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# Sensory, physico-chemical, hardness and microbiological changes in whey protein concentrate based protein-rich dairy spread during storage

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**Abstract**
Protein-rich dairy spread is a functional dairy product having higher nutritional benefits with enhanced protein content. The spread is formulated using a combination of butter, Greek yoghurt and whey protein concentrate (WPC-80) to achieve a desirable texture and improved spreadability at room temperature. The developed spread was analyzed for sensory, physico-chemical, hardness and microbial quality during storage. The storage study was conducted at 7±2 °C for 90 days using polypropylene cups with continuous monitoring at regular interval of 10 days. Sensory evaluation indicated a significant difference ($P$<0.05) in flavour, body and texture, spreadability, and overall acceptability between the control ($T_1$) and the protein-rich dairy spread ($T_2$). Physicochemical analysis showed a decrease in moisture content, hardness, and water activity over time, whereas acidity, free fatty acid (FFA), peroxide value, tyrosine value, oiling off and wheying off increased. In microbial analysis, both samples $T_1$ and $T_2$, had visible mold growth on the 100$^{th}$ day of storage at 7±2 °C.

**Keywords:** Protein-rich dairy spread, white butter, WPC-80

## Introduction
Dairy spreads are increasingly gaining attention as convenient, value-added products that combine desirable flavour with improved spreadability at refrigeration temperature. Unlike butter, which becomes firm at low temperatures and is prone to oiling-off at higher temperatures, dairy spreads maintain a softer, more uniform texture during storage. This has encouraged the development of functional and low-fat spread formulations (Prajapati, 1988; Deshmukh *et al*., 2002) [17, 2].

Protein enrichment in spreads is of particular interest due to current consumer demand for healthier alternatives and nutritional recommendations i.e. for healthy men and women, the recommended dietary allowance (RDA) is 0.83g protein/kg body weight (ICMR-NIN, 2024) [8]. Whey protein concentrate (WPC) is widely recognized for its high biological value, favourable amino acid profile, and excellent functional properties such as emulsification, gelling, and water binding, making it suitable for improving the structural and nutritional quality of dairy-based spreads (Walstra *et al*., 2005; Suthar *et al*., 2017; Smithers, 2008) [27, 22, 20]. Similarly, Greek yoghurt, a concentrated fermented milk product, contributes additional protein and enhances viscosity and creaminess, making it a compatible ingredient in spread formulations (Tamime & Robinson, 2007; Campos *et al*., 2018) [28, 29].

Protein-rich dairy spreads during refrigerated storage may influence moisture distribution, acidity development, proteolysis, lipid stability, texture, and microbial quality factors that collectively define the product's acceptability and shelf life. Earlier studies on dairy spreads and related products have highlighted that protein-fat interactions, fat crystallization behaviour, and moisture migration can considerably alter their physicochemical and rheological attributes over time (Mishra *et al*., 2019; Pavithra *et al*., 2024) [13, 15]. Given the increasing market interest in healthier spreads and the behaviour of protein-rich dairy spreads during storage, the present study was undertaken to develop a protein-rich dairy spread using WPC-80 and Greek yoghurt, and to evaluate the effect of refrigerated storage (7±2 °C) on its physicochemical, textural, and microbiological properties.

## Materials and Methods

### Preparation of Protein-Rich Dairy Spread

The protein-rich dairy spread was prepared using 38.10% white butter, 18.10% WPC-80, and 10% Greek yogurt, along with common salt, $Na_2HPO_4$, CMC, soya lecithin, annatto colour, and starter distillate, as per the optimized procedure described in Prajapati *et al*. (2025) [16]. The product was hot filled into polypropylene cups at 50 °C and the filled cups were stored at 7±2 °C.

### Sensory Analysis of Spread

Sensory evaluation was conducted by a trained panel of seven judges using a 9-point hedonic scale (Meilgaard *et al*., 1999) [12]. Judges assessed the spread's flavour, body and texture, colour and appearance, spreadability, and overall acceptability. Sensory responses were recorded on evaluation scorecards, and panelists used saline water for palate cleansing between samples.

### Physico-Chemical Analysis of Spread

The physicochemical properties of the protein-rich dairy spread were evaluated using established methods. The moisture content of spread was determined as per the method described in FSSAI manual (2022) [3] for butter. Titratable acidity was determined as per the FSSAI manual (2022) [3], while Free fatty acid (FFA) content was analyzed following the procedure of Thomas *et al*. (1954) [24], and peroxide value was estimated according to AOAC (1981). Protein breakdown, assessed in terms of tyrosine content, was evaluated using the method described by Hull (1947) [7]. Water activity ($a_w$), crucial for microbial stability, was measured at 25 °C using a water activity probe. Oiling off and wheying off were determined using the method of Sethi (2017) [18], where 5g of spread was placed on pre-weighed Whatman No. 1 filter paper and stored at 20±1 °C for 48 hours, followed by a short cooling period at 8 °C. The absorbed oil and moisture were quantified after drying the filter paper at 100±2 °C for 3 hours.

