bulgaricus* and *S. salivarius* ssp. *thermophilus* and was used as control (Sarabhai 2012). 100 ml of milk was pre-heated to 60 °C. Eight gram of sugar was added to this milk and was again heated at 90–95 °C for 5 min and cooled down to 40–45 °C. After that pure culture of the bacteria was inoculated to the mixture at a rate of 2% level each and stirred well. The mixture was filled in 50 ml cups and sealed using cling film. The yogurt package was incubated without further agitation at 42 °C for about 4–5 h. Then it was cooled down to 37 °C and kept at 4 °C. In rice-based yogurt, rice slurry was added to the milk at different proportions after adding sugar. And they are Treatment 0 (T_0) with 100% milk, Treatment 1 (T_1) with 50% milk and 50% rice slurry, Treatment 2 (T_2) with 75% milk, 25% rice slurry and Treatment 3 (T_3) with 25% milk and 75% rice slurry.

Enumeration of viable starter culture cells

Microbial quality of the rice-based yogurt was evaluated during alternative days (2nd, 4th, 6th, 8th and 10th day). The enumeration of bacteria was done using serial dilution pour plate method (Aggarwal and Hasija 1986) in M-17 agar and MRS agar for *S. salivarius* ssp. *thermophilus* and *L. delbrueckii* ssp. *bulgaricus* respectively. Colonies of bacteria were counted and was expressed as cfu/ml.

Organoleptic evaluation and selection of best scored

Organoleptic evaluation was done in the morning using a 5-point hedonic scale. Judges are selected based on their availability and are provided with a score card and are asked to evaluate the sensory parameters like appearance, colour, odour, flavor, texture, taste, after-taste, and overall acceptability. The evaluation was carried out during the alternative days (2nd, 4th and 6th day). The high scored rice-based yogurt was selected for further studies (Tamime and Robinson 1999).

Enrichment of rice-based yogurt with fruit

The yogurt receiving the highest score in the sensory analysis was selected and enriched with the fruits of *A. muricata, P. guajava* and *C. papaya*. The pulp of the fruit

was extracted using a mixer grinder and was filtered. Then it was added to the milk while preparing rice-based yogurt. *A. muricata* was added at 20 and 30% levels, *C. papaya* and *P. guajava* were added at 5 and 10% levels for both. It was represented as treatments 4, 5, 6, 7, 8 and 9 (T_4, T_5, T_6, T_7, T_8, T_9) respectively and a rice-based yogurt without any fruit enrichment (T_2) was considered as control for further studies. The concentration of the fruit pulp was decided based on a prior study and the results were not shown.

Organoleptic evaluation of fruit enriched yogurt and selection of best scored

The fruit enriched yogurts and the control were organoleptically evaluated. The judges were asked to evaluate the parameters like appearance, colour, odour, flavor, texture, taste, after-taste, and overall acceptability and the best scored was selected (Tamime and Robinson 1999).

Quality evaluation

Microbial quality of the highest scored fruit enriched yogurts was evaluated. The methodology followed is similar as the earlier case. Syneresis of undisturbed yogurts was estimated using a drainage test according to Atamer and Sezgin (1986). 25 g of yogurt sample was weighed and filtered at 4 °C and after 2 h of drainage; the volume of filtrate collected in a graduated cylinder was measured and was used as an index of syneresis. The titratable acidity was determined as described by Hooi et al (2004). Ten gram of the sample was placed in a beaker and titrated with 0.1 N NaOH solution using phenolphthalein as indicator. End point was the transition from colourless to pink. The pH of the yogurts was determined by using a pH meter (pH electrode combined CL 51B of MISEL).

Cost analysis

The cost analysis of the selected fruit enriched rice-based yogurts was carried out using the market rate of the ingredients used for preparation of yogurt and was compared with the price of yogurts available in the market.

Statistical analysis

The results were tabulated and analysis of Variance was performed (using Microsoft Excel with XLSTAT). The critical difference (CD) was determined by using the formula $CD = t_\alpha \sqrt{\frac{2Ve}{r}}$ where t_α—from student's t Table Ve—MS within group, r—df within group.

