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Study on effect of refrigerated storage on physicochemical, microbiological and sensory attributes of lutein incorporated calcium and vitamin D fortified *Shrikhand*

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Abstract

Shrikhand was prepared from buffalo milk fortified with tricalcium citrate tetrahydrate (162ppm), vitamin D_3 (558 IU/L) and lutein (0.5mg/100g). Effect of refrigerated storage on physico-chemical, microbiological and sensory attributes of fortified Shrikhand was studied and compared with control. Physico-chemical parameters such as titratable acidity, pH, total solids, free fatty acid value, tyrosine value and thiobarbituric acid value of both control and optimised sample were evaluated. Titratable acidity of both samples increased during storage. A significant increase in free fatty acid value and tyrosine value were also noted. Microbiological parameters such as standard plate count and yeast and mold count of experimental Shrikhand was lower than control Shrikhand during storage. Throughout the storage period all attributes of sensory scores obtained for experimental Shrikhand remained higher. Both control and experimental Shrikhand were kept unspoiled for 28 days at refrigeration.

Keywords: *Shrikhand, fortified, storage, free fatty acid, tyrosine.*

Milk and milk products are widely chosen for micro nutrient fortification as they are frequently consumed and liked by all age groups. Preservation using lactic fermentation is one of the oldest and efficient methods to preserve milk and its valuable nutrients. Fermented dairy products act as an excellent medium to generate an array of products that fit into the current consumer demand for healthy foods.

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It has been reported that during the period of 2016 to 2020, the market size of curd has grown by 14.4 per cent which indirectly figures out the contribution of fermented dairy products to the revenue of Indian dairy industry (Gandhi *et al.,* 2020). *Shrikhand* is a semi solid, sweetish-sour fermented dairy product, popular in the states of Gujarat, Maharashtra and Karnataka. Traditionally, it is prepared by expulsion of whey from dahi (curd) to yield chakka followed by mixing with sugar and other optional ingredients. It has high nutritive value, characteristic flavour, taste and is highly palatable with good therapeutic properties.

Lutein, a member of the *Xanthophylls* family of carotenoids, is mainly isolated from the petals of Marigold flowers *(Tagetes erecta).* Many studies have proved its antioxidant and pigmentation properties which can be utilised in the formulation of nutritional supplements, cosmetics and pharmaceutical industry (Landrum and Bone, 2001).

Calcium being an important component of bone, accounts for about 30 to 35 per cent of its mass and much of its strength. Lower calcium intake could adversely affect the development of peak bone mass in adolescents and young adults and the retention of bone mass in older adults. In India, according to the International Osteoporosis Foundation, the average calcium intake is only 429 mg against the recommended value of 800-1000 mg per day. During pregnancy and lactation calcium requirement is further enhanced to 1200 mg per day.

Vitamin D is a secosteroid which is synthesised endogenously when human skin is exposed to ultraviolet blue rays from the sun. During insufficient sun exposure, especially in winters and at higher latitudes, the true vitamin needs to be taken up in the diet, such as fatty fish or fortified milk, or by direct administration. Moreover, vitamin D is an important factor that promotes calcium absorption in the gut, minimises its excretion through the kidney and enhances its re-absorption. Therefore, a combination of vitamin D and calcium during food fortification is more preferable to combat their deficiencies (Satyanarayana, 2006). Hence, the current study was aimed to evaluate the effect of refrigerated storage on physico-chemical, microbiological and sensory attributes of lutein incorporated calcium and vitamin D fortified *Shrikhand*.

Materials and methods

Fresh whole buffalo milk was collected from University Dairy Plant, Kerala Veterinary and Animal Sciences University. Lutein was procured from Plant Lipids Pvt. Ltd, Kolenchery, Cochin. Vitamin D_3 premix was supplied by Pd-Navkar, Pvt. Ltd, Karnataka. Tricalcium citratetetrahydrate purchased from Hi-Media Laboratories Mumbai was used as a calcium source. Yoghurt Culture (*Streptococcus thermophilus: Lactobacillus delbrueckii* subsp. *bulgaricus*) was procured from Dept. of Dairy Microbiology, Verghese Kurien Institute of Dairy and Food Technology, Mannuthy. Polystyrene cups (125ml) with lids were obtained from Lyka Packaging Pvt. Ltd, Ernakulam.

