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Effect of Levels of Nisin on Quality of Custard Apple (*Annona squamosa* L.) Enriched *Shrikhand*

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A B S T R A C T

The chemical and microbiological changes brought about by microorganisms and their enzymes during storage have influence on the keeping quality and acceptability of fermented products. The shelf life is therefore determined by microbiological and physico-chemical quality. The present investigation was therefore carried out to examine the effect of levels of nisin on the physico-chemical and microbial quality of custard apple enriched *shrikhand* prepared by using stevia during storage period. There was progressive increase in acidity and decrease in moisture, fat, protein, reducing sugar, pH, ash and viscosity at different rates in all the samples of custard apple enriched *shrikhand* preserved at 6 ± 1°C. The standard plate count, yeast and mould count and lactic acid bacteria count in *shrikhand* was increased as storage period progressed and coliform count was absent.The shelf life of custard apple enriched *shrikhand* prepared by using stevia was preserved with nisin preservative at different levels viz. 20 IU, 25 IU and 30 IU and packed in pre-sterilized plastic cup was found 15, 21 and 24 days at 6 ± 1°C, respectively, as against 12 days at 6 ± 1°C without preservative *shrikhand*.

Introduction

Shrikhand is a well-known Indian delicacy made from milk fermentation. Shelf life of *shrikhand* is claimed to be higher than milk and *dahi* due to increased acidity, reduced water content (Garg *et al.*, 1983) and addition of sugar (Patel and Chakraborty, 1988). Different antimicrobial substances elaborated by lactic acid bacteria such as hydrogen peroxide, diacetyl, bacteriocins especially nisin (Daeshel, 1989) could be used to enhance the shelf life of *shrikhand*. Nisin is a food preservative produced by *Lactococcus lactis* subsp. *lactis* widely being used for preservation of wide range of dairy products. Higher the temperature of storage less will be the keeping quality, lower the temperature of storage

more will be shelf life of the product. Even at low temperature also the contamination do grow but their rate of multiplication is slow. The microorganisms bring about deterioration in the product leading to unacceptability of the product. Several decomposition and formation processes of chemical and biochemical nature are initiated from the onset of the storage which makes the product less acceptable.

Moisture loss whether originating from exchanges with the atmosphere or with another component of the food, always cause deterioration in the overall quality of the food through softening, toughening, breakdown and swelling or shrinkage. These changes are much of significance because they affect not only various constituents but also the chemical acceptability of the product.

Materials and Methods

Materials/ Equipment's

The fresh, clean whole buffalo milk was procured from local market. Good quality custard apple pulp was procured from local market in a single lot. Good quality readymade market stevia leaf extract manufactured by Anubhav Biotech Ltd. permitted by FSSAI was procured from ayurvedic medical at ahemednagar district in a single lot. The freeze-dried culture of LF-40 was procured from National Collection of Dairy culture (NCDC) unit, N.D.R.I., Karnal (Haryana) and used @ 1 per cent of milk for preparation of *dahi* for every trial. Nisin manufactured by Freda Company was used as a preservative in the *shrikhand*. Clean, suitable size muslin cloth was used for straining of whey. Polypropylene cup was used for storing of *shrikhand*, was procured from local market in single lot.

All the glassware's *viz.* petriplates, dairy microbiological pipette, test tubes, glass beakers, conical flasks, desiccators etc. Of Borosil make was used to analyze *shrikhand* for different parameters throughout the study. All the chemicals required for the analytical work were used of Analytical Reagent (AR) or Guaranteed reagent (GR) grade manufactured by Merk India Ltd/Glaxo India Ltd. Microbiological media made by M/SHi-media laboratories used for preparation of media and microbial examination of *shrikhand.* Different equipment's were used viz. Electronic weighing balance, Hot air oven, Muffle furnace, pH meter, Incubator, Autoclave, Rotational viscometer, Colony counter and laminar air flow.

Treatment

In all the treatment the custard apple pulp (10 per cent) and readymade market stevia leaf extract (RMSLE 1.5 per cent W/V) of *chakka* was kept constant.

T_0 - Control sample
T_1 - *Shrikhand* with 20 IU nisin
T_2 - *Shrikhand* with 25 IU nisin
T_3 - *Shrikhand* with 30 IU nisin

Chemical analysis

Moisture content of custard apple enriched *shrikhand* was determined as per SP:18 (Part XI), 1981.Total solid content of custard apple enriched *shrikhand* was determined as per the method described in IS 1479 (Part- II) 1961. Fat in *shrikhand* was determined by Gerber method described in IS: 1224 (Part II) 1977. Protein content of *shrikhand* was estimated by Micro-Kjeldhal method, AOAC, (1992).

