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Efficacy of flavored *Aloe vera* cubes in probiotic yogurt

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Abstract
Efficacy of flavored *Aloe vera* cubes as a replacement of fruit jelly cubes in Probiotic Yogurt (APY) was investigated. *Aloe vera* is widely used as functional raw material in food especially in dairy products. *Aloe vera* probiotic yogurt was fermented with yogurt cultures contained *Streptococcus thermophilus* (NCDC074), *Lactobacillus Bulgaricus* (NCIM2057) starter cultures and *Lactobacillus Acidophilus* (NCIM2903) probiotic were incubated at 37 °C for 24 hours. The *Aloe vera* Probiotic Yogurt was produced using standardized milk incorporating *Aloe vera* cubes soaked in pineapple juice and litchi juice incubated at 42 °C for 4.30 hrs. Quantitative investigation of physico- chemical attributes like protein 2.64 to 2.6, fat 4.78 to 4.7, moisture 68.4 to 64.5, ash 1 to 0.83, Carbohydrate 19.81 to 19.45, Total Solids 28.21 to 27.94, Total soluble solids° Brix (refractometer) 35 to 31, pH 4.24 to 4.04,Titratable acidity 1.16 to 0.39,Probiotic count 0^{th} day $9.1x10^7$ to $7.4 x10^7$, Probiotic count 7^{th} day $8.8 x10^7$ to $6.32 x10^7$, probiotic count 14^{th} day $8.55 x10^7$ to $6.15x10^7$, Yeast and mold count 45 to15 maximum and minimum value respectively of *Aloe vera* cube probiotic yogurt. The study shows that *Aloe vera* probiotic Yogurt can be used as a good carrier of probiotic bacteria with bacterial counts more than the suggested level.

Keywords: probiotic, streptococcus thermophilus, lactobacillus bulgaricus, lactobacillus acidophilus, *Aloe vera* barbadensis

1. Introduction
Yogurt is one of the most consumed fermented dairy products all around the world because yogurt contains more nutrition as compared to milk. Yogurt means a coagulated product from pasteurized milk by a process of lactic acid fermentation through Streptococcus Thermophilus and lactobacillus bulgaricus upon addition of some probiotic cultures like lactobacillus acidophilus and Bifidobacterium Bifidum which gives it a probiotic value. Yogurt has a very good smooth texture and characteristic acid flavor. Quality of the final product always depends upon the quality of the raw product.

Probiotic foods enhanced the health after its consumption as because it contains microorganisms which are viable and effective on human system. Fermented milks have been used for the carriers of probiotic strains. Several factors must be considered when using probiotic bacteria in yogurt such it should be high in count at the time of consumption, thus a probiotic dairy product should contain >106 cfu/mL and should be consumed on regular basis more than 100g per day or at least 9 log cfu per day (codex, 2003). One of the most popular dairy product for the delivery of lactobacillus acidophilus and Bifidobacterium Bifidum is yogurt. An increasing commercial interest in the addition of probiotic bacteria (Lactobacillus acidophilus, bifidobacteria, Lactobacillus casei) to the different dairy products have been a recent trend (Guldas & Irkin, 2010).

Dairy products incorporating *Aloe vera* are also gaining popularity. *Aloe vera* powder and *Aloe vera* juice were utilized for the preparation of yogurt. The yogurt prepared with *Aloe vera* juice was reported to have a better quality retention compared to that prepared using dried skim milk. The phenolic compounds are minimized in *Aloe vera* juice processing but these ingredients will present in the right proportions in the *Aloe vera* cubes that will be incorporated into probiotic yoghurt. Anthraquinones, which is a phenolic compound and are used for medicinal purposes. Phenolic compounds are the second major substances found in *Aloe vera* it helps better functioning of the digestive system as they have analgesic, antibacterial, antifungal and antiviral properties. The Anthraquinones and chromones are responsible for the anti-cancer activity, anti-inflammatory (swelling).

2. Material and Methods

2.1 Material

Freeze dried culture Streptococcus Thermophilus (NCDC074), Lactobacillus bulgaricus (NCIM2057) starter culture and Lactobacillus acidophilus (NCIM2903) probiotic were procured from NDRI-Karnal and National Collection of industrial microorganisms-Pune, Amul brand milk (6% fat) and sugar crystals were purchased from local market of Allahabad. *Aloe vera* plant was purchased from local nursery of Allahabad.

