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Storage-induced changes in the physico-chemical characteristics of soy-fortified *shrikhand*#

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Abstract

This study aims to investigate the impact of storage duration on the physico-chemical properties of soy-fortified Shrikhand, a traditional Indian fermented dairy product. The experimental Soy-fortified Shrikhand (SS) and control Shrikhand (CS) was stored under refrigeration temperature at 4±1°C. The physico-chemical analysis viz., fat, protein, ash, pH, moisture, acidity and total solids content for the product was done at 7-day intervals for up to 28 days of storage. Results showed an increase in protein, fat and ash percentages (P<0.05) in both sample types (SS and CS) over the storage period, except for the moisture percentage. With extended storage, the pH levels decreased while the acidity content increased (P<0.05) in both varieties of shrikhand.

Keywords: *Soy milk, shrikhand, physico-chemical properties, soy-fortified shrikhand*

"Food as medicine" philosophy that is the core of functional foods is gaining popularity. Functional foods are food product that provides specific health benefits beyond basic nutrition (Jooyandeh, 2011; Ambili and Singh, 2023). Interest in functional foods has recently increased among consumers due to greater consciousness of health and nutrition as well as the need to cure diseases and also the increasing scientific evidence of their effectiveness (Opara *et al*., 2013; Singh *et al*., 2018; Ambili *et al*., 2023; Snigdha *et al*., 2023).

Soybean (*Glycine max L*.) serves as an outstanding protein source, offering a cost-effective means to address protein deficiency in the diet compared to other agricultural products (Ur-Rehman *et al*., 2007; Hajirostamloo, 2009; Amanze and Amanze, 2011; Ikya *et al*., 2013; Singh and Singh, 2013). Soy milk is designed for consumption by individuals unable to digest milk due

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to reasons such as lactose intolerance, milk protein allergies, or adherence to a vegetarian diet (Božanić *et al*., 2011). The use of lactic acid fermentation can be employed to diminish beany flavours (Wang *et al*., 2006) and reduce anti-nutritional factors like phytic acid in soybean products (Donkor *et al*., 2007).

The word *Shrikhand* derives its name from the Sanskrit word "*Shrikharini*" meaning curd preparation with added sugar, flavouring agent, fruits and nuts. *Shrikhand* is an indigenous semi-soft, sweetish-sour, whole milk delicious and healthful dessert, particularly in western part of India and prepared from lactic fermented curd. It is made with chakka (strained yoghurt/curd) which is finely mixed with sugar and flavoring agents (Nigam *et al*., 2009; Singh *et al*., 2014; Singh and Singh, 2014; Singh *et al*., 2016).

Hence, this study aimed to evaluate the impact of storage on the physico-chemical profile of soy-fortified *shrikhand*, which was developed as a functional food.

Materials and methods

The study was carried out in the Department of Animal Husbandry & Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India.

Raw materials

The standard yoghurt culture (*Lactobacillus delbrueckii ssp.bulgaricus* NCDC 009 and *Streptococcus thermophilus* NCDC 074) were obtained separately from National Collection of Dairy Culture (NCDC), Dairy Microbiology Division at NDRI Karnal, Haryana, India. The rest of the materials including cow milk were procured from the Department of Animal Husbandry & Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India.

Manufacturing of soy milk and soy fortified shrikhand

Soy milk and soy fortified *shrikhand* was manufactured as per the methods suggested by Singh *et al.* (2014).