Reducing sugars of *shrikhand* sample were estimated by Lane and Eynon method (1932) with slight modification suggested by Ranganna (1986). The acidity of *shrikhand* was determined as per the procedure SP:18 (Part-XI) 1981. The pH was measured by Oroion-3 star pH bench top pH meter. The ash content of custard apple enriched *shrikhand* was determined as per method described in IS:1479 (Part-II), 1961. Viscosity of the *Shrikhand* samples was determined by rotational viscometer using disk spindle RV_3 and RV_4 at different speed, with 10 rpm increment and in a continuous run mode.

Microbiological analysis

All the samples of custard apple enriched *shrikhand* were subjected to microbial analysis at 3 days regular interval. Microbial analysis included determination of standard plate count (SPC), yeast and mould count (YMC), lactic acid bacteria (LAB) and coliform count (CC). Plate Count Agar (Hi-media) was used to enumerate the standard plate counts in the *shrikhand* samples. Potato Dextrose Agar (PDA) of Hi- media was used to enumerate yeast and mould counts in the *shrikhand* samples. The De man Rogosa Sharpe (MRS) agar having pH 6.4 was used for enumeration of lactic acid bacteria in the *shrikhand* samples. Violet Red Bile Agar (VRBA) of Hi-media was used to enumerate the coliform counts in the *shrikhand* samples.

Statistical Analysis

Data generated during the course of investigation was analyzed with the help of statistical design namely Factorial Completely Randomized Design (FCRD) as per Snedecor and Cochran (1967).

Results and Discussion

Effect of Levels of Nisin on Physico-Chemical Quality of Custard Apple Enriched *Shrikhand* Prepared by Using Stevia Stored at 6 ± 1℃

Moisture

The moisture content of fresh custard apple enriched *shrikhand* remained more or less the same for all the treatments. The moisture content of custard apple enriched *shrikhand* of T_0, T_1, T_2 and T_3 reduced from 56.94 ± 0.01 to 56.65 ± 0.06 on 12 days, 56.68 ± 0.02 to 56.40 ± 0.02 on 15 days, 56.59 ± 0.04 to 56.18 ± 0.01 on 21 days and 56.52 ± 0.04 to 56.20 ± 0.02 on 24 days of storage period, respectively. Several workers have observed that during storage of *shrikhand* there is considerable loss of moisture which results the product unacceptable. However, in the present case, T_0 was acceptable to 12 days of storage while the samples T_1, T_2 and T_3 became more acceptable to 15, 21 and 24 days of storage period, even there was slight loss of moisture.

There was an inverse relationship between moisture content and storage period. Present results are in accordance with the results of Mehrotra *et al.*, (2014), they prepared *shrikhand* by 30 per cent sugar replacement with stevia. *Shrikhand* stored at 7℃ and found that in 0 to 21 days the moisture content was decreased with increased storage period. Decrease in moisture content of *shrikhand* samples during storage was also reported by Sonawane *et al.*, (2007).

Fat

Fat containing fermented dairy products usually undergo lipolysis, an important storage change, which affects acceptability of the product. Lipolysis under laboratory condition within limit is desirable, but it has a detrimental effect on the keeping quality beyond certain limit. The extent of lypolytic changes during storage of *shrikhand* is an indication of shelf life of the product. The changes in fat content of *shrikhand* during storage are depicted in Table 1. It was noticeable from the Table 1 that fat content was gradually decreased in all the samples irrespective of treatment. The rate of decrease was maximum in control sample (T_0) wherein fat content decreased from initial value of 22.85 ± 0.01 to 22.68 ± 0.01 per cent on 12 days of storage. The degradation of fat was considered to be primarily affected by growth of yeast and mould.

The present results are accordance with Ghube (2016) who found that fat per cent was decrease with increase storage period of *shrikhand*. This effect might due to oxidation of fat in *shrikhand* during storage. Such trend of decrease in fat was also noticed during storage of stevia added *shrikhand* by Mehrotra *et al.*, (2014) and *shrikhand* prepared by using *L. acidophilus* by Jagtap (1997). From the consideration of least fat cleavage, it can be concluded that amongst all the treatments, T_3 was proved best followed by T_2, T_1 and T_0 *shrikhand* samples suggesting suppression of lipolytic activity

by the additives. Significant differences were found towards the quantum of fat degradation during the period of storage and levels of preservative. Interaction between period and preservative was also significant.