Preparation of Shrikhand

Shrikhand was prepared as per the procedure described by Aneja *et al.* (2002) with slight modification. Fresh whole buffalo milk was received, filtered and standardised to 6 g fat/ 100 ml milk and 9 per cent SNF. Tricalcium citrate tetrahydrate and vitamin D_3 were added to milk at the rate of 162 ppm and 558 IU/ L, respectively followed by thorough mixing. Milk was then heated at 85 ºC for 30 min and then cooled to 45 ºC for inoculation with starter culture at the rate of 1 per cent and incubated at 45ºC for 4 to 5 h until a firm coagulum was formed. Coagulum was then broken and transferred to a muslin cloth and hung for complete whey drainage. The obtained semi solid mass, called as chakka, was mixed with sugar (40 per cent) and lutein (0.59 mg/ 100g). The mixture was kneaded well to obtain a desirable consistency. It was then filled into polystyrene cups and kept for refrigerated storage. *Shrikhand* was analysed at a regular interval of 7 days for physicochemical microbiological and sensory parameters.

The treatment combinations were:

T0: Control *Shrikhand* (40 per cent sugar)

T1: *Shrikhand* (40 per cent sugar) prepared usingbuffalo milk fortified with selected levels of calcium, vitamin D3 and lutein.

Physico-chemical analysis

Thiobarbituric acid values were determined according to the method recommended by modified Sidwell *et al*. (1955). The free fatty acid content was determined by extraction and titration method as suggested by Deeth *et al.* (1975). The extent of protein breakdown (proteolysis) in terms of tyrosine was estimated as per the modified method of Juffs (1973).

Microbiological analysis

Standard plate count of *Shrikhand* samples was estimated by pour plate technique, as described by Morton (2001). Coliform and yeast and mold count was estimated as per IS: SP: 1224 (Part I and II), 1981.

Sensory evaluation of Shrikhand

The samples were organoleptically evaluated for quality attributes like flavour, colour and appearance, body and texture, sweetness and overall acceptability by a selected panel of six judges. A hedonic scale score card was used for evaluation.

Statistical analysis

Data was statistically analysed using SPSS software

Results and discussion

The effect of refrigerated storage on physicochemical, microbiological and sensory attributes of lutein incorporated calcium and vitamin D fortified *Shrikhand* are presented in Table 1-3.

Changes in chemical quality of lutein incorporated calcium and vitamin D fortified Shrikhand

During refrigerated storage (7±1°C), the titratable acidity of control and optimised *Shrikhand* increased from a mean value of 1.07 to 1.40 per cent and 1.00 to 1.38 per cent LA, respectively, during 28 days of storage. An increase in acidity of functional *Shrikhand* incorporated extract of pomegranate fruit peel during storage at 5ºC was observed by Pugazhenthi *et al.* (2020).

Changes in the FFA value of *Shrikhand* samples during storage at 7± 1°C are depicted in Table 1. The FFA value of control and optimised *Shrikhand* showed a significant ($p< 0.01$) increase from a mean value of 0.63 to 3.98 µg/gand 0.53 to 3.43µg/g respectively during 28 days of storage. When compared with control, optimised *Shrikhand* samples showed a significantly ($p< 0.05$) lower FFA values throughout the storage. Kuttabadkar *et al.* (2014) observed that increase in free fatty acid content of *Shrikhand* prepared from safflower milk was faster when stored at higher temperatures.

During refrigerated storage (7±1°C), the TBARS value of control and optimised *Shrikhand* were found to have significantly ($p<0.01$) increased from 0.42 to 0.90 and 0.32 to 0.71, respectively. When compared with control, optimised *Shrikhand* samples showed a significantly ($p< 0.05$) lower TBA values from 7^{th} to 28^{th} days of storage. The results observed were in close resemblance with the findings of Sunilkumar *et al.* (2011).