2.2 Methods

2.2.1 Preparation of *Aloe vera* cube probiotic yogurt

Standardized milk was heated to 100 °C for 10 min followed by immediate cooling to 40 °C and then added with 2.5mm cube size of processed *Aloe vera* cubes at 10g/100g level, 5mm cube size of processed *Aloe vera* cubes at 15g/100g level and 7.5 mm cube size of processed *Aloe vera* Cubes at 22g/100g level. This milk- *Aloe vera* cube mixture was inoculated with culture combination comprising of NCDC074, NCIM2057 and NCIM2903(1:1:1) at 2g/100g level, filled in pre-sterilized glass beakers followed by incubation at 42 °C for 4.30 hours to obtain a firm yogurt. The yogurt samples were refrigerated at 5±1 °C until subjected for various analysis Starter cultures (NCDC074), (NCIM2057) and probiotic strain (NCIM2903) were maintained in sterilized skim milk tubes. The propagation of the cultures was done at weekly intervals.

2.2.2 Physico chemical analysis of *Aloe vera* probiotic yogurt

2.2.2.1 Determination of Fat Content

2g of the sample was weighed into a flat bottom flask of known weight with the extractor mounted on it. The thimble was held half way into the extractor and the weighed sample was carefully transferred into the thimble and the thimble was plugged with cotton wool.

Temperature for extraction was at 40 –60 °C for 8hours. The solvent was removed by evaporation and then, the remaining part of the flask was dried in the oven at 80 °C for 30minutes and was finally cooled in a desiccator. The flask was reweighed and the percentage fat was calculated as;

$$\% \text{ Fat} = \frac{\text{Weight of extracted fat}}{\text{Weight of sample used}} \times 100$$

2.2.2.2 Determination of ash content

2g each of the samples was measured into a crucible of known weight, the sample was burnt to ash in a muffle furnace for 3hours at 550 °C. It was then cooled in a desiccators and the weight of the ash was finally determined. The % Ash content was calculated as;

$$\frac{W1\text{-}W2}{W1} \text{ X } 100$$

Where;
W1 = Initial weight of the sample
W2= Weight of the dried sample

2.2.2.3 Determination of protein content

2g of the sample was introduced into the digestion flask. 10g of copper sulphate and sodium sulphate in the ratio of 5:1and 25ml of concentrated sulphuric acid was added to the digestion flask. The flask was placed into digestion block in fume cupboard and heated until frothing ceased given a clear and light blue coloration. The mixture was allowed to cool and was diluted with distilled water until it reached 25ml of volumetric flask.10ml of the mixture was poured into the distillation apparatus and 10ml of 40% sodium hydroxide was added. The released ammonia by boric acid was allow to continue until 10ml of boric acid is treated with 0.02m of hydrochloric acid until the green color change to purple. The nitrogen in the sample was then determined. The percentage nitrogen of the sample was calculated as;

% Nitrogen = molar mass of N2 × acid conc. (0.02m) × volume made × titre value × 100

$$\% \text{ Crude protein} = \% \text{ Nitrogen} \times 6.25 \quad \frac{10 \times 1000 \times \text{sample weight (2g)}}{}$$

2.2.2.4 Determination of moisture in yogurt

2g of the sample was dried in a hot air oven for 24h at 100°C. The lost in weight was determined and recorded as the moisture content and expressed as;

$$\% \text{ moisture} = \frac{W1 - W2*100}{W1}$$

Where;
W1 = Initial weight of the sample
W2= Weight of the dried sample

2.2.2.5 Determination of Total soluble Solids percent in yogurt

The total soluble solids (TSS) was determined by using digital bench refractometer. Before use the instrument was cleaned and adjust to zero at 20 °C using distilled water. An appropriate quantity of sample of each product prepared was placed on the prism plate of the refractometer with the help of a glass rod and folding back the cover, for each of the sample the refractometer was calibrated using distilled water. The reading appeared on the screen was directly recorded as total soluble solids as brix.

2.2.2.6 Determination of percentage of Total Titratable Acidity in yoghurt

The sample was dissolved in distilled water and mixed thoroughly. 1ml of phenolphthalein indicator was introduced into 10ml of the mixed solution. It was titrated against standard sodium hydroxide solution until pink color persisted for about 10-15 seconds for complete neutralization.

2.2.2.7 Determination of pH

The pH of yogurt was measured using using a digital ph meter at 20°C using a combined glass electrode fitted in association with a temperature probe. Before use the pH meter was calibrated using standard buffers of pH 4.0 and 9.0 at 20 °C.