### Hardness of Spread

The hardness of the spread was assessed using a cone penetrometer as per Verma (1996) [26]. The spread, set at 4°C overnight, was subjected to a penetration test, where a cone assembly freely penetrated the sample for 5 seconds. The penetration depth (0.1 mm units) was recorded, and hardness (kg/cm²) was calculated using the standard equation:

$$\text{Hardness (kg/cm}^2) = \frac{G \times 10^{-3}}{\{h\pi (\tan\alpha / \cos\alpha)(h + 2r / \tan\alpha) + \pi 2r\} \times 10^{-4}}$$

Where,
G = Weight of cone assembly
h = Depth of penetration (mm)
α = Half of cone angle
r = Radius of flat top of cone

### Microbiological Analysis of Spread

Microbial safety was assessed by determining aerobic plate count (APC), coliform count, and yeast and mold count. Spread samples (11g) were diluted in 99 mL citrate buffer (1:10 dilution), with subsequent serial dilutions prepared. APC was determined as per FSSAI (2023) [4], coliform count was evaluated using IS: 5401 (Part I, 2002) [9], and yeast and mold counts were determined following IS: 5403 (1999).

### Statistical Analysis

The data related to physico-chemical, rheological, sensory and microbial quality of protein-rich dairy spread samples were analysed using Completely Randomized Design (Steel and Torrie, 1980) [21].

## Results and Discussion

### Changes in the Sensory Analysis of Spread During Storage

The flavour scores of the dairy spread during refrigerated storage (7±2 °C) is presented in Table 1. The initial flavour scores of $T_1$ and $T_2$ were 8.03 and 8.20, which declined to 6.27 and 7.03, respectively, by the 90$^{th}$ day. Statistical analysis revealed significant effects ($p$<0.05) of treatment, storage period, and their interaction on flavour scores. Throughout storage, $T_2$ maintained significantly higher ($p$<0.05) flavour scores than $T_1$, and both products remained acceptable (score >6.0) until day 90. Verma (1996) [26] noted a reduction in flavour scores of low-fat spreads over 30 days, while Patange (2011) [14] also found a steady decline in ghee-based low-fat spreads during 77 days of storage. Comparable reductions were also documented in chakka spreads by Tambe (2020) [23] and omega-enriched spreads by Hamid (2023) [6]. In the present investigation, the declining flavour scores appear associated with progressive proteolysis and lipolysis, as supported by increasing tyrosine values and FFA content (Table 2).

The body and texture scores of $T_1$ and $T_2$ (Table 1) declined from 8.17 to 6.04 and from 8.29 to 6.66, respectively, by the 90$^{th}$ day of refrigerated storage. Treatment, storage period, and their interaction exerted significant effects ($p$<0.05). The protein-rich spread $T_2$ consistently received higher scores ($p$<0.05) across all storage intervals, and both samples remained within the acceptable range (score >6.0). A sharper reduction in short-period studies involving WPC-incorporated chakka spreads were seen in Tambe (2020) [23]. Omega-enriched spreads studied by Hamid (2023) [6] exhibited comparable behavior as well. In the current study, reduced body and texture coherence may be linked to weakening of the protein matrix, also reflected in rising tyrosine values over storage (Table 2).

The colour and appearance scores (Table 1) showed a gradual decline from 8.27 to 6.74 for $T_1$ and from 8.21 to 7.09 for $T_2$ by day 90. Treatment and storage period significantly influenced the scores ($p$<0.05), while their interaction remained non-significant. Both samples retained acceptable colour and appearance (score >6.0) throughout the testing period. Comparable observations have been previously reported. In low-fat spreads, Verma (1996) [26] documented slight colour deterioration within 30 days, whereas Patange (2011) [14] also observed reductions in ghee-based spreads. Tambe (2020) [23] reported more reduction within 8 days in WPC-based chakka spreads. Omega-enriched spreads examined by Hamid (2023) [6] showed consistent reductions during 35 days. The observed decline in the present study may be attributed to pigment breakdown and mild proteolytic effects during refrigeration.

Spreadability scores (Table 1) declined from 8.23 to 5.93 in $T_1$ and from 8.33 to 6.59 in $T_2$ over 90 days. Treatment, storage period, and their interaction showed significant effects ($p$<0.05). $T_2$ maintained higher spreadability scores

throughout, and both samples remained acceptable (score >6.0) except $T_1$, on 90th day. Studies on similar products report comparable declines. Verma (1996) [26] identified decreasing spreadability in low-fat spreads during 30 days, and Patange (2011) [14] also saw a similar decline in ghee-based spreads. Hamid (2023) [6] observed comparable declining spreadability in omega-enriched spreads as well. In the current study, the drop in spreadability likely relates to changes in micro texture and gradual moisture loss during storage.

