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Changes in physico-chemical, microbial and organoleptic attributes of Shrikhand fortified with litchi pulp and lactulose during storage at refrigerated temperature

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Abstract
Shrikhand is a semi solid, sweetish-sour, whole milk product, prepared by lactic fermentation of milk. The curd (dahi) obtained is partially strained through a muslin cloth to remove the whey to yield Chakka. In the present study shrikhand was prepared by adding 15% litchi pulp and 4.5% lactulose which was stored at 5-7 ºC. During the storage period organoleptic evaluation, physico-chemical and microbial analysis of the product was carried out. The sensory evaluation of optimized product during the storage showed that there was continuous decrease in scores of all the sensory parameters. The physico-chemical analysis of shrikhand during storage showed that carbohydrate, protein, fat, ash, total solids, titratable acidity, antioxidant and crude fiber was increased and pH was decreased. Based on the organoleptic scores and the physico-chemical aspects that were assessed, it was concluded that the product retained its aesthetic value and was acceptable upto 42 days.

Keywords: Shrikhand, lactulose, litchi pulp, storage study, physico-chemical

Introduction

Shrikhand is a traditional fermented dairy product which is mainly consumed in western part of the country. The curd is partially strained through a muslin cloth to remove the whey to produce a solid mass called Chakka. This Chakka is mixed with the required amount of sugar to yield Shrikhand (De, 1982) [9]. Because of the change in the economic status, the food habit of consumers are also changing and a plethora of the other varieties of Shrikhand such as fruit Shrikhand are also in great Demand.

Out of the total milk production in India, only 7% of total milk is used for manufacturing of traditional fermented dairy products like dahi, shrikhand and lassi which are western counterpart of yogurt, quarg and stirred yogurt respectively. Fermented milk products constitute a vital component of the human diet in many regions of the world. In the Indian sub-continent such products are also classified as “indigenous fermented milk products” like dahi (curd), lassi, shrikhand etc. which is a prominent part of people’s diet. Value addition of shrikhand with fruit pulp is being accepted at a large by consumers. Litchi is one of the most delicious, refreshing and perishable subtropical fruit of India. Litchi has sweet white appearance with translucent, juicy flesh. The flavour of the fresh pulp is musky. It is a rich source of sugars, vitamins and minerals like magnesium, iron, phosphorous, potassium, calcium and copper (Marisa, 2006) [19]. It has been reported that litchi plays a therapeutic role in fighting blood pressure, arthiritis, asthma and cardiovascular disease.

Lactulose is a disaccharide and is produced by the isomerization of lactose. It is widely established as a laxative agent in the treatment of constipation and reduces the risk of colon cancer. Lactulose has also an important application in lowering blood glucose level and reduces the pancreatic insulin production and thus lactulose shows anti-diabetic effect. Unlike many other carbohydrates such as lactose, lactulose is not hydrolyzed by digestive enzymes in the small intestine. Instead, it is transported to the large intestine where it is hydrolyzed by resident saccharolytic microflora. Using lactulose as an energy source, some gram positive bacteria such as bifidobacteria species produce lactic and acetic acids, lowering pH and raising osmotic pressure. These in turn soften intestinal contents and may prevent the proliferation of some gram negative, putrefactive and pathogenic species such as clostridium, bacteroides, salmonella, shigella and *Escherichia coli*. The present investigation was conducted to study the effect of litchi pulp and lactulose addition on shelf life of shrikhand.

Materials and Methods

Fresh buffalo milk was collected from Prayagraj. Litchi pulp was collected from National Litchi Research Institute, Bihar. Lactulose was procured from Azelis (India) Private Limited, Navi Mumbai**.** Mesophilic mixed culture NCDC-167 (*Lactococcus lactis ssp. lactis, L. lactis ssp. cremoris, L. lactis ssp. diacetylactis and Leuconostoc citrovorum*) was procured from National Collection of Dairy Cultures, NDRI, Karnal. Polystyrene cups (100/ml) with lids were obtained from AGGIES Dairy of Warner College of Dairy Technology, S.H.U.A.T.S., Prayagraj and used for packaging and storage of shrikhand.

