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Changes in Sensory, Physico-chemical and Microbial Quality of Yoghurt Samples during Storage

R. P. Bhosale, B. D. Patil and D. K. Kamble
Department of Animal Husbandry and Dairy Science, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri - 413 722, Ahmednagar, Maharashtra, India
e-Mail : ravirajbhosale2000@gmail.com

Authors Contribution

R. P. Bhosale : Investigation, data curation and manuscript preparation

B. D. Patil : Conceptualization, result facilitation and supervision

D. K. Kamble : Manuscript editing and guidance

Corresponding Author :
R. P. Bhosale

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Abstract

The present investigation with a view to standardize the process for preparation of dragon fruit yoghurt with improved therapeutic value using dragon fruit pulp and sugar. Initially, preliminary trials were conducted to finalize the levels of dragon fruit pulp and sugar in the yoghurt. Yoghurt samples were prepared with 4, 6, 8, 10 and 12 per cent dragon fruit pulp and 6, 8 and 10 per cent sugar. It was observed that, all the sensory attributes *viz*., colour and appearance, flavor, body and texture and overall acceptability of fresh yoghurt samples under different treatment combinations were significant. On the basis of results of sensory, chemical, microbial evaluation, treatment T_3 was best quality and used to carry out the storage study. The samples were kept at refrigerated temperature (5 ± 1°C) and compared to fresh control samples (T_0). The samples were evaluated for sensory, chemical and microbiological qualities during storage on 1st, 4th, 8th, 12th and 16th day at 4 days interval period using two types of packaging materials *i.e.,* polypropylene (PP) cups and polystyrene (PS) cups.

Keywords : Quality of yoghurt, Dragon fruit, Storage study of yoghurt

Yoghurt is a fermented product made from fresh milk and or reconstituted milk by using bacteria, such as *Lactobacillus bulgaricus* and *Streptococcus thermophiles* with or without any additional food ingredients and permitted food additives (Mukhekar and Dasale, 2018).

Yoghurt's appeal has significantly increased globally with the introduction of health foods. It is among the most distinctive and versatile dairy products. Yoghurt's distinctive flavour stems from the symbiotic fermentation process *Lactobacillus bulgaricus* and *Streptococcus thermophiles* used in its production. In addition, it is a fermented milk product that is distinct from other fermented milk products due to its semisolid qualities and custard-like consistency. Due to its health benefits, yoghurt is appreciated every where in the world. Live lactic acid bacteria in yoghurt provide several health benefits, including reducing the risk of cancer, protecting against gastrointestinal distress, improving lactose digestion by maldigesters, boosting the immune system and assisting the body in assimilating calcium and protein. (Marona and Pedrigon, 2004).

The pitaya (*Hylocereus polyhizus*), a native of Southeast Asia and South America, is an edible cactus that is grown in tropical climates. They are becoming more and more well-known all over the world as foods, snacks and dietary supplements. Their high concentration of bioactive phytochemical compounds particularly betalains has recently piqued scientific interest. Red pitaya supplements reduce liver and cardiovascular damage brought on by a diet heavy in fat and carbohydrates, according to *in-vivo* research. Furthermore, the advantageous properties of pitaya have been documented in rodents and linked to its components, including flavonoids and betalains. The authors ascribed these beneficial effects to the plant's antioxidant components and as a result to their capacity to reduce oxidative stress-induced damage. Thus, we study pitaya's hypolipidemic action in an animal model of *hyper cholesterolemia* in order to prevent and control chronic diseases. In contrast to its fruit, the skin of dragon fruit is an unexplored resource. The weight of a leather dragon fruit is between 30 and 35 per cent that of a fruit that is primarily wasted and not used extensively. The skin of the dragon fruit has roughly 46.7 per cent fibre, which is highly beneficial to health (Rijal, 2019).

The fresh dragon fruit contains 83.00 to 88.00 per cent moisture, 0.16 to 0.23 g protein, 0.21 to 0.61g fats and 0.70 to 0.90 per cent fibre. Every 100 g of fresh fruit pulp contains 6.30 to 8.80 mg of calcium, 30.20 to 36.10 mg of phosphorous, 0.50 to 0.65 mg of iron, 8.00 to 9.00 mg of vitamin-C and the pink-fleshed fruit contain a pigment called betacyanin containing up to 150 to 200 mg per 100 g of fruit (Ayesha *et al.*, 2021).

Material and Methods

Starter Culture

The freeze dried pure cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were procured from the National Collection of Dairy Cultures (NCDC), Division of Dairy Microbiology, National Dairy Research Institute, Karnal (India).

Dragon Fruit Pulp

The Dragon fruit was purchased from local market of Rahuri city. Dragon fruit pulp was prepared in laboratory of Department of Animal husbandry and Dairy Science, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri (M.S.).

Sugar

Food grade sugar was obtained from the local market.

