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Development and characterization of cheese spread fortified with flaxseed oil

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ABSTRACT

This study aimed to develop a healthier processed cheese spread by replacing milk fat with flaxseed oil, a rich source of omega-3 fatty acids. The research tested flaxseed oil substitution levels of 25%, 50%, 75%, and 100% to find the optimal balance between health benefits and product quality. Key analyses included titratable acidity, moisture content, pH, fat content, protein, ash content, microbiological growth, and sensory evaluation. The major finding includes that increasing flaxseed oil led to decreased initial acidity, but acidity increased during storage. Moisture content rose over 21 days initial moisture ranged 35.05–36.55%, slightly increasing during 21 days, with T4 and T3 maintaining the highest levels (36.77%) at later stages, and pH increased with more flaxseed oil, as Fresh samples displayed pH values ranging from 6.16 (T0) to 6.31 (T4), though 25% substitution showed similar pH to the control. Fat contents and ash contents significantly increased with flaxseed oil fortification. Microbial counts also rose, likely due to typical cheese breakdown organisms, as Fresh samples had initial TBC values ranging from 83 CFU/g (T0) to 91 CFU/g (T4). By Day 21, the TBC for T0 rose to 101 CFU/g, while T4 showed a more pronounced increase, reaching 116 CFU/g. Sensory tests showed colour changes to yellow and brown hues, but overall good acceptance (T3, T4), yielding lower initial scores due to the oil's distinct colour and flavor profile. T0 (control) and T1 (25% oil) maintained superior acceptability throughout. The study concludes that flaxseed oil can produce a healthier cheese spread with lower levels of saturated and trans fats while maintaining acceptable sensory qualities.

Keywords: flaxseed oil, cheese spread, omega-3 fortification, physicochemical properties, sensory evaluation

INTRODUCTION

Modern nutritional science increasingly emphasizes the role of functional foods and bioactive dietary components in preventing and managing chronic non-communicable diseases by improving metabolic health, reducing inflammation, and supporting optimal physiological functions beyond basic nutrient provision [1]. Organizations like the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) advocate limiting intake of fats, saturated fatty acids, and trans fatty acids to prevent health issues such as obesity, a growing concern in developing countries. Excessive consumption of high-calorie foods, particularly those rich in lipids, can lead to elevated triglyceride levels, increasing the risk of cardiovascular diseases, type 2 diabetes, and colon cancer. Consequently, consumers are increasingly seeking low-fat food options [2]. Fats and oils provide a high energy density (9 kcal/gram), nearly double that of carbohydrates and proteins. This has driven food manufacturers to innovate in reducing product fat content while preserving desirable sensory qualities. A prime example is the dairy industry, where significant efforts have been made to reduce the calorific value of products by lowering their fat content [2].

A crucial aspect of a healthy diet is the adequate intake of essential fatty acids, particularly omega-3 fatty acids, which the body cannot produce on its own. Research highlights numerous benefits, including reduced risk of cardiovascular disease, improved cognitive function, and decreased inflammation [3]. The Food and Drug

Administration (FDA) recommends a daily intake of 2 grams of omega-3 fatty acids, and the food industry is actively developing functional foods — products that offer health benefits beyond basic nutrition — to incorporate these vital components. These functional foods often include additional nutrients or bioactive substances to address dietary needs and metabolic disorders [3]. Therefore, public health research increasingly urges the food industry to advance the reformulation of food products by reducing total fat, saturated fat, and industrial trans fatty acids—for example through the adoption of healthier fat sources and improved product standards—as these changes have been shown in population-level modelling to significantly lower intakes of nutrients linked to chronic disease risk and can help move diets closer to recommended guidelines [4].

The pursuit of a nutrient-rich diet to enhance quality of life is driving innovation, particularly in the dairy sector, leading to the creation of functional and appealing products. Table/fat spreads, for instance, represent a cost-effective, nutritionally balanced option that can be fortified with both lipid- and water-soluble compounds [5]. Spreads are widely used culinary ingredients that enhance the flavour and texture of various foods [6]. Among these, cheese spread has seen consistent demand growth due to its affordability, versatility, and longer shelf life compared to natural cheeses. Fat content significantly influences the energy and functional properties of cheese spreads, prompting researchers and the food industry to collaborate on developing low-fat functional alternatives [7]. Cheese analogues formulated with plant-based proteins and non-dairy lipids—such as zein and chickpea protein blends—have been shown to successfully mimic many textural and functional characteristics of traditional cheese, offering a cost-effective and accessible alternative for consumers seeking dairy-free options while addressing sustainability and nutritional considerations [6].

Plant-derived omega-3 fatty acids play an important role in human health, particularly for individuals following plant-forward or vegetarian diets. Alpha-linolenic acid (ALA), the primary plant-based omega-3 found in flaxseed, chia seeds, and walnuts, has been associated with reduced risk factors for cardiovascular disease and cognitive decline, highlighting its significance as a dietary source of omega-3s [8]. In addition, stearidonic acid (SDA), another plant-derived omega-3 present in select seed oils, has emerged as a complementary omega-3 that can enhance overall omega-3 status without relying on marine sources. SDA contributes to the biosynthesis of eicosapentaenoic acid (EPA) in the body and may offer additional health benefits, including supporting cardiovascular and metabolic health [9]. Together, these findings underscore the importance of incorporating plant-based omega-3s into the diet to achieve optimal fatty acid balance and promote long-term health. Omega-3 polyunsaturated fatty acids (PUFAs) are essential for maintaining cardiovascular health, supporting retinal and brain function, and modulating inflammatory responses [10]. Adequate omega-3 intake is especially crucial for pregnant and nursing women to support optimal fetal brain and retinal development [11].

Primary sources of omega-3 PUFAs include fish, marine oils, and certain plant-derived precursors such as α -linolenic acid (ALA) found in flaxseed, walnuts, chia seeds, and canola oil; dietary intake of ALA — the major plant form of ω -3 PUFA — has been associated with reduced cardiovascular disease risk and improved lipid profiles [8]. Flaxseed (*Linum usitatissimum*), a globally cultivated oilseed crop, contains a high proportion of essential fatty acids, with α -linolenic acid (ALA) accounting for approximately 39–54 % of total fatty acids in different flaxseed varieties, making it one of the richest plant sources of omega-3 PUFAs alongside other beneficial fatty acids like linoleic and oleic acids [12]. Its high nutritional value, including protein, dietary fiber, lignans, flavonoids, and phenolic acids, makes flaxseed a highly desirable functional food ingredient [13]. Flaxseed oil added to the diet of diabetic mice (at 7% or 10.5% levels) lowers liver inflammation by blocking harmful pathways and changes gut bacteria for the better—increasing helpful types like *Bacteroidetes* and *Lactococcus*—which supports overall gut health [14]. Its oil is a significant source of ALA, which can be converted to long-chain omega-3 fatty acids in the body, and contains natural antioxidants like tocopherols, phenolic acids, and lignans [15]. These antioxidants, along with vitamin E found in sesame oils, contribute to flaxseed's protective effects against cