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SHELF LIFE STUDIES OF "CARROT JUICE ENRICHED PROBIOTIC SHRIKHAND"

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ABSTRACT

Shrikhand is a Maharashtrian dessert popularly known in common household and it is fermented product among the available milk products which are prepared locally. Shrikhand contains acid and sugar, thus exhibiting longer shelf life. The present study aims to study the shelf life of Shrikand by adding carrot juice, to enhance its health benefits. Further, the study used two different wild strains of non-probiotic and probiotic cultures in the room and controlled (refrigeration) temperature. The findings show that, Shrikhand with probiotic features was prepared by heating whole milk, which was used as a control and another one was added with carrot juice with a ratio of 7.5:1 proportion, this has yielded 27.8 per cent and 29.3 per cent of chakka respectively. The findings also revealed that, the overall acceptability was more in Shrikhand with probiotic features enriched with carrot juice. The results show that, the keeping quality of carrot enriched probiotic juice was comparatively revealed to be 4 days duration, than whole milk which lasts only for 3 days, at the prevailing temperature (29+10C). In addition to this, the study also focused on the viability of cultures, it was found to be about 6 log10cfu/g. Thus the study concludes that, Shrikhand enriched probiotic with carrot juice reveals increased keeping quality till 30 days when compared to other milk products which are not enriched.

KEYWORDS: Shrikhand, Carrot Juice, Optimization, Probiotic Cultures Shelf Life of Shrikand

INTRODUCTION

Fermented milk products are the most popular milk products in the world, in view of their high nutritional and therapeutic values. The subtropical climate of India made is essential to devise India's own modes of milk preservation. Consequently, various fermented milk products which suited the needs and tastes of its inhabitants were developed. Over the years, fermented milk became an indispensable supplement to the staple food, consumed every day. Shrikhand is one of the indigenous fermented milk products of India (Aneja *et al.*, 2002).

In modern Indian society, there is an increase in the awareness among consumers, towards the health promoting probiotic foods. Shrikhand being the most popular indigenous fermented product of India, attempts has been made to make it more attractive for consumer acceptance by value addition into Shrikhand (Shrivastava and Goyal, 2007). Carrot is a variable biennial root crop containing nutrients like carbohydrates, proteins and dietary fiber. It is rich in ß–Carotene, vitamin $B_{3,}$ Panthothenic acid and potassium and also has probiotic components that enhance the growth of probiotic cultures (Gopalan *et al.*, 1989).

Patel and Chakraborthy (1987) conducted studies on the storage stability of Shrikhand manufactured from different sources of milk solids. Based on the data with respect to organoleptic evaluation, changes in acidity, pH, soluble nitrogen, total fatty acids and microflora, they reported the average shelf-life of the Shrikhand.

MATERIALS AND METHODS

The Shrikhand samples were packed in sterile polysterine cups (rinsing in boiling water; in 200ppm of hypochlorite solution and exposing to UV rays in laminar air flow for 15 min.) and control and carrot juice enriched Shrikhand were stored at ambient (29 ± 1^0C) and refrigeration temperature (7 ± 1^0C) and were examined for sensory, chemical and microbiological analysis.

Coli form and Yeast & Mold Count of Shrikhand Samples

Control and carrot juice enriched probiotic Shrikhand samples stored at ambient and refrigeration condition were examined at an interval of each day and once in 3 days respectively till the defect was noticed in the sample for coliform, yeast & mold count to know the safety of the product. The pour plate technique was adopted for the enumeration of Coliforms and yeast & molds by adopting the serial dilution technique and using violet red bile agar and malt extract agar (pH 3.5 at the time of pouring) respectively.

Viability of Probiotics and Non-Probiotics in Shrikhand During Storage

Control and carrot juice enriched probiotic Shrikhand samples containing probiotic and non-probiotic cultures separately stored at ambient and refrigeration condition were examined at an interval of each day and 3 days for their viability respectively till the defect was noticed in the sample. The pour plate technique was adopted for the enumeration of lactic cultures present in control and carrot enriched Shrikhand by adopting the serial dilution technique and using M17 agar for *Lactococci* and S. *themophillus*; de Mann Rogosa Sharpe (MRS) agar was used for *L. fermentum* and leuconostoc and M17 with the addition of sodium citrate of 0.2% L. *lactis* ssp. *lactis* bv. *Diacetylactis.*