Preparation of Dragon Fruit Yoghurt

The Yoghurt was prepared by using the procedure prescribed by Sharma and Singh (1981) with some minor modifications. Fresh good quality cow milk (3.5% fat) was taken and skim milk powder @ 4 per cent was added and then subjected to filtration/ clarification. The mix was pre-heated to 60°C and homogenized single stage at 2000-2500 psi, the milk was heated to 85°C for 30 min and then cooled to 43 ± 1^{0}C and the sugar was added. It was then inoculated @ 2 per cent with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (1:1 ratio) which were mixed well and incubated at 43 ± 1^{0}C for 5 hours in plastic cups/containers. When the curd was set firmly, it was transferred to the refrigerator and stored at 5 ± 1^{0}C.

Chemical Properties Analysis

Fat : As per the method described in IS: 1224, Part I, 1977. Protein : As per the procedure described in IS: 1479 (part-II) (1961). Total solids: As per the method given in ISI: 1479 Part-II (1961). Titratable acidity (% L. A.) : As per the method given in IS: 1479, Part–I (1960). pH: As per the method given in IS: 1465 (1967). Part –II (1961). Total soluble solids: determined with the help of Erma hand refractometer (Range 0-32^{0} Brix) IS: 1479 (Part-II), 1961. pH: pH of the dragon fruit pulp was measured by Elico digital pH meter. Reducing sugar and total sugar: Determined by the method of Ranganna (1986).

Statistical Analysis

The data generated during the course of this investigation was tabulated and analyzed using Completely Randomized Design (CRD) for pre-experimental trials and to compare control with other treatments. However, effect of dragon fruit pulp and sugar levels and their interaction effect was analyzed by Factorial Completely Randomized Design (FCRD) with four replications (Snedecor and Corchan, 1967).

Treatment details :	
T_0	Control without addition of dragon fruit pulp and sugar in Yoghurt
T_1	Yoghurt + 4 % dragon fruit pulp + 8 % sugar (P_1,S_1)
T_2	Yoghurt + 4 % dragon fruit pulp + 10 % sugar (P_1,S_2)
T_3	Yoghurt + 6 % dragon fruit pulp + 8 % sugar (P_2,S_1)
T_4	Yoghurt + 6 % dragon fruit pulp + 10 % sugar (P_2,S_2)
T_5	Yoghurt + 8 % dragon fruit pulp + 8 % sugar (P_3,S_1)
T_6	Yoghurt + 8 % dragon fruit pulp + 10 % sugar (P_3,S_2)

Storage Studies

These samples were examined every fourth day until the product's were found sensorily acceptable.

A_1 : Control sample (T_0 treatment combination)

A_2 : Optimized sample (T_3 treatment combination)

B_1 : 1st Day

B_2 : 4th Day

B_3 : 8th Day

B_4 : 12th Day

B_5 : 16th day

C_1 : Polypropylene (PP) cup

C_2 : Polystyrene (PS) cup

Results and Discussion

Change in Sensory Qualities of Yoghurt Samples during Storage at 5 ± 1⁰C

Colour and Appearance : The colour and appearance scores of yoghurt as affected by dragon fruit pulp concentrations have been presented in Table 1 and Fig.1. For the control sample, the colour and appearance score decreased from 7.18 to 5.89 in PP cup and from 7.16 to 5.90 in PS cup packaging. In comparison, the optimized sample's colour and appearance scores decreased from 8.21 to 7.07 in PP cup and from 8.19 to 7.00 in PS cup packaging. Overall, during the storage period, the treatment was significant and their interaction showed a significant ($P<0.05$) effect on colour and appearance scores. The decrease in the colour and appearance scores during storage may be attributed to the increase wheying off in yoghurt.

Table 1

Change in sensory qualities of yoghurt samples during storage at 5 ± 1⁰C (score out of 9)

Treatment	Colour and appearance	Flavour	Consistency	Overall acceptability
$A_1B_1C_1$	7.18	7.75	7.93	7.58
$A_1B_1C_2$	7.16	7.63	7.84	7.57
$A_1B_2C_1$	6.74	7.03	7.03	6.93
$A_1B_2C_2$	6.63	7.00	6.98	6.87
$A_1B_3C_1$	6.50	6.43	6.44	6.46
$A_1B_3C_2$	6.30	6.41	6.40	6.37
$A_1B_4C_1$	6.14	6.01	6.01	6.05
$A_1B_4C_2$	5.94	6.00	6.00	5.98
$A_1B_5C_1$	5.89	5.90	5.91	5.90
$A_1B_5C_2$	5.90	5.89	5.87	5.88
$A_2B_1C_1$	8.21	8.46	8.46	8.38
$A_2B_1C_2$	8.19	8.43	8.41	8.34
$A_2B_2C_1$	7.99	8.04	8.04	7.97
$A_2B_2C_2$	7.90	7.98	7.95	7.99
$A_2B_3C_1$	7.69	7.63	7.63	7.65
$A_2B_3C_2$	7.58	7.60	7.60	7.59
$A_2B_4C_1$	7.28	6.98	6.98	7.08
$A_2B_4C_2$	7.19	6.95	6.95	7.03
$A_2B_5C_1$	7.07	6.90	6.89	6.93

Mean of four replications

This trend is consistent with the results of Roy *et al.* (2016) where it was observed that the colour and appearance scores of papaya yoghurt, banana yoghurt and watermelon yoghurt decreased during the storage. The results of the present study during storage were in agreement with the Shalini (2006) in sweetened yoghurt blended with lichi, plum and jamun.

