

DEVELOPMENT OF ALOE VERA (*Aloe barbadensis* Miller) INCORPORATED DRINKING YOGHURT

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ABSTRACT

The present study investigated the possibility of developing a novel drinking yoghurt incorporated with Aloe Vera juice and evaluated its sensory quality parameters. The experiment was conducted as Complete Randomized Design with four replicates. Juices of Aloe Vera was extracted using cold extraction method and drinking yoghurt prepared by incorporating pasteurized Aloe Vera juice with four levels (10%, 15%, 20% and 25%) and compared with the control (0%). Developed product was stored at 4 °C for 20 days. Nutritional and physicochemical properties of the developed products were analyzed. Sensory evaluation was done with 33 untrained panelists using nine point hedonic scales. Titratable acidity and pH of the developed products were tested at 0, 4, 8, 12, 16, 20 days and microbial counts (Total coliform, yeast and mold) were tested at 0, 5, 10, 15, 20 days of storage. Parametric data were analyzed using one way Analysis of Variance in SAS (Ver. 9.0) and sensory data were analyzed by Friedman test in MINITAB. Results revealed that brix values were significantly different ($p < 0.05$) among treatments. Protein, ash and dry matter contents were not significantly different ($p > 0.05$) among treatments. However, Fat content among treatments differed ($p < 0.05$) significantly. Sensory analysis revealed that the drinking yoghurt incorporated with 15% of Aloe Vera juice had the best sensory qualities. Titratable acidity and pH showed the significant difference ($p < 0.05$) among developed products. The significant interaction was observed between treatment and storage time on pH and acidity for the developed products ($p < 0.05$) and it was within the acceptable range up to 16 days of storage period at 4°C. Yeast and mold counts were within the recommended values of Sri Lanka Standards Institute (SLSI) up to 15 days of storage. In conclusion, 15% of Aloe Vera juice can be incorporated to produce drinking yoghurt with the best sensory attributes and the same can be stored up to 15 days at 4 °C without any quality deterioration.

Keywords-Aloe vera, Drinking yoghurt, Storage, Sensory quality

1. INTRODUCTION

The demand for the fermented dairy products such as curd, set and drinking yoghurts is growing up due to its numerous health benefits. These products provide and preserve nutrients, different flavors, aromas and textures, enhance organoleptic properties and increase economic value [1]. Yoghurt is a coagulated milk product obtained by lactic acid fermentation through the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Yoghurt drinks are categorized under stirred yoghurt which contains low viscosity due to high agitation after formation of fermented coagulum [2][3]. The use of herbal products has been growing rapidly in the general public. Aloe vera has a long history of providing lots of health benefits, and is one of the most frequently used herbal treatments throughout the world. Out of 400 species of Aloe, most popular and widely used species is *Aloe barbadensis* Miller [4], commonly referred as Aloe Vera. Processing of Aloe Vera leaf pulp has become a large industry in the world. In the food industry, it is used as a source of functional foods such as yoghurt and as an ingredient in other food products,

for the production of gel-containing health drinks and beverages [5]. Today, consumer demand is moved towards the health and nutrition. Therefore, fortifications of food products with various functional ingredients have been used to fulfill above requirements [6]. If the probiotic products are fortified with the herbal additives then the formulated products can be unique and provide more health benefits. Therefore, the main objective of the study was to produce Aloe Vera incorporated drinking yoghurt and to get functional properties of both Aloe Vera and milk by including in to one product while satisfying the interest of consumers.

2. MATERIALS AND METHODS

Manufacturing of Aloe Vera incorporated drinking yoghurt, sensory tests, chemical analysis and microbial analysis were carried out at Dairy Science Laboratory, Department of Animal and Food Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka. Bacterial culture was provided by J.L. Morison Son. Fresh cow milk samples were collected from Dairy farm of Faculty of Agriculture, Rajarata University of Sri Lanka.