RESULTS AND DISCUSSIONS

Effect of Storage on the Titratable Acidity and Viability of Probiotic and non Probiotic Cultures in Whole Milk Shrikhand Stored at Ambient Temperature (29 ± 1^0C)

In order to determine the effect of ambient temperature on the viability of control and carrot juice enriched Shrikhand, every day the titratable acidity and viable counts of the individual cultures present in probiotic culture and non-probiotic culture was determined by serial dilution of whole milk Shrikhand made using a PC (probiotic culture) and NPC (Non probiotic culture) and adopting pour plate technique. On the 0th day of storage of ambient temperature the acidity of probiotic Shrikhand was 0.82 and reached 1.12% lactic acid with the viable count of $7.90\log_{10}$/g (*L. lactis* ssp. *lactis* LC1), $7.20\log_{10}$/g(*S.thermophillus* ST1),$7.00\log_{10}$/g (*L.mesenteroides* ssp. *mesenteroides* LEU1), $7.40\log_{10}$/g (*L.fermentum* LB4). Shrikhand made using non- probiotic culture showed TA of 0.72% lactic acid with the viable count of $7.60\log_{10}$/g (*L. lactis* ssp. *lactis* LC3), $7.00\log_{10}$/g (*S.thermophillus* ST2), $6.90\log_{10}$/g (*L.lactis* ssp. *lactis* bv. *diacetylactis* LC5), $7.10\log_{10}$/g (*L. fermentum* LB2). More reduction viable count of both probiotic and non- probiotic cultures occurred in whole milk Shrikhand and was more prominent among non-probiotic cultures rather than probiotic cultures. The reduction in viable count maybe because of the growth of lactic cultures at ambient temperature and that might have caused a reduction in viability. The probiotic cultures used in the preparation of whole milk Shrikhand, showed significant difference in TA as well as viable count compared to non-probiotic cultures with each day of storage at ambient temperature (Table 2). Srinivasa (2008) showed reduction in viability of probiotic cultures (*L. acidophillus* and *B. bifidum*) in enriched Shrikhand stored at room temperature.

Coliform and Yeast & Mold Counts of Shrikhand Stored at Ambient Temperature

In order to determine the safety as well as an indication of spoilage of the control and carrot juice enriched Shrikhand, samples were subjected every day to coliform count and yeast & mold count by serially diluting the samples and using sterile molten Violet Red Bile Agar (VRBA) and Malt Extract agar respectively. Up to 4 days of storage at room temperature, Coliforms were absent in control Shrikhand and carrot juice enriched made of NPC (non-probiotic culture) and showed mold growth on the surface with counts of 20-30 cfu/g on 4^{th} day. Whole milk Shrikhand (control) and carrot enriched Shrikhand made using a PC (Probiotic culture) also did not show the presence of Coliforms but showed the growth of mold on the 5th day of storing between 20-30 cfu/g (Table 1). The growth of Coliforms may be inhibited by one more day in Shrikhand prepared using the probiotic culture that might have produced antimicrobial compound. Rao, (1979) could able to store Shrikhand up to 3 days at ambient temperature. Patel and Chakraborthy, (1988) indicated the average shelf life of Shrikhand stored at room temperature (32±1) was 2 days as mold growth was noticed on 3^{rd} day of storage.

Effect of Storage on the Titratable Acidity and Viability of Probiotic and Non-Probiotic Cultures in Carrot Juice Enriched Shrikhand Stored at Ambient Temperature (29±1°C)

On the 0^{th} day of storage at room temperature, the acidity of carrot juice enriched probiotic Shrikhand was 0.96% lactic acid with the viable count of 8.40$\log_{10}$/g (LC1), 7.50 $\log_{10}$/g (ST1), 7.00 $\log_{10}$/g (LEU1), 8.10 $\log_{10}$/g (LB4) and carrot juice enriched, non-probiotic Shrikhand showed % lactic acid of 0.81 with the viable count of 7.80 $\log_{10}$/g (LC3), 7.20 $\log_{10}$/g (ST2), 7.10 $\log_{10}$/g (LC5), 7.90 $\log_{10}$/g (LB2). At the end of every day of storage, samples were subjected to viable counts of individual cultures of probiotic and non-probiotic combination (Table 3). On the 3^{rd} day, the acidity of carrot juice enriched, non-probiotic Shrikhand was 1.21 with the viable count of 6.40$\log_{10}$/g (LC3), 5.80$\log_{10}$/g (ST2), 5.40$\log_{10}$/g (LC5), 6.20 $\log_{10}$ cfu/ml (LB2). The next day of storage, i.e., 4^{th} day the same sample showed mold growth.