Flavour : The changes in flavour scores of yoghurt during storage at 5 ± 1°C, using PP cup and PS cup packaging, are shown in Table 1 and graphically in Fig. 1. For the control sample, the flavour scores decreased from 7.75 to 5.90 in PP cup and from 7.63 to 5.89 in PS cup packaging. In comparison, the optimized sample's flavour scores decreased from 8.46 to 6.90 in PP cup and from 8.43 to 6.89 in PS cup packaging. Overall, during the storage period, the treatment was significant and their interaction showed

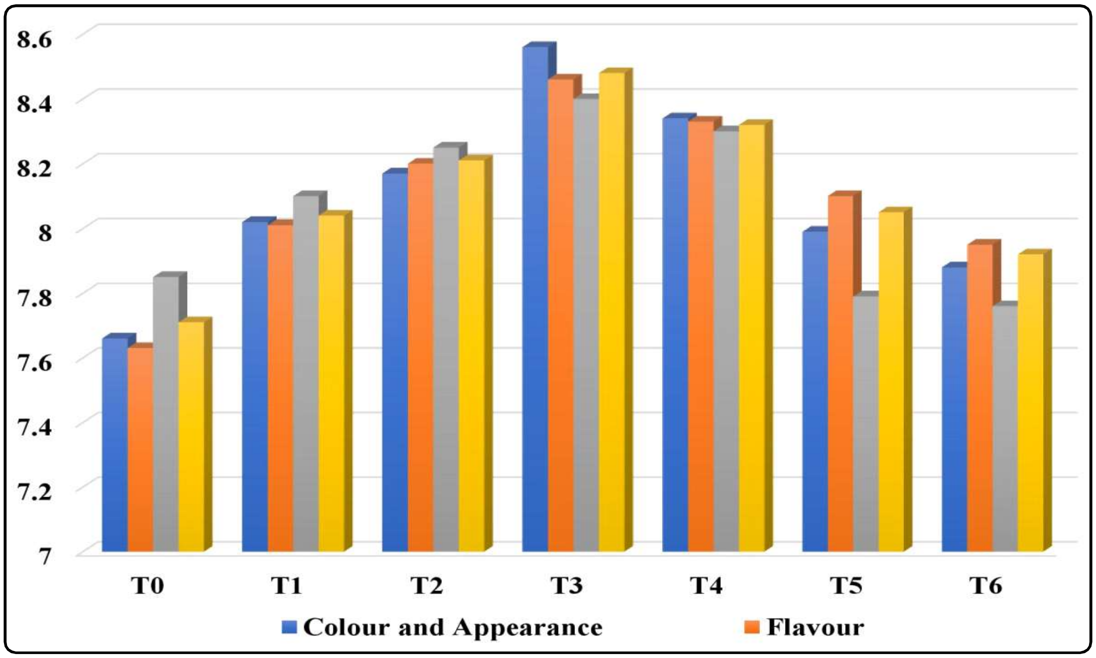

Fig. 1 : Changes in sensory qualities of yoghurt samples during storage at refrigerated temperature (5±1^{0}C)

a significant (P<0.05) effect on flavour scores. The decrease in the flavour scores during storage may be attributed to the increased acidity in yoghurt sample.

This trend is similar to the study conducted by Roy *et al.* (2016) and they observed that the flavour score of papaya yoghurt, banana yoghurt and watermelon yoghurt decreased during the storage. Also the results of the present study were in agreement with the Shalini (2006) in sweetened yoghurt blended with lichi, plum and jamun due to the increased acidity during storage.

Consistency : The consistency scores of yoghurt that were kept in PP and PS cups are displayed graphically in Fig. 1 and in Table 1. The consistency scores dropped for the control sample in the PP cup packaging from 7.93 to 5.91 and in the PS cup packaging from 7.84 to 5.87. In contrast, the optimised sample's consistency scores dropped in the PP cup packaging from 8.46 to 6.89 and in the PS cup packaging from 8.41 to 6.83. A rise in moisture content was given credit for the consistency score's notable decline.