The fat content was decreased with increase in the storage period. This may be due to increase in free fatty acid content of *shrikhand* during storage. Kuttabadkar *et al.*, (2014) found that the fat content was decreased during storage in *shrikhand* prepared from safflower milk, the decrease in fat content was mainly due to the metabolic activity of micro-organism during storage. Radha (2014) noticed, a marginal decline in fat per cent of both control and nisin added samples of pasteurized milk as storage period progressed.

Protein

The protein content of fresh *shrikhand* remained more or less the same for all the treatments. The protein content of custard apple enriched *shrikhand* of T_0, T_1, T_2 and T_3 reduced from 13.36 ± 0.01 to 13.16 ± 0.01, 13.36 ± 0.01 to 13.13 ± 0.01, 13.36 ± 0.01 to 13.08 ± 0.01, 13.37 ± 0.01 to 13.05 ± 0.01 per cent, respectively over storage period of 12, 15, 21 and 24 days. From that data it observed that, the product T_3 had minimum protein decreasing rate followed by T_2, T_1 and T_0. Though the difference in protein content were very narrow but were statistically significant.

Several workers have observed that during storage of *shrikhand* there is considerable decrease in protein content because of proteolysis. However, in the present study, due to preservative level the custard apple enriched *shrikhand* became acceptable to 12 (T_0), 15 (T_1), 21 (T_2) and 24 days (T_3) of storage period, even though there was decrease in protein content. Mehrotra *et al.*, (2014) prepared *shrikhand* by 30 per cent sugar replacement with stevia and they observed that *shrikhand* stored at 7^0C, in 0 to 21 days protein was decreased with increased storage period. Decrease in protein content of *shrikhand* during storage period is also reported by Ghube (2016) and Dandile (2010).

Acidity

The development of titratable acidity (% lactic acid) is an important degradative chemical change, which occur in *shrikhand*. The level of acidity may also serve as an indicator to know the extent of microbial spoilage in dairy products. Too high acidity may even influence the consumer acceptability of the product.

The initial mean value for the titratable acidity (% lactic acid) of custard apple enriched *shrikhand*, increased from 0.99 ± 0.01 to 1.41 ± 0.01, 0.99 ± 0.01 to 1.40 ± 0.01, 0.99 ± 0.01 to 1.41 ± 0.01 and 0.99 ± 0.01 to 1.40 ± 0.01 in T_0, T_1, T_2 and T_3 treatment on 12, 15, 21 and 24 days of storage period, indicating that the product T_3 had minimum acidity followed by T_2, T_1 and T_0. The increase in acidity was much slower in nisin added *shrikhand* samples as compared to control. This may be due to the addition of nisin has controlled the bacterial multiplication and thereby significantly reduced the development of acidity. The significant difference in the values of titratable acidity of custard apple enriched *shrikhand* in different treatments were perhaps due to the conversion of lactose into lactic acid and other organic acids by the starter culture, as reported by Ghatak and Dutta (1998). During storage increase in the titratable acidity was also reported by Karthikeyan *et al.*, (2000); Dandile (2010); Mehrotra *et al.*, (2014); Srinivas *et al.*, (2017); Jadhav (2019) and Yadav *et al.*, (2021).

Lower development of acidity was also reported in *dahi* samples containing nisin in comparison to those without nisin, reported by Sarkar *et al.*, (1996). Similar results were reported in *lassi* samples containing nisin by Kumar and Prasad (1996).

pH

The pH of all the fresh custard apple enriched *shrikhand* samples was 4.19 ± 0.01. The pH of the samples significantly ($P<0.05$) decreased during storage period. The pH values for T_0, T_1, T_2 and T_3 was decreased from 4.19 ± 0.01 to 3.98 ± 0.01, 4.19

± 0.01 to 3.97 ± 0.01, 4.19 ± 0.01 to 3.98 ± 0.01 and 4.19 ± 0.01 to 3.97 ± 0.01, respectively over a storage period of 12, 15, 21 and 24 days.

Although changes in pH cannot be directly correlated with changes in acidity, still it was found that with increase in acidity the pH was proportionately decreased. During storage, the factors that control changes in acidity may also control the changes in pH. During storage, decrease in the pH of *shrikhand* was also reported by Karthikeyan *et al.,* (2000); Mehrotra *et al.,* (2014); Srinivas *et al.,* (2017) and Yadav *et al.,* (2021).

Table.1 Effect of Levels of Nisin on Physico-Chemical Quality of Custard Apple Enriched *Shrikhand* Prepared by Using Stevia Stored at 6 ± 1°

Physico-chemical constituents	Treatment	Storage period (Days)								
		0	3	6	9	12	15	18	21	24
Moisture (%)	T_0	56.94 ± 0.01	56.91± 0.01	56.87± 0.01	56.79 ± 0.02	56.65 ± 0.06	-	-	-	-
	T_1	56.68 ± 0.02	56.66 ± 0.01	56.62± 0.01	56.59 ± 0.01	56.51 ± 0.02	56.40± 0.02	-	-	-
	T_2	56.59 ± 0.04	56.57 ± 0.01	56.55± 0.03	56.49± 0.01	56.39± 0.01	56.35 ± 0.01	56.30 ± 0.03	56.18 ± 0.01	-
	T_3	56.52 ± 0.04	56.52± 0.01	56.50 ± 0.02	56.47 ± 0.01	56.43 ± 0.03	56.36 ± 0.05	56.32 ± 0.01	56.26 ± 0.02	56.20 ± 0.02
Fat (%)	T_0	22.85 ± 0.01	22.83 ± 0.01	22.79 ± 0.01	22.74 ± 0.01	22.68 ± 0.01	-	-	-	-
	T_1	22.85 ± 0.01	22.84 ± 0.01	22.80 ± 0.01	22.77 ± 0.01	22.73 ± 0.01	22.68 ± 0.01	-	-	-
	T_2	22.85 ± 0.01	22.84 ± 0.01	22.82 ± 0.01	22.80 ± 0.01	22.75 ± 0.01	22.70± 0.01	22.65 ± 0.01	22.58 ± 0.01	-
	T_3	22.86 ± 0.01	22.85 ± 0.01	22.84 ± 0.01	22.82 ± 0.01	22.77 ± 0.01	22.73 ± 0.01	22.67 ± 0.01	22.59 ± 0.01	22.51± 0.01
Protein (%)	T_0	13.36 ± 0.01	13.33 ± 0.01	13.29 ± 0.01	13.23 ± 0.01	13.16 ± 0.01	-	-	-	-
	T_1	13.36 ± 0.01	13.35 ± 0.01	13.32 ± 0.01	13.25 ± 0.01	13.18 ± 0.01	13.13 ± 0.01	-	-	-
	T_2	13.36 ± 0.01	13.35 ± 0.01	13.33 ± 0.02	13.30 ± 0.01	13.24 ± 0.01	13.20 ± 0.01	13.14 ± 0.01	13.08 ± 0.01	-
	T_3	13.37 ± 0.01	13.36 ± 0.01	13.35 ± 0.02	13.32 ± 0.01	13.26 ± 0.01	13.22 ± 0.01	13.17 ± 0.01	13.11 ± 0.01	13.05 ± 0.01
Acidity (% LA)	T_0	0.99 ± 0.01	1.04 ± 0.01	1.20 ± 0.01	1.34 ± 0.01	1.41 ± 0.01	-	-	-	-
	T_1	0.99 ± 0.01	1.03 ± 0.01	1.17 ± 0.01	1.26 ± 0.01	1.37 ± 0.01	1.40 ± 0.01	-	-	-
	T_2	0.99 ±	0.99 ±	1.06 ±	1.18 ±	1.25 ±	1.31 ±	1.38	1.41 ±	-