Carrot juice enriched probiotic Shrikhand on 4^{th} day of storage at ambient temperature had 1.40% lactic acid with viable counts of 6.00, 5.30, 5.00 and 5.45$\log_{10}$ cfu/ml for LC3, ST2, LC5 and LB2 respectively, and on the next day of storage of the same sample showed growth of mold on the surface of the product. The probiotic cultures used in the preparation of carrot juice enriched Shrikhand, showed significant difference in TA as well as viable count compared to non-probiotic cultures with each day of storage at ambient temperature.

Coliform and Yeast & Molds Counts of Shrikhand Stored at Refrigeration Temperature (7±1°C)

In order to determine the safety of the control and carrot juice enriched Shrikhand, samples were subjected to coliform count and yeast and mold count by serially diluting the samples and using sterile molten Violet Red Bile Agar (VRBA) and Malt Extract agar (MEA) respectively. On every 3^{rd} day of storage till mold growth was visualized on the samples. Up to 21 days of storage at refrigeration temperature, Coliforms were absent in all the variants of Shrikhand made of PC (Probiotic culture) and NPC (Non probiotic culture). Even on the 30th day of storage the Coliforms were absent in carrot juice enriched probiotic Shrikhand. Mold growth on the surface with a viable count of 1.0 $\log_{10}$/g on 24^{th}day of refrigeration storage was in observed in whole milk Shrikhand made with NPC. Whole milk Shrikhand made using a PC; carrot juice enriched non-probiotic Shrikhand showed the presence of mold on the 27th day of storage. On the 30th day of storage even carrot juice enriched probiotic Shrikhand showed the presence of mold.

Increased shelf life of the carrot juice enriched probiotic Shrikhand may be attributed to the low temperature storage and the presence of antimicrobial compounds compared to non-probiotic enriched as well as whole milk Shrikhand (Table 4).

Effect of Storage on the Titratable Acidity and Viability of Probiotic and Non-Probiotic Cultures in Whole Milk Shrikhand Stored at Refrigeration Temperature (7±1^{0}C)

On the 0th day of refrigeration storage the acidity of probiotic Shrikhand was 0.82 with the viable count of 7.90 (*L. lactis* ssp. *lactis* LC1), 7.20 (*S. thermophillus* ST1), 7.00 (*Leuconostoc mesenteroides* ssp. *mesenteroides* LEU1), 7.40log$_{10}$cfu/g (*L. fermentum* LB4) while nonprobiotic Shrikhand showed 0.72% lactic acid with the viable count of 7.60 (*L. lactis* ssp. *lactis LC3), 7.00 (S. thermophillus* ST2), 6.90 (*L. lactis* ssp. *lactis* bv. *diacetylactis* LC5), 7.10log$_{10}$cfu/g (*L. fermentum* LB2). Increased production of lactic acid in the case of probiotic culture is the main characteristic of them. On the 3rd day of storage of the refrigeration temperature the acidity of probiotic Shrikhand was 0.84 with the viable counts of 7.70, 7.00, 6.90 and 7.20log$_{10}$/g for LC1, ST2, LEU1 and LB4 respectively. The non- probiotic whole milk Shrikhand had 0.74% lactic acid with the viable count of 7.40for LC3, 6.80 for ST2, 6.70for LC5 and 7.00log$_{10}$/g for the LB2.On 21st day, the acidity of non-probiotic Shrikhand was 0.79 with the count of 5.00 log$_{10}$/g (LC3), 5.20 log$_{10}$/g (ST2), 4.60 log$_{10}$/g (LC5), 4.80 log$_{10}$/g (LB2). The sample showed the mold growth on the 24th day of storage at refrigeration temperature. The acidity of probiotic Shrikhand was 0.90 with the viable count of 4.90, 4.80, 4.50 and 4.60 log$_{10}$cfu/g for LC1, ST1, LEU1 and LB4 respectively on the 27th day of refrigeration storage and later on 30th day the sample revealed the signs of deterioration with mold growth on the surface. The average viable counts of probiotic culture in whole milk Shrikhand were around 4.70 at the end of refrigeration storage and reduction in viability is low temperature as well as the action of lactic acid on the cells. The probiotic cultures used in the preparation of whole milk Shrikhand, showed significant difference in TA as well as viable count compared to non-probiotic cultures with each day of storage at refrigeration temperature (Table 5).