This observation is on par to the study reported by Shalini (2006) who reported a decrease in consistency score with the addition of plum, lichi and jamun to *yoghurt* during storage. This can be attributed to reduction in viscosity and consistency of yoghurt with increase in storage period. Tarakci and Kucukoner (2004) also observed an average decrease in the body and texture scores during storage from 4.21 to 3.99 on 9-point Hedonic scale.

Overall Acceptability : The indicator criteria for the overall sensory quality of the items were overall acceptance. For the control sample, the overall acceptability scores dropped from 7.58 to 5.90 in PP cup packaging and from 7.57 to 5.88 in PS cup packaging (as per Table 1 and Fig. 1). The overall acceptability score decreased for the optimised samples in the PP cup packaging from 8.38 to 6.93 and in the PS cup packaging from 8.34 to 6.93. The decrease in overall acceptability scores was significant during storage.

This trend is consistent with the findings of Roy *et al.* (2016) observed that the overall acceptability score of papaya yoghurt, banana yoghurt and watermelon yoghurt goes on decreasing during the storage. Khote (2021) also reported declining trend of overall acceptability in dragon fruit *lassi* during the storage period.

Change in Chemical Qualities of Yoghurt Samples During Storage at 5 ± 1°C

Moisture : The yoghurt samples' moisture content was measured; the results are displayed in Table 2. The moisture level of the control sample influenced in the PP cup from 85.81 to 86.02 per cent and in the PS cup packaging from 85.79 to 86 per cent. Comparatively, the optimised sample's moisture content influenced in the PP cup from 78.75 to 79.24 per cent and in the PS cup packaging from 78.75 to 79.19 per cent. A decrease in total solids (TS) during storage could be the cause of this increase in moisture content.

These findings are consistent with Sameem *et al.* (2018), who reported a similar increase in moisture content during the storage of *shrikhand* blended with dragon fruit pulp.

Fat : In both samples and packaging materials, it was found that the fat content of the yoghurt dropped significantly from the beginning to the sixteen day of storage (Table 2). The fat content of the control sample dropped in the PP cup (from 3.48 to 3.08 %) and the PS cup packaging (from 3.48 to 3.07 %). The fat content of the optimised sample dropped in the PP cup packaging from 3.24 to 2.97 per cent and in the PS cup packaging from 3.24 to 2.95 per cent. The modest lipolysis that occurs during storage could be the cause of this drop in fat content.

Similar results have been reported by Shalini (2006), who also noted a slight decrease in fat content in yoghurt incorporated with plum, lichi and jamun. The decrease in fat per cent by the addition of fruit pulp in yoghurt is due to dilution effect. Johny *et al.* (2021) observed the same trend of decrease in fat content in sapota and guava yoghurt during storage of 15 days with every five days interval. A decrease in fat content during storage which is due to lipolytic activity of enzymes lipase and lipoxidase produced by microorganisms.

Table 2

Changes in Fat, Protein content (per cent) of yoghurt samples during storage at 5 ± 1°C

Treatment Interaction	*Mean (per cent)		
	Moisture	Fat	Protein
$A_1B_1C_1$	85.81	3.48	3.46
$A_1B_1C_2$	85.79	3.48	3.47
$A_1B_2C_1$	85.86	3.36	3.41
$A_1B_2C_2$	85.85	3.35	3.42
$A_1B_3C_1$	85.91	3.28	3.38
$A_1B_3C_2$	85.89	3.26	3.37
$A_1B_4C_1$	85.97	3.19	3.26
$A_1B_4C_2$	85.96	3.16	3.28
$A_1B_5C_1$	86.02	3.08	3.19
$A_1B_5C_2$	86.00	3.07	3.19
$A_2B_1C_1$	78.75	3.24	2.98
$A_2B_1C_2$	78.75	3.24	2.99
$A_2B_2C_1$	78.88	3.17	2.91
$A_2B_2C_2$	78.85	3.16	2.94
$A_2B_3C_1$	78.97	3.11	2.85
$A_2B_3C_2$	78.94	3.09	2.81
$A_2B_4C_1$	79.05	3.02	2.74
$A_2B_4C_2$	79.03	3.00	2.74
$A_2B_5C_1$	79.24	2.97	2.67
$A_2B_5C_2$	79.19	2.95	2.66

*Mean of four replications

Protein : The control sample's protein content dropped from 3.46 to 3.19 per cent in the PP cup and from 3.47 to3.19 per cent in the PS cup packaging, as shown in Table 2. The protein content of the optimised sample dropped from 2.98 to 2.67 per cent in PP cup packaging and from 2.99 to 2.66 per cent in PS cup. Over the course of the storage period, the protein content in both packaging materials and in both samples considerably dropped. This decrease can be the result of microbial development-induced proteolysis.