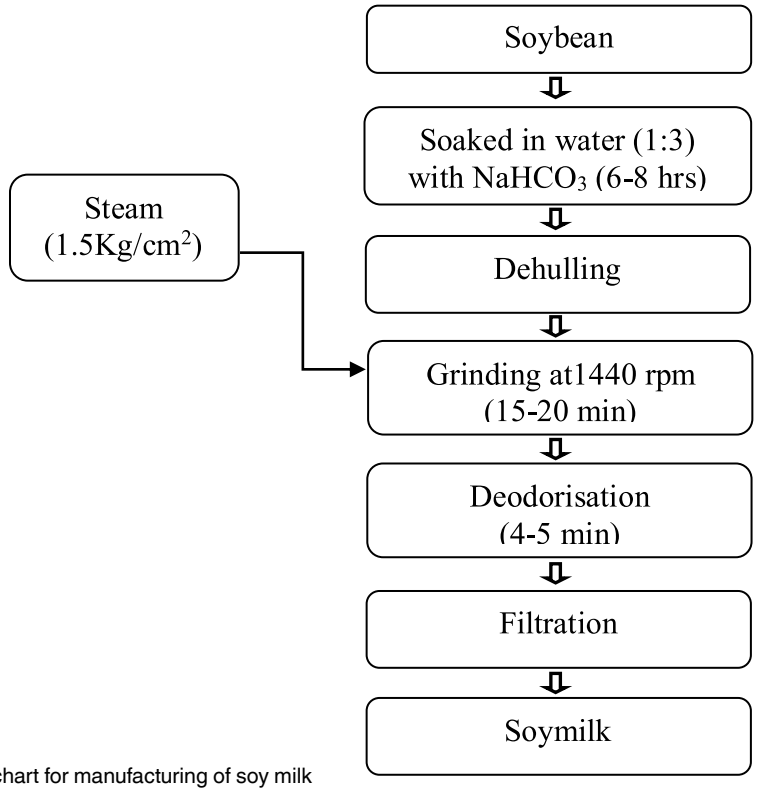

Fig. 1. Flow chart for manufacturing of soy milk

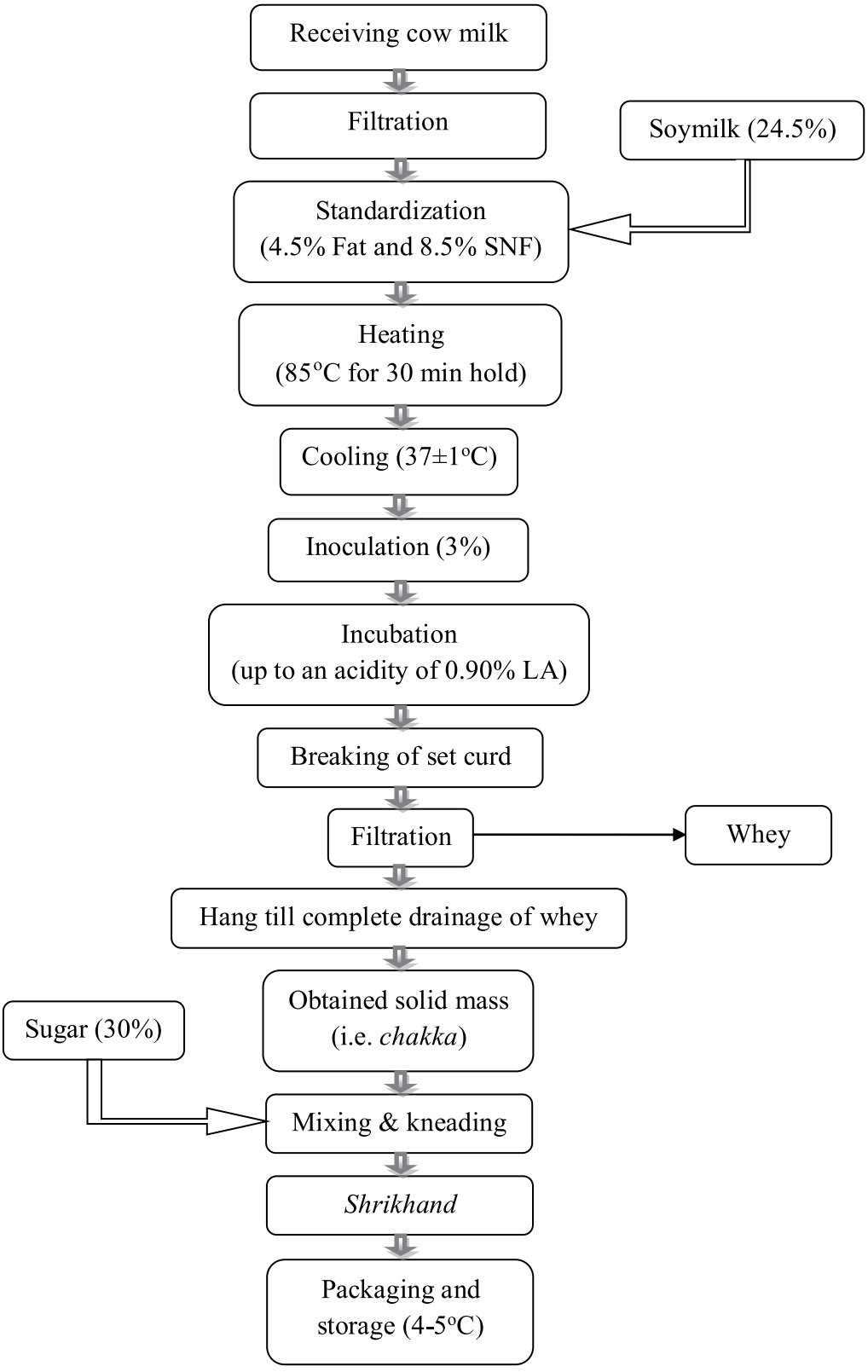

Fig. 2. Flow chart for manufacturing of soy fortified *shrikhand*

Physico-chemical analysis

Proximate analysis of soy milk, control *shrikhand* and soy fortified *shrikhand* (fat, protein, moisture, total solids, ash, pH and titratable acidity) were analysed using standard procedures (AOAC, 2005).

The samples of control *shrikhand* (CS) and soy fortified *shrikhand* (SS) were stored in paper board boxes at 4±1°C temperature. The samples were analysed every 7th day of interval during storage.

Statistical analysis

Data were analysed using Statistical Analysis Software package (SAS, 2006). Duncan's multiple range test (Montgomery, 1997) was used to detect differences between treatment means.

Results and discussion

Chemical composition of soy milk

Soy milk contained 4.2 per cent protein, 2.2 per cent fat, 0.59 per cent ash, 9.89 per cent total solid and 90.11 per cent moisture (Table 1). Liu (1997) have reported similar values (3.6 per cent protein, 2.0 per cent fat, 2.9 per cent carbohydrates, 0.5 per cent ash, 8-10 per cent total solids and 90-92 per cent moisture) in soy milk. Ur-Rehman *et al.* (2007) have also reported similar values (4.03 per cent protein, 2.28 per cent fat, 0.58 per cent ash, 10.40 per cent total solids, 89.60 per cent moisture and pH 6.74) in soy milk.

Proximate composition of shrikhand

The proximate composition of *shrikhand* as control (CS) and soy fortified *shrikhand* (SS) was evaluated and represented in Table (2).

Physico-chemical changes during refrigerated storage

