International Journal of Food Science and Nutrition
www.foodsciencejournal.com
ISSN: 2455-4898
Received: 19-03-2026, Accepted: 19-04-2026, Published: 04-05-2026
Volume 11, Issue 2, 2026, Page No. 77-82

Storage studies of functional yoghurt developed by incorporation of probiotic, Aloe vera and honey

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Abstract
Yoghurt is a widely consumed fermented dairy product known for its nutritional richness and health-promoting properties.In recent years, functional yogurt formulations incorporating natural sweeteners and aloe vera have gained significant attention due to consumer demand for healthier alternatives.Hence, the present study was envisaged to assess the changes in quality of functional yoghurt prepared by using probiotic culture, aloe vera and honey at refrigerated storage. The control yoghurt with (0.004%) probiotic culture, treatment yoghurt 1 (T1) without aloe vera (7%) and treatment 2 yoghurt (T2) with aloe vera (7%) and honey (7%) were subjected to storage studies under refrigeration (5±1^{0}C).The control and treated products were evaluated for physico-chemical, sensory and microbiological quality on 1, 5, 9, 12 and 14 days of storage.As the storage days progress,there was significant (P<0.05) reduction in pH and proximate composition, whereas, significant (P<0.05)increase in titratable acidity (%) of yoghurt were observed within and between product.There was gradual but significant (P<0.05) increase in standard plate count and yeast and mould count, whereas, lactobacillus count shows significant reduction as the days progress. The microbiological values were much below the permissible limit for all the products. All the products had very good sensory score for all the sensory attributes throughout the storage period.Based on the findings of the present study it may be concluded that yoghurt containing 0.004% probiotic, 7% aloe vera and 7% honey was found to be optimum.It was found to be acceptable upto 14 days at refrigerator (5±1^{0}C). The present study shows that probiotic culture, aloe vera and honey can be used in yoghurt for developing functional food.

Keywords: Functional yoghurt, probiotic culture, aloe vera, honey, storage study, sensory score

Introduction

Yoghurt has been recognized as healthy food. Yoghurt is one of the most favoured fermented dairy products obtained by adding starter culture containing lactic acid bacteria, *Lactobacilllusbulgaricus,* and *Streptococcus thermophillus*to milk; which have appreciable consumer demand due to its nutritional benefits (Shiby and Mishra, 2013) [27].Yoghurt contains good quality protein, B vitamins and calcium (Sullivan *et al.,* 2016 [30]; Nguyen *et al.,* 2017[18]). Several therapeutic and medicinal effects such as antibacterial, anti-mutagenic, anti-proliferative, hepato-protective, hypoglycaemic, and antioxidant effects have been ascribed to honey (Ghashm*et al*., 2010) [5].Yoghurt should contain at least 3.25% milk fat and 8.25% of milk solids not-fat (IDFA, 2021) [9].

Aloe vera scientifically known as *Aloe barbadensis*, belongs to the family Lilaceae. The term “Aloe “originated from the Arabic word "aloeh," which means "bitter" (Sheikh *et al.* 2021) [26]. Because of its extraordinary abilities, the aloe vera plant is preferred for herbal and Ayurvedic medicines (Machado *et al.,* 2017) [12].

Honey not only enhances flavour but also contributes antimicrobial and antioxidant benefits, while aloe vera pulp improves nutritional value and consumer acceptability.Honey contains fructooligosaccharides (FOS), which selectively promote the growth of beneficial bacteria such as *Bifidobacteriumlongum*(BB 536). Previous studies have shown that exogenously supplemented prebiotics, including FOS, galactooligosaccharides (GOS), and inulin, can improve the viability and activity of *Lactobacilli* and *Bifidobacteria* in yogurt cultures (Mohan *et al*. 2017) [14].

The functional foods comprises of probiotics, prebiotics and synbiotics. Though yoghurt serves as the most popular fermented milk products, now-a-days probiotic cultures, honey, aloe vera are also incorporated into the yoghurt to achieve extra beneficial effect to the human beings as a functional food. Hence, a functional yoghurt was developedincorporating probiotic cultures, honey, aloe vera and its storage study at refrigerator was conducted.