The overall acceptability scores (Table 1) of the spreads decreased from 8.09 to 6.22 in $T_1$ and from 8.23 to 6.92 in $T_2$ over 90 days at 7±2 °C. Treatment, storage duration, and their interaction exhibited significant effects ($p$<0.05). $T_2$ consistently outperformed $T_1$, and both remained acceptable (score >6.0) up to 90 days. Verma (1996) [26] observed a lesser reduction in overall acceptability of low-fat spreads over 30 days, while Patange (2011) [14] reported a more gradual decrease during a 77-day study. More pronounced decreases over short storage was recorded by Tambe (2020) [23] in WPC-based spreads. Hamid (2023) [6] also reported declining overall acceptability in omega-enriched spreads during storage. In the present study, $T_2$ consistently showed superior acceptability, indicating that incorporation of WPC-80 and Greek yoghurt enhanced the sensory integrity and stability of the protein-rich dairy spread.

**Table 1:** Changes in the sensory attributes of dairy spread during storage

| **Flavour score (out of 9)** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Spread Sample** | **Storage day** | | | | | | | | | | **T** |
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | |
| $T_1$ | 8.03 | 7.99 | 7.96 | 7.84 | 7.66 | 7.4 | 7.17 | 6.98 | 6.73 | 6.27 | 7.4 |
| $T_2$ | 8.2 | 8.13 | 8.1 | 8.05 | 8 | 7.91 | 7.76 | 7.52 | 7.24 | 7.03 | 7.8 |
| P | 8.12 | 8.06 | 8.03 | 7.95 | 7.83 | 7.66 | 7.46 | 7.25 | 6.99 | 6.65 | - |
| CD (0.05) | T = 0.07 P = 0.15 T×P = 0.21 | | | | | | | | | | |
| **Body and texture score (out of 9)** | | | | | | | | | | | |
| **Spread Sample** | **Storage day** | | | | | | | | | | **T** |
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | |
| $T_1$ | 8.17 | 8.1 | 8.01 | 7.94 | 7.7 | 7.32 | 7.16 | 6.93 | 6.55 | 6.04 | 7.39 |
| $T_2$ | 8.29 | 8.21 | 8.11 | 8.07 | 8 | 7.83 | 7.36 | 7.23 | 7.13 | 6.66 | 7.69 |
| P | 8.23 | 8.16 | 8.06 | 8.01 | 7.85 | 7.57 | 7.26 | 7.08 | 6.84 | 6.35 | - |
| CD (0.05) | T = 0.08 P = 0.18 T×P = 0.26 | | | | | | | | | | |
| **Colour and apperance score (out of 9)** | | | | | | | | | | | |
| **Spread Sample** | **Storage day** | | | | | | | | | | **T** |
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | |
| $T_1$ | 8.27 | 8.21 | 8.08 | 8.02 | 7.91 | 7.81 | 7.66 | 7.21 | 6.95 | 6.74 | 7.68 |
| $T_2$ | 8.21 | 8.19 | 8.12 | 8.05 | 7.97 | 7.89 | 7.75 | 7.54 | 7.26 | 7.09 | 7.81 |
| P | 8.24 | 8.2 | 8.1 | 8.04 | 7.94 | 7.85 | 7.71 | 7.38 | 7.11 | 6.91 | - |
| CD (0.05) | T = 0.10 P = 0.22 T×P = NS | | | | | | | | | | |
| **Spreadability score (out of 9)** | | | | | | | | | | | |
| **Spread Sample** | **Storage day** | | | | | | | | | | **T** |
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | |
| $T_1$ | 8.23 | 8.18 | 8.13 | 7.99 | 7.87 | 7.42 | 7.2 | 6.98 | 6.52 | 5.93 | 7.45 |
| $T_2$ | 8.33 | 8.26 | 8.22 | 8.1 | 8.02 | 7.82 | 7.62 | 7.39 | 7.15 | 6.59 | 7.75 |
| P | 8.28 | 8.22 | 8.18 | 8.05 | 7.94 | 7.62 | 7.41 | 7.19 | 6.83 | 6.26 | - |
| CD (0.05) | T = 0.10 P = 0.22 T×P = 0.31 | | | | | | | | | | |
| **Overall acceptability score (out of 9)** | | | | | | | | | | | |
| **Spread Sample** | **Storage day** | | | | | | | | | | **T** |
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | |
| $T_1$ | 8.09 | 8.05 | 8 | 7.89 | 7.72 | 7.43 | 7.21 | 6.99 | 6.69 | 6.22 | 7.43 |
| $T_2$ | 8.23 | 8.17 | 8.13 | 8.06 | 8 | 7.88 | 7.7 | 7.48 | 7.22 | 6.92 | 7.78 |
| P | 8.16 | 8.11 | 8.06 | 7.98 | 7.86 | 7.66 | 7.46 | 7.24 | 6.95 | 6.57 | - |
| CD (0.05) | T = 0.07 P = 0.16 T×P = 0.22 | | | | | | | | | | |

$T_1$ = Control spread, $T_2$ = Protein-rich dairy spread
The values are mean; n=3.
T = Treatment mean, P = Period mean
NS = non-significant.