Extraction of Aloe Vera gel and preparation of juice

Aloe Vera gel was extracted using cold extraction method and juice was prepared. Freshly harvested Aloe Vera leaves were dipped into 500ppm potassium meta-bisulfite (KMS) solution and washed thoroughly with tap water and kept for flash cooling to 5°C for gel stabilization. Treating after bactericide the Aloe Vera leaves was cut vertically into two halves and gel was separated using stainless steel knife, allowed to settle for 12 hours and then finally homogenized using mixer grinder and enzymatically treated with 1% pectolytic enzyme at 50°C for 20 minutes. Then it was filtered and pH was adjusted to 3.0 by adding Citric acid and Ascorbic acid to control browning and improve flavor. Further it was deaerated, pasteurized, flash cooled and stored [7].

Preparation of Aloe Vera incorporated drinking yoghurt

Fresh cow milk was standardized up to 2.5% fat level. Then milk was pasteurized at 80°C/30 min. While heating, sugar (10%) and gelatin (0.25%) was added. Then the milk was cooled and starter culture was inoculated at 45°C (3g for 100kg) and incubated for about 4 hrs. until pH drops to 4.6. Coagulum was then immediately broken with the help of high speed home scale electric blender while adding of Aloe Vera juice with different levels (Table 1). Finally, yoghurt drink was refrigerated at 4°C.

Table 1: Different Formula used for the development of Aloe Vera incorporated yoghurt drink

Treatment	Yoghurt (W/V)	Aloe Vera Juice
T1	90%	10%
T2	85%	15%
T3	80%	20%
T4	75%	25%
T5	100%	0%

Physicochemical analysis of drinking yoghurt made with Aloe Vera juice

The pH and acidity of the samples was measured at 4 days intervals during storage period up to 20 days using a pH meter (sensION+PH1, Hach, USA). Acidity was estimated by titration with 0.1% NaOH. Total solids, total soluble solids (brix value), crude protein, ash and fat of the developed yoghurt were estimated according to AOAC[8] procedure.

Microbiological analysis of developed drinking yoghurt

The developed drinking yoghurt samples were analyzed for undesirable (Total coliform, yeast and mold) microorganisms at 5 days intervals using pour plate methods. Required amount of culture media (PDA for yeast and mold, Macconkey agar for Total coliform) was taken and sterilized by autoclaving at 121°C for 15 min. One milliliter of drinking yoghurt was added in to 9 mL peptone water and mixed to make up 10⁻¹ dilution. One milliliter from 1st dilution was transferred in to the 2nd dilution tube with 9 mL peptone water and prepared 10⁻² dilution. This process was repeated up to 10⁻⁵ dilution. One milliliter samples from each and every dilution were transferred in to the petri dishes and 15mL of prepared media was poured into each and allowed to solidify at ambient temperature. Then dishes were inverted and transferred in to the incubator (For coliform count request 37 ± 1°C for 24 hours and yeast and mold request normal room temperature for 4-5 days). Colony counter was used for the enumeration of microorganisms.

Sensory evaluation of developed drinking yoghurt

Sensory evaluation was done with 33 untrained panelists along with ballot paper prepared as nine-point hedonic scale. Sensory qualities such as appearance, taste, odor, color and overall acceptance were evaluated. The samples were given three digit random numbers and placed in plastic cartons. These were served in random order to panelists.

Data Analysis

Treatment was arranged according to Complete Randomized Design (CRD) with four replicates. Parametric data was analyzed by one way Analysis of Variance (ANOVA) in Statistical Analysis Software (SAS) ver. 9.0 [9]. Mean separation was done by using Tukey's Studentized Range Test (TSRT). Sensory evaluation data were analyzed by Friedman non-parametric test in MINITAB software package with 95% confidence interval. Microbial data were presented by comparing with SLS 824: part 2: 1989 yoghurt standards. Statistical significance was declared at $P < 0.05$.