The physico-chemical attributes for CS and SS were investigated up to the 28th day of refrigerated storage (4±1°C). The findings related to fat, protein, ash, pH, moisture, acidity and total solids content were assessed (Fig.1 to 7).

Fat

The percentage of fat varied from 8.62±0.017 to 8.93±0.017 per cent in samples SS and from 8.59±0.003 to 8.83±0.0058 per cent in CS samples (Table 3; Fig. 1). Figure (1) makes it abundantly evident that the fat percentage of the samples SS increased significantly (P<0.05) up until the 21st day of storage, after which it was non-significant (P>0.05) as the sample storage periods extended. As the storage periods continued, the fat percentage significantly increased (P<0.05) in CS samples, while the difference

Table 1. Proximate composition of soy milk

	Fat (%)	Protein (%)	Ash (%)	Total Solid (%)	Moisture (%)
Soy milk	2.2±0.1	4.2±0.21	0.59±0.03	9.89±0.06	90.11±0.06

Table 2. Compositional properties of optimised soy fortified *shrikhand* (SS) and control *shrikhand* (CS)

Treatments	Fat (%)	Protein (%)	Ash (%)	Total Solid (%)	Moisture (%)	pH	Acidity
CS	8.59± 0.0033^{a}	8.51± 0.016^{a}	0.78±0^{a}	58.34± 0.0058^{a}	42.66± 0.0058^{a}	4.68± 0.0033^{a}	1.21± 0.0033^{a}
SS	8.62± 0.017^{a}	10.14± 0.01^{b}	0.80±0^{b}	60.26± 0.075^{b}	39.74± 0.075^{b}	4.39±0^{b}	1.28± 0.0033^{b}

Values bearing different superscripts (a, b, c) in a column differ significantly (Duncan test, P<0.05)

CS=Control *shrikhand* samples

SS= Soy fortified *shrikhand* samples (optimised level)

Table 3. Effect of storage on chemical characteristics of soy fortified *shrikhand* (SS) and control *shrikhand* (CS) samples