2.2.2.8 Determination of Total Solids

3g of the sample was weighed into a dry Petri dish of a known weight. The total portion was pre-dried for 25minutes on steam bath and then dried for 3hours at 100 °C in forced draft air oven. The Total Solid sample is the weight of the dried sample residue and was
Calculated as:

$$\% \text{ Total Solid} = \frac{W2-W1*100}{W3-W1}$$

Where:
W1 = Weight of the dish
W2= Weight of dish and sample test portion
W3 = Weight of dish and dry sample

2.2.2.9 Determination of Carbohydrate
Carbohydrate content was determined by difference
CHO= 100- % (ash+protein+moisture+fat)

2.2.3 Microbiological Analysis
2.2.3.1 Probiotic count
After homogeneous mixing of the *Aloe vera* Probiotic Yogurt1ml of the sample was transferred into a test tube containing 9 ml of distilled water and mixed well. Further 1/7th serial dilutions were prepared in distilled water. Appropriate sample diluents were pour plated using MRS agar for probiotic count. The Lactobacillus colonies were counted after incubating the plates for 24 hours at 37°C.The results were expressed as log cfu/ml.

2.2.3.2 Yeast and mold count
For Yeast and mold count 1ml of the sample was transferred to a test tube containing 9 ml of distilled water and mixed well.1ml of the sample was transferred into a Petri dish and then a media prepared using potato dextrose agar was poured into the sample. Petri dish was incubated for 24 hours at 26°C.The colonies were counted then after.

2.2.3.3 Coliform count
For Coliform count 1ml of the sample was transferred to a test tube containing 9 ml of distilled water and mixed well. 1 ml of the sample was transferred into a Petri dish and then a media prepared using Mconkey Agar (Hi media lab. Bombay) was poured into the sample. Plates were kept at 37°C for 48 hours. The colonies were counted then after.

2.2.4 Sensory evaluation
Sensory evaluation of *Aloe vera* Probiotic Yogurt was carried out using 9-point hedonic scale. The Yogurt samples were drawn from refrigerator and was served for the panelists immediately. Sensory evaluation panel was consist of ten judges having adequate knowledge of sensory evaluation method and product characteristics were chosen from Department of Food Process Engineering and Warner College of Food and Dairy Technology, SHUATs, Allahabad. Sensory attributes were evaluated for appearance (observe for any possible whey separation and layered appearance on its surface), Flavor and Taste (place adequate amount of probiotic Yogurt in mouth and perceive the taste and odors like acidity and off flavors if any), Body and Texture(place adequate amount of probiotic Yogurt in mouth and perceive firmness, ease of dissolving, adhesiveness to the tongue and palate) and overall acceptability (conclusion based on the above attributes).Sensory score card consist of following hedonic ratings. 1-Dislike very much, 2-Dislike Much, 3-Dislike Moderately, 4-Slightly dislike, 5-Neither like nor dislike, 6-Like slightly, 7-Like Moderately, 8-Like much, 9-Like very much.

3. Result and Discussion

Table 3.1 Quality Parameters of physico chemical attributes of *Aloe vera* probiotic Yogurt

No. of Treatments	%Fat	%Protein	%Ash	% carbohydrate	%TS	%Moisture
T1 (PA)	4.73	2.62	0.83	19.81	27.99	65
T2 (PA)	4.7	2.6	0.89	19.65	28	66.4
T3 (PA)	4.77	2.61	1	19.60	28.19	68.4
T4 (L)	4.75	2.59	0.83	19.71	27.98	67.8
T5 (L)	4.78	2.63	0.89	19.56	28.11	67.4
T6 (L)	4.72	2.58	1	19.48	28.11	66.52
T7	4.74	2.56	0.83	19.45	27.94	66.19
T8	4.71	2.6	0.89	19.50	28.01	65.83
T9	4.76	2.64	1	19.52	28.21	64.5

Table 3.1: Quality Parameters of physico chemical attributes of *Aloe vera* probiotic Yogurt

No. of Treatments	Tss (Refractometer) °Brix	pH	% Titratable acidity
T1 (PA)	31	4.07	0.5
T2 (PA)	33	4.24	1.16
T3 (PA)	35	4.07	0.4
T4 (L)	32	4.18	0.46
T5 (L)	34	4.04	0.39
T6 (L)	35	4.07	0.72
T7	33	4.08	0.47
T8	33	4.16	0.42
T9	34	4.14	0.39