3. RESULTS AND DISCUSSION

Nutritional properties of Developed Aloe Vera incorporated drinking yoghurt

Protein, ash, dry matter and moisture contents of drinking yoghurts prepared with different percentages of Aloe Vera juice were not significantly different from control (Table 2, $p > 0.05$). The drinking yoghurt incorporated with 20% Aloe Vera juice showed numerically highest dry matter content but highest ash content was observed in 10% Aloe Vera incorporated drinking yoghurt. Numerically, highest protein content was observed in 15% Aloe Vera juice incorporated drinking yoghurt. Jenness [10] reported that difference in animal breed, weather, type of feed, age of animal, stage of lactation are the major reasons of changing composition of raw milk and those changes may be affected on the nutritional composition of prepared drinking yoghurt. But fat percentage was significantly higher ($p < 0.05$) in control treatment and 10% Aloe Vera juice incorporated drinking yoghurt than other treatments. The observed fat % in the study was significantly different ($p < 0.05$) among treatments. The highest fat percentage was reported in the control and 10% Aloe Vera juice incorporated drinking yoghurt. Fat percentage was gradually decreased with the increasing level of Aloe Vera incorporation in drinking yoghurt. The lowest fat percentage was observed in drinking yoghurt incorporated with 25% Aloe Vera. Reduction of yoghurt percentages while increasing Aloe Vera juice percentages in drinking yoghurt can be the reason for the changes in fat percentages.

Table 2: Fat, protein, ash and dry matter (DM) contents in developed Aloe Vera incorporated drinking yoghurt

Treatments	Fat%	Protein%	Ash%	Dry matter%
T1 (10%)	3.45±0.14 ^{ac}	2.70±0.25	2.157±0.39	26.83±2.10
T2 (15%)	2.91±0.24 ^{ade}	3.01±0.10	1.517±0.19	25.79±2.16
T3 (20%)	2.59±0.04 ^{bd}	2.95±0.37	1.455±0.31	29.29±2.02
T4 (25%)	2.76±0.22 ^{be}	2.7±0.04	1.205±0.45	24.75±1.76
T5 (0%)	3.62±0.02 ^c	2.78±0.14	0.787±0.14	28.05±3.63

Data are presented as means±SE

a,b,c,d,e means within the same column with different superscripts are significantly different ($p<0.05$).

Physicochemical properties of developed Aloe Vera incorporated drinking Yoghurts

Brix values of treatment drinking yoghurts were significantly different ($p<0.05$). Brix value decreased while increasing Aloe Vera juice percentage in drinking yoghurts. The brix value of milk drinks ranged from 13.26 to 26.30 (Figure 1). Brix values of treatment drinking yoghurts in this study agreed with above finding [11].