The results showed that, during the refrigerated storage (7±1°C), tyrosine content of control *Shrikhand* significantly ($p < 0.05$) increased from 48.10 to 90.00 mg/ 100ml. A significant ($p<0.01$) increase from 40.10 to 86.10 mg/100 ml was also observed in optimised *Shrikhand*. When compared with control, optimised *Shrikhand* samples showed a significantly ($p< 0.01$) lower tyrosine content throughout the storage. The results obtained were in accordance with the results of Kuttabadkar *et al.* (2014).

Changes in microbiological quality of lutein incorporated calcium and vitamin D fortified Shrikhand

During storage at 7±1°C, the SPC of control and optimised *Shrikhand* significantly ($p<0.01$) increased from 3.63 $\log_{10}$ cfu/ gm to 4.32 $\log_{10}$ cfu/ gm and from 3.24 $\log_{10}$ cfu/ gm

to 4.43 log_{10} cfu/ gm, respectively, whereas the YMC significantly ($p<0.01$) increased from 0.95 log_{10} cfu/ gm to 1.65 log_{10} cfu/ gm and from 0.92 log_{10} cfu/ gm to 1.67 log_{10} cfu/ gm, respectively, over 28 days of storage. Coliforms were absent in both control and optimised *Shrikhand* throughout the storage period.

When compared with control, the SPC of optimised *Shrikhand* was remained lesser throughout the storage period. The difference in SPC between the control and optimised *Shrikhand* were found to be significant at one per cent level from 1st to 21st days of storage and after that the change was non-significant.

Table 1. Effect of refrigerated storage on physico-chemical attributes of *Shrikhand*

Sample	Days of storage						
	1st	7th	14th	21th	28th	35th	F-value
Changes in acidity (% Lactic acid)							
Control	1.07 ± 0.01	1.13 ± 0.02	1.27 ± 0.02	1.37 ± 0.01	1.40 ± 0.01	Spoiled	60.38**
Optimised	1.00 ± 0.02	1.05 ± 0.01	1.18 ± 0.01	1.30 ± 0.02	1.38 ± 0.02	Spoiled	120.48**
T- value	3.33**	3.02**	3.37**	3.05**	1.04 ns		
Changes in total solids (%)							
Control	59.55 ± 0.38	60.13±0.42	60.69± 0.41	61.12± 0.46	61.52 ± 0.55	Spoiled	3.02ns
Optimised	61.43 ± 0.31	61.89 ± 0.34	62.35 ±0.29	62.90± 0.39	63.52 ± 0.41	Spoiled	5.55**
T - value	3.82**	3.23*	3.33*	2.97*	2.92*		
Changes in pH							
Control	4.27 ± 0.02	4.17 ± 0.02	4.01 ± 0.02	3.93 ± 0.02	3.88 ± 0.02	Spoiled	92.70**
Optimised	4.43± 0.03	4.37 ± 0.02	4.23 ± 0.02	4.17± 0.02	4.03 ± 0.03	Spoiled	41.45**
T- value	4.47**	8.49**	10.61**	9.90**	4.02*		
Changes in FFA (µg/g)							
Control	0.63 ± 0.02	1.13 ± 0.07	2.27 ± 0.14	3.03 ± 0.03	3.98 ± 0.07	Spoiled	285.04**
Optimised	0.53 ± 0.02	0.85 ± 0.02	1.23 ± 0.20	1.9 ± 0.40	3.43 ± 0.12	Spoiled	30.17**
T- value	4.30*	4.04*	4.14*	2.79*	3.85*		
Changes in tyrosine (mg/100ml)							
Control	48.10 ± 0.06	55.4 ± 0.11	67.23± 0.09	78.60± 0.58	91.00± 0.58	Spoiled	4142.40*
Optimised	40.1 ± 0.09	43.67 ± 0.88	55.16± 0.12	71.47± 0.74	86.1± 0.05	Spoiled	1380.00**
T- value	75.58**	13.19**	80.95**	9.58**	8.45**		
Changes in TBA							
Control	0.42 ± 0.02	0.52 ± 0.02	0.63 ± 0.02	0.72 ± 0.02	0.90 ± 0.04	Spoiled	53.82**
Optimised	0.32 ± 0.01	0.42 ± 0.01	0.54 ± 0.02	0.62 ± 0.01	0.71 ± 0.02	Spoiled	125.12**
T - value	5.30**	4.24*	3.18*	4.22*	4.37*		

