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Development of functional yoghurt by incorporation of probiotics and Aloe Vera

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Abstract
The main objective of the present investigation was to develop functional yoghurt using suitable level of probiotic culture and aloe vera, to evaluate its physico-chemical, sensory and microbial quality under refrigerated storage. In experiment-I probiotic culture was added at the levels of 0.002, 0.003 and 0.004% and the products were subjected to physico-chemical, microbial and sensory quality to select the suitable level of probiotic culture. Among the three levels of probiotic culture 0.004% was selected as the best level based on the sensory quality and other parameters (pH, TA and lactobacillus count). In experiment-II selected level of probiotic culture (0.004%) in experiment-I, was used to prepare yoghurt with three different levels of aloe vera at 7, 10, 13% with a control (without aloe vera) and the products were subjected to physico-chemical, microbial and sensory quality analysis. Among the three levels, 7% aloe vera was selected as the suitable level based on the sensory evaluation and other parameters. Both control and treated samples were found to be acceptable on 5 point Hedonic scale. Based on the physico-chemical, sensory and microbilogical quality, 0.004% probiotic culture, 7% aloe vera was found to be ideal combination for preparing functional yoghurt. Prebiotic activity of aloe vera could be found as indicated by the better growth of lactobacillus.

Keywords: Yoghurt, probiotic culture, aloe-vera, titratable acidity, sensory score.

Introduction

India is the largest producer and also the largest consumer of milk in the world. In India around 247.87 million tonnes of milk produced and the per capita availability of milk in India was 485 grams per day (DAHD, 2025) [6]. Around 60-65% of total milk produced in the country is consumed as liquid milk with remaining 35-40% is converted to milk products *viz* 8% as ghee, 8% as curd (yoghurt), 10% as butter, cream, cheese (including paneer) and dairy whitener, 4% as ice cream and other products account for the remaining percentages, etc (IDMRF, 2025) [12]. Yoghurt is one of the most favoured fermented dairy products obtained by adding starter culture containing lactic acid bacteria, *Lactobacilllus bulgaricus*, and *Streptococcus thermophillus* to milk; which have appreciable consumer demand due to its nutritional benefits (Shibby *et al.*, 2013) [27]. Yoghurt is classically characterised as a shine, viscous gel with a taste of sharp acidic and a green apple flavour. These flavours play an important role for acceptability and liking of yoghurt among the consumers (Cheng, 2010) [3]. Codex regulation for yoghurt indicates that minimum milk protein content of 2.7% and 5.6% of concentrated yoghurt and the maximum fat content is 15% (Codex, 2008) [4]. Yoghurt should contain at least 3.25% milk fat and 8.25% of milk solids not-fat (IDFA, 2021) [11]. Yoghurt is considered as a healthy food due to its high digestibility and bioavailability of nutrients and also can be advised to the people with lactose intolerance, gastrointestinal disorders such as inflammatory bowel disease, and irritable bowel disease, and aids in immune function and weight control (Kinley, 2005) [16]. The functional foods are those "designed to have physiological benefits and/or reduce the risk of chronic illness beyond primary nutritional functions, and may be close in appearance to conventional food and taken as part of a regular diet (USDA, 2010) [31]. The functional foods comprises of probiotics, prebiotics and synbiotics. According to Heydari *et al.* (2018) [10], fermented milk, are good vehicle for probiotics delivery in the human body due to their good compatibility. Probiotics are live microorganisms which can exert health benefits on the host when taken in adequate level (García-Burgos *et al.* 2020) [7]. Aloe vera is a clear thin gelatinous material that comes from inside the aloe vera leaves (Neal, 1965) [20]. There are over 200 types of aloe vera. Out of these only 4 or 5 are commonly used for medicinal purpose and food. The most widely used variety for food and medicinal purpose is *Aloe barbadensis* referred as aloe vera. Various parts of the plant contains approximately 75 nutrients as well as 200 active compounds including aminoacids, salicylic acid, lignin, anthraquinones, saponins, vitamins, minerals, enzymes (Misir *et al.*, 2014) [18]. Though yoghurt serves as the most popular fermented milk products, now-a-days probiotic cultures, aloe vera are also incorporated into the yoghurt to achieve extra beneficial effect to the human beings as a functional food. But combined form of these two ingredients incorporated yoghurt is not available in the market. Hence, this study was done to develop functional yoghurt with probiotic and aloe vera.

Materials and Methods

The present research work was carried out in the Department of Livestock Products Technology, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry. Details about the ingredients and chemicals used, plan of experiments and methodology are presented below.

Dairy ingredients: Pasteurized cow milk was obtained from The Pondicherry Co-operative Milk Producers Union Ltd,

(PONLAIT) Puducherry and skim milk powder, *viz.* Everyday dairy whitener (Nestle), was used for the preparation of functional yoghurt by incorporation of probiotic culture and aloe vera.

Non-dairy ingredients: Cane sugar (Parry's, White label), liquid Vanilla essence/ flavour (Top) brand were purchased from local market of Puducherry. Aloe vera (*Aloe barbadensis*) was obtained from alocal 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. Yoghurt was prepared with specific DVI starter culture (Direct Vat Inoculation Cultures). The LAB starter cultures (Commercially available - Lactoferm YA- 1 Pro-Tek) with probiotic organisms comprising of *Streptococcus salivarius sub sp. Thermophillus, Lactobacilllus delbrueckii subsp. Bulgaricus* and *Lactobacilllus acidophilus*. Concentrated lyophilized lactic starter culture (DVI) was obtained from Biochem (Biochemical research centre), Ahmedabad.

Experiment-I: Selection of suitable level of probiotic culture for yoghurt:

To determine the optimum level of direct vat inoculation (DVI) culture, three batches of yoghurt were prepared with different levels (0.002, 0.003, and 0.004 %) of the culture (table-1). The optimum level of culture required in the yoghurt was assessed by the physico-chemical quality, sensory evaluation and lactobacilllus count.

Table 1: Different level of DVI culture with standard recipe

Ingredients	Treatment 1 (0.002%)	Treatment 2 (0.003%)	Treatment 3 (0.004%)
Milk	1000g	1000g	1000g
Skim milk powder (3%)	30g	30g	30g
Sugar (10%)	100g	100g	100g
DVI culture	0.02g	0.03g	0.04g
Flavour	5ml	5ml	5ml

Based on the result of experiment-I, 0.004% level of DVI culture was selected for further study in experiment-2.

Experiment-II: Selection of suitable level of aloe vera in yoghurt

Three batches of yoghurt were prepared with selected level of culture 0.004% in Experiment –2 and by addition of aloe vera at 0, 7, 10, and 13% (table-2).

Table 2: Different level of aloe vera in yoghurt

Ingredients	Control (0%)	Treatmen-1 (7%)	Treatment-2 (10%)	Treatmen-3 (13%)
Milk	1000g	930g	900g	870g
Skim milk powder (3%)	30g	30g	30g	30g
Sugar (10%)	100g	100g	100g	100g
DVI-culture	0.04g	0.04g	0.04g	0.04g
Aloe vera	0g	70g	100g	130g
Flavour	5ml	5ml	5ml	5ml

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) [30]. After cooling the batch was inoculated with commercially available starter culture (Lactoferin YA- 1 Pro- Tek) 0.04 g in one kg milk and mixed thoroughly for even distribution of culture. The inoculated yoghurt mix was filled in steel vessels and incubated at 42- 44°C for 4-5 hours in the pre-set incubator. During incubation, the progress of fermentation of yoghurt mix was observed every hour up to three hours then every 30 minutes till completion of fermentation. 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) [15]. 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) [24]. Yoghurt samples were tested for fat by Gerber method (IS 1223, 2001) [14] and was expressed as percentage.

Microbial quality

Lactobacillus count of yoghurt sample was 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

The data generated from various trials under each experiment were pooled, processed and analyzed by statistical method of one 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 observation on the progress of fermentation using commercial DVI culture and DVI culture with aloe vera is presented in table 3. A such there was no difference in the fermentation process, means addition of alo vera has not interfered with the function of the yoghurt culture.

Table 3: Observation on fermentation with DVI culture and DVI Culture+Aloe vera

Treatment	2 hrs	3hrs, 30min	4hrs, 20min	5hrs, 20min
DVI-culture	Started fermentation	Partial fermentation	Complete fermentation	-
DVI-culture+ Aloe vera	Started fermentation	Partial fermentation	Complete fermentation	-

Physico-chemical, microbiological and sensory quality of yoghurt with probiotic culture:

The mean values of physico-chemical parameters namely pH, titratable acidity (%) and lactobacillus count of yoghurt incorporated with 0.002 (T1), 0.003 (T2) and 0.004 (T3) percent levels of probiotic are presented in table 4.

Table 4: Physico-chemical and microbiological quality of yoghurt with probiotic culture (Mean±S.E)

Parameters	Treatment 1 (0.002%)	Treatment 2 (0.003%)	Treatment 3 (0.004%)
pH	4.41±0.01^{a}	4.37±0.01^{ab}	4.33±0.02^{b}
TA (%)	0.71±0.00^{a}	0.73±0.00^{b}	0.77±0.00^{c}
Lactobacillus count (Log cfu/g)	9.01±0.28^{a}	9.24±0.51^{b}	9.84±0.37^{c}

$^{a\,b\,c}$ Means bearing different superscripts in a row differ significantly (P<0.05)

Addition of probiotic culture resulted in significant (P<0.005) decrease in pH values of functional yoghurt and significant (P<0.05) differences were found between T1 and T3. The results revealed that lower pH in the T3 sample might be due to higher acid production because of increased level of probiotic addition leading to lower pH. Similar findings were reported in an earlier study by Pagthinathan *et al.* (2018) [21] while evaluating the physical, chemical and sensory parameters of probiotic yoghurt during storage. Cutrim *et al.* (2016) [5] reported that lactic acid fermentation leads to food matrix of reduced pH creating harsh condition for food borne pathogens in yoghurt and other fermented products.

There was a gradual increase in titratable acidity (%) with increasing levels of probiotic culture incorporation. The titratable acidity (%) of T3 sample increased significantly (P<0.05) when compared to T1 and T2 samples. The results revealed that acidity increased with increasing level of probiotic culture due to more lactic acid production from lactose by higher level of bacteria which is matching with similar decline in pH in an earlier study by Ikram *et al.* (2020) [13] while studying the factors affecting the ability of high beta galactosidase yoghurt to enhance lactose absorption. Pagthinathan *et al.* (2018) [21] reported that the change in titratable acidity of yoghurt is due to the fermentation process by micro-organism and degradation of lactose into lactic acid.

The lactobacillus count (log cfu/g) increased significantly (P<0.05) with increasing level of probiotic culture. The results revealed that a little higher level of culture has given better growth of lactobacillus which is also correlated with higher level of acidity or lower pH. Similar findings were observed by Pagathinathan *et al.* (2018) [28] while evaluating the physical, chemical and sensory parameters of probiotic yoghurt during storage.

Sensory attributes: The mean values of various sensory parameters of yoghurt incorporated with 0.002 (T1), 0.003 (T2) and 0.004 (T3) percent levels of probiotic are presented in table 5.

Table 5: Sensory quality of yoghurt with probiotic culture (Mean ± SE)

Parameters	Treatment 1 (0.002%)	Treatment 2 (0.003%)	Treatment 3 (0.004%)
Colour	4.31± 0.09^{a}	4.16± 0.01^{a}	4.25 ± 0.09^{a}
Texture	4.18± 0.01^{a}	4.08 ± 0.10^{a}	4.14 ± 0.11^{a}
Flavour	4.25 ± 0.10^{b}	3.94 ± 0.11^{a}	4.08 ± 0.10^{ab}
Sweetness	4.18 ± 0.12^{a}	3.98 ± 0.12^{a}	4.16 ± 0.10^{a}
Overall acceptability	4.35 ± 0.08^{b}	3.90 ± 0.12^{a}	4.20 ± 0.09^{b}

$^{a\,b\,c}$ Means bearing different superscripts in a row differ significantly (P<0.05)