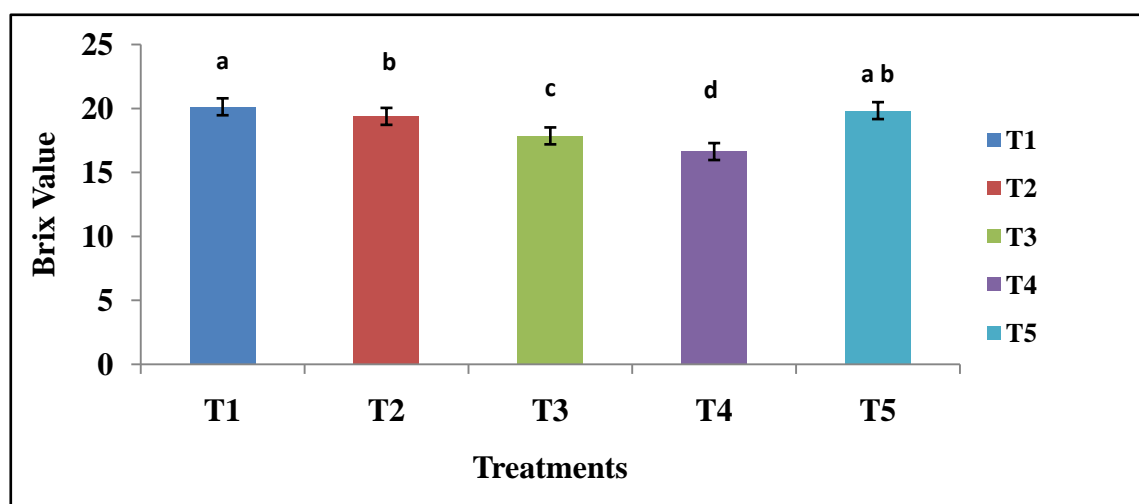


Figure 1: Brix values of developed Aloe Vera incorporated drinking yoghurts

pH value in each developed Aloe Vera incorporated drinking yoghurts were significantly different ($p<0.05$) among treatments and also with the storage (Figure 2). Initial pH value of Aloe Vera juice incorporated drinking yoghurts were significantly lower ($p<0.05$) than control drinking yoghurt. During the storage period, pH of yoghurt gradually decreased in all treatments. According to the standard of SLSI normally pH value of drinking yoghurt ranged between 4.6-4.2. In this study pH value of developed drinking yoghurts were varied between SLSI standard up to 16th day of storage. The final pH values of developed treatment drinking yoghurts ranged between 4.0-4.1 after 20 days of storage time may be due to post acidification. Lactic strains have the

ability to ferment lactose into lactic acid. With the time accumulation of lactic acid which was produced by lactic acid bacteria and other living organisms can be the reason for reduction of pH value [12] (Figure 2).

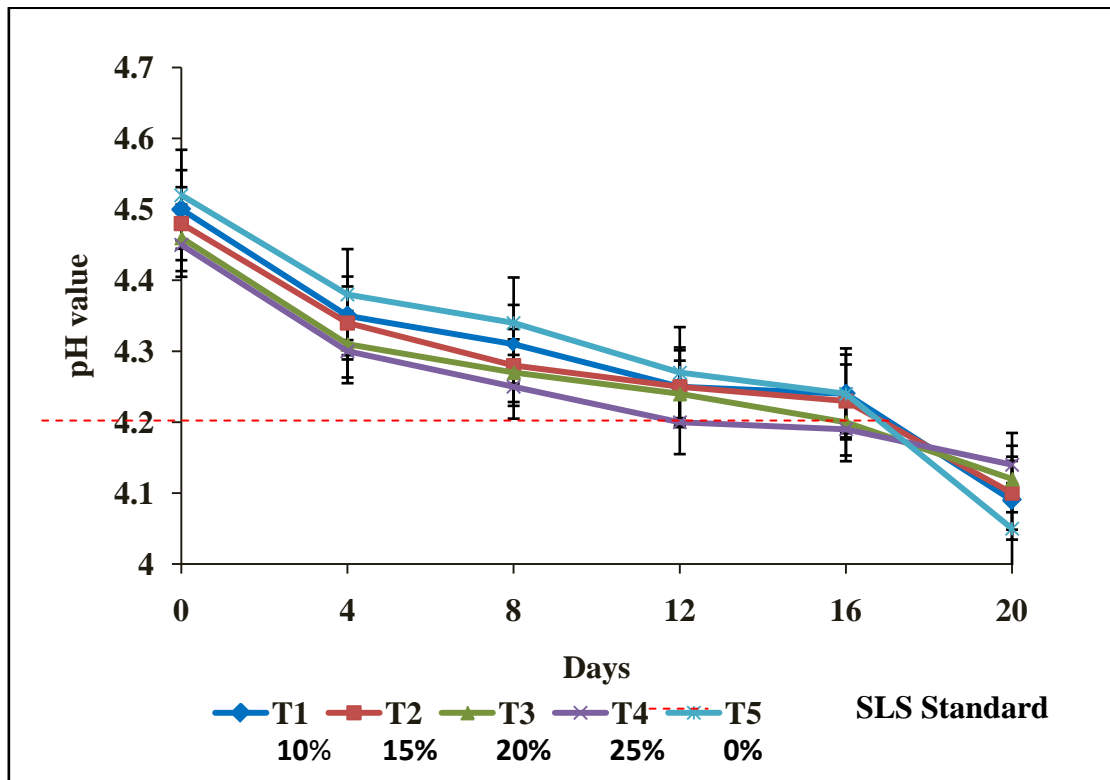


Figure 2: pH variation in developed Aloe Vera incorporated drinking yoghurts with storage period