Effect of Storage on the Titratable Acidity and Viability of Probiotic and Non-Probiotic Cultures in Carrot Juice Enriched Shrikhand Stored at Refrigeration Temperature (7±1^{0}C)

On the 0th day of refrigeration storage of carrot juice enriched probiotic Shrikhand the acidity was 0.96% lactic acid with the viable count of 8.40 (LC1), 7.50 (ST1), 7.00 (LEU1), 8.10log$_{10}$/g (LB4) while non-probiotic Shrikhand showed 0.81% lactic acid with the viable count of 7.80 (LC3), 7.20 (ST2), 7.10 (LC5), 7.90 log$_{10}$/g (LB2). On the 3rd day the acidity of carrot juice enriched probiotic Shrikhand was 0.99 with the viable counts of 8.20, 7.30, 6.80 and 8.00log$_{10}$/g for LC1, ST2, LEU1 and LB4 respectively. Carrot juice enriched, non-probiotic had 0.82% lactic acid with the viable count of 7.60for LC3, 7.00 for ST2 & LC5 and 7.70 log$_{10}$/g for the LB2.On 24th day, the acidity of carrot juice enriched, non-probiotic Shrikhand was 0.86 with the count of 5.15 log$_{10}$/g (LC3), 4.00 log$_{10}$/g (ST2), 4.55 log$_{10}$/g (LC5), 4.85 log$_{10}$/g (LB2). The sample showed the mold growth on the 27th day of storage at refrigeration temperature. The acidity of carrot juice enriched probiotic Shrikhand was 1.04 with the viable count of 4.48,3.80,3.76and 5.00log$_{10}$/g for LC1, ST1, LEU1 and LB4 respectively on the 30th day of refrigeration storage and later on 33rdday the mold growth on the surface was observed on the same variant of Shrikhand at refrigerated storage (Table 6). The probiotic cultures used in the preparation of carrot juice enriched Shrikhand, showed significant difference in TA as well as viable count compared to non-probiotic cultures with each day of storage at refrigeration temperature.

CONCLUSIONS

The milk products are widely used for health benefits in day to day life, this usefulness is more orderly benefits when the milk products are having more shelf life than other products. The fermented milk products have recently been used on a large scale due to health benefits which are labeled as "probiotic", this signifies the difference between other products with reference to health benefits. Due to these benefits, the study made an attempt to assess the impact of carrot juice with the probiotic nature of milk products especially which are fermented in nature. Thus, the study used carrot juice, this juice is helpful for building Immunity, Vitamin-A and antioxidant in nature, these qualities when enriched with probiotic shrikand features always revealed good shelf life apart from flavored taste than non-enriched milk products.

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APPENDICES

Table 1: Coliform and Yeast & Mold Counts of Shrikhand Stored at Ambient Temperature (29±1^{0}C)

A) Coliform count – Nil throughout the storage period

B) Yeast and molds

Day of Storage	Whole Milk		WM: Carrot Juice	
	Probiotic	Non-Probiotic	Probiotic	Non- Probiotic
Viable Count Log_{10}cfu/g				
0	Nil	Nil	Nil	Nil
1	Nil	Nil	Nil	Nil
2	Nil	Nil	Nil	Nil
3	Nil	Nil	Nil	Nil
4	Nil	30	Nil	20
5	50	Spoiled	30	Spoiled

Table 2: Effect of Storage on Titra Table Acidity and Viability of Probiotic and Non-Probiotic Cultures of Whole Milk Shrikhand Stored at Room Temperature (29±1^{0}C)

Day of Storage	TA(%LA)		Viable Count Expressed as Log_{10} cfu/g							
			LC1	LC3	ST1	ST2	LEU1	LC5	LB4	LB2
	PC	NPC	PC	NPC	PC	NPC	PC	NPC	PC	NPC
0	0.82	0.72	7.90	7.60	7.20	7.00	7.00	6.90	7.40	7.10
1	0.94	0.84	6.90	7.00	7.00	6.50	6.78	6.20	7.10	6.80
2	1.06	0.99	6.40	6.20	6.20	6.00	6.16	5.90	6.64	6.10
3	1.12	1.09	6.00	5.98	5.98	5.70	5.90	5.10	6.00	5.85
4	1.18	Spoiled	5.50	Spoiled	5.40	Spoiled	5.50	Spoiled	5.60	Spoiled
5	Spoiled		Spoiled		Spoiled		Spoiled		Spoiled	