## Changes in the Physico-Chemical Properties of Spread During Storage

The moisture content of dairy spread samples ($T_1$ and $T_2$) showed a gradual decline over the 90-day storage period at 7±2 °C. Initially, $T_1$ and $T_2$ had moisture contents of 47.99% and 46.87%, respectively, which decreased significantly ($p$<0.05) to 47.11% and 45.91% by the 90$^{th}$ day (Table 2). This decline was attributed to evaporative losses during storage in PP cups. Similar trends have been reported in avocado pulp based functional spread (Sethi, 2017) [18] where moisture loss occurred gradually during storage from 44.94 to 42.88% on 90$^{th}$ day of storage at 5±1 °C in PP cups. The titratable acidity of the dairy spread samples ($T_1$ and $T_2$) increased progressively during the 90-day storage at 7±2 °C. Initially, $T_1$ and $T_2$ recorded acidity values of 0.310% and 0.542% LA, respectively, which significantly increased

($p$<0.05) to 0.389% and 0.649% LA by the end of storage (Table 2). The higher acidity observed in $T_2$ throughout the storage period was likely due to the incorporation of Greek yogurt, which itself had an average acidity of 1.16% LA. The increase in acidity during storage could also be attributed to lactic acid production and release of acidic amino acids by proteolytic enzymes, as well as the rise in microbial load over time. Similar observations were reported in mixed fat spread with avocado pulp (Sethi, 2017) [18], where a gradual increase in titratable acidity was noted during refrigerated storage from 0.38 to 0.94%LA on 90th day of storage at 5±1 °C in PP cups and in functional creamy vegetable spread Tuyakbayeva *et al*. (2022) [25] from 2 to 2.4%LA on 90$^{th}$ day of storage at 4±2 °C.

The free fatty acid (FFA) content of dairy spread samples ($T_1$ and $T_2$) showed a gradual increase during the 90-day storage at 7±2 °C. The initial FFA values of $T_1$ and $T_2$ were 0.260% and 0.344% oleic acid, respectively, which increased significantly ($p$<0.05) to 0.312% and 0.403% oleic acid by the 90$^{th}$ day (Table 2). $T_2$ consistently exhibited higher FFA levels compared to $T_1$, which can be attributed to the inclusion of Greek yogurt, known to contain free fatty acids and active lipolytic cultures. The increase in FFA content during storage may be due to triglyceride hydrolysis by microbial lipases and the action of yeast and mould, contributing to fat breakdown. Similar trends of increasing FFA during storage were observed in other studies, Patange (2011) [14] observed an increase in FFA levels during the storage of ghee-based low-fat spread 23.6 to 29.4 µequivillqent/g on storage at 77$^{th}$ day at 5±1 °C and in mixed fat spreads with avocado pulp (Sethi, 2017) [18] from 0.098 to 0.484%oleic acid on 90$^{th}$ day indicating the common occurrence of lipid hydrolysis over time in refrigerated dairy-based spreads.

The peroxide value of dairy spread samples ($T_1$ and $T_2$) showed a steady increase during 90 days of storage at 7±2 °C. Initially, both $T_1$ and $T_2$ had peroxide values of 0.079 meq of $O_2$/kg fat, which increased to 0.147 and 0.144 meq of $O_2$/kg fat, respectively, by the end of storage (Table 2). Although the peroxide values increased over time, no significant difference ($p$>0.05) was observed between the two treatments, indicating that the protein enrichment in $T_2$ did not adversely affect oxidative stability. The rise in peroxide value may be attributed to the oxidative degradation of lipids, forming primary oxidation products such as peroxides and hydroperoxides. Shakerardekani *et al*. (2015) [19] reported a marked increase in peroxide values during storage of pistachio spreads from 0.05 to 4.90 meq of $O_2$/kg by 25$^{th}$ day at elevated temperature of 60 °C.

The tyrosine value of dairy spread samples ($T_1$ and $T_2$) exhibited a consistent increase throughout the 90-day storage period at 7±2 °C. Initially, $T_1$ and $T_2$ recorded tyrosine values of 12.05 and 20.76 µg of tyrosine/5 ml filtrate, respectively, which increased significantly ($p$<0.05) to 17.53 and 28.82 µg by the 90th day (Table 2). $T_2$ consistently showed higher tyrosine levels than $T_1$, likely due to the inclusion of Greek yogurt, which contains proteolytic enzymes that enhance protein hydrolysis during storage. Similarly, Kharab *et al*. (2022) [11] showed that even fortified low-fat spreads with preservatives exhibited notable increases during storage from 53.57 to 78 µg/g on 90$^{th}$ day. The observed rise in tyrosine value during storage reflects ongoing proteolysis, resulting from both heat stable enzymes present in the ingredients and microbial proteolytic activity.

The water activity ($a_w$) of dairy spread samples ($T_1$ and $T_2$) exhibited a gradual decrease during the 90-day storage period at 7±2 °C. Initially, $a_w$ values were 0.926 for $T_1$ and 0.922 for $T_2$, which declined to 0.912 and 0.906, respectively, by the end of the storage period (Table 2). Although changes during storage were non-significant ($p$>0.05), the difference between treatments was statistically significant ($p$<0.05). This decline in water activity is indicative of moisture loss or water binding within the matrix, reducing the amount of free water available for microbial or enzymatic activity. This trend is consistent with earlier findings. Gulzar *et al*. (2015) [5] observed a significant reduction in water activity from 0.76 overtime in spreads due to surface moisture loss over time. The decline in $a_w$ in the present study aligns with the observed reduction in moisture content (Table 2), suggesting that water loss and increased water-binding interactions contributed to the lower aw values. The decrease in water activity during storage may thus enhance microbial stability and extend shelf life without adversely affecting product quality.