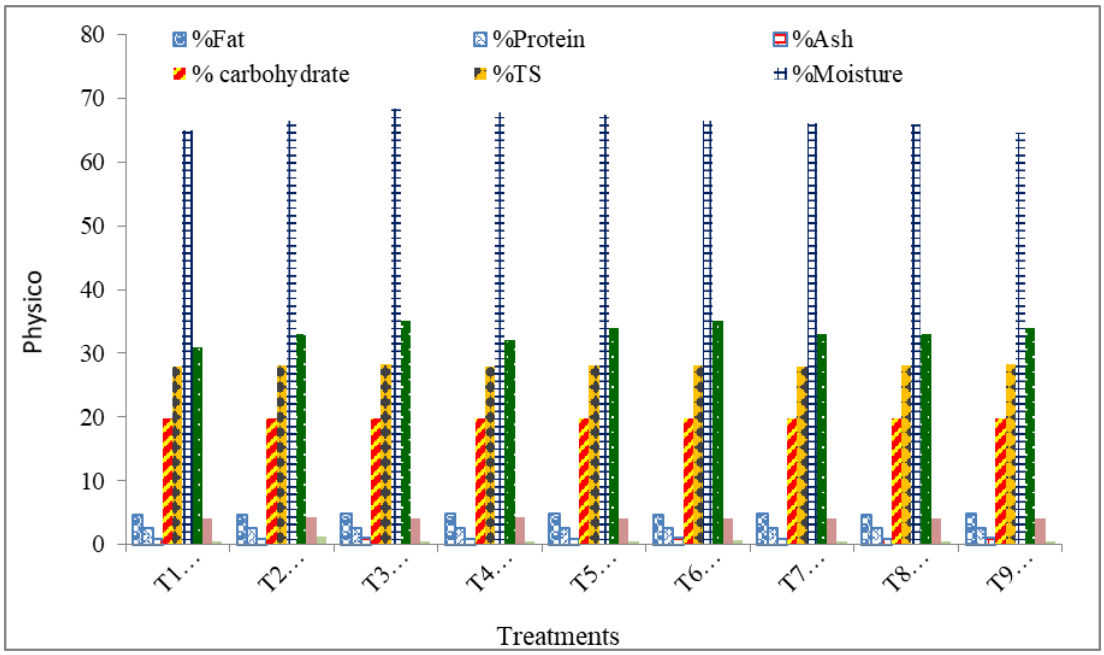

Fig 3.1: Effect of treatments on physico-chemical attributes of *Aloe vera* probiotic yogurt

Table 3.1 shows that effect of treatments on fat, protein, ash, carbohydrate, total solid, moisture, total soluble solids (refracto meter), pH and acidity of control and experimental samples (T1, T2, T3, T4, T5, T6, T7, T8 and T9). The fig 3.1 indicates that fat, protein, ash, carbohydrate, total solid, moisture, total soluble solids (refracto meter), pH and acidity (T1, T2, T3, T4, T5, T6, T7, T8 and T9) was found to decrease with increasing substitution. This is due to *Aloe vera* is rich in moisture content and other chemical attributes are almost negligible. i.e. per 100 gm of fresh *Aloe vera* gel contains moisture-98.93±0.06, protein-0.12±0.01, fat-0.01±0.02, ash-0.16±0.02, carbohydrates-0.66, pH-4.74±0.01, acidity-0.06±0.02 glucose-25.20±0.06, fructose-9.30±0.01. There was slight changes in chemical attributes of the control and experimental samples (T1, T2, T3, T4, T5, T6, T7, T8 and T9).

Table 3.2: Quality parameters of treatments on probiotic count, yeast and mold count and Coliform count of *Aloe vera* probiotic Yogurt.

No. of Treatments	Probiotic count 0 day cfu/ml	Probiotic count 7 day cfu/ml	Probiotic count 14 day cfu/ml	Yeast and mold count	Coliform
T1 (PA)	7.43 x10^7	6.41x10^7	6.25 x10^7	15	Nil
T2 (PA)	8.46 x10^7	8.30 x10^7	7.35 x10^7	20	Nil
T3 (PA)	7.4 x10^7	6.32 x10^7	6.2 x10^7	35	Nil
T4 (L)	7.65 x10^7	7.45 x10^7	7.10 x10^7	25	Nil
T5 (L)	7.70 x10^7	7.30 x10^7	6.15 x10^7	45	Nil
T6 (L)	8.6 x10^7	8.40 x10^7	7.40 x10^7	30	Nil
T7	9.1 x10^7	8.8 x10^7	8.55 x10^7	35	Nil
T8	7.82 x10^7	7.70 x10^7	7.52 x10^7	40	Nil
T9	8.5 x10^7	8.10 x10^7	7.85 x10^7	15	Nil