Result of Trend Analysis

Coefficient	0.09	-0.119	-0.57	-1.622	-0.462	-1.48	-0.388	-1.49	-0.47	-1.515
SE	0.0091652	0.1415003	0.0640312	0.6114976	0.049085	0.6007773	0.0313262	0.5325098	0.0311341	0.6187555
R2	0.9698276	0.1907771	0.9635231	0.7010695	0.9672455	0.6691922	0.9808193	0.7229712	0.9870067	0.6664803
T	9.8198051	-0.8409876	-8.9019044	-2.6525042	-9.4122517	-2.4634753	-12.385783	-2.7980707	-15.095979	-2.4484632
T*	2.2621572	2.2621572	2.2621572	2.2621572	2.2621572	2.2621572	2.2621572	2.2621572	2.2621572	2.2621572

Note: If T value is greater than T* value, it indicates significant difference

Table 3: Effect of Storage on Carrot Juice Enriched Shrikhand (7.5:1 – Milk: Juice) Stored at Room Temperature (29±1^{0}C)

Day of Storage	TA(%LA)		Viable Count Expressed as Log_{10} cfu/g							
			LC1	LC3	ST1	ST2	LEU1	LC5	LB4	LB2
	PC	NPC	PC	NPC	PC	NPC	PC	NPC	PC	NPC
0	**0.96**	**0.81**	**8.40**	**7.80**	**7.50**	**7.20**	**7.00**	**7.10**	**8.10**	**7.90**
1	1.08	0.97	7.50	7.00	6.80	6.90	6.20	6.40	7.40	7.00
2	1.20	1.09	6.90	6.10	6.00	6.00	5.83	5.80	7.00	6.10
3	1.32	1.21	6.40	5.40	5.80	5.40	5.40	5.10	6.20	5.40
4	1.40	Spoiled	6.00	Spoiled	5.30	Spoiled	5.00	Spoiled	5.45	Spoiled

All the values are average of 3 trials.

Note:* PC-Probiotic culture; ** NPC- Non-probiotic culture

Result of Trend Analysis

Coefficeint	0.112	-0.138	-0.59	-1.72	-0.54	-1.59	-0.48	-1.55	-0.65	-1.74
SE	0.004619	0.155936	0.055076	0.525611	0.061101	0.556147	0.044692	0.513907	0.037859	0.52
R2	0.994924	0.207018	0.974524	0.781158	0.963012	0.73151	0.974652	0.752003	0.989925	0.788684
T	24.24871	-0.88498	-10.7125	-3.27238	-8.83782	-2.85895	-10.7403	-3.01611	-17.1688	-3.34615
T*	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157

Note: If T value is greater than T* value, it indicates significant difference

Table 4: Coliform and Yeast & Mold Counts of Shrikhand Stored at Refrigeration Temperature (7±1^{0}C)

A. Coliform count was nil for control and carrot enriched shrikhand through out the storage period

B. Yeast and mold count:

Day of Storage	Whole Milk		WM: Carrot Juice	
	Probiotic	Non Probiotic	Probiotic	Non Probiotic
0	Nil	Nil	Nil	Nil
3	Nil	Nil	Nil	Nil
6	Nil	Nil	Nil	Nil
9	Nil	Nil	Nil	Nil
12	Nil	Nil	Nil	Nil
15	Nil	Nil	Nil	Nil
18	Nil	Nil	Nil	Nil

Table 4: Contd.,				
Day of Storage	Whole Milk		WM: Carrot Juice	
	Probiotic	Non Probiotic	Probiotic	Non Probiotic
21	Nil	Nil	Nil	Nil
24	Nil	30	Nil	20
27	30	Spoiled	Nil	Spoiled
30	Spoiled		Nil	
33			10	

All the values are average of 3 trials.