Preparation of shrikhand

Shrikhand was prepared by following the procedure of David (2015) [8] with slight modification. Fresh buffalo milk was received. Required quantity of milk was standardized to 6 percent fat and 9 percent SNF and filtered. Milk was heated at 85 ºC for 30 minutes. It was cooled to 30 ºC and inoculated by the starter culture i.e. NCDC 167 (combination of *Lactococcus lactis ssp. lactis, Lactococcus lactis ssp. cremoris and Lactococcus lactis ssp. diacetylactis*) at the rate of 2 percent and incubated at 32 ºC for 10-12 hours until a firm coagulam was formed. Coagulam was then broken and transferred to a muslin cloth and hanged for expulsion of whey for 8 to 10 hours. The semi solid mass left after drainage of whey is called chakka which is the base for shrikhand. Chakka was mixed with litchi pulp and lactulose (as mentioned in treatment). 35 percent sugar was added. The mixture was well kneaded to smooth paste. It was then filled into sanitized polystyrene cups, sealed and kept for storage at 5-7 ºC.

Physico chemical analysis

Carbohydrate was determined by Lane Eynon method described in SP: 18, Part XI, 1981.The fat percentage of shrikhand supplemented with lactulose and litchi pulp was determined as per procedure described in A.O.A.C. (1990) [3]. Determination of protein was done as per the procedure of Maneffee and Overman (1940) [18]. Determination of ash content was done as per the procedure to A.O.A.C. (1975) [4]. Total solids of Shrikhand supplemented with lactulose and litchi pulp was determined by gravimetrically as per the procedure for milk laid down in IS 2802, 1964. Titratable acidity of shrikhand sample was carried out by titration method. The pH of shrikhand samples was determined by potentiometric method using a digital pH meter at a temperature of 25 ºC. Determination of crude fiber contents was done as per A.O.A.C. (1995) [2]. Antioxidant activity was determined by the method of Brand-Williams *et al*., (1995) [7].

Microbiological analysis

Lactic acid bacteria count, Yeast and mould count and Coliform count were carried out as per the procedure given by (APHA), standard method for the examination of Dairy products (1992) [5].

Sensory evaluation of shrikhand

Shrikhand fortified with litchi pulp and lactulose was evaluated for organoleptic properties by using 9-point Hedonic scale (Amerine *et al.,* 1965) [1].

Storage Study

Storage study of shrikhand was carried out only for the highly accepted levels of lactulose and litchi pulp. The shrikhand sample prepared using 4.5 percent lactulose and 15 percent litchi pulp was superior over rest of the treatments which lead to higher overall acceptability among the consumer. Storage study was carried out at 5-7 ºC for 42 days and the sample was analysed at a regular interval of 7 days for physico-chemical microbiological and sensory parameters.

Statistical analysis

Data was analysed using Analysis of Variance (ANOVA) and Critical difference (C.D) in WASP software (RBD) and MS office, 2007.

Results and Discussion

Changes in physico-chemical characteristics during storage

The physico chemical characteristics of the samples were studied upto 42th days of storage at 5-7 ºC (Table 1). The results of the samples in respect of total solids, fat, protein, carbohydrate, ash, titratable acidity, pH, antioxidant and crude fiber content are discussed here under the following heads:

Table 1: Effect of storage on physico-chemical characteristics of shrikhand supplemented with lactulose and litchi pulp.

	0th Day	7th Day	14th Day	21th Day	28th Day	35th Day	42th Day
Total Solid (%)	59.22	59.23	59.30	59.35	59.85	60.26	60.81
Fat (%)	11.88	11.89	11.91	11.92	12.00	12.06	12.17
Protein (%)	7.05	7.05	7.06	7.06	7.14	7.20	7.28
Carbohydrate (%)	39.59	39.59	39.62	39.66	40.00	40.28	40.64
Ash (%)	0.69	0.69	0.70	0.70	0.70	0.71	0.71
Acidity (%LA)	0.87	0.88	0.91	0.94	0.96	0.99	1.03
pH	5.58	5.51	5.33	5.13	5.02	4.86	4.67
Antioxidant (mg ascorbic acid)	4.83	4.83	4.84	4.84	4.86	4.89	4.94
Crude Fiber (%)	0.26	0.26	0.27	0.27	0.27	0.28	0.28