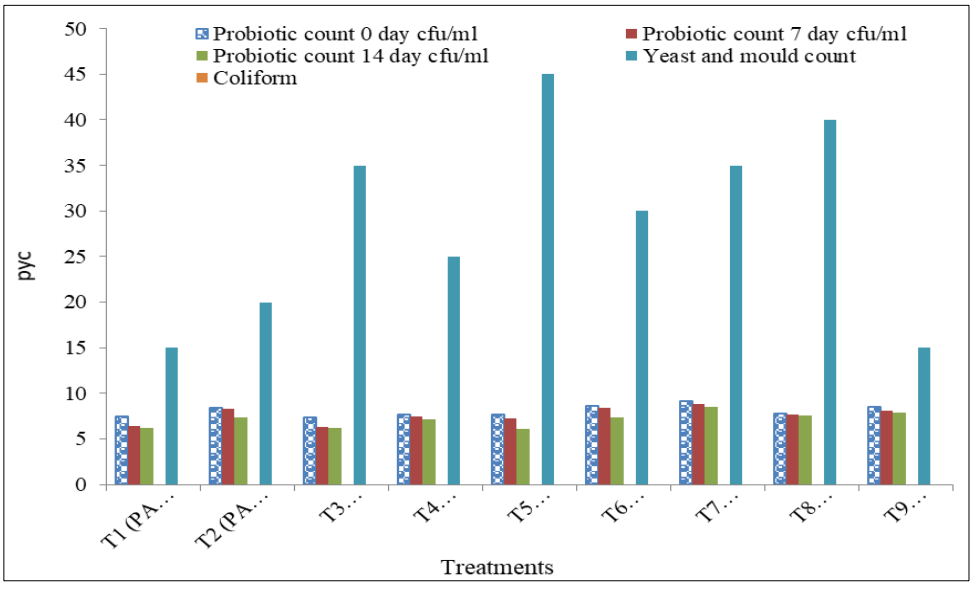

Fig 3.2: Effect of Treatments on Probiotic count, Yeast and Mold Count and Coli form Count of *Aloe vera* Probiotic Yogurt.

Table 3.2 shows that effect of treatments on probiotic count, yeast and mold count and coliform count of control and experimental samples (T1, T2, T3, T4, T5, T6, T7, T8 and T9). The fig 3.2 indicates probiotic count, yeast and mold count (T1, T2, T3, T4, T5, T6, T7, T8 and T9) was found to decrease with increasing substitution. As per Panesar and Shinde (2011) [13] the decrease in probiotic bacteria is due to antagonistic relationship between Yogurt bacteria and probiotic strains and dissolved oxygen content. Oxygen may affect probiotic cultures due to direct toxicity to cells. Some of the probiotic cultures are sensitive to oxygen and generally die in its presence due to the intracellular production of hydrogen peroxide.

Table 3.3: Sensory parameters of *Aloe vera* Probiotic Yogurt

Treatment	Appearance	Flavor and Taste	Body and Texture	Overall Acceptability
T1 (PA)	7.55	7.81	7.61	7.58
T2 (PA)	7.83	7.68	7.41	7.55
T3 (PA)	7.38	7.56	7.08	7.38
T4 (L)	7.75	7.65	7.45	7.71
T5 (L)	8	8.06	7.63	7.91
T6 (L)	7.35	7.8	7.25	7.48
T7	7.78	7.73	7.46	7.5
T8	7.78	8	7.43	7.6
T9	7.55	7.55	7.38	7.46