Johny *et al.* (2021) observed the same trend of decrease in protein content in sapota and guava yoghurt during storage of 15 days with every five days interval. Protein content decreased in T_0 (Control) from 4.59 to 3.35, T_1 (Sapota yoghurt) from 3.77 to 2.71, T_2 (Guava yoghurt) from 3.23 to 2.61 during storage. These findings are consistent with Widyastuti *et al.* (2017) which showed that during yoghurt fermentation, proteins can undergo proteolysis, leading to a decrease in protein content. Additionally, vitamins and organic acids in yoghurt can be degraded over time, especially during extended storage, due to metabolic activities of probiotics and lactic acid bacteria.

Ash : The changes are given in Table 3. Over the course of storage, it was found that the ash content reduced significantly. Ash content dropped for the control sample in the PP cup from 0.68 to 0.57 per cent and in the PS cup packaging from 0.68 to 0.56 per cent. By contrast, the optimised sample's ash concentration dropped in PP cups from 0.64 to 0.49 per cent and in PS cups from 0.64 to 0.48 per cent. One possible explanation for the decline in ash content during storage is a decrease in mineral content.

Table 3

Changes in ash and total solid content (per cent) of yoghurt samples during storage at 5 ± 1⁰C

Treatments Interaction	Mean (Per cent)	
	Ash	Total solids
$A_1B_1C_1$	0.68	12.19
$A_1B_1C_2$	0.68	12.19
$A_1B_2C_1$	0.66	12.14
$A_1B_2C_2$	0.67	12.13
$A_1B_3C_1$	0.65	12.08
$A_1B_3C_2$	0.64	12.07
$A_1B_4C_1$	0.61	12.03
$A_1B_4C_2$	0.59	12.01
$A_1B_5C_1$	0.57	11.96
$A_1B_5C_2$	0.56	11.95
$A_2B_1C_1$	0.64	21.36
$A_2B_1C_2$	0.64	21.36
$A_2B_2C_1$	0.61	21.19
$A_2B_2C_2$	0.59	21.14
$A_2B_3C_1$	0.56	20.98
$A_2B_3C_2$	0.55	20.01
$A_2B_4C_1$	0.53	20.87
$A_2B_4C_2$	0.51	20.91
$A_2B_5C_1$	0.49	20.70
$A_2B_5C_2$	0.48	20.73

Mean of four replications

The recorded trend of decreased ash content in yoghurt was similar to the findings of Isanga and Zhang (2009). This study examined the impact of storage on nutrient degradation in yoghurt and found a reduction in ash content over time. The reduction was due to the precipitation of minerals, especially calcium, as a result of the acidic environment produced during the fermentation process.

Total Solid : At the 5 per cent level of significance, it was discovered that the reduction in total solids (TS) content was significant across treatments, storage time, packaging materials and their interactions. The TS content of the control sample dropped from 12.19 to 11.96 per cent in the PP cup and from 12.19 to 11.95 per cent in the PS cup packaging, as shown in Table 3. Comparatively, the TS content of the optimised sample dropped in the PP cup from 21.36 to 20.70 per cent and in the PS cup packaging from 21.36 to 20.73 per cent. The total solids in both samples significantly decreased, most likely as a result of the control sample having a greater moisture content.

Johny *et al.* (2021) observed the same trend of decrease in total solid content in sapota and guava yoghurt during storage of 15 days with every five days interval. T_0 (Control) from 14 to 13.24 per cent, T_1 (Sapota yoghurt) from 16 to 15.10 per cent, T_2 (Guava yoghurt) from 15 to 13.99 per cent.

Sugar

Reducing Sugar : As given in Table 4 for control sample, the reducing sugar level was reduced in the PP cup from 4.43 to 4.09 per cent and in the PS cup packaging from 4.43 to 4.07 per cent. IN contrast, the reducing sugar content of the optimised sample dropped from 4.38 to 4.08 per cent in PP cups and from 4.37 to 4.07 per cent in PS cup packaging. The possible cause of the decrease in sugar content could be the bacteria's utilisation of lactose, which during storage transforms into lactic acid.

The present investigation is comparable to the findings of Tamime and Robinson (2007). This study discusses how reducing sugars such as glucose and fructose are rapidly fermented by lactic acid bacteria during the storage of yoghurt, leading to a significant decrease in their concentration. This fermentation process results in the conversion of sugars into lactic acid and other metabolites, contributing to the decline in sugar content. Shukla *et al.* (2017) who assessed the effect of storage on the quality of a functional dairy beverage made with milk, sugar and 30 per cent mango paste. Their study observed a significant ($P < 0.05$) loss of reducing sugar content during 15 days of storage.

Total Sugar : Over the course of the storage period, a considerable drop in the total sugar concentration was observed as given in Table 4. Regardless of the packaging material, this decrease was caused by reductions in both reducing and non-reducing sugars in both the optimised and control samples. In particular, the total sugar level in the control sample dropped in the PP cup from 4.22 to 4.03 per cent and in the PS cup packaging from 4.21 to 4.01 per cent. By contrast, the optimised sample's total sugar content dropped in the PP cup packaging from 12.68 to 12.04 per cent and in the PS cup packaging from 12.66 to 12.02 per cent.