Total solids percentage was found to be increasing for optimized shrikhand from 0th day to 42th day of storage. The mean values for stored shrikhand ranged from 59.22 to 60.81. There was significant difference (P<0.05) in total solids percentage of optimized shrikhand during different storage intervals at 5-7 ºC. The present results are in accordance the findings of Nigam *et al.,* (2009) [20] and Kumar *et al.,* (2011) [17] who reported similar trends of increase in TS % when shrikhand were stored for longer duration.

Fat percentage was observed to be increasing for optimized shrikhand from 0th day to 42th day of storage. The mean values for stored shrikhand ranged from 11.88 to 12.17. There was significant difference (P<0.05) in fat percentage of optimized shrikhand during different storage intervals at 5-7 ºC. The increase in the levels of fat may be due to loss of moisture content in the samples during storage. The findings of Nigam *et al.,* (2009) [20] is at par with the result of this investigation. Kumar *et al.,* (2011) [17] also reported similar increase in fat content of apple pulp and *Celosia argentea* fortified shrikhand during storage under refrigerated temperature.

Protein percentage was also found to be increasing for optimized shrikhand from 0th day to 42th day of storage. The mean values for stored shrikhand ranged from 7.05 to 7.28. There was significant difference (P<0.05) in protein percentage of optimized shrikhand during different interval of storage at 5-7 ºC. The similar findings have also been reported by Nigam *et al.,* (2009) [20] and Kumar *et al.,* (2011) [17] who studied the effect of storage period on protein content of apple pulp and *Celosia argentea* fortified shrikhand during storage under refrigerated temperature.

Carbohydrate percentage was found to be increasing for optimized shrikhand from 0th day to 42th day of storage. The mean values for stored shrikhand ranged from 39.59 to 40.64. There was significant difference ($P<0.05$) in carbohydrate percentage of optimized shrikhand during different interval of storage at 5-7 ºC.

Ash percentage was increasing for optimized shrikhand from 0th day to 42th day of storage. The mean values for stored shrikhand ranged from 0.69 to 0.71. There was no significant difference ($P>0.05$) in ash percentage of optimized shrikhand during different interval of storage at 5-7 ºC. The findings of Nigam *et al.*, (2009) [20] and Kumar *et al.*, (2011) [17], were in confirmity with our findings who studied the changes in physico-chemical aspects of shrikhand at refrigerated tempeature.

Titratable acidity percentage was found to be increasing for optimized shrikhand from 0th day to 42th day of storage. The mean values for stored shrikhand ranged from 0.87 to 1.03 (% LA). There was significant difference ($P<0.05$) in acidity percentage of optimized shrikhand during each interval of storage at 5-7 ºC. This may be due to growth of microorganisms responsible for spoilage of milk and milk products. Similar observations were also recorded by Patel *et al.*, (1993), Jain (2003) [21], Sonawane *et al.*, (2007) [26], Nigam *et al.*, (2009) [20], Bhat *et al.*, (2010) [6] and Kumar *et al.*, (2011) [17] when various dairy products were stored for longer duration under refrigerated temperature. Gandhi and Jain (1977) [11] also reported the keeping quality of shrikhand to be 12 to 14 days at temperature less than 18 °C.

pH was observed to be decreasing for optimized shrikhand from 0th day to 42th day of storage. The mean values for pH of stored shrikhand ranged from 4.67 to 5.58. There was significant difference ($P<0.05$) in pH values of optimized shrikhand on each interval of storage at 5-7 ºC. Nigam *et al.*, (2009) [20] and Kumar *et al.*, (2011) [17] have also reported similar findings when dairy products were stored at refrigerated temperature. Although antioxidant activity was found to be increasing for optimized shrikhand from 0th day to 42th day of storage but there was no significant difference ($P>0.05$) between antioxidant activity of optimized shrikhand that was analyzed at different intervals during storage at 5-7 ºC. The mean values for antioxidant activity ranged from 4.83 to 4.94 (expressed in terms of mg ascorbic acid).