Titrateable acidity of developed Aloe Vera incorporated drinking yoghurts were significantly different ($p < 0.05$) among the treatments and it was increased during the storage period (Figure 3). Initial titrateable acidity value of Aloe Vera juice incorporated drinking yoghurts were significantly higher ($p < 0.05$) than control. Highest initial titrateable acidity value was reported in 25% of Aloe Vera juice incorporated drinking yoghurt. Higher acidity values observed for developed Aloe Vera incorporated drinking yoghurts compared to control drinking yoghurt is in agreement with findings of [13] on Aloe Vera gel enriched dahi and Shin, Lee [14] on Aloe Vera gel enriched yoghurt. Authors [15] reported that the titrateable acidity was increased with the storage period. It happened due to the microbial activity during storage. Titrateable acidity of developed Aloe Vera incorporated drinking yoghurts were increased with the Aloe Vera juice percentage in drinking yoghurt and during the storage period. This is in agreement with other studies [13, 16] indicated that Aloe gel polysaccharides have a stimulative effect on the metabolic activity of dahi microorganisms.

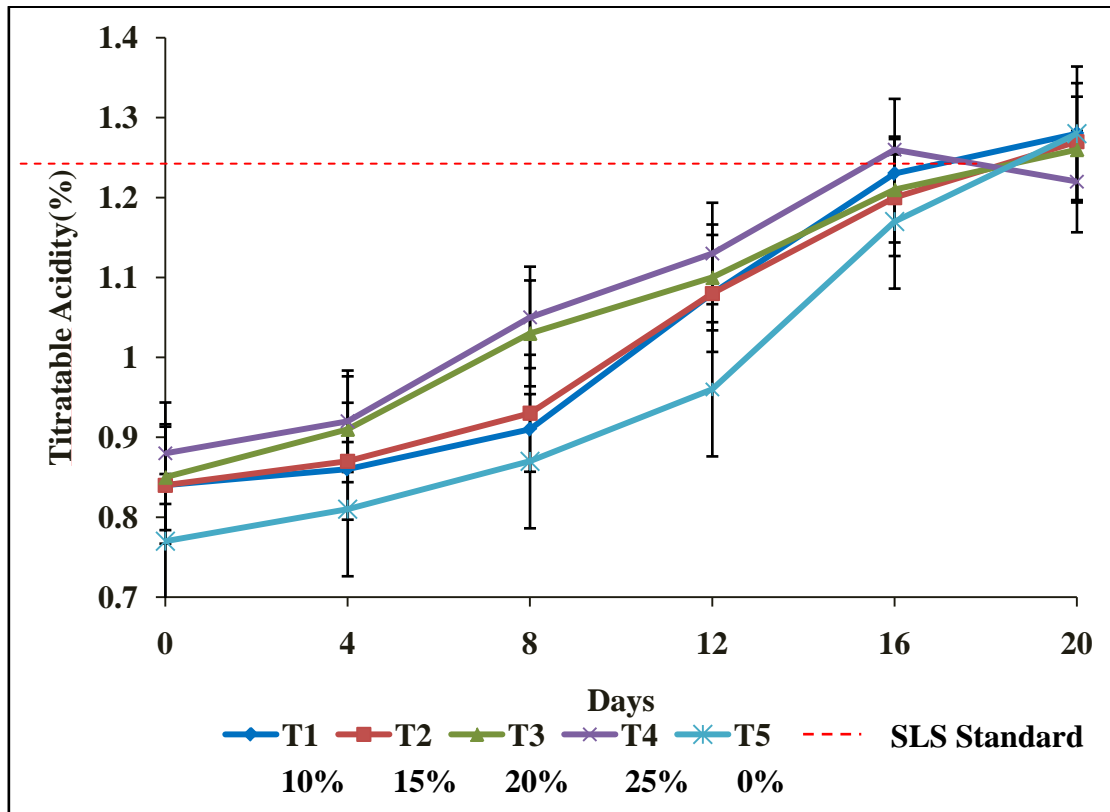


Figure 3: Titratable acidity variation in developed Aloe Vera incorporated drinking yoghurts with storage period