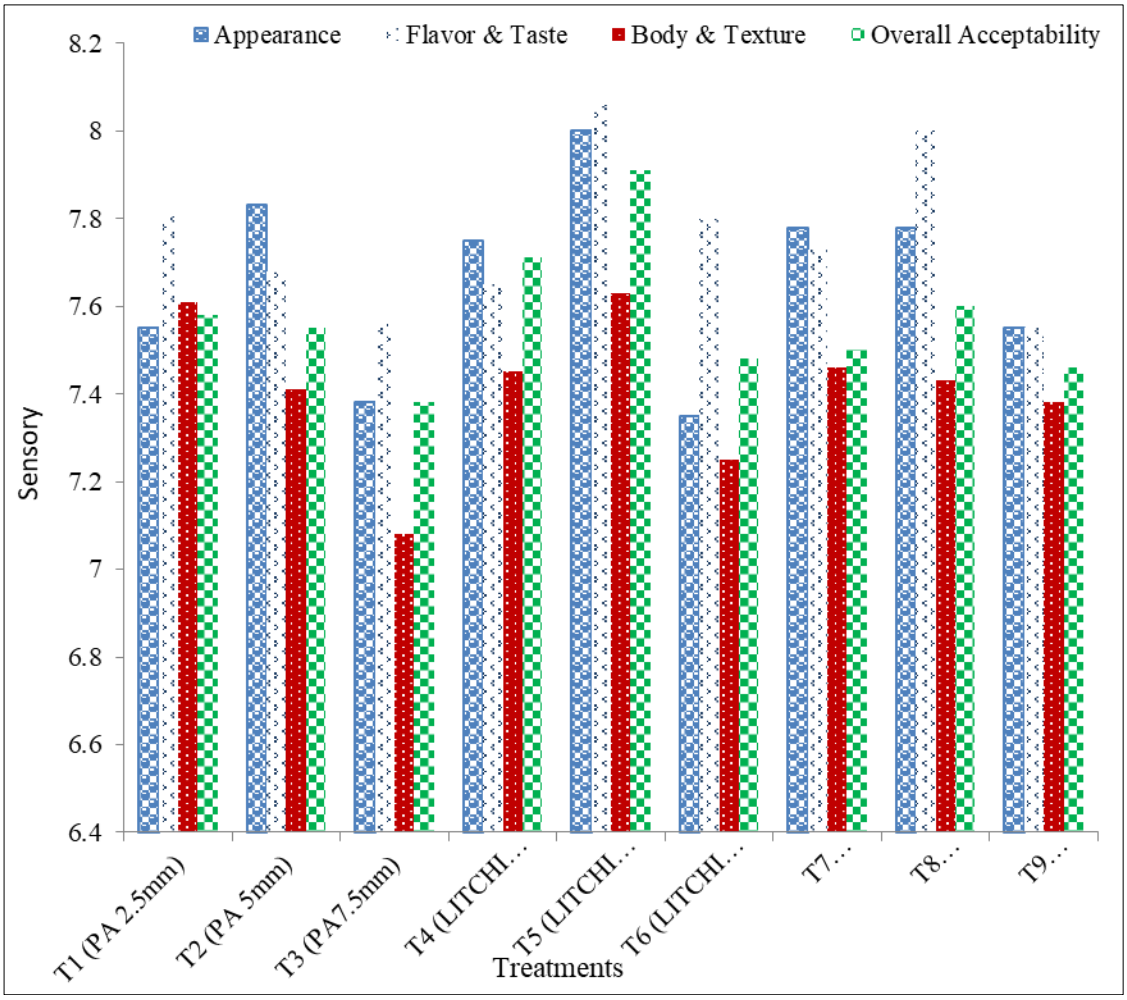

Fig 3.3: Effect of treatments on sensory parameters *Aloe vera* probiotic yogurt

Table 3.4: Comparison of flavored samples with control sample

Treatment	Flavor & Taste			Body & Texture			Overall Acceptability		
	pine apple	litchi	Control	pine apple	litchi	Control	pine apple	litchi	Control
T1	7.65	8.81	7.73	7.45	8.61	7.46	7.71	8.58	7.5
T2	8.06	8.68	8.00	7.63	8.41	7.43	7.91	8.55	7.6
T3	7.8	8.56	7.55	7.25	8.08	7.38	7.48	8.38	7.46
T. cal	-0.43	-6.18		-0.18	6.4		-1.37	13.13	
T. tab	2.78	2.78		2.78	2.78		2.78	2.78	
Results	NS	S		NS	S		NS	S	

Aloe vera probiotic yogurt supplemented by different flavors were sensory evaluated and compared with control samples (10°Brix sugar solution in 100gm). As shown in table 4.5 and which shows there was significant effect of treatments on the appearance, flavor and taste, body and texture and overall acceptability. Fig. 4.17 shows comparison of flavored samples (T1, T2, T3, T4, T5, T6) with control samples (T7, T8, T9) of which T5 Litchi flavored sample shows significant effect.

4. Conclusion

On the basis of above findings it can be concluded that *Aloe vera* gel cube can be successfully incorporated with probiotic

yogurt. It contains all the qualities of good probiotic yogurt. The probiotic count remains above 106 cfu/ml suggested level at 14 day storage period therefore this product has better viability of beneficial microorganisms. Thus overall the sample T5 Litchi flavor with 5mm *Aloe vera* cube size was more acceptable.

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