Storage	Fat		Protein		Moisture		TS		Ash		pH		Acidity	
	SS	CS	SS	CS	SS	CS	SS	CS	SS	CS	SS	CS	SS	CS
0 Day	8.62± 0.017^{aAB}	8.59± 0.0033^{aB}	10.14± 0.01^{aA}	8.51± 0.016^{aB}	39.74± 0.075^{aA}	41.66± 0.0058^{aB}	60.26± 0.075^{aA}	58.34± 0.0058^{aB}	0.80± 0^{aA}	0.78± 0^{aB}	4.39± 0^{aA}	4.68± 0.0033^{aB}	1.28± 0.0033^{aA}	1.21± 0.0033^{aB}
7th Day	8.73± 0.017^{bC}	8.64± 0.0066^{bA}	10.23± 0.017^{bC}	8.64± 0.010^{bD}	39.42± 0.0067^{bC}	41.05± 0.065^{bD}	60.58± 0.0067^{bC}	58.95± 0.065^{bD}	0.80± 0^{aC}	0.78± 0^{aD}	4.36± 0.0033^{bC}	4.60± 0.0033^{bD}	1.30± 0.0033^{bC}	1.23± 0.0033^{bD}
14th Day	8.82± 0.017^{cD}	8.69± 0.0058^{cE}	10.33± 0.017^{cE}	8.75± 0^{cF}	39.32± 0.0088^{bE}	40.37± 0.012^{cF}	60.68± 0.0088^{bE}	59.63± 0.012^{cF}	0.81± 0^{aE}	0.79± 0^{aF}	4.34± 0.0033^{cE}	4.54± 0.0033^{cF}	1.34± 0.0033^{cE}	1.26± 0^{cF}
21st Day	8.90± 0^{dF}	8.73± 0.012^{dG}	10.43± 0.016^{dG}	8.8± 0.0033^{cH}	39.083± 0.044^{cG}	49.88± 0.0058^{dH}	60.92± 0.044^{cG}	60.12± 0.0058^{dH}	0.81± 0^{aG}	0.80± 0^{aH}	4.31± 0.0033^{dG}	4.42± 0.0033^{dH}	1.38± 0.0058^{dG}	1.29± 0.0033^{dC}
28th Day	8.93± 0.017^{dH}	8.83± 0.0058^{eI}	10.57± 0.033^{eI}	8.91± 0.0033^{dJ}	38.68± 0^{dI}	39.46± 0.0033^{eC}	61.35± 0^{dI}	60.54± 0.0033^{eC}	0.81± 0^{aI}	0.80± 0^{aJ}	4.30± 0.0033^{eI}	4.38± 0.0058^{eA}	1.41± 0.0033^{eH}	1.35± 0.0058^{eI}

Values bearing different small superscripts (a, b, c) in a column differ significantly (Duncan test, P<0.05)

Values bearing different capital superscripts (A, B, C) in between column differ significantly (Duncan test, P<0.05)

CS=Control *shrikhand* samples,

SS= Soy fortified *shrikhand* samples (optimised level)

between SS and CS samples at 0 day and on the 7th day was not significant (P>0.05). The rise in fat levels is likely attributed to moisture loss in the samples during storage. Nigam *et al*. (2009) reported findings consistent with our investigation when studying refrigerated storage of papaya-fortified *shrikhand*. Similarly, studies on apple pulp and *Celosia argentea* fortified *shrikhand* (Kumar *et al.,* 2011) and soy cake-fortified *burfi* (Verma, 2013) reported an increase in fat content during storage. Snigdha *et al*. (2023) also noted a steady increase in fat content in herbal paneer spread under storage. On the other hand, Kumar (2013) reported contrasting results in a study on herbal ice cream, where the fat content decreased during storage.

Protein

The values presented in Table 3 (Fig. 2) clearly depicts that as the storage periods increases the protein content also increased (P<0.05) in both type of samples. The protein content of samples CS increased significantly (P<0.05) during storage except at 14th and 21st day. The protein content was higher in the sample SS than CS (Figure 2), which may me due to presence of higher protein content in soyabeen. Consistent with our findings, Nigam *et al.* (2009) reported an increase in protein content in papaya-fortified *shrikhand* under cold storage conditions. Similar outcome was observed by Sonawane *et al.* (2007) and Verma (2013) in their studies on the protein content changes during storage of strawberry fortified *shrikhand*, and soy cake enriched *burfi*, respectively.