Crude fibre percentage was observed to be increasing for optimized shrikhand from 0th day to 42th day of storage. The mean values for crude fibre in stored shrikhand ranged from 0.26 to 0.28. There was no significant difference ($P>0.05$) between crude fibre percentage of optimized shrikhand that was analyzed at different intervals during storage at 5-7 ºC.

Changes in microbial characteristics during storage

The microbial characteristics of the samples were studied upto 42th days of storage at 5-7 ºC. The results of lactic acid bacteria count, yeast and mould count and coliform count of the shrikhand samples are presented in fig. 1.

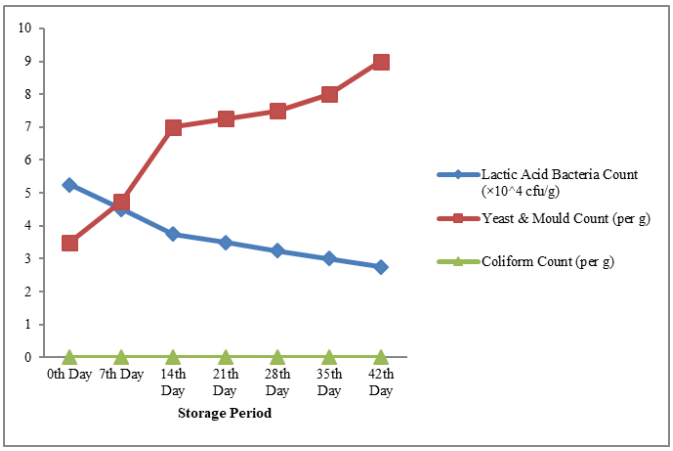

Fig 1: Effect of storage on microbial characteristics of shrikhand supplemented with lactulose and litchi pulp.

Lactic acid bacteria count was found to be decreasing for stored shrikhand samples from 0th day to 42th day of storage. The mean values for LAB count in stored shrikhand samples ranged from 2.75 to 5.25 ($\times 10^4$ cfu/g). There was significant difference ($P<0.05$) in lactic acid bacteria count of optimized shrikhand during different intervals of storage at 5-7 ºC. The viability of microorganisms decreased during storage which may be due to death of cells. Yeast and mould count was found to be increasing in shrikhand samples from 0th day to 42th day of storage. The mean values for Yeast and mold count for stored shrikhand samples ranged from 3.50 to 9.00 (per g). There was significant difference ($P<0.05$) in yeast and mould count of optimized shrikhand during different intervals of storage at 5-7 ºC. No coliform count was recorded during storage. 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 indicates that there was no source of faecal contamination.

Changes in sensory characteristics during storage

The sensory characteristics of the samples were studied upto 42th days of storage at 5-7 ºC. The results of the shrikhand samples in respect of flavour, colour and appearance, consistency and overall acceptability are presented in fig. 2.

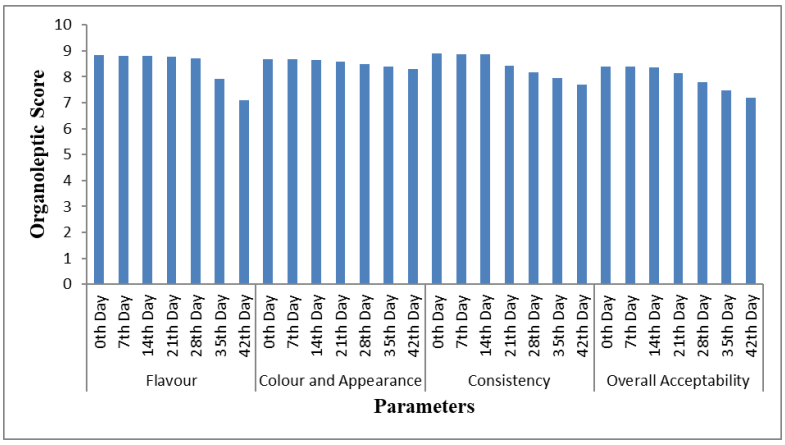

Fig 2: Effect of storage on sensory characteristics of shrikhand supplemented with lactulose and litchi pulp.