Sensory evaluation of probiotic yoghurt revealed that there was no significant (P>0.05) difference between T1, T2 and T3 in respect of colour and texture of the yoghurt. The mean values of sensory parameters such as colour, texture, flavour, sweetness and overall acceptability of T1 sample recorded higher scores than T2 and T3, indicating very good to good sensory scores. But no significant (P>0.05) differences were found among the treatments indicating that the different levels of culture did not affect the colour, texture and sweetness of the products. Mahmoudi *et al.* (2016) [17] reported that probiotic incorporated yoghurt samples were similar to original sensory properties of yoghurt. For flavour no significant (P>0.05) differences were found between T1 and T3 and between T2 and T3, but significant (P<0.05) difference were found between T1 and T2 samples indicating that higher culture level had significant (P<0.05) impact on flavour but score were within the commercially acceptable range. No significant (P>0.05) differences were observed between T1 and T3 on overall acceptability scores. The results indicated that incorporation of probiotics has not significantly altered the sensory properties of yoghurt except flavour. Similar reports were mentioned in earlier study made by Hekmat and Reid (2006) [9].

Physico-chemical, microbiological and sensory quality of yoghurt with aloe vera

The mean values of physico-chemical parameters namely pH, proximate composition and lactobacillus count of selected probitic added yoghurt (0.004%) incorporated with 0, 7, 10 & 13 percent levels of Aloe vera are represented in Table 6.

Table 6: Physico-chemical and microbiological quality of yoghurt with aloe vera (Mean±S.E)

Parameters	Control (0%)	Treatment 1 (7%)	Treatment 2 (10%)	Treatment 3 (13%)
TA (%)	0.73± 0.00^{a}	0.76±0.00^{b}	0.78±0.00^{c}	0.79 ±0.00^{d}
pH	4.49 ± 0.01^{d}	4.41 ±0.00^{c}	4.36 ± 0.01^{b}	4.30 ± 0.01^{a}
Moisture (%)	77.58±0.38^{a}	79.41±0.18^{b}	80.32±0.15^{c}	81.40±0.12^{d}
Protein (%)	3.43±0.03^{c}	3.32±0.00^{c}	3.26±0.03^{b}	3.10±0.03^{a}
Fat (%)	3.53±0.03^{c}	3.48±0.04^{bc}	3.36±0.03^{b}	3.23±0.04^{a}
Lactobacillus count (Log cfu/g)	10.65±0.11^{a}	11.12±0.07^{b}	11.62±0.02^{c}	11.83±0.01^{d}

$^{ab\,cd}$ Means bearing different superscripts in a row differ significantly (p<0.05)

The pH of yoghurt decreased significantly (P<0.05) with increasing level of aloe vera. The decrease in pH with increased level of aloe vera suggests that the aloe vera is synergistic to the growth of the culture which might be due to presence of some prebiotic polysaccharides effect. Yadav *et al.* (2018) [33] found that pH decreased with increase in aloe vera concentration in aloe vera cubes flavoured probiotic yoghurt.

The titratable acidity (%) of yoghurt increased significantly (P<0.05) with increasing level of aloe vera. Numerically, T3 sample had the higher acidity value than control sample, due to acidic nature of aloe vera gel. This was in accordance with the findings of Pushkala (2014) [23] reported in formulation and quality evaluation of aloe vera enriched functional foods. Wijesundara and Adikari (2017) [32] evaluated the titratable acidity of yoghurt and reported that the acidity of yoghurt increased with increased aloe vera juice percentage in the preparation of yoghurt.

The moisture content of yoghurt increased significantly (P<0.05) in all the treatments with increasing level of aloe vera when compared to control. Numerically the highest moisture content was observed in T3 sample. The result revealed that increased moisture content in aloe vera incorporated yoghurt might be due to the high moisture content (90-95%) of aloe vera. A similar increase in moisture percentage was observed by Samah *et al.* (2020) [26] in case of ultra-filtrate soft cheese with aloe vera pulp and reported that aloe vera pulp had an ability to hold more water (Rodriguez *et al.,* 2010) [25].

In protein content, significant (P<0.05) differences were found between control, T2 and T3, but no significant (P>0.05) differences were noticed between control and T1. The results revealed that protein content of yoghurt decreased significantly (P<0.05) with increasing level of aloe vera in T2 and T3. The decreased protein content in aloe vera incorporated yoghurt might be due to negligible amount of protein (0.12%) in aloe vera. A similar decrease in protein percentage was reported by Yadav *et al.* (2018) [33] in case of aloe vera cubes flavoured probiotic yoghurt.