Results and discussion

Rice based yogurt with different combinations of rice slurry were prepared and its microbial count was evaluated during alternative days (2nd, 4th, 6th, 8th and 10th days). (Table 1).

It was observed that the count of both *S. salivarius* ssp. *thermophilus* and *L. delbrueckii* ssp. *bulgaricus* bacteria were higher in the combination having less amount of milk (i.e. 25% milk and 75% rice slurry) (T_3). Both the bacteria showed the highest count on the 2nd day of analysis and then the count show a decrease in the storage period. These findings corroborate the observations made by Cakmakci et al. 2012. They reported that the probiotic properties of the yogurt containing banana marmalade decreased after 7 days and the count of both the bacteria generally decreased during the storage period.

The organoleptic evaluation of rice-based yogurt was carried out on alternative days (2nd, 4th and 6th day) and the observations were given in Table 2. The organoleptic evaluation selected the combination with 75% milk (T_2). It scored high in all the characters under study. Even though the microbial quality was higher in the combination with 25% milk and 75% rice slurry, the organoleptic quality was lower. So, the former combination was selected and was enriched with fruit pulp of different fruits in different combinations. *A. muricata* was added at 20 and 30% levels (T_4, T_5), *C. papaya* and *P. guajava* were added at 5 and 10% levels (T_6, T_7, T_8, T_9).

The organoleptic evaluation of rice-based yogurts enriched with three different fruits in different combinations

Table 1 (a) Count of *Lactobacillus delbrueckii* ssp. *bulgaricus* in four different combinations of the rice-based yogurt in alternate storage days, (b) Count of *Streptococcus salivarius* ssp. *thermophiles* in four different combinations of the rice-based yogurt in alternate storage days

Treatments	log cfu/ml*				
	2nd day	4th day	6th day	8th day	10th day
a					
T_0	8.18	7.83	7.68	7.88	8.21
T_1	7.40	7.89	7.43	7.48	6.98
T_2	7.93	7.67	8.10	8.13	7.75
T_3	8.35	7.27	8.16	8.14	8.05
b					
T_0	8.12	7.78	7.48	7.98	8.44
T_1	7.36	8.23	7.78	7.90	7.51
T_2	7.83	7.78	8.34	8.24	7.78
T_3	8.06	7.98	7.95	8.06	7.99

*Means of 2 dilutions in 3 replication

Table 2 Overall scores of eight organoleptic characteristics of rice-based yogurt

Characteristics	T_0	T_1	T_2	T_3	CD
Appearance	8.93	7.60	8.60	2.86	0.17
Colour	8.60	7.00	8.60	4.20	0.19
Odour	9.20	6.60	8.20	4.53	0.21
Flavour	9.13	7.20	8.13	2.33	0.16
Texture	8.93	5.53	9.46	2.26	0.17
Taste	9.40	6.40	8.26	2.26	0.16
After taste	8.53	5.93	7.60	2.60	0.18
Over all acceptability	9.20	6.60	8.86	3.00	0.19

was done in three alternative days (2nd, 4th and 6th day) and the critical difference was estimated. T_2 was taken as control (Tables 3 and 4).

The organoleptic evaluation results in the selection of the rice-based yogurt enriched with 20% of Annona fruit pulp (T_4) and the one with 5% of Papaya fruit pulp (T_6). According to the judges the after-taste and taste of these yogurts were very good when compared to the other treatments and control having no fruit flavor. Also, these two has got the highest overall acceptability score i.e. 9.33 for 20% Annona enriched yogurt and 4.8 for 5% Papaya enriched yogurt. This supports the result of Tarakci (2010) that the yogurts containing kiwi marmalade fruit were found to be acceptable with respect to overall acceptability. Findings of Othman et al (2019), Senadeera et al (2018) and Illupapalayam et al (2014) also comparable to the present results. Othman et al (2019) stated that yogurt with papaya puree got higher ratings than the pure yogurt and Senadeera et al (2018) reported that soursop enriched yogurt had better sensory scores than other treatments. Illupapalayam et al (2014) reported that probiotic-yogurt products containing spices have good sensory parameters.