		0.01	0.01	0.02	0.01	0.01	0.01	± 0.01	0.01	
	T_3	0.99 ± 0.01	0.99 ± 0.01	1.00 ± 0.02	1.14 ± 0.01	1.20 ± 0.01	1.24 ± 0.01	1.29 ± 0.01	1.35 ± 0.01	1.40 ± 0.01
pH	T_0	4.19 ± 0.01	4.17 ± 0.01	4.13 ± 0.01	4.05 ± 0.01	3.98 ± 0.01	-	-	-	-
	T_1	4.19 ± 0.01	4.18 ± 0.01	4.16 ± 0.01	4.08 ± 0.01	4.03 ± 0.01	3.97 ± 0.01	-	-	-
	T_2	4.19 ± 0.01	4.19 ± 0.01	4.17 ± 0.02	4.14 ± 0.01	4.07 ± 0.01	4.05 ± 0.01	4.00 ± 0.01	3.98 ± 0.01	-
	T_3	4.19 ± 0.01	4.19 ± 0.01	4.18 ± 0.02	4.16 ± 0.01	4.11 ± 0.01	4.06 ± 0.01	4.03 ± 0.01	3.99 ± 0.01	3.97 ± 0.01
Reducing sugar (%)	T_0	5.50 ± 0.01	5.46 ± 0.01	5.41 ± 0.01	5.32 ± 0.01	5.25 ± 0.01	-	-	-	-
	T_1	5.50 ± 0.01	5.48 ± 0.01	5.44 ± 0.01	5.38 ± 0.01	5.30 ± 0.01	5.26 ± 0.01	-	-	-
	T_2	5.50 ± 0.01	5.50 ± 0.01	5.47 ± 0.02	5.41 ± 0.01	5.35 ± 0.01	5.29 ± 0.01	5.22 ± 0.01	5.16 ± 0.01	-
	T_3	5.50 ± 0.01	5.50 ± 0.01	5.48 ± 0.02	5.44 ± 0.01	5.38 ± 0.01	5.31 ± 0.01	5.26 ± 0.01	5.19 ± 0.01	5.11 ± 0.01
Ash (%)	T_0	1.21 ± 0.01	1.20 ± 0.01	1.18 ± 0.01	1.15 ± 0.01	1.13 ± 0.01	-	-	-	-
	T_1	1.21 ± 0.01	1.21 ± 0.01	1.19 ± 0.01	1.17 ± 0.01	1.16 ± 0.01	1.15 ± 0.01	-	-	-
	T_2	1.21 ± 0.01	1.21± 0.01	1.19 ± 0.02	1.18 ± 0.01	1.17 ± 0.01	1.16 ± 0.01	1.15 ± 0.01	1.13 ± 0.01	-
	T_3	1.22 ± 0.01	1.22 ± 0.01	1.20 ± 0.02	1.19 ± 0.01	1.17 ± 0.01	1.17 ± 0.01	1.15 ± 0.01	1.14 ± 0.01	1.12 ± 0.01
Viscosity(mPa*s)	T_0	233 ± 1.11	230 ± 0.97	222 ± 3.23	217 ± 1.62	213 ± 2.01	-	-	-	-
	T_1	233 ± 1.11	231 ± 1.93	226 ± 3.54	222 ± 0.80	218 ± 3.49	213 ± 1.54	-	-	-
	T_2	235 ± 1.85	234 ± 1.15	232 ± 2.15	226 ± 0.92	222 ± 2.15	217 ± 0.72	211 ± 2.64	205 ± 0.95	-
	T_3	235 ± 1.94	235 ± 1.42	233 ± 1.41	229 ± 1.00	226 ± 2.72	222 ± 1.00	216 ± 1.46	209 ± 0.90	204 ± 0.93

Table.2 ANOVA for effect of levels of nisin on physico-chemical constituents of custard apple enriched *shrikhand* prepared by using stevia stored at 6 ± 1°

Physico-chemical constituents	Source of variation	DF	MSS	F value	CD
Moisture	Between period	8	3298.101	107187.5	**0.14**
	Between Treatment	3	3469.633	112762.3	**0.09**
	Interaction	24	951.818	30933.88	**0.28**
	Error	72	0.03	--	--
Fat	Between period	8	537.004	10638.62	**0.18**
	Between Treatment	3	571.687	11325.74	**0.12**
	Interaction	24	152.301	3017.254	**0.36**
	Error	72	0.05	--	--
Protein	Between period	8	184.109	3011.598	**0.20**
	Between Treatment	3	193.834	3170.689	**0.13**
	Interaction	24	51.099	835.867	**0.40**
	Error	72	0.06	--	
Acidity	Between period	8	1.1112	69.2300	**0.10**
	Between Treatment	3	1.4947	93.1201	**0.06**
	Interaction	24	0.6329	39.4324	**0.20**
	Error	72	0.01	--	--
pH	Between period	8	18.280	1186.92	**0.10**
	Between Treatment	3	18.601	1207.77	**0.06**
	Interaction	24	4.6763	303.621	**0.20**
	Error	72	0.01	--	
Reducing sugar	Between period	8	31.8728	1678.66	**0.11**
	Between Treatment	3	31.2511	1645.91	**0.07**
	Interaction	24	7.9512	418.77	**0.22**
	Error	72	0.01	--	--
Ash	Between period	8	1.5261	54.144	**0.13**
	Between Treatment	3	1.5359	54.489	**0.09**
	Interaction	24	0.3787	13.436	**0.27**
	Error	72	0.02	--	--
Viscosity	Between period	8	58985.17	63703.98	**0.78**
	Between Treatment	3	55110.06	59518.86	**0.52**
	Interaction	24	12560	13564.8	**1.56**
	Error	**72**	**0.9259**	--	

Table.3 Effect of Levels of Nisin on Microbial Quality of Custard Apple Enriched *Shrikhand*prepared by Using Stevia Stored at 6 ± 1°