Materials and Methods

This research work was carried out in the Department of Livestock Products Technology, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry. Pasteurized cow milk was obtained from The Pondicherry Co-operative Milk Producers Union Ltd Puducherry (PONLAIT).Milk containing 3.5% fat and 8.5% SNF (as per the label) and was used for the preparation of functional yoghurt by incorporation of probiotic culture and aloe vera. Skim milk powder, *viz.* Everyday dairy whitener (Nestle), cane sugar (Parry's, White label) and liquid Vanilla essence/ flavour (Top) brand were purchased from local market of Puducherry.

Aloe vera (*Aloe barbadensis*) was obtained from a local garden in Puducherry. The fresh aloe vera was brought to laboratory from the garden. Then aloe vera leaf was placed in water for some time to remove yellow-brown coloured bitter compound aloin. The obtained aloe gel was washed with portable water for 4-5 times and made into smaller pieces and was ground using home mixer grinder. Finally the aloe gel was heated to 90°C for 5 minutes under gas stove with low flame to achieve pasteurization. After cooling, processed aloe gel was used for making the yoghurt.

Preparation of yoghurt

Yoghurt was prepared (Thivya *et al.,* 2026) [33] with specific DVI starter culture (Direct Vat Inoculation Cultures). Honey (Apis) was purchased from local market of Puducherry. The LAB starter cultures (Commercially available - Lactoferm YA- 1 Pro- Tek) with probiotic organisms comprising of*Streptococcus salivariussubsp. Thermophillus*, *Lactobacilllusdelbrueckii subsp. Bulgaricus* and *Lactobacilllus acidophilus.*Concentrated lyophilized lactic starter culture (DVI) was obtained from Biochem (Biochemical research centre), Ahmedabad. In our earlier study an ideal level of the culture was selected as 0.004% level and ideal level of aloe vera was selected as 7% (Thivya *et al.,* 2026) [33].

Selection of suitable level of honey in yoghurt

Three batches of yoghurt were prepared by adding selected level of DVI culture and aloe vera (7%) and honey at 0, 5, 7, 10% levelsto select the best level of honey required for yoghurt preparation (table-1). The quality of the yoghurt was assessed by the physico-chemical quality, lactobacillus count and sensory evaluation and based on the result, the level of 7 % honey was selected as ideal.

Table 1: Different level of Honey in yoghurt

Ingredients	Control (0%)	Treatment 1 (5%)	Treatment 2 (7%)	Treatment 3 (10%)
Milk	1000g	1000g	1000g	1000g
Honey	0g	50g	70g	100g
Skim milk powder (3%)	30g	30g	30g	30g
Sugar (10%)	100g	50g	30g	0g
DVI culture	0.04g	0.04g	0.04g	0.04g
Aloe vera	0g	70g	70g	70g
Flavour	5ml	5ml	5ml	5ml

Storage study of functional yoghurt at refrigerator (5±1^{0}C)

Three kind of yoghurt were prepared for storage study: Control (with selected levels DVI culture), Treatment-1 (control with aloe vera @7% and Treatment 2 (Control with aloe vera @7 % and honey @7%). All the products were stored at 5±1°C for 14 days and quality assessment of yoghurt was done on 1, 5, 9, 12 & 14thday of storage. The quality monitoring was done by monitoring physico-chemical, microbial and sensory analysis of the products during storage.

Preparation of yoghurt

The pasteurized milk containing 3.5% fat and 8.5% SNF was taken as per the requirement by weighing. Then the milk was transferred to the stainless steel vessel and pre-heated to 35-40°C. At this temperature, 3% skim milk powder was added and it was mixed well with the help of stirrer. The required quantity of sugar was added and heated to 90°C for 5 mins and cooled to 45°C (Tamime, 1980) [32]. After cooling the batch was inoculated with commercially available starter culture (Lactoferm YA- 1 Pro- Tek) 0.04 g in one kg milk and mixed thoroughly for even distribution of culture; followed by addition of aloe vera and honey. The inoculated yoghurt mix was filled in steel vessels and incubated at 42- 44°C for 4-5 hours in the pre-set incubator. After complete fermentation (setting) product was transferred from incubator to refrigerator at 5± 1°C until further use.