Table 2. Effect of refrigerated storage on microbiological attributes of lutein incorporated calcium and vitamin D_3 fortified *Shrikhand*

Sample	Days of storage						
	1st	7th	14th	21th	28th	35th	Chi-square value
Changes in standard plate count (log10 cfu/g)							
Control	3.63 ± 0.01	3.79 ± 0.02	3.91 ± 0.02	4.15 ± 0.01	4.32± 0.01	Spoiled	50.79**
Optimised	3.24 ± 0.02	3.37 ± 0.01	3.54± 0.01	3.83 ± 0.02	4.43±0.02	Spoiled	120.48**
T- value	116**	14.02**	9.76**	5.97**	1.70 ns		
Changes in yeast and mold count (log10 cfu/g)							
Control	0.95± 0.01	0.98± 0.01	1.07± 0.02	1.25± 0.05	1.65± 0.05	Spoiled	71.25**
Optimised	0.92± 0.01	0.94± 0.01	1.10± 0.05	1.22± 0.02	1.67± 0.03	Spoiled	119.72**
T - value	2.12 ns	4.02 ns	0.45 ns	0.45 ns	0.45 ns		

Table 3. Effect of refrigerated storage on sensory attributes of lutein incorporated calcium and vitamin D_3 fortified *Shrikhand*

Sample	Days of storage						
	1st	7th	14th	21th	28th	35th	Chi-square value
Changes in flavour							
Control	7.75 ± 0.11	7.45 ± 0.05	7.25 ± 0.11	7.10 ± 0.17	7.05 ± 0.15	Spoiled	12.94*
Optimised	8.05 ± 0.05	7.70 ± 0.09	7.55 ± 0.09	7.25 ± 0.11	7.15 ± 0.06	Spoiled	18.79**
U- value	21.00ns	21.00 ns	20.50 ns	15.00 ns	15.50 ns		
Changes in colour and appearance							
Control	7.70 ± 0.09	7.60 ± 0.06	7.55 ± 0.09	7.40 ± 0.06	7.30 ± 0.09	Spoiled	10.26*
Optimised	8.10 ± 0.06	8.00 ± 0.11	7.65 ± 0.06	7.55 ± 0.09	7.40 ± 0.06	Spoiled	18.05**
U - value	23.50*	23.00*	16.00 ns	18.00 ns	16.00 ns		
Changes in body and texture							
Control	7.85 ± 0.06	7.55 ± 0.09	7.50 ± 0.08	7.35 ± 0.13	6.95 ± 0.09	Spoiled	16.61**
Optimised	8.20 ± 0.09	7.95 ± 0.09	7.60 ± 0.06	7.50 ± 0.11	7.10 ± 0.06	Spoiled	20.14**
U - value	23.00*	23.00*	16.5 ns	16.5 ns	18.00		
Changes in sweetness							
Control	7.85 ± 0.06	7.75 ± 0.08	7.60 ± 0.06	7.35 ± 0.20	7.20 ± 0.17	Spoiled	11.61*
Optimised	8.05 ± 0.09	7.80 ± 0.09	7.65 ± 0.06	7.55 ± 0.15	7.25 ± 0.14	Spoiled	14.05**
U- value	19.50 ns	14.50 ns	15.00 ns	16.00 ns	13.50 ns		
Changes in overall acceptability							
Control	7.80 ± 0.09	7.60 ± 0.06	7.55 ± 0.09	7.45 ± 0.09	7.25 ± 0.14	Spoiled	10.75*
Optimised	8.25 ± 0.08	8.00 ± 0.11	7.65 ± 0.06	7.50 ± 0.08	7.30 ± 0.09	Spoiled	19.44**
U- value	24.00*	23.00*	16.00 ns	14.5 ns	13.00 ns		