Microbial counts (log_{10}/g)	Treatment	Storage period (Days)								
		0	3	6	9	12	15	18	21	24
Standard plate count (SPC)	T_0	1.34 ± 0.01	1.67 ± 0.02	2.00 ± 0.05	3.29 ± 0.01	4.19 ± 0.03	-	-	-	-
	T_1	1.22 ± 0.01	1.30 ± 0.02	1.95 ± 0.01	2.19 ± 0.03	3.68 ± 0.02	4.17 ± 0.06	-	-	-
	T_2	1.18 ± 0.02	1.21 ± 0.02	1.81 ± 0.02	2.10 ± 0.03	2.52 ± 0.03	2.99 ± 0.02	3.51 ± 0.08	4.31 ± 0.02	-
	T_3	1.10 ± 0.06	1.16 ± 0.02	1.60 ± 0.04	1.99 ± 0.02	2.31 ± 0.03	2.80 ± 0.03	3.25 ± 0.06	3.86 ± 0.01	4.36 ± 0.06
Yeast and mould count (YMC)	T_0	ND	ND	1.15 ± 0.01	2.04 ± 0.04	2.61 ± 0.01	-	-	-	-
	T_1	ND	ND	1.11 ± 0.03	1.84 ± 0.01	2.29 ± 0.02	2.93 ± 0.01	-	-	-
	T_2	ND	ND	1.07 ± 0.03	1.65 ± 0.01	2.03 ± 0.02	2.74 ± 0.02	3.00 ± 0.02	3.19 ± 0.07	-
	T_3	ND	ND	1.03 ± 0.04	1.54 ± 0.02	1.95 ± 0.01	2.23 ± 0.04	2.84 ± 0.01	3.01 ± 0.04	3.28 ± 0.01
Lactic acid bacteria count (LAB)	T_0	1.28± 0.03	1.42 ± 0.04	2.02 ± 0.02	2.53± 0.05	3.12 ± 0.08	------	------	------	------
	T_1	1.21 ± 0.02	1.33 ± 0.02	1.85 ± 0.07	2.39 ± 0.03	2.97 ± 0.04	3.22 ± 0.03	--------	-----	-----
	T_2	1.16 ± 0.01	1.29 ± 0.04	1.72 ± 0.01	2.34 ± 0.03	2.90 ± 0.02	3.17 ± 0.01	3.23 ± 0.02	3.87 ± 0.02	------
	T_3	1.13 ± 0.01	1.24 ± 0.02	1.61 ± 0.01	2.22 ± 0.01	2.85 ± 0.05	3.08 ± 0.02	3.19 ± 0.01	3.31 ± 0.06	3.76 ± 0.03
Coliform count (CC)	T_0	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
	T_1	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
	T_2	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
	T_3	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL

Reducing sugar

Initial average values of reducing sugar of the control custard apple enriched *shrikhand* (T_0) on 3, 6, 9 and 12 days were found to be 5.50 ± 0.01, 5.46 ± 0.01, 5.41 ± 0.01, 5.32 ± 0.01 and 5.25 ± 0.01 per cent respectively, the reducing sugar values of 20 IU nisin added samples (T_1) on 0, 3, 6, 9, 12 and 15 days were 5.50 ± 0.01, 5.48 ± 0.01, 5.44 ± 0.01, 5.38 ± 0.01, 5.30 ± 0.01 and 5.26 ± 0.01per cent, respectively, Reducing sugar values of 25 IU nisin added samples (T_2) on 0, 3, 6, 9, 12, 15, 18 and 21 were 5.50 ± 0.01, 5.50 ± 0.01, 5.47 ± 0.02, 5.41 ± 0.01, 5.35 ± 0.01, 5.29 ± 0.01, 5.22 ± 0.01 and 5.16 ± 0.01 per cent, respectively and 30 IU nisin added *shrikhand* samples (T_3) had reducing sugar values on 0, 3, 6, 9, 12, 15, 18, 21 and 24 days were 5.50 ± 0.01, 5.50 ± 0.01, 5.48 ± 0.02, 5.44 ± 0.01, 5.38 ± 0.01, 5.31 ± 0.01, 5.26 ± 0.01, 5.19 ± 0.01 and 5.11 ± 0.01 per cent, respectively. The effect of level of nisin on reducing sugar content was found to be significant among the treatments, storage periods as for the interaction between the treatment and storage periods at 5 per cent level of significance. The

results presented in this investigation are in agreement with Sharma and Zariwala (1980) and Nigam *et al.*, (2009).

Ash

The initial ash content of T_0, T_1, T_2 and T_3 was 1.21 ± 0.01, 1.21 ± 0.01, 1.21 ± 0.01 and 1.22 ± 0.01, respectively which was decreased to 1.13 ± 0.01, 1.15 ± 0.01, 1.13 ± 0.01 and 1.12 ± 0.01, respectively on 12, 15, 21 and 24 days of storage. According to Jagtap (1997), there was a slight decrease in ash content of *shrikhand* during storage period. Similar trend was reported, by Sharma and Zariwala (1980) during storage of *shrikhand* at 10° and 37°. They reported that ash content of *shrikhand* samples stored remained more or less constant throughout the storage period.