Table 3 Overall scores of organoleptic qualities of rice-based yogurt enriched with two different concentrations of *Annona muricata* fruit juice

Characteristics	T_2	T_4	T_5
Appearance	9.00	8.80	8.60
Colour	8.66	8.60	7.93
Odour	8.33	9.33	9.20
Flavour	8.13	9.40	5.00
Texture	9.33	7.60	7.40
Taste	8.06	9.26	4.30
After taste	7.60	9.20	2.86
Over all acceptability	8.86	9.33	5.73

Table 4 Overall scores of organoleptic qualities of rice-based yogurt enriched with three different concentrations of *Carica papaya* fruit juice

Characteristics	T_2	T_6	T_7
Appearance	4.66	4.38	3.47
Colour	4.61	4.20	4.90
Odour	4.21	4.58	3.53
Flavour	4.63	4.97	3.47
Texture	4.21	3.95	3.27
Taste	4.69	4.80	3.37
After taste	4.10	4.58	3.53
Over all acceptability	4.90	4.77	3.63

Thus, the formulation containing 75% milk and 25% rice flour enriched with 20% Annona fruit pulp and the one with 5% Papaya fruit were selected.

When the microbial count of the selected fruit enriched rice-based yogurts were evaluated, the count of both the bacteria were very high in these combinations when compared to the one without any fruit enrichment. It suggests that the fruit enrichment was not affecting the viability of the bacteria in the yogurt. This result was supported by Ranadheera et al (2012). They reported that the addition of fruit juice appeared to support the viability of *Lactobacilli* and higher microorganism numbers observed in fruit yogurts than in plain yogurt throughout the shelf life. The results published by Illupapalayam et al (2014) are also justifying this view. They reported that addition of spices in yogurt did not affect the probiotic population. But Senadeera et al (2018) reported that in soursop enriched yogurt, the counts of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* were found to be insignificant.

Then the quality parameters like syneresis, titratable acidity and pH were assessed. The syneresis and the pH of the yogurt showed a decrease in the days of analysis were as the titratable acidity showed an increase in those days (Fig. 1).

In this study the value of syneresis of fruit enriched yogurt decreases as similar in the case of Tarakci (2010). He stated that the increasing amount of marmalade in yogurt resulted in a decrease in syneresis. A study by Ersan and Kurdal (2014) also supported the view that the syneresis decreases during the storage and they came into a conclusion that the culture combinations and the storage time significantly influenced the properties of the yogurt.

But Tarakci and Kucukoner (2003) reported that the syneresis and the titratable acidity increased over the storage period. Garcia-Perez et al (2005) also supported the view of Tarakci and Kucukoner (2003). The study by Ranadheera et al (2012) does not corroborate the result of