Microbiological analysis of developed Aloe Vera incorporated drinking yoghurts

Yeast and mold counts were increased with the storage (Table 3). According to the Sri Lanka Standards [17], yeast and mold counts in drinking yoghurts were in acceptable range within 15 days of storage (< 10³cfu/1mL). During first 10 days, the yeast and molds were not developed in the treatment drinking yoghurts. Yeast and molds were shown in samples up on 10th day of storage. The highest yeast and mold counts were observed in control drinking yoghurt up to 15th day of storage. From 15th day onwards, yeast and mold counts were exceeded the acceptable limit in all treatment levels and highest count was reported in drinking yoghurt prepared with incorporating 20% of Aloe Vera juice.

In the yoghurt drinks, lactic acid produced by the starter culture bacteria prevents the growth undesirable microorganisms. Several metabolic products produced by these bacteria have antimicrobial effects including organic acids, fatty acids, hydrogen peroxide and relatively small diacetyl. Soomroconcluded that the combination of specific *Lactobacillus* and *Propionibacterium* strains used in normal starter cultures to inhibit the growth of yeasts, molds, *Bacillus spp* and *Clostridium*. This is resulted due to bio preservation activity of *Lactobacillus acidophilus* in drinking yoghurts. Increasing the yeast and mold count of drinking yoghurt samples due to increment of acidity (Table 3) and reduction of oxygen during fermentation process may offer proper conditions for growth of yeasts and molds. Total coliform counts were zero for all treatments during storage period of 20 days (table 4), probably due to good hygienic practices adapted during manufacturing process.

Table 3: Yeast and mold count during storage period

Treatment	0 day	5 day	10 day	15 day	20 day
T1 (10%)	Nil	Nil	3.2×10 ¹ cfu/1ml	8.70×10 ² cfu/1ml	1.56×10 ³ cfu/1ml

T2 (15%)	Nil	Nil	1×10^1 cfu/1ml	9.20×10^2 cfu/1ml	1.27×10^3 cfu/1ml
T3 (20%)	Nil	Nil	5×10^1 cfu/1ml	9.65×10^2 cfu/1ml	1.84×10^3 cfu/1ml
T4 (25%)	Nil	Nil	7×10^1 cfu/1ml	1.02×10^3 cfu/1ml	1.73×10^3 cfu/1ml
T5 (0%)	Nil	Nil	8×10^1 cfu/1ml	7.90×10^2 cfu/1ml	1.24×10^3 cfu/1ml

Table 4: Total Coliform Count during storage period

Treatment	0 day	5 day	10 day	15 day	20 day
T1 (10%)	Nil	Nil	Nil	Nil	Nil
T2 (15%)	Nil	Nil	Nil	Nil	Nil
T3 (20%)	Nil	Nil	Nil	Nil	Nil
T4 (25%)	Nil	Nil	Nil	Nil	Nil
T5 (0%)	Nil	Nil	Nil	Nil	Nil