Viscosity

A viscosity of custard apple enriched *shrikhand* of T_0, T_1, T_2 and T_3 decreased from 233 ± 1.11 to 213 ± 2.01, 233 ± 1.11 to 213 ± 1.54, 235 ± 1.85 to 205 ± 0.95 and 235 ± 1.94 to 204 ± 0.93 mPa*s, respectively over storage period of 12, 15, 21 and 24 days. It was observed from Table 4.35 that viscosity decreases as the storage period progressed. The rate of decrease viscosity was maximum in control sample T_0 followed by, T_1, T_2 and T_3. ANOVA in Table 2 revealed that storage period, level of preservative and their interaction had significant ($P<0.05$) effect on viscosity. According to Rahayu and Christani (1991), the longer a product is stored, the protein will form a heavy aggregate that easily settles. The decrease of viscosity was probably due to the degradation of protein during long storage.

Effect of Levels of Nisin on Microbial Quality of Custard Apple Enriched *Shrikhand* by Using Stevia Stored at 6 ± 1°

Standard plate count (SPC)

The SPC values are given in log form. It is revealed from Table 3 that as the storage period increased the SPC count also increased. The mean SPC count of T_0, T_1, T_2 and T_3 ranged from 1.34 ± 0.01 to 4.19 ± 0.03, 1.22 ± 0.01 to 4.17 ± 0.06, 1.18 ± 0.02 to 4.31 ± 0.02 and 1.10 ± 0.06 to 4.36 ± 0.06 $\log_{10}$/g, respectively over a storage period of 12, 15, 18, 21 and 24 days of storage, respectively.

Table.4 ANOVA for Effect of Levels of Nisin on Microbial Quality of Custard Apple Enriched *Shrikhand* prepared by Using Stevia Stored at 6 ± 1°

Microbial counts	Source of variation	DF	MSS	F value	CD
Standard plate count (SPC)	Between period	8	5.6028	329.743	**0.10**
	Between Treatment	3	6.9593	409.576	**0.07**
	Interaction	24	6.0931	358.598	**0.21**
	Error	72	0.01	--	--
Yeast and mould count (YMC)	Between period	8	7.7761	481.967	**0.10**
	Between Treatment	3	7.3172	453.521	**0.06**
	Interaction	24	3.0986	192.052	**0.20**
	Error	72	0.01	--	--
Lactic acid bacteria count (LAB)	Between period	8	5.0506	328.83	**0.10**
	Between Treatment	3	10.5339	685.83	**0.06**
	Interaction	24	3.8897	253.24	**0.20**
	Error	**72**	**0.01**	--	--

Flow Chart.1 Flow diagram for manufacture of custard apple enriched *shrikhand* by using stevia

Receiving of fresh buffalo milk
↓
Preheating (38-40°C)
↓
Filteration
↓
Standardization (6 % fat)
↓
Pasteurization (90°C for 15 minutes)
↓
Cooling 30°C
↓
Inoculation of culture (LF-40 @ 1% of milk)
↓
Incubation (30°C for 8 to 10 hrs.)
↓
Curd
↓
Breaking of curd
↓
Drainage of whey (8 hr)
↓
Chakka
↓
Addition of custard apple pulp @10 per cent and readymade market stevia leaf extract @ 1.5 per cent (W/V) of *chakka*)
↓
Kneading
↓
Packaging in polypropylene cups
↓
Storage (6 ± 1°C)

The increase in SPC was observed lower in T_3 followed by T_2, T_1 and T_0 and reached to a maximum 4.36 ± 0.06 on 24 days of storage. From that data it looks that, as compare to T_0 the T_1, T_2 and T_3 had more shelf life because of nisin preservative, which inhibit the bacterial growth. The increase in SPC count may be due to growth of undesirable microorganism during storage.

Delves-Broughton (2005) reported that the addition of 12.5 and 5.20 mg/kg of nisin inhibited the bacterial growth in *cheese*. Radha (2014) reported that, as compare to control pasteurized milk the nisin added pasteurized milk samples had slower rate of increase in SPC count during storage period and had more shelf life. Pawar *et al.*, (2010) found that the control *lassi* showed higher SPC count as compared to *lassi* added with nisin during storage period.

Srinivas *et al.*, (2018) also studied the microbial changes in value added *shrikhand* and found that the SPC count was significantly increased as storage period progressed.