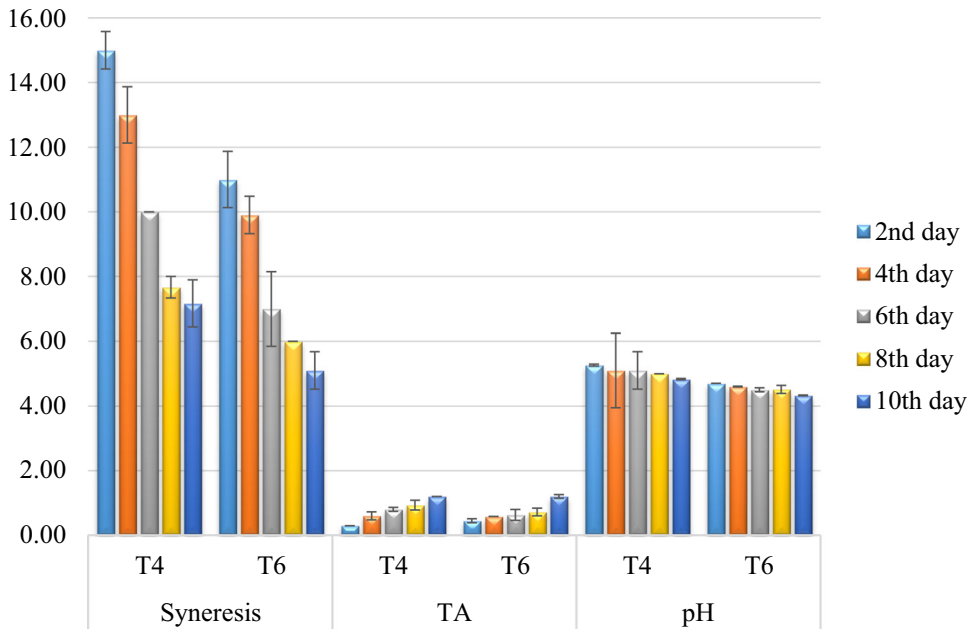

Fig. 1 Syneresis, titratable acidity and pH of the fruit enriched rice-based yogurt

the present study because they stated that the addition of fruit juice significantly increased the syneresis and water holding capacity of yogurt. Similarly Barakat and Hassan (2017) reported that the water holding capacity of pumpkin yogurt improved by adding pumpkin fruit pulp.

In this study the titratable acidity was increasing during the days of analysis and as the titratable acidity increases the pH of the yogurt decreases. This result was similar to the findings of the study by Barakat and Hassan (2017). They reported that pH gets decreased whereas the acidity gets increases when pumpkin pulp was added to yogurt. Similarly Celik et al (2006) reported that addition of cornelian cherry paste and sugar to the yogurt resulted in an increase in the titratable acidity and decrease in the pH during storage. Guler-akin (2005), Tarakci (2010) and Ersan and Kurdal (2014) also comparable to the result of the present study. The cost analysis of the selected rice-based yogurt and of the fruit enriched rice-based yogurt was done. It was obtained that the total cost for the production of one litre of Annona flavored rice-based yogurt was 398.50 rupees and of Papaya flavored rice-based yogurt was 243 rupees and if it was a plain rice-based yogurt it can be made at rupees 188.5 for one litre. Whereas in the market one litre of fruit flavored (strawberry, mango) yogurt was obtained at 320 rupees. The higher cost of these fruit flavored yogurts can be reduced by collecting the fruit from wild, and thus the rice based yogurt with and without fruit enrichment can be made available in the same price. Also, in a price lower than the market price. i e. a fully natural product without any synthetic flavors in lower cost. Hence, it is possible to make available Annona and Papaya, which are medicinally very important, in the form of healthy probiotic yogurt to normal people in a tastier way in low cost.

Conclusion

Rice based yogurt containing 75% milk and 25% of rice slurry is selected by organoleptic evaluation and microbial quality analysis. It has better count of both the bacteria and the highest score in organoleptic characters. It is then enriched with fruit pulp of different fruits in different combinations and selected the yogurt with 20% Annona fruit pulp and the one with 5% Papaya fruit pulp by organoleptic evaluation. The Bacterial counts of both strains were higher in the fruit enriched rice-based yogurt than the rice-based yogurt without any fruit enrichment. In the quality evaluation, syneresis and pH shows a decrease whereas the titratable acidity shows an increase during the analysis days. And in cost analysis it is found that these fruits (Annona and Papaya) can be made available in the form of probiotic yogurt in lower cost than the current market value.

In general yogurt with 62.5% milk, 20.8% rice flour slurry, and 16.6% Annona fruit pulp and 71.4% milk, 23.8% rice flour slurry and 4.7% Papaya fruit pulp are the best formulations and can be released as a healthy probiotic food product.

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Authors' contribution KN designed, carried out the experiments and wrote the MS; PSB carried out some experiments; MTT conceived, supervised the research and edited the manuscript.

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Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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