Johny *et al.* (2021) observed the same trend of decrease in total sugar content in sapota and guava yoghurt during storage of 15 days with every five days interval. T_0 (Control) from 11.88 to 11.71 per cent, T_1 (Sapota yoghurt) from 15.14 to 14.94 per cent, T_2 (Guava yoghurt) from 14.14 to 14.02 per cent. Singh and Wani (2020) reported a reduction in the total sugar content of fruit yoghurt during refrigerated storage. The sugar reduction was attributed to fermentative metabolism of added fruits by yoghurt cultures, where sugars such as glucose and fructose were consumed by the LAB over time.

TABLE 4

Changes in sugar content, titrable acidity (per cent) and pH of yoghurt samples during storage at 5 ± 1^{0}C

Treatments Interaction	Mean (Per cent)			
	Reducing sugar	Total sugar	Titrable acidity	pH
$A_1B_1C_1$	4.43	4.22	0.66	4.38
$A_1B_1C_2$	4.43	4.21	0.67	4.40
$A_1B_2C_1$	4.32	4.18	0.70	4.26
$A_1B_2C_2$	4.31	4.17	0.71	4.27
$A_1B_3C_1$	4.24	4.13	0.74	4.10
$A_1B_3C_2$	4.22	4.11	0.76	4.14
$A_1B_4C_1$	4.16	4.07	0.79	3.98
$A_1B_4C_2$	4.15	4.06	0.80	3.99
$A_1B_5C_1$	4.09	4.03	0.86	3.89
$A_1B_5C_2$	4.07	4.01	0.84	3.90
$A_2B_1C_1$	4.38	12.68	0.72	4.07
$A_2B_1C_2$	4.37	12.66	0.72	4.07
$A_2B_2C_1$	4.31	12.53	0.75	3.97
$A_2B_2C_2$	4.30	12.51	0.76	3.99
$A_2B_3C_1$	4.23	12.36	0.80	3.82
$A_2B_3C_2$	4.22	12.33	0.81	3.83
$A_2B_4C_1$	4.15	12.19	0.85	3.67
$A_2B_4C_2$	4.13	12.15	0.87	3.68
$A_2B_5C_1$	4.08	12.04	0.92	3.61
$A_2B_5C_2$	4.07	12.02	0.91	3.63

Mean of four replications

Titrable Acidity : The changes in acidity across the storage period, treatments and packaging materials were significant. As per Table 4, the acidity content of the control sample increased from 0.66 to 0.86 per cent in PP cup and from 0.67 to 0.84 per cent in PS cup packaging. In contrast, the acidity of the optimized product increased from 0.72 to 0.91 per cent in PP cup and from 0.72 to 0.92 per cent in PS cup packaging. Increased acidity during the storage period could be due to the post process fermentation.

Johny *et al.* (2021) observed the same trend of increase in titrable acidity content in sapota and guava yoghurt during storage of 15 days with every five days interval. T_0 (Control) from 0.68 to 0.76 per cent, T_1 (Sapota yoghurt) from 0.61 to 0.65 per cent, T_2 (Guava yoghurt) from 0.66 to 0.89 per cent. Hittiarachchi *et al.* (2015) also observed that titrable acidity of the dragon fruit yoghurt increased during the storage period due to the post fermentation process.

pH : Significant variations in pH were observed depending on the storage duration, treatments and packaging materials. In particular, as per Table 4 the control sample's pH dropped in the PP cup from 4.38 to 3.89 and in the PS cup packaging from 4.40 to 3.90. On the other hand, in the PP cup and PS cup packaging, the pH of the optimised product dropped from 4.07 to 3.61 and from 4.07 to 3.63, respectively. The development of acid-forming bacteria throughout the storage period was probably attributed to drop in the pH.

Present investigation was similar to the finding of Hittiarachchi *et al.* (2015) where they reported that the pH values of dragon fruit yoghurt products in their study decreased from 4.06 to 3.90 during the storage time period. The reduction of pH observed in the present study was due to the growth of acid forming bacteria during the storage period. Johny *et al.* (2021) observed the same trend of decrease in pH of sapota and guava yoghurt samples during storage of 15 days with every five days interval. T_0 (Control) from 4.52 to 4.27, T_1 (Sapota yoghurt) from 4.68 to 4.37, T_2 (Guava yoghurt) from 4.56 to 4.15.