Physico-chemical analysis

The pH values of yoghurt samples were determined using a digital pH meter.The titratable acidity (%) of yoghurt sample was estimated by titrating 10 ml of yoghurt sample with standardized 0.1 N NaOH and phenolphthalein was used as an indicator according to the method of Jayamanne and Adams, (2004) [11]. The moisture content of the functional yoghurt was determined according to the AOAC (1995) [1]. The protein content of yoghurt sample was estimated by the method of (Pynes, 1932*)* [21].Yoghurt samples were tested for fat by Gerber method (IS 1223, 2001) [10] and was expressed as percentage (%).

Microbial quality

All the microbial parameters *viz.,* standard plate count (SPC), Lactobacillus count and yeast and mould count (YMC) of yoghurt sample were determined following procedures recommended by APHA (1984) [2]. Colonies appeared on the plates were counted and multiplied by the reciprocal of the respective dilution and expressed as log cfu/g.

Sensory evaluation

Semi-trained panelists consisting of faculty and PG students of the Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry were involved in conducting the sensory evaluation of the product. The organoleptic attributes namely colour, texture, sweetness and overall acceptability were evaluated using a 5-point hedonic scale, where (5- Excellent, 4- very good, 3- Good, 2- Fair, 1- Poor) for any attributes.

Statistical analysis

Three trial were done for storage study. The data generated from various trials were pooled, processed and analyzed by statistical method of two way ANOVA (SPSS software package version 17.0). Significant effects were tested using the least significant difference (LSD) test (Snedecor and Cochran, 1994) [29].

Results and Discussion

The results on pH, titratable acidity, proximate composition of functional yoghurt at refrigerator storage (5±1^{0}C) are presented in Table- 2.

pH

The pH significantly (P<0.05) decreased in treatment groups than control on all the days of storage. The values significantly (P<0.05) decreased in case of all groups as the storage period increased. However, on day 1, control group showed a significantly (P<0.05) higher value than treatment groups. The results revealed that lower pH of treated groups might be due to increased acidity which could have been contributed by prebiotic agents present in aloe vera and

honey. Similar to our findings, Coskun and Dirican (2019) [3] also reported that the pH of the honey added yoghurt samples was lower than the honey free yoghurt samples during storage. Vijayalakshmi *et al.* (2010) [35] found that a significant decrease in pH was noticed in low fat yoghurt during the storage period but within the permissible levels. Selvamuthu (2014) [24] also reported that pH of artificial sweetened yoghurt decreased as the storage days increased. Similar findings by Gonzalez *et al*., (2000) [6] reported that the decline in pH value during the storage is probably due to residual enzymes released by lactic acid bacteria during the fermentation.

Titratable acidity

Titratable acidity was significantly (P<0.05) lower in case of control group than treatment groups on all the days (Table-2). However, on day 1, the treatment groups showed significantly (P<0.05) higher value than control group. This might be due to presence of prebiotic agents in aloe vera and honey. Das *et al.* (2015) [4] reported that increased acidity in yoghurt may be associated with the presence of prebiotic oligosaccharides in honey which used to promote the growth and metabolic activity of lactic acid bacteria.The values increased significantly (P<0.05) in case of all groups as the storage period progressed. Muhammad *et al.* (2009) [15] also reported that acidity increased with the storage period which might be due to the microbial activity during storage. Govindammal *et al.* (2017) [7] reported that acidity percentage increased gradually during storage period because of lactic acid fermentation and also slight acidity contributed by aloe vera gel. Selvamuthu (2014) [24] also reported that titratable acidity of artificial sweetened yoghurt increased with the advancement of storage period. Tahra *et al.* (2015) [31] reported that titratable acidity values of all treatment and control samples of yoghurt increased due to activity of starter culture during storage.

Moisture content

The moisture content of control, T-1 and T-2 samples varied between 78.62±0.21 to 74.56±0.21%; 79.79±0.21 to 75.90±0.21% and 80.70±0.21 to 76.58±0.21, respectively during storage(Table-2). The moisture contents were significantly (P<0.05) lower in control group than treatment groups on all the days of storage. The moisture content significantly (P<0.05) decreased in case of all groups as the storage period increased. On day 1, treatment groups showed a significantly (P<0.05) higher moisture content than control. Selvamuthu (2014) [24] also reported that moisture content of artificial sweetened yoghurt decreased as the storage days increased. Rodriquez *et al.* (2010) [22] found that moisture content in the cheese increased with the increase in aloe vera pulp concentration in the cheese due to the ability of aloe vera pulp to hold more water.