Table 5: Effect of Storage on Whole Milk Shrikh and Stored at Refrigeration Condition (7±1^{0}C)

Day of Storage	TA(%LA)		Viable Count Expressed as log_{10}cfu/g							
			LC1	LC3	ST1	ST2	LEU1	LC5	LB4	LB2
	PC*	NPC**	PC	NPC	PC	NPC	PC	NPC	PC	NPC
0	0.82	0.72	7.90	7.60	7.20	7.00	7.00	6.90	7.40	7.10
3	0.84	0.74	7.70	7.40	7.00	6.80	6.90	6.70	7.20	7.00
6	0.85	0.75	7.50	7.20	6.80	6.60	6.70	6.60	7.00	6.80
9	0.85	0.75	7.30	7.00	6.60	6.40	6.50	6.40	6.80	6.60
12	0.86	0.76	6.80	6.40	6.00	6.00	6.00	5.90	6.00	6.10
15	0.86	0.76	6.60	5.90	5.80	5.90	5.50	5.40	5.80	5.70
18	0.88	0.78	6.00	5.40	5.40	5.60	5.00	5.00	5.10	5.05
21	0.89	0.79	5.10	5.00	5.00	5.20	4.70	4.60	4.90	4.80
24	0.90	Spoiled	4.90	Spoiled	4.80	Spoiled	4.50	Spoiled	4.60	Spoiled
27	0.90	Spoiled	4.70	Spoiled	4.50	Spoiled	4.32	Spoiled	4.26	Spoiled

All the values are average of 3 trials.

Note: * PC-Probiotic culture; ** NPC- Non-probiotic culture

Whole milk shrikhand made using NPC showed spoilage on 24th day of storage, while Pc showed spoilage on 30th day.

Result of Trend Analysis

Coefficient	0.002889	-0.02297	-0.1303	-0.27576	-0.10606	-0.24263	-0.11297	-0.2499	-0.12578	-0.25848
SE	0.000207	0.009416	0.00934	0.054228	0.004035	0.058367	0.006578	0.050513	0.006524	0.052337
R2	0.960724	0.426532	0.960519	0.763724	0.988552	0.683542	0.973589	0.753657	0.978928	0.753028
T	13.98872	-2.4393	-13.951	-5.08515	-26.2829	-4.1569	-17.1726	-4.94723	-19.278	-4.93887
T*	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157

Note: If T value is greater than T* value, it indicates significant difference

Table 6: Effect of Storage on Carrot Juice Enriched Shrikhand Stored at Refrigeration Temperature (7±1^{0}C)

Day of Storage	TA(%LA)		Viable Count Expressed as log_{10} cfu/g							
			LC1	LC3	ST1	ST2	LEU1	LC5	LB4	LB2
	PC	NPC	PC	NPC	PC	NPC	PC	NPC	PC	NPC
0	0.96	0.81	8.40	7.80	7.50	7.20	7.00	7.10	8.10	7.90
3	0.99	0.82	8.20	7.60	7.30	7.00	6.80	7.00	8.00	7.70
6	0.99	0.82	8.02	7.46	7.17	6.85	6.65	6.80	7.80	7.50
9	1.00	0.82	7.40	7.00	6.90	6.45	6.45	6.00	7.60	7.00
12	1.00	0.83	7.00	6.42	6.12	6.00	6.00	5.80	7.00±	6.40
15	1.02	0.83	6.50	6.00	5.90	5.58	5.60	5.60	6.40	6.00
18	1.02	0.85	6.00	5.75	5.10	5.00	5.00	5.00	6.00	5.10
21	1.02	0.86	5.60	5.45	4.90	4.60	4.80	4.75	5.82	4.90
24	1.04	0.86	5.10	5.15	4.60	4.00	4.25	4.55	5.52	4.85
27	1.04	0.86	4.80	Spoiled	4.24	Spoiled	4.00	Spoiled	5.24	Spoiled
30	1.04	Spoiled	4.48	Spoiled	3.80	Spoiled	3.76	Spoiled	5.00	Spoiled

All the values are average of 3 trials. **Note:** * PC-Probiotic culture; ** NPC- Non-probiotic culture

Result of Trend Analysis

Coefficient	0.002455	-3.14286	2.29712	1.691734	0.408551	1.74512	0.416635	1.865904	0.390665	2.127637
SE	0.000243	3.13647	1.693584	0.337966	0.081433	0.287882	0.064442	0.340876	0.080731	0.384519
R2	0.918908	0.100367	0.169721	0.735732	0.736616	0.803265	0.822834	0.769011	0.722369	0.772824
T	10.09874	-1.00204	1.356366	5.005637	5.01703	6.061921	6.46528	5.473845	4.83913	5.533246
T*	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157	2.262157

Note: If T value is greater than T* value, it indicates significant difference