Changes in Microbial Qualities of Yoghurt Samples during Storage at 5 ± 1^{0}C

Lactobacillus bulgaricus Counts : It was observed that significant decrease in the *Lactobacillus bulgaricus* counts of both samples in both packaging material during entire period of storage as given in Table 5 and Fig. 2. The optimised sample's *Lactobacillus bulgaricus* counts in the PP cup dropped from 2.39 to 2.01 x10^8 cfu/ml, while indropped from 2.32 to 2.03 x10^8 cfu/ml. The *Lactobacillus bulgaricus* counts for the control sample in PP cup packaging droped from 2.19 to 2.02 x 10^8 cfu/ml up to the 16th day, while the control sample PP cup packaging

Table 5

Changes in *Lactobacillus bulgaricus* counts, *Strptococcus thermophiles* counts, Yeast and mould counts and Coliform counts of yoghurt samples during storage at 5 ± 1^{0}C

Treatments Interaction	*Lactobacillus bulgaricus* Count (10^8cfu/ml)	*Strptococcus thermophiles* count (10^8cfu/ml)	Yeast and Mould Count (10^1cfu/ml)	Coliform count
$A_1B_1C_1$	2.19	2.21	0	ND
$A_1B_1C_2$	2.21	2.20	0	ND
$A_1B_2C_1$	2.19	2.18	1.46	ND
$A_1B_2C_2$	2.20	2.17	1.47	ND
$A_1B_3C_1$	2.14	2.15	1.56	ND
$A_1B_3C_2$	2.16	2.14	1.58	ND
$A_1B_4C_1$	2.09	2.09	1.66	ND
$A_1B_4C_2$	2.10	2.08	1.67	ND
$A_1B_5C_1$	2.02	2.01	1.78	ND
$A_1B_5C_2$	2.05	1.99	1.79	ND
$A_2B_1C_1$	2.39	2.41	0	ND
$A_2B_1C_2$	2.41	2.40	0	ND
$A_2B_2C_1$	2.32	2.33	1.27	ND
$A_2B_2C_2$	2.34	2.31	1.29	ND
$A_2B_3C_1$	2.26	2.24	1.37	ND
$A_2B_3C_2$	2.28	2.20	1.38	ND
$A_2B_4C_1$	2.13	2.14	1.48	ND
$A_2B_4C_2$	2.15	2.13	1.49	ND
$A_2B_5C_1$	2.01	2.04	1.56	ND
$A_2B_5C_2$	2.03	2.01	1.58	ND

Mean of four replications
ND-Not detected

dropped from 2.21 to 2.05 x 10^8 cfu/ml. The development of all aerobic mesophilic bacteria was inhibited by the drop in pH and increase in acidity, which may have contributed to the *Lactobacillus bulgaricus* counts decline.

Ghuge *et al.* (2023) observed that addition of mango, dragon fruit and apple to yoghurt result in the significant decrease in *Lactobacillus bulgaricus* counts and gave following result; the LAB count in control and fruit yoghurt sample on 1st day of storage was 57 x 10^{10} and 42×10^{12} cfu/ml, respectively which decreased to 9×10^8 and 21×10^8 on 14th day. When *Hibiscus sabdariffa* L. flowers were added to yoghurt,