This finding was also supported by Karche *et al.,* (2015); Hole *et al.,* (2016) and Para (2015) who reported that the SPC count was increased in *shrikhand* during storage period. ANOVA in Table 4 revealed that storage period, level of preservative and their interaction had significant ($P<0.05$) effect on standard plate count.

Yeast and mould count (YMC)

Yeast and mould perhaps an important group of micro-organism responsible for spoilage of dairy products. The occurrence of yeast and mould in dairy products is significant because they can cause spoilage, affect the bio-chemical changes and adversely affect public health. There were no signs of growth of yeast and mould up to 3 days of storage period in control and preservative added samples. The growth of yeast and mould was 1.15 ± 0.01, 1.11 ± 0.03, 1.07 ± 0.03 and 1.03 ± 0.04 log_{10}/g observed on 6 days of storage in T_0, T_1, T_2 and T_3, respectively.

At condition T_0, the average YMC on 6, 9 and 12 days of storage was observed to be 1.15 ± 0.01, 2.04 ± 0.04 and 2.61 ± 0.01 log_{10}/g, respectively. At condition T_1, the average YMC increased from 1.11 ± 0.03, 1.84 ± 0.01, 2.29 ± 0.02 and 2.93 ± 0.01 log_{10}/g on 6, 9, 12 and 15 days of storage, respectively. Similarly at condition T_2, it increased from 1.07 ± 0.03 to 3.19 ± 0.07 log_{10}/g from 6 to 21 days of storage. At last condition in T_3, the YMC was increase from 1.03 ± 0.04 to 3.28 ± 0.01 log_{10}/g from 6 to 24 days of storage.

The rate of increase of YMC were significantly ($P<0.05$) higher in T_0 followed by T_1, T_2 and T_3. The preservative added samples showed lower yeast and mould count as compared to control sample at the end of storage period. This might may be due to the samples treated with nisin which inhibit the microbial spoilage.

The increase in the count of yeast and mould can be attributed mainly due to post pasteurization contamination. Pawar *et al.,* (2010) reported that as the level of nisin increases microbial load decreases. Sarkar *et al.,* (1996) also observed that the *shrikhand* added with nisin had lower YMC on 20 days of storage as compare to control *shrikhand* on the same day. The results obtained in agreement with Upadhyay *et al.,* (1985); Karthikeyan (1993); Dandile (2010); Karche (2015); Ghube (2016); Hole *et al.,* (2016) and Venkatesh *et al.,* (2018), they observed that the yeast and mould counts increases in the *shrikhand* samples as the storage period progresses.

Lactic acid bacteria count (LAB)

The data presented in Table 3 revealed that the control sample (T_0) had highest LAB count as compare to nisin added samples and as the level of nisin increases the LAB count decreases. The LAB count increased in T_0, T_1, T_2 and T_3 samples ranged from 1.28 ± 0.03 to 3.12 ± 0.08, 1.21 ± 0.02 to 3.22 ± 0.03, 1.16 ± 0.01 to 3.87 ± 0.02 and 1.13 ± 0.01 to 3.76 ± 0.03, respectively on 12, 15, 21 and 24 days of storage. The highest level of preservative added sample (T_3) showed lower LAB count as compare to T_0, T_1 and T_2.

Statistical analysis revealed that the preservative levels, storage days and their interactions significantly ($P<0.05$) affected the changes in LAB count of all samples during storage.
The finding of the present study also supported by report of Sarkar *et al.,* (1996) who reported that LAB count in *shrikhand* was increases with storage period progressed.

Coliform Count (CC)

Presence of coliform in any dairy products indicates unhygienic conditions prevailing during production and packaging. In the present study coliforms were absent in all the fresh as well as stored samples during storage at 6 ± 1°C. This indicates that proper hygienic precautions were taken during the production and packaging of custard apple enriched *shrikhand* in the laboratory.

According to Kumar *et al.,* (2019) and Pathrikar *et al.,* (2021) fermented milk products are not suitable

for the growth of coliform because the low pH and acidity of the fermented milk inhibits the growth of these microorganisms. The absence of coliform is mostly indicated that there was no source of faecal contamination. Absence of coliform was also reported by Upadhyay *et al.,* (1985); Karthikeyan (1993); Nadaf *et al.*, (2012); Ghube (2016) and Para (2015) in *shrikhand* during storage.

From the present study it was concluded that, during storage the moisture, fat, protein, viscosity and ash decreased with increase in storage period. While, pH decreased with acidity increased. The standard plate count, yeast and mould count and lactic acid bacteria count in *shrikhand* was increased as storage period progressed. Coliform count was absent. Nisin at 30 IU was quite good in controlling the microbial changes. Hence, use of 30 IU nisin in preservation of custard apple enriched *shrikhand* is recommended.

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