The oiling off of dairy spread samples ($T_1$ and $T_2$) exhibited a gradual increase over the 90-day storage period at 7±2 °C (Table 2). Initially, oiling off values were 2.71% for $T_2$ and 3.18% for $T_1$, which increased significantly ($p$<0.05) to 3.11% and 3.67%, respectively, by the 90$^{th}$ day. The increase in oiling off during storage can be attributed to emulsion breakdown, temperature-induced phase separation, and the destabilization of fat-water interfaces over time. Notably, the protein-rich dairy spread ($T_2$) consistently showed lower oiling off values than the control ($T_1$), which may be due to the improved emulsifying properties imparted by WPC-80. Whey protein concentrates are known to enhance emulsion stability by forming interfacial films around fat globules, thus reducing oil separation during storage. Further, Balasaheb *et al*. (2019) [1] recorded oiling off increases in low-fat spreads with strawberry powder and synthetic preservatives from 3.73% to 3.88% and 3.70% to 4.04%, respectively on 100$^{th}$ day of storage in plastic cups at 5±1 °C.

The wheying off of dairy spread samples ($T_1$ and $T_2$) showed a gradual increase over the 90-day storage period at 7±2 °C (Table 2). Initially, wheying off values were 5.19% for $T_2$ and 5.36% for $T_1$, which increased significantly ($p$<0.05) to 5.98% and 6.04%, respectively, by the 90$^{th}$ day. The increase in wheying off is attributed to protein aggregation and syneresis, leading to moisture separation from the protein matrix during storage. The protein-rich dairy spread ($T_2$) consistently exhibited slightly lower wheying off compared to the control ($T_1$), likely due to the water-binding and stabilizing properties of WPC-80. Whey protein concentrate forms a gel matrix that retains moisture and reduces phase separation. Balasaheb *et al*. (2019) [1] noted wheying off rising from 7.56% to 7.93% in spreads with strawberry powder, and from 7.58% to 7.83% in those with synthetic preservatives during extended storage at 5±1 °C on 100$^{th}$ day.