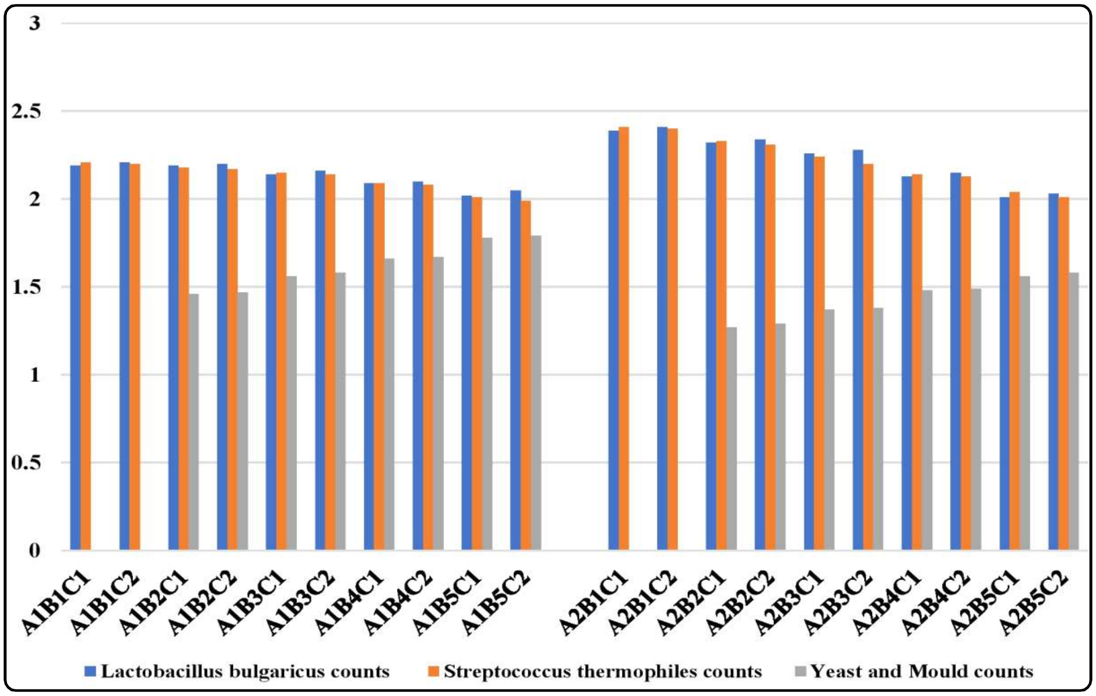

Fig. 2 : Changes in *Lactobacillus bulgaricus* counts (10^8cfu/ml), *Streptococcus thermophilus* counts (10^8cfu/ml) and Yeast and mould (10^1cfu/ml) of yoghurt samples during storage at refrigerated temperature (5 ± 1^0C)

Arslaner *et al.* (2021) showed a similar outcome of decreasing *Lactobacillus bulgaricus* count in yoghurt and noted that the addition of HM generally decreased the *Lactobacillus bulgaricus* counts of yoghurt samples. Comparably, during storage, this group of microbes decreased in both the optimised sample (HM20) and the control for up to 14 days. Due to the higher marmalade concentration, this was linked to a drop in pH as well as an increase in acidity in the samples.

Streptococcus thermophiles Counts : It was observed that there was a significant decrease in the *Streptococcus thermophiles* counts of both samples in both packaging material during entire period of storage. The optimised sample's *Streptococcus thermophiles* count in the PP cup ranged from 2.41 to 2.04 x 10^8 cfu/ml, while the control sample's *Streptococcus thermophiles* counts in PP cup dropped from 2.21 to 2.01 x 10^8 cfu/ml until the sixteenth day of storage. The *Streptococcus thermophiles* counts for the optimised sample in PS cup packaging ranged from 2.40 to 2.02 x 10^8 cfu/ml up to the 16th day, while the control sample's *Streptococcus thermophiles* counts in PS cup dropped from 2.20 to 2.05 x 10^8 cfu/ml. The development of all aerobic mesophilic bacteria was inhibited by the drop in pH and increase in acidity, which may have contributed to the *Streptococcus thermophiles* counts decline.

Similar trend was recorded by Priyanka *et al.* (2013). During initial stage, viable count of *S. thermophilus* for control and spirulina yoghurt were 7.57 and 7.87, respectively. With progress of storage up to 15 days the viable count decreased to 5.08 and 6.87 for control and spirulina yoghurt, respectively. Research by Bourrie *et al.* (2016) highlighted how the production of lactic acid during fermentation creates an acidic environment, which can negatively affect the viability of *Streptococcus thermophilus* over time. The study indicated that the pH of yoghurt decreased from 4.5 to 4.0 during storage, leading to a significant decline in the counts of *Streptococcus thermophilus*, from 10^8 CFU/mL to 10^5 CFU/mL after 14 days.

Yeast and Mould Count : It was observed that there was significant ($P<0.05$) increase in the yeast and mould count of both samples in both packaging material during entire period of storage at 5 ± 1^0C as indicated in Table 5 and Fig. 2. The yeast and mould counts of optimized sample in the PP cup ranged from 0 to 1.56 (x10^1 cfu/ml) until the sixteenth day, while the control sample's grew from 0 to 1.78 (x10^1 cfu/ml). The optimised sample's yeast and mould counts increased from 0 to 1.58 (x10^1 cfu/ml) until the sixteenth day in PS cup packaging, while the control sample's increased from 0 to 1.79 (x10^1 cfu/ml). One possible explanation for the increase in yeast and mould counts during storage is cross-contamination.

Hittiarachchi *et al.* (2015) reported that the yeast and mould population in the developed dragon fruit yoghurts increased but did not exceed 10^1 cfu/g until the 15 day of storage period. Akin *et al.* (2001) performed a study on fruit yoghurt made of cow and goat milk in which they reported that the development of yeast and mould was lower in peach yoghurt than in strawberry yoghurt and that the counts of yeast and mould increased throughout the storage period in all the fruit yoghurts.

Coliform Count : During the storage period of 16 days, no coliform growth was observed. It indicates that proper hygienic conditions were followed throughout the manufacture of yoghurt.

Thus the sensorily superior treatment combination T_3 packaged in polypropylene cups maintained the overall acceptability score of 6.93 upto 16 days at refrigerated temperature (5 ± 1^0C). Over the course of the 16-day storage period, there was a notable rise in moisture and acidity and a decrease in fat, protein, total solid, ash, pH, reducing sugar and non-reducing sugar. During storage, there was an increase in yeast and mould counts and a drop in *Lactobacillus bulgaricus* counts and *Streptococcus thermophiles* counts; coliform counts were not observed during the storage.

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