Table 2: Effect of aloe vera and honey on the pH, titratable acidity (%) and proximate composition of yoghurt during storage (5±1°C)

	Storage period (days)				
Sample	**1**	**5**	**9**	**12**	**14**
pH					
Control	4.48±0.03^{Ec}	4.38±0.03^{Dc}	4.32±0.03^{Ca}	4.27±0.03^{Bb}	4.18±0.03^{Ab}
T1	4.42±0.03^{Eb}	4.33±0.03^{Db}	4.28±0.03^{Ca}	4.22±0.03 ^{Bb}	4.12±0.03^{Ab}
T2	4.38±0.03^{Da}	4.29±0.03^{CDa}	4.18±0.03^{BCa}	4.12±0.03^{Ba}	3.84±0.03^{Aa}
Titrable acidity (%)					
Control	0.75±0.0^{Aa}	0.77±0.0^{Ba}	0.79±0.0^{Ca}	0.79±0.0^{Ca}	0.80±0.00^{Ca}
T1	0.78±0.0^{Ab}	0.79±0.0^{ABb}	0.81±0.0^{BCb}	0.82±0.0^{CDab}	0.83±0.00^{Db}
T2	0.79±0.0^{Ab}	0.81±0.00^{Bc}	0.82±0.0^{CBb}	0.82±0.0^{CBb}	0.83±0.00^{Cb}
Moisture (%)					
Control	78.62±0.21^{Ea}	77.61±0.21^{Da}	76.83±0.21^{Ca}	75.78±0.21^{Ba}	74.56±0.21^{Aa}
T1	79.79±0.21^{Eb}	78.51±0.21^{Db}	77.66±0.21^{Cb}	76.64±0.21^{Bb}	75.9±0.21^{Ab}
T2	80.70±0.21^{Ec}	79.88±0.21^{Dc}	78.68±0.21^{Cc}	77.77±0.21^{Bc}	76.58±0.21^{Ac}
Protein (%)					
Control	3.49±0.06^{Bb}	3.37±0.06^{Ab}	3.33±0.06^{Ac}	3.24±0.06^{Ab}	3.19±0.06^{Ac}
T1	3.36±0.06^{Da}	3.32±0.06^{DCb}	3.23±0.06^{BCb}	3.08±0.06^{Aa}	3.04±0.06^{Ab}
T2	3.27±0.06^{Ca}	3.19±0.06^{Ca}	3.07±0.06^{Ba}	2.98±0.06^{ABa}	2.9±0.06^{Aa}
Fat (%)					
Control	3.48±0.02^{Ec}	3.41±0.02^{Db}	3.33±0.02^{Cb}	3.21±0.02^{Bb}	3.1±0.02^{Ab}
T1	3.40±0.02^{Eb}	3.32±0.02^{Da}	3.28±0.02^{DCb}	3.06±0.02^{Ba}	2.95±0.02^{Aa}
T2	3.30±0.02^{Da}	3.25±0.02^{Da}	3.10±0.02^{Ca}	3.03±0.02^{BCa}	2.9±0.02^{Aa}

(T1 with 7% aloe vera; T2 with 7% aloe vera and 7% honey)

Means with different superscripts in a row (upper case letters) and in a column (lower case letters) differ significantly (P<0.05).

Protein content

Protein contents of functional yoghurt ranged from 3.49±0.06 to 3.19±0.06% for control, 3.36±0.06 to 3.04±0.06% for T-1 and 3.27±0.06 to 2.90±0.06 for T-2 and theywere significantly (P<0.05) lower in treatment groups than control on all the days of storage(Table-2). The protein content significantly (P<0.05) decreased in case of all groups as the storage period increased. However, on day 1, control group showed a significantly (P<0.05) higher protein values than treatment groups. Lower protein content in treatment groups might be due to negligible amount of protein in aloe vera and honey. Similar to the results of present study, Ikram*et al.* (2020) [8] also reported that protein content in all the aloe vera yoghurt samples decreased as the storage period increased.