**Table 2:** Changes in the physico-chemical properties of dairy spread during storage

| Spread Sample | Storage day | | | | | | | | | | T |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | |
| **Moisture (%)** | | | | | | | | | | | |
| $T_1$ | 47.99 | 47.9 | 47.82 | 47.74 | 47.65 | 47.57 | 47.49 | 47.4 | 47.28 | 47.11 | 47.59 |
| $T_2$ | 46.87 | 46.79 | 46.72 | 46.6 | 46.49 | 46.39 | 46.2 | 46.15 | 46.05 | 45.91 | 46.42 |
| P | 47.43 | 47.35 | 47.27 | 47.17 | 47.07 | 46.98 | 46.85 | 46.78 | 46.66 | 46.51 | - |
| CD (0.05) | T = 0.10 P = 0.23 T×P = NS | | | | | | | | | | |
| **Titrtable acidity (%LA)** | | | | | | | | | | | |
| $T_1$ | 0.31 | 0.313 | 0.313 | 0.315 | 0.317 | 0.317 | 0.335 | 0.353 | 0.371 | 0.389 | 0.334 |
| $T_2$ | 0.542 | 0.543 | 0.553 | 0.559 | 0.569 | 0.577 | 0.606 | 0.623 | 0.631 | 0.649 | 0.585 |
| P | 0.426 | 0.428 | 0.433 | 0.437 | 0.443 | 0.447 | 0.471 | 0.488 | 0.501 | 0.519 | - |
| CD (0.05) | T = 0.007 P = 0.015 T×P = NS | | | | | | | | | | |
| **Free fatty acids (% oleic acid)** | | | | | | | | | | | |
| $T_1$ | 0.26 | 0.263 | 0.263 | 0.271 | 0.275 | 0.278 | 0.301 | 0.302 | 0.308 | 0.312 | 0.283 |
| $T_2$ | 0.344 | 0.346 | 0.352 | 0.357 | 0.367 | 0.375 | 0.378 | 0.39 | 0.395 | 0.403 | 0.371 |
| P | 0.302 | 0.305 | 0.308 | 0.314 | 0.321 | 0.326 | 0.34 | 0.346 | 0.351 | 0.357 | - |
| CD (0.05) | T = 0.004 P = 0.009 T×P = NS | | | | | | | | | | |
| **Peroxide value (meq of $O_2$/kg fat)** | | | | | | | | | | | |
| $T_1$ | 0.079 | 0.081 | 0.085 | 0.09 | 0.093 | 0.103 | 0.113 | 0.119 | 0.135 | 0.147 | 0.105 |
| $T_2$ | 0.079 | 0.081 | 0.088 | 0.092 | 0.095 | 0.107 | 0.114 | 0.122 | 0.138 | 0.144 | 0.106 |
| P | 0.079 | 0.081 | 0.087 | 0.091 | 0.094 | 0.105 | 0.113 | 0.121 | 0.137 | 0.146 | - |
| CD (0.05) | T = NS P = 0.004 T×P = NS | | | | | | | | | | |
| Tyrosine value (µg of tyrosine/5 ml filtrate) | | | | | | | | | | | |
| $T_1$ | 12.05 | 12.38 | 13.24 | 13.34 | 13.45 | 14.1 | 14.85 | 15.82 | 16.46 | 17.53 | 14.32 |
| $T_2$ | 20.76 | 21.52 | 21.63 | 21.73 | 21.95 | 22.27 | 23.24 | 24.53 | 25.92 | 28.82 | 23.24 |
| P | 16.41 | 16.95 | 17.43 | 17.54 | 17.7 | 18.18 | 19.04 | 20.17 | 21.19 | 23.18 | - |
| CD (0.05) | T = 0.14 P = 0.32 T×P = 0.46 | | | | | | | | | | |
| Water activity ($a_w$) | | | | | | | | | | | |
| $T_1$ | 0.926 | 0.926 | 0.926 | 0.924 | 0.922 | 0.922 | 0.919 | 0.918 | 0.916 | 0.912 | 0.921 |
| $T_2$ | 0.922 | 0.921 | 0.919 | 0.917 | 0.916 | 0.915 | 0.914 | 0.911 | 0.91 | 0.906 | 0.915 |
| P | 0.924 | 0.924 | 0.922 | 0.921 | 0.919 | 0.919 | 0.916 | 0.915 | 0.913 | 0.909 | - |
| CD (0.05) | T = 0.005 P = NS T×P = NS | | | | | | | | | | |
| Oiling off (%) | | | | | | | | | | | |
| $T_1$ | 3.18 | 3.23 | 3.26 | 3.34 | 3.38 | 3.39 | 3.48 | 3.54 | 3.62 | 3.67 | 3.41 |
| $T_2$ | 2.71 | 2.74 | 2.79 | 2.81 | 2.85 | 2.85 | 2.9 | 3.01 | 3.05 | 3.11 | 2.88 |
| P | 2.94 | 2.99 | 3.03 | 3.07 | 3.11 | 3.12 | 3.19 | 3.28 | 3.34 | 3.39 | - |
| CD (0.05) | T = 0.02 P = 0.05 T×P = NS | | | | | | | | | | |
| **Wheying off (%)** | | | | | | | | | | | |
| $T_1$ | 5.36 | 5.44 | 5.46 | 5.49 | 5.56 | 5.63 | 5.72 | 5.84 | 5.93 | 6.04 | 5.65 |
| $T_2$ | 5.19 | 5.26 | 5.3 | 5.36 | 5.45 | 5.55 | 5.63 | 5.72 | 5.85 | 5.98 | 5.53 |
| P | 5.28 | 5.35 | 5.38 | 5.43 | 5.5 | 5.59 | 5.67 | 5.78 | 5.89 | 6.01 | - |
| CD (0.05) | T = 0.03 P = 0.08 T×P = NS | | | | | | | | | | |

$T_1$ = Control spread, $T_2$ = Protein-rich dairy spread
The values are mean; n=3.
T = Treatment mean, P = Period mean
NS = non-significant.

### Changes in the Hardness of Spread During Storage

The hardness of dairy spread samples ($T_1$ and $T_2$) showed a progressive decline during 90 days of storage at 7±2 °C (Table 3). Initial hardness values were 1.181 g/cm² for $T_1$ and 1.225 g/cm² for $T_2$, which gradually decreased to 0.993 g/cm² and 1.029 g/cm², respectively, by the end of storage. The reduction in hardness was statistically significant across storage days ($p$<0.05), with both treatments responding differently to storage conditions. The decline in firmness can be attributed to protein network weakening, moisture redistribution, and proteolytic activity, all of which contribute to a softer structure over time. The protein-rich spread ($T_2$) maintained slightly higher hardness values than the control ($T_1$), likely due to the stronger structural matrix

formed by WPC-80. Whey proteins enhance initial firmness through gel formation but later undergo gradual breakdown, which aligns with decreasing hardness. Sethi (2017) [18] documented a fall in firmness during 90-day storage in mixed fat spreads from 429.3 to 398.8 in PP cups at 5±1 °C. In another study, Hamid (2023) [6] noted a similar decline 288.4 to 264.7 on 21st day followed by a late increase to 316.2 on 35th day of storage in PP cups at 4±1 °C in omega-enriched spreads.