Flavour score was found to be decreasing in stored shrikhand samples from 0th day to 42th day of storage. The mean values for flavour score in stored shrikhand ranged from 7.10 to 8.83. There was significant difference (P<0.05) in flavour score of optimized shrikhand that were obtained during different interval of storage at 5-7 ºC. Shrikhand prepared with fortification of papaya pulp (Nigam *et al*., 2009) [20] and apple pulp and *Celosia argentea* (Kumar *et al*., 2011) [17] also showed decreased in flavour score (P<0.05) during storage periods.

Colour and appearance score was observed to be decreasing for optimized shrikhand from 0th day to 42th day of storage. The mean values of colour and appearance scores for stored shrikhand ranged from 8.28 to 8.67. There was significant difference (P<0.05) in colour and appearance score of optimized shrikhand that were obtained during different interval of storage at 5-7 ºC. Nigam *et al*., (2009) [20] have also reported that the incorporation of papaya pulp in the manufacture of shrikhand shows declining trend in colour and appearance score during storage.

Consistency score was found to be decreasing for optimized shrikhand from 0th day to 42th day of storage. The mean values for consistency scores in stored shrikhand ranged from 7.70 to 8.90. There was significant difference (P<0.05) in consistency score of optimized shrikhand that were obtained during different interval of storage at 5-7 ºC. Nigam *et al*., (2009) [20] have also reported that the consistency score decreased during storage periods in papaya pulp fortified *Shrikhand*. Kumar *et al.*, (2011) [17] also reported a similar decline in consistency score during storage when apple pulp and *Celosia argentea* were added in Shrikhand.

Overall acceptability score was observed to be decreasing for optimized shrikhand from 0th day to 42th day of storage. The mean values for overall acceptability scores in stored shrikhand ranged from 7.72 to 8.93. There was significant difference (P<0.05) in overall acceptability score of optimized shrikhand that were obtained during different interval of storage at 5-7 ºC. Patel *et al.*, (1993) [21] reported that the overall acceptability score of chakka decreased with increase in storage periods due to deterioration of flavor. Verma (2013), Kumar *et al.*, (2011) [17], Nigam *et al.*, (2009) [20] and Sonawane *et al.*, (2007) [26] also reported similar findings (decline in the sensory parameters) when various dairy products were stored for longer duration at refrigerated temperature.

Conclusion

The physico-chemical analysis of optimized shrikhand during storage shows that carbohydrate, protein, fat, ash, total solids, acidity, antioxidant and crude fiber increased and pH decreased. The sensory evaluation of optimized shrikhand during storage showed that there was continuous decrease in all the sensory parameters. The microbial analysis of optimized shrikhand revealed that there was no growth of coliform during storage, while there was effective growth of lactic acid bacteria which gradually decreased with increase in storage period. Storage study suggested that the shrikhand was acceptable upto 42 days under refrigeration condition at 5-7 ºC. Sharma and Zariwala (1980) [24] have reported that shrikhand stored at 10 ± 3 °C developed off flavour and unpleasant odour in 40 days. The observations obtained in this study are in accordance with the data reported by Sharma and Zariwala (1980) [24] who stated that the titratable acidity and protein were found to increase, while pH was decreased. Upadhyay *et al*., (1985) [27] reported that the storage life of shrikhand as 40 days at 7 ± 2 °C. Similar findings were reported by Patel and Chakraborthy (1985) who studied the keeping quality of shrikhand stored at 10 °C and reported that *shrikhand* can be stored up to 42 days. However, the shrikhand prepared form homogenized milk showed the shelf life of only 30 days at 10 °C (Desai *et al*. 1985). Patel and Abd-El-Salam (1986) [22] reported that shrikhand had shelf life of 35 to 40 days at 8 °C. From this study it can be concluded that lactulose and litchi pulp fortified shrikhand has similar shelf life period as that of shrikhand and addition of litchi pulp and lactulose do not affect storage period of the product.

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