Significant (P<0.05) differences were found between control, T2 and T3, but no significant (P>0.05) were observed between control and T1 and between T1 and T2 in fat content. The result revealed that fat content of yoghurt decreased significantly (P<0.05) in T2 and T3 samples with increasing levels of aloe vera. The decrease in fat content in aloe vera incorporated yoghurt may be due to the negligible fat content of aloe vera. This is in agreement with studies reported by Siddharth (2012) [28] on yoghurt preparation characteristics and recent advancement. A similar decrease in fat percentage was also observed by Govindammal *et al.* (2017) [8] in case of aloe vera gel fortified yoghurt.

The results revealed that lactobacillus counts increased significantly (P<0.05) with increasing level of aloe vera. T3 had significantly (P<0.05) higher counts compared to control, T1 and T2 samples. Since aloe vera contains polysaccharides having prebiotic property that might have enhanced the lactobacilli growth rate in treated yoghurts compared to control. Similar findings were recorded by Samah *et al,* (2020) [26] who reported that 15% aloe vera additive increased the progress of probiotic bacteria content of cheese compared to control and other treatments due to high content of polysaccharides.

The mean values of sensory parameters of selected probitic added yoghurt (0.004%) incorporated with 0, 7, 10 & 13 percent levels of Aloe vera are represented in Table 7.

Table 7: Sensory quality of yoghurt with aloe vera (Mean ±SE)

Parameters	Control (0%)	Treatment 1 (7%)	Treatment 2 (10%)	Treatment 3 (13%)
Colour	4.73 ± 0.09^{c}	4.57 ± 0.11^{bc}	4.37 ± 0.11^{ab}	4.13 ± 0.09^{a}
Texture	4.57 ± 0.11^{b}	4.47 ± 0.11^{b}	3.97 ± 0.13^{a}	3.67 ± 0.13^{a}
Flavour	4.77 ± 0.09^{b}	4.37 ± 0.13^{a}	4.20 ± 0.14^{a}	4.07 ± 0.14^{a}
Sweetness	4.57 ± 0.11^{b}	4.33 ± 0.11^{b}	3.93 ± 0.12^{a}	3.67 ± 0.13^{a}
Overall acceptability	4.73 ± 0.09^{c}	4.37 ± 0.12^{b}	4.07 ± 0.10^{ab}	3.83 ± 0.11^{a}

[ab c]Means bearing different superscripts in a row differ significantly (P<0.05)

Sensory evaluation of aloe vera incorporated yoghurt revealed significant (P<0.05) differences between control and treatments 2 & 3. The colour, texture and sweetness scores of T1 were comparable to scores for control sample. The results revealed that T3 had significantly (P<0.05) lower texture score compared to control and T1 which might be due to higher moisture content (90-95%) in aloe vera which significantly altered the yoghurt texture. Flavour scores decreased significantly (P<0.05) with increased addition of aloe vera. Control and T1 samples had significantly higher overall acceptability scores compared to T2 and T3. Similarly Mukhekar *et al.* (2018) [19] reported that higher level of aloe vera in yoghurt imparted into greenish/ whey tinge to the product resulting in lower colour/appearance scores, harsh flavour. Mahmoudi*et al.* (2016) [17] reported that with the addition of aloe vera extract, sensory properties of probiotic yoghurt samples differed significantly from other samples. Pakbin*et al.* (2015) [22] reported that adding any vegetable or herbal extract in yoghurt cause change in sensory properties of yoghurt that leads to be ranked low by consumers.

Conclusion

The purpose of this study was to select the suitable level of probiotic culture, aloe vera for the incorporation in the yoghurt to develop functional yoghurt. For preparation of functional yoghurt different levels of probiotic culture, aloe vera were tried and were found to be acceptable up to 0.004% probiotic culture, 13% aloe vera individually. Based on the physico-chemical, sensory and microbiological quality, 0.004% probiotic culture, 7% aloe vera was found to be ideal combination for preparing functional yoghurt. Prebiotic activity of aloe vera could be found as indicated by the better growth of lactobacillus. The present study shows that probiotic culture, aloe vera can be used in yoghurt for developing functional foods.

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