**Table 3:** Changes in the hardness of dairy spread during storage

| Hardness (g/cm$^2$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Spread Sample** | **Storage day** | | | | | | | | | | **T** |
| | **0** | **10** | **20** | **30** | **40** | **50** | **60** | **70** | **80** | **90** | |
| $T_1$ | 1.181 | 1.173 | 1.172 | 1.145 | 1.119 | 1.116 | 1.1 | 1.067 | 1.034 | 0.993 | 1.11 |
| $T_2$ | 1.225 | 1.223 | 1.217 | 1.257 | 1.303 | 1.248 | 1.172 | 1.148 | 1.094 | 1.029 | 1.191 |
| P | 1.203 | 1.198 | 1.194 | 1.201 | 1.211 | 1.182 | 1.136 | 1.107 | 1.064 | 1.011 | - |
| CD (0.05) | T = 0.009 P = 0.020 T×P = 0.028 | | | | | | | | | | |

$T_1$ = Control spread, $T_2$ = Protein-rich dairy spread
The values are mean; n=3.
T = Treatment mean, P = Period mean

**Changes in the Microbiological Analysis of Spread During Storage**

The aerobic plate count (APC) of dairy spread samples ($T_1$ and $T_2$) remained absent up to 70 days of storage at 7±2 °C, reflecting good-quality raw materials and hygienic manufacturing conditions (Table 4). Detectable growth appeared only after 70 days, with APC reaching 143.33 cfu/g in $T_1$ and 118.33 cfu/g in $T_2$ by the 80th day, further increasing to 280.00 and 193.33 cfu/g, respectively, by day 90. Patange (2011) [14] noted APC rising substantially in ghee-based low-fat spreads by day 77 from 2.12 to 4.68 log cfu/g in PS tubs at 5±1 °C.

**Table 4:** Changes in the microbial quality of dairy spread during storage

| Aerobic plate count (cfu/g) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Spread Sample** | **Storage day** | | | | | | | | | |
| | **0** | **10** | **20** | **30** | **40** | **50** | **60** | **70** | **80** | **90** |
| $T_1$ | Absent/g | | | | | | | | 143.33 | 280.00 |
| $T_2$ | Absent/g | | | | | | | | 118.33 | 193.33 |
| **Yeast and mould count (cfu/g)** | | | | | | | | | | |
| **Spread Sample** | Storage day | | | | | | | | | |
| | **0** | **10** | **20** | **30** | **40** | **50** | **60** | **70** | **80** | **90** |
| $T_1$ | Absent/g | | | | | | | | 10.00 | 13.33 |
| $T_2$ | Absent/g | | | | | | | | 6.67 | 16.67 |

$T_1$ = Control spread, $T_2$ = Protein-rich dairy spread
The values are mean; n=3.

Coliforms remained absent throughout the 90-day storage period in both the control spread ($T_1$) and the protein-rich dairy spread ($T_2$), indicating hygienic processing, effective heat treatment, and the absence of post-processing contamination. The complete absence of coliforms is consistent with earlier findings: Patange (2011) [14] reported no coliforms in ghee-based spreads up to 77 days, while Hamid (2023) [6] also documented zero coliform counts in omega-enriched spreads during refrigerated storage.

Yeast and mould counts were undetectable in both $T_1$ and $T_2$ until the 70th day of refrigerated storage (Table 4). On day 80, counts reached 10.00 cfu/g in $T_1$ and 6.67 cfu/g in $T_2$, increasing to 13.33 and 16.67 cfu/g, respectively, by day 90. The fungal growth after prolonged storage reflects environmental favourability. Patange (2011) [14] observed counts rising significantly in ghee-based spreads by day 77 from 1 to 33 cfu/g in PS tubs at 5±1 °C and Hamid (2023) [6] reported rapid fungal growth in omega-enriched spreads from 5.83 to 77 cfu/g on 35th day of storage in PP cups at 4±1 °C. The delayed fungal growth in the present study can be attributed to the preservative action of nisin and sorbic acid. Their combined effect likely contributed to the absence of APC and fungal counts until the 70th day of storage.

**Conclusion**

The protein-rich dairy spread formulated using WPC-80 and Greek yoghurt exhibited better physicochemical stability, reduced syneresis, and controlled microbial growth compared to the control during 90 days of storage at 7±2 °C. The improved stability can be due to the functional properties of whey proteins, which enhanced moisture retention and emulsion stability. Rheological and microstructural studies would further help in understanding the protein-fat interactions responsible for these stability improvements during long-term storage. Overall, the product remained microbiologically safe and sensorially acceptable throughout the evaluated storage period.

**References**

1. Balasaheb LP, Patange D, Gaondhare S. Effect of level of strawberry powder on sensory qualities of low-fat spread. The Pharma Innovation Journal. 2019;8(10):25-29.
2. Deshmukh MS, Patil GR, Sontakke AT, Mitkari KR. Development of low-fat spread from safflower milk blended with buffalo milk. Indian Journal of Dairy and Biosciences. 2002;13(2):60-64.