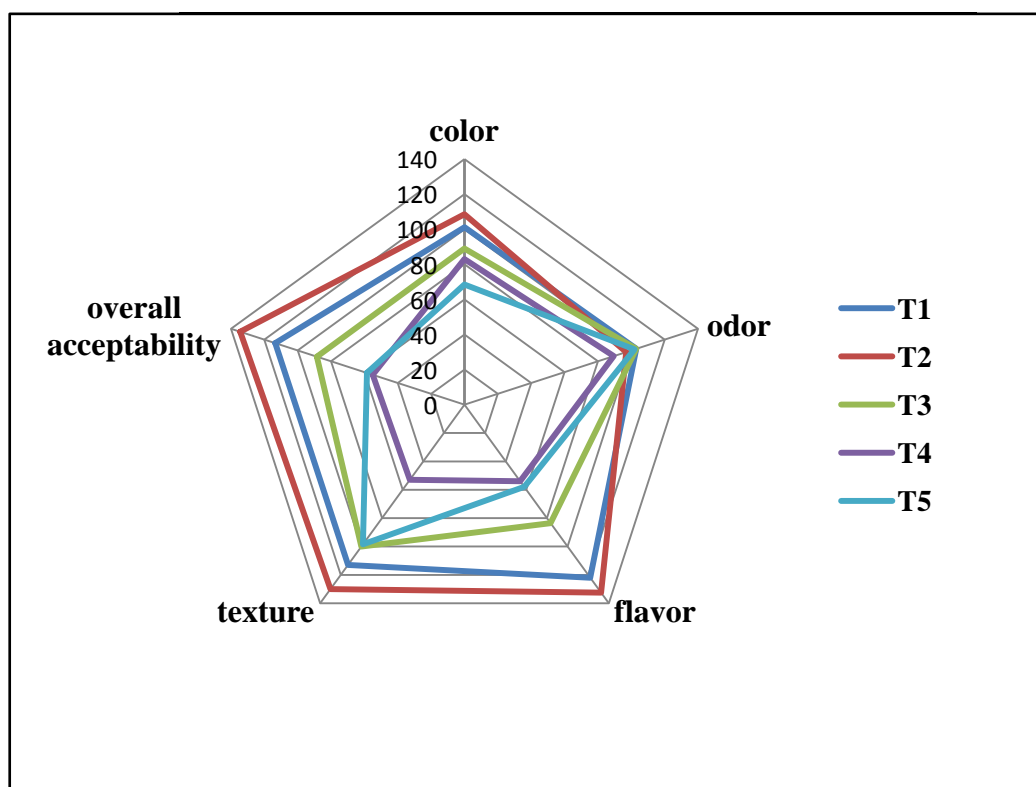


Figure 4: Sensory Evaluation analysis of Aloe Vera incorporated drinkingyoghurt

Sensory qualities of developed Aloe Vera incorporated drinking yoghurt

The results of sensory tests for the color, taste, odor, texture and overall acceptability of developed drinking yoghurt are shown in Figure 4. The panelists were able to differentiate a significant difference ($P < 0.05$) for color, taste, texture and overall acceptability among different types of yoghurt. A higher average rank values for color (118), taste(144), texture (130) and overall acceptability (143.5) were observed in drinking yoghurt developed by incorporating 15% Aloe Vera juice. However, odor did not differ significantly among developed drinking yoghurt ($P = 0.67$). Therefore, the level of incorporation of Aloe Vera juice had an influence on the sensory properties of developed yoghurt samples. Drinking yoghurt developed from adding 15% Aloe Vera juice showed the best sensory qualities with the highest score for overall acceptability.

Shelf life of developed Aloe Vera incorporated drinking yoghurt

Shelf life of the developed drinking yoghurt was 15 days. According to the yoghurt standard SLS 824: part 2: 1989 [18], pH value should not be more than 4.2 in yoghurt. pH value was not in acceptable range after 16 days of production and also titratable acidity was exceeded acceptable limit. According to the pH variation, product could be stored up to 16 days.

IV. CONCLUSIONS

Aloe Vera incorporated drinking yoghurt can be developed by incorporating 15% of Aloe Vera juice to 85% of yoghurt with acceptable sensory and nutritional qualities. Developed 15% of Aloe Vera incorporated drinking yoghurt can be stored 15 days in refrigerator at 4°C with acceptable limit of yeast and mold count and without any quality deterioration.

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