The reduction of SPC in optimised *Shrikhand* may be attributed to the antioxidant activity of lutein added and the inhibition starter growth as a result of calcium fortification. The difference in YMC between the control and optimised *Shrikhand* were found to be non-significant during the storage. The obtained results were comparable with those of Kumar *et al.* (2011)

Changes in sensorial quality of lutein incorporated calcium and vitamin D fortified Shrikhand during refrigerated storage

The reduction in flavour scores during refrigerated storage (7±1°C) of control *Shrikhand* was significant at five per cent level whereas that of optimised *Shrikhand* was observed to be significant at one per cent level. The mean flavour scores of control and optimised samples ranged from 7.75 to 7.05 and 8.05 to 7.15, respectively, during 28 days refrigerated storage. However, the difference between flavour scores of control and optimised *Shrikhand* were non-significant throughout the storage period.

The reduction in colour and appearance scores during refrigerated storage (7±1°C) of control *Shrikhand* was significant at five per cent level whereas that of optimised *Shrikhand* was observed to be significant at one per cent level. The mean colour and appearance scores of control and optimised samples ranged from 7.70 to 7.30 and 8.10 to 7.40, respectively, during 28 days refrigerated storage. During first and seventh days, colour and appearance scores between control and optimised treatment were significantly ($p<0.05$) different, whereas a non-significant difference in scores were observed between control and optimised samples from 14th day of storage. This may be due to the gradual fading of yellowness imparted by lutein to the optimised product during storage.

The results showed that the body and texture scores of control and optimised *Shrikhand* reduced significantly ($p<0.01$) from 7.85 to 6.95 and 8.20 to 7.10, respectively, during refrigerated storage. The body and texture scores between control and optimised

Shrikhand differed significantly ($p<0.05$) during first and seventh days of storage. Afterwards, the difference in scores between control and optimised products were non-significant.

The reduction in sweetness scores during refrigerated storage (7±1°C) of control *Shrikhand* was significant at five per cent level whereas that of optimised *Shrikhand* was observed to be significant at one per cent level. The mean sweetness scores of control and optimised samples ranged from 7.85 to 7.20 and 8.05 to 7.25, respectively. However, the difference between sweetness scores of control and optimised *Shrikhand* were non-significant throughout the storage period.

The reduction in overall acceptability scores during refrigerated storage (7±1°C) of control *Shrikhand* was significant at five per cent level whereas that of optimised *Shrikhand* was observed to be significant at one per cent level. The mean overall acceptability scores of control and optimised samples ranged from 7.80 to 7.25 and 8.25 to 7.30, respectively. The overall acceptability scores between control and optimised *Shrikhand* differed significantly ($p<0.05$) during first and seventh days of storage. Afterwards, the difference in scores between control and optimised product were non-significant.

From the results of sensory evaluation of control and optimised product, it can be concluded that there is noticeable change in the sensory attributes of both the samples during refrigerated storage. It is also noted that, throughout the storage period, all attributes of sensory scores obtained for optimised *Shrikhand* remained higher when compared to control *Shrikhand*. The results obtained were comparable with the mean sensory scores of goat milk *Shrikhand* added with kiwi fruit, optimised by Pathrikar *et al*. (2021)

Conclusion

From the present study, it is concluded that *Shrikhand* prepared from buffalo milk fortified with tricalcium citrate tetrahydrate, vitamin D_3 and lutein is more acceptable than control *Shrikhand* during refrigerated storage in terms of sensory attributes, physico-chemical parameters and microbiological quality.

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Conflicts of interest

There were no conflicts of interest reported by the authors.

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