Fat content

The fat content of functional yoghurt was significantly higher for control (3.48±0.02 to 3.10±0.02%) followed by

T-1 (3.40±0.02 to 2.95±0.02%) andT-2 (3.30±0.02 to 2.90±0.02%) during storage period(Table-2).The fat content decreased significantly (P<0.05) in case of all groups as the storage period increased. However, on day 1, control group had significantly (P<0.05) higher value than both the treatment groups (T1 and T2). The results revealed that treatment groups had lower fat content and this might be due to negligible amount of fat in aloe vera and honey. Govindammal*et al.* (2017) [7] reported a decrease in fat percentage in yoghurt containing aloe vera gel. Wijesundara and Adikari (2017) [36] also reported that the fat percentage gradually decreased with aloe vera incorporation in drinking yoghurt.

Sensory quality

Control group showed significantly (P<0.05) higher colour score than treatment groups. The lower colour scores in treatment group might be due to addition of honey and aloe vera, which caused a slight change in colour. Mukhekar*et al.* (2018) [16] reported that aloe vera added into yoghurt resulted into greenish or wheyish tinge to the product. Nazni and Komathi (2014) [17] reported that colour and appearance score of yoghurt ranged from 7.60 to 7.70 on 9 point hedonic scale. Control group showed significantly (P<0.05) higher texture score than treatment groups with good to very good texture score. Mahmoudi *et al*., (2016) [13] reported that due to addition of aloe vera extract, sensory properties of probiotic yoghurt sample differed significantly (P<0.05) from other samples. Samah and Hoda (2020) [23] reported a decrease in textural parameters of cheese supplemented with aloe vera pulp during storage. A significantly (P<0.05) higher flavour score was found for control samples compared to treatment groups with very good flavour score. Similar to the results of present study, Tahra *et al.* (2015) [31] reported that all the yoghurt samples containing honey gave a good total impression, were medium sour and did not have any marked off-flavour during storage period. Control group showed significantly (P<0.05) higher sweetness scores than treatment groups with very good sweetness score.Treatment groups showed significantly (P<0.05) lower overall acceptability scores than control group, but still scores were in acceptable range. Similar findings were reported by Tahra *et al.* (2015) [31] who revealed that all yoghurt samples containing honey were acceptable during storage. Aloe vera and honey added functional yoghurt were very well acceptable for sensory quality *viz.* colour, texture, flavour, sweetness and overall acceptability for storage period of 14 days under refrigerator (5±1°C).Similarly, Selvamuthu (2014) [24] also reported the shelf life of aspartame added yoghurt under refrigerator for 13 days.

Table 3: Effect of aloe vera and honey on the sensory quality of yoghurt during storage (5±1°C)