3. FSSAI. Manual of methods of analysis of foods: Dairy and Dairy Products. New Delhi: Food Safety and Standards Authority of India; 2022.
4. FSSAI. Methods of analysis: microbiological examination of food and water. New Delhi: Food Safety and Standards Authority of India; 2023.
5. Gulzar N, Sameen A, Khan MI, Huma N, Murtaza M, Rafiq S. Nutritional and functional properties of fruited cream cheese spread as influenced by hydrocolloids. Journal of Food and Nutrition Research. 2015;3(3):191-195. https://doi.org/10.12691/jfnr-3-3-10
6. Hamid AQ. Development of technology for omega enriched fat spread. M.Tech. Thesis, Kamdhenu University, Gandhinagar; 2023.
7. Hull M. Studies on milk proteins. II. Colorimetric determination of the partial hydrolysis of the proteins in milk. Journal of Dairy Science. 1947;30(11):881-884. https://doi.org/10.3168/jds.s0022-0302(47)92412-0
8. ICMR-National Institute of Nutrition. Dietary guidelines for Indians. Telangana: National Institute of Nutrition; 2024. https://www.nin.res.in
9. Bureau of Indian Standards. IS 5401 (Part 1): Microbiology of food and animal feeding stuffs - Horizontal method for detection and enumeration of coliforms. Part 1: Colony count technique. New Delhi: BIS; 2002.
10. Bureau of Indian Standards. IS 5403: Yeast and mold count of foodstuffs and animal feeds (first revision). New Delhi: BIS; 1999. p. 209.
11. Kharb S, Thompkinson DK, Kumari H. Stability study of fortified low-fat spreads with preservatives. Food Quality and Safety. 2022;6(1):1-9. https://doi.org/10.1093/fqsafe/fyac027
12. Meilgaard MC, Carr BT, Civille GV. Sensory evaluation techniques. CRC Press; 1999. https://doi.org/10.1201/9781003040729
13. Mishra VK, David J, Rani R, Bharti BK, Dixit NK. Storage study of filled milk chhana spread. Journal of Pharmacognosy and Phytochemistry. 2019;8(5):1567-1571.
14. Patange DD, Patel AA, Singh RRB, Patil GR, Bhosle DN. Storage related changes in ghee-based low-fat spread. Journal of Food Science and Technology. 2011;50(2):346-352. https://doi.org/10.1007/s13197-011-0339-7
15. Pavithra S, Praveen AR, Devaraju R, Shilpashree BG, Malashree L, Kumar A. Development of functional dairy spread. Journal of Scientific Research and Reports. 2024;30(6):690-700. https://doi.org/10.9734/jsrr/2024/v30i62087
16. Prajapati DR, Patel AM, Balakrishnan S, Mallik JM, Dharaiya CN, Patel DH. Analysis of sensory, textural and compositional attributes of protein-rich dairy spread using response surface methodology. Indian Journal of Dairy Science. 2025;78(2):165-172. https://doi.org/10.33785/IJDS.2025.v78i02.009
17. Prajapati PS. Technology of low-fat spread manufacture. Doctoral dissertation, Kurukshetra University, Kurukshetra; 1988. http://krishikosh.egranth.ac.in/handle/1/65706
18. Sethi N, Balasubramanyam BV. Optimization of butter fruit incorporated fat spread using instrumental textural analysis: a response surface methodology. Journal of Food Measurement and Characterization. 2017;12(2):859-866. https://doi.org/10.1007/s11694-017-9700-5
19. Shakerardekani A, Karim R, Ghazali HM, Chin NL. Oxidative stability of pistachio (*Pistacia vera* L.) paste and spreads. Journal of the American Oil Chemists' Society. 2015;92(7):1015-1021. https://doi.org/10.1007/s11746-015-2668-6
20. Smithers GW. Whey and whey proteins from 'gutter to gold'. International Dairy Journal. 2008;18(7):695-704. https://doi.org/10.1016/j.idairyj.2008.03.008
21. Steel RD, Torrie JH. Principles and procedures of statistics: a biometrical approach. New York: McGraw-Hill; 1980.
22. Suthar J, Jana A, Balakrishnan S. High protein milk ingredients a tool for value-addition to dairy and food products. Journal of Dairy, Veterinary & Animal Research. 2017;6(1):259-265. https://doi.org/10.15406/jdvar.2017.06.00171
23. Tambe. Preparation of functional chakka spread enriched with dragon fruit. M.Sc. Thesis, Mahatma Phule Krishi Vidyapeeth, Rahuri; 2020. https://krishikosh.egranth.ac.in/handle/1/5810170568
24. Thomas W, Harper W, Gould I. Free fatty acid content of fresh milk as related to portions of milk drawn. Journal of Dairy Science. 1954;37(6):717-723. https://doi.org/10.3168/jds.s0022-0302(54)91317-x
25. Tuyakbayeva Z, Alzhaxina N, Zhadrassyn Z, Matai M, Muslimov N. Investigation of qualitative indicators of a functional creamy vegetable spread during storage. Journal of Hygienic Engineering & Design. 2022;40(1):73-78.
26. Verma. A study on technological aspects for development of low-fat butter spread. M.Sc. Thesis, Anand Agricultural University, Anand; 1996. http://krishikosh.egranth.ac.in/handle/1/5810100575
27. Walstra P, Wouters JTM, Geurts TJ. Dairy Science and Technology. CRC Press; 2005. https://doi.org/10.1201/9781420028010
28. Tamime AY, Robinson RK. Tamime and Robinson's yoghurt: science and technology. Elsevier; 2007 Mar 22.
29. Campos AC, Mendes J, Valle PO, Scott N. Co-creation of tourist experiences: A literature review. Current Issues in Tourism. 2018 Mar 4;21(4):369-400.