Ash

The average ash content in *shrikhand* samples at 0th day was 0.80±0.0 per cent for SS and 0.78±0.0 per cent for CS samples (Table 3; Fig. 3). The difference in the values were significant (P<0.05). These values slightly increased as storage periods prolonged, but the differences in those values were not significant. The ash content in SS group was 1.2 per cent higher than CS group at 28th day of storage whereas the difference in the value at 0 day was 2.6 per cent. The findings of Nigam *et al.* (2009), Kumar *et al.* (2011), Kumar (2013) Verma (2013) and Snigdha *et al.* (2023) studied the effect of refrigerated storage on various dairy products were similar with our findings.

pH

The pH of the samples was the highest when tested at day 0 in both the groups (SS and CS) (Table 3; Fig. 6). These values clearly

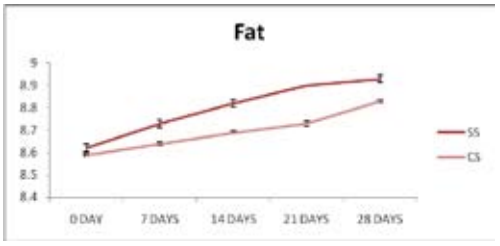

Fig. 1. Changes in fat per cent of *shrikhand* during storage

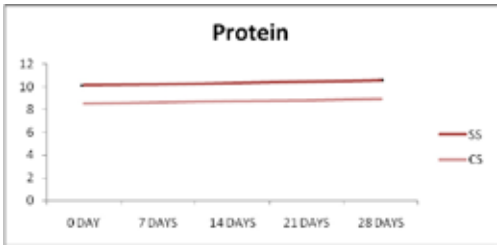

Fig. 2. Changes in protein per cent of *shrikhand* during storage

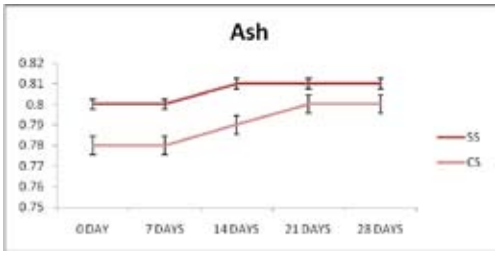

Fig. 3. Changes in ash per cent of *shrikhand* during storage

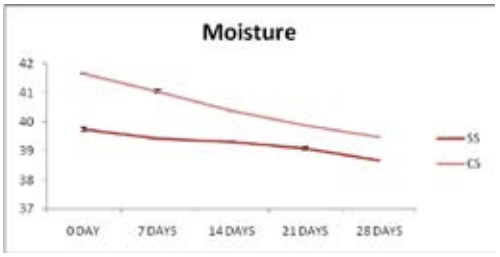

Fig. 4. Changes in moisture per cent of *shrikhand* during storage

depicts that the pH content of both product (SS and CS) was decreasing significantly (P<0.05) as the storage periods increased. The differences in the intensity of pH deterioration between SS and CS samples were significant (P<0.05) except at 0 day of storage in SS and at 28th day of CS sample. This may be due to increase in microbial activity during storage. Kumar *et al.* (2011), Kumar (2013) and Verma (2013) had also reported the same during storage for a variety of dairy products.