	Storage period (days)				
Sample	**1**	**5**	**9**	**12**	**14**
Colour					
Control	4.97 ± 0.07^{ABb}	4.93 ± 0.07^{ABb}	4.90 ± 0.07^{Bc}	4.87 ± 0.07^{ACc}	4.80 ± 0.07^{ABc}
T1	4.90 ± 0.07^{Cb}	4.87 ± 0.07^{BCa}	4.70 ± 0.07^{Ab}	4.67 ± 0.07^{ACb}	4.60 ± 0.07^{ABb}
T2	4.57 ± 0.07^{Ca}	4.37 ± 0.07^{Ba}	4.27 ± 0.07^{BCa}	4.20 ± 0.07^{ABa}	4.03 ± 0.07^{ABa}
Texture					
Control	4.93 ± 0.04^{ABb}	4.93 ± 0.04^{ABc}	4.83 ± 0.04^{BCb}	4.80 ± 0.04^{BCb}	4.73 ± 0.04^{Ab}
T1	4.73 ± 0.04^{Cb}	4.4 ± 0.04^{Bb}	4.26 ± 0.04^{Aa}	4.13 ± 0.04^{Aa}	4.13 ± 0.04^{Aa}
T2	4.20 ± 0.04^{Aa}	4.16 ± 0.04^{Aa}	3.93 ± 0.04^{Aa}	3.96 ± 0.04^{Aa}	3.93 ± 0.04^{Aa}
Flavour					
Control	4.96 ± 0.08^{Eb}	4.80 ± 0.08^{DEb}	4.63 ± 0.0^{DCb}	4.50 ± 0.08^{BCb}	4.26 ± 0.08^{Ab}
T1	4.86 ± 0.08^{Db}	4.80 ± 0.08^{DCb}	4.53 ± 0.08^{Bb}	4.50 ± 0.08^{ABb}	4.26 ± 0.08^{Ab}
T2	4.46 ± 0.08^{Ca}	4.30 ± 0.08^{BCa}	4.10 ± 0.08^{ABa}	4.00 ± 0.08^{ABa}	3.90 ± 0.08^{Aa}
Sweetness					
Control	4.90 ± 0.09^{Bb}	4.93 ± 0.09^{Bb}	4.86 ± 0.09^{Bc}	4.83 ± 0.09^{Bb}	4.56 ± 0.09^{Ab}
T1	4.86 ± 0.09^{Db}	4.80 ± 0.09^{CDb}	4.43 ± 0.09^{Bb}	4.16 ± 0.09^{Aa}	4.00 ± 0.09^{Aa}
T2	4.50 ± 0.09^{Ca}	4.43 ± 0.09^{Ca}	4.03 ± 0.09^{Ba}	4.03 ± 0.09^{ABa}	3.80 ± 0.09^{ABa}
Overall acceptability					
Control	4.97 ± 0.07^{Ab}	4.93 ± 0.07^{Ab}	4.93 ± 0.07^{Ab}	4.93 ± 0.07^{Ac}	4.90 ± 0.07^{Ac}
T1	4.83 ± 0.07^{Ab}	4.80 ± 0.07^{Ab}	4.73 ± 0.07^{Ab}	4.67 ± 0.07^{Ab}	4.63 ± 0.07^{Ab}
T2	4.60 ± 0.07^{Aa}	4.53 ± 0.07^{ABa}	4.43 ± 0.07^{ABa}	4.37 ± 0.07^{ABa}	4.23 ± 0.07^{Ba}

(T1 with 7% aloe vera; T2 with 7% aloe vera and 7% honey)

Means with different superscripts in a row (upper case letters) and in a column (lower case letters) differ significantly (P<0.05).

Effect of refrigerated (5±1^{0}C) storage on microbial quality of yoghurt with aloe vera and honey

Lactobacillus count (log cfu/g)

The lactobacillus counts decreased in control samples compared to treatment groups throughout the storage period. A significant (P<0.05) decrease in lactobacillus counts in case of all the groups as the storage period increased. This is in accordance with Selvamuthu (2014) [24] who found that the lactobacillus count for both control and aspartame added yoghurt showed progressive decrease during storage. Turgut and Calmaki (2018) [34] stated that interaction between starter organisms (*S. thermophilus* and *L. delbruceki ssp. bulgaricus*) and probiotic bacteria could be regarded as an important aspect in yoghurt production, because, the activities of the yoghurt starter bacteria during fermentation process and the cold storage period may sometimes threaten the survival of the probiotic bacteria. Shori (2015) [28] reported that production of lactic acid during fermentation and cold storage, and hydrogen peroxide generation by *L. bulgaricus*in yoghurt could adversely affect probiotic bacteria like *Bifidobacterium* and *L. acidophilus.* Panesar and Shinde (2011) [20] reported that the decrease in probiotic bacteria might be due to antagonistic relationship between yoghurt bacteria and probiotic strains and dissolved oxygen content. Tahra *et al.* (2015) [31] reported that counts of Bifidobacterium gradually declined during refrigeration storage of yoghurt.

Table 4: Effect of aloe vera and honey on the microbiological quality of yoghurt during storage (5±1°C)

	Storage period (days)				
Sample	**1**	**5**	**9**	**12**	**14**
Lactobacillus count (log cfu/g)					
Control	10.22 ± 0.09^{Ea}	9.75 ± 0.09^{DEa}	8.82 ± 0.09^{Ca}	7.61 ± 0.09^{Ba}	6.60 ± 0.09^{Aa}
T1	10.77 ± 0.09^{Eab}	9.80 ± 0.09^{Da}	8.94 ± 0.09^{Ca}	7.83 ± 0.09^{Ba}	6.66 ± 0.09^{Ab}
T2	10.86 ± 0.09^{Eb}	9.95 ± 0.07^{Db}	8.95 ± 0.09^{Ca}	7.86 ± 0.09^{Ba}	6.76 ± 0.09^{Ac}
Standard plate count (log cfu/g)					
Control	10.81 ± 0.03^{Aa}	10.92 ± 0.03^{Aa}	10.94 ± 0.03^{Aa}	11.494 ± 0.03^{Ba}	12.34 ± 0.03^{Ca}
T1	10.93 ± 0.03^{Ab}	10.94 ± 0.03^{Ab}	10.97 ± 0.03^{Aa}	11.62 ± 0.03^{Bab}	12.40 ± 0.03^{Ca}
T2	10.96 ± 0.04^{Ab}	10.97 ± 0.03^{Bc}	10.98 ± 0.03^{Ba}	11.73 ± 0.03^{Cb}	12.53 ± 0.03^{Db}
Yeast and Mould count (log cfu/g)					
Control	Nil	3.5 ± 0.11^{Ab}	4.30 ± 0.11^{Bb}	4.47 ± 0.11^{Ba}	4.62 ± 0.11^{Ba}
T1	Nil	2.3 ± 011^{Aa}	3.30 ± 0.11^{Ba}	4.30 ± 0.11^{Cab}	4.4 ± 0.11^{Ca}
T2	Nil	2.7 ± 0.11^{Aa}	3.3 ± 0.11^{Ba}	4.1 ± 0.11^{Cb}	4.4 ± 0.11^{Ca}

(T1 with 7% aloe vera; T2 with 7% aloe vera and 7% honey)

Means with different superscripts in a row (upper case letters) and in a column (lower case letters) differ significantly ($P<0.05$).

Standard plate count (log cfu/g)

The counts were significantly ($P<0.05$) lower in control samples compared to treatment groups throughout the storage period. The results revealed that standard plate count increased significantly ($P<0.05$) in case of all groups with the advancement of storage period. Pal *et al.* (2012) [19] also reported that standard plate count increased on addition of aloe vera juice. This is in accordance with Selvamuthu (2014) [24] who found that the standard plate count for both control and aspartame added yoghurt showed progressive increase during storage.

Yeast and Mould count (log cfu/g)

The counts significantly ($P<0.05$) decreased in treatments groups compared to control throughout the storage period. The yeast and mould count increased significantly ($P<0.05$) in case of all the groups as the storage period increased. Yeast and mould count could not be detected on first day of storage which might be due to hygienic measures during the processing of yoghurt. Selvamuthu (2014) [24] also observed that yeast and mould count in aspartame added yoghurt has risen gradually as the storage period progressed. Wijesundara and Adikari (2017) [36] also reported that yeast and mould count increased with the storage of yoghurt and the highest yeast and mould count were observed in control sample on 15th day of storage. Senarathna *et al.* (2020) [25] reported that yeast and mould growth was not noticed in osmotically dehydrated aloe gel treated stirred yoghurt till 35 days of storage at 4^0C, and counts were still below the recommended value.

Conclusion

Based on the findings of the present study it may be concluded that yoghurt containing 0.004% probiotic, 7% aloe vera and 7% honey was found to be optimum. Use of probiotic, aloe vera and honey in functional yoghurt has not only imparted shelf life stability to the product but also helped in reducing the level of microbial load in the functional yoghurt thereby promising additional health promoting effect in the human. Based on the physico-chemical, sensory and microbiological quality, 0.004% probiotic culture, 7% aloe vera and7% honey were found to be ideal combination for preparing functional yoghurt. It was found to be acceptable upto 14 days at refrigerated temperature (5 ± 1^0C). The present study shows that probiotic culture, aloe vera and honey can be used in yoghurt for developing functional food.

Acknowledgements

We extend our sincere gratitude to Rajiv Gandhi institute of Veterinary Education and Research (RIVER), for providing necessary facilities to carry out this work.

Competing interests

The authors have no competing interests; technical, financial or personal between themselves or others that might bias the work.

Ethics statement Not applicable

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