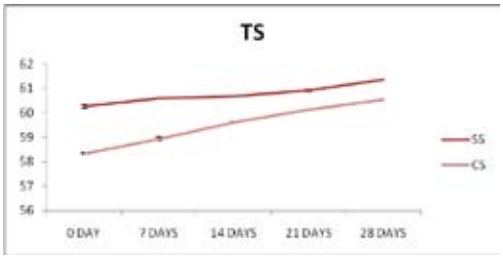

Fig. 5. Changes in TS content of *shrikhand* during storage

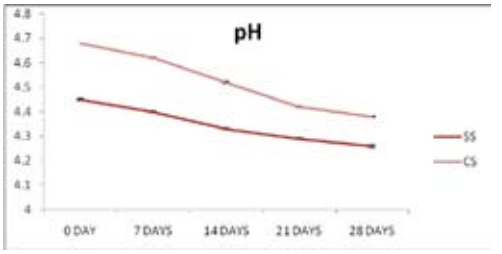

Fig. 6. Changes in pH content of *shrikhand* during storage

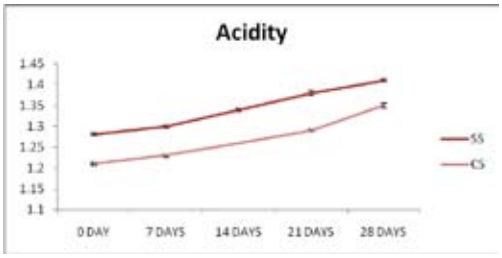

Fig.7. Changes in acidity content of *shrikhand* during storage

Moisture

The moisture content varied from 39.74±0.075% to 38.68±0% in SS samples and from 41.66±0.005839% to 0.46±0.0033% in the CS samples during storage (Table 3; Fig. 4). The moisture content of the sample CS decreases significantly (P<0.05) during storage. The figure (4) clearly depicts that the average moisture content was higher in the samples CS than SS. The difference in the values between SS and CS samples were not significant (P>0.05) except at 7th day for SS and 28th day for CS sample. There was an inverse relationship between moisture content and storage periods. The findings of Sonawane *et al.* (2007), Nigam *et al.* (2009), Kumar *et al.* (2011), Verma (2013) and Snigdha *et al.* (2023) were at par with the results of present investigation.

Acidity (% Lactic Acid)

The acidity of *shrikhand* samples varied from 1.28±0.0033 to 1.41±0.0033 per cent in SS and from 1.21±0.0033 to 1.35±0.0058 per cent in CS groups during storage (Table 3; Fig. 7). The acidity content in both the groups significantly (P<0.05) inceased as the storage periods increased (Fig. 7). There was a significant (P<0.05) increase in acidity content for both SS and CS products during storage. The differences in the values between SS and CS samples during storage were significant (P<0.05) except at 7th day in SS and at 21st day in CS samples. This may be due to growth of microorganisms responsible for spoilage of milk and milk products. Similar findings regarding the increase in acidity (% lactic acid) during extended refrigerated storage of a variety of dairy products were also reported by Jain (2003), Sonawane *et al*. (2007), Bhat *et al.* (2010), Kumar *et al*. (2011), and Verma (2013).

Total solid (TS)

We observe a steady rise in the total solids content of the SS sample over prolonged storage times, showing statistical significance (P<0.05), apart from the observations made on the 7th and 14th days (Table 3 and Fig. 5). The TS content varied from 60.26±0.075 to 61.35±0 per cent for SS and from 58.34±0.0058 to 60.54±0.0033 per cent for CS samples during storage (upto 28 days) at refrigeration temperature. The difference between SS and CS sample was significant (P<0.05) except in between at 7th day of SS and 28th day of CS sample during storage. The TS content in the samples CS increases significantly (P<0.05) as the storage duration increased. The present results validates the findings of Nigam *et al.* (2009), Kumar *et al.* (2011) and Snigdha *et al.* (2023) when dairy products were stored for longer duration.

Conclusion

The highest protein level in groups SS (10.57± 0.033%) and CS (8.91± 0.0033%) was recorded at 28th day of storage. The increase in protein was high (P<0.05) in both types of samples during storage except from 14th to 21st days in CS samples. The fat per cent increased (P<0.05) in both types of *shrikhand* except from 21st to 28th day of SS samples. At 0 day, the difference in the values between SS and CS samples were not significant. The ash content in all the samples apparently increased as storage periods increased. The ash content in the SS samples were very high (P<0.05) than CS. As the storage periods prolonged, the pH decreased and acidity content increased (P<0.05) in both types of *shrikhand*. The comparison between SS and CS samples reveals significant differences (P<0.05) in their composition during storage. Specifically, the moisture percentage was significantly (P<0.05) higher in CS samples compared to SS samples throughout the storage period. However, as storage time increased, both groups experienced a decrease in moisture content, with the exception of the period from 7th to 14th day in SS samples, where this trend was not observed. This observation suggests an inverse relationship between moisture content and total solids in the samples.

Given these findings, we can conclude that the content of protein, fat, ash, acidity and total solids were significantly (P<0.05) higher in SS samples compared to CS samples, indicating a richer nutrient profile in SS. Conversely, moisture content and pH values were lower in SS samples, highlighting a difference in preservation. These differences underline the impact of storage conditions on the chemical composition of the products, affecting their quality over time.

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Conflict of interest

The authors declare that they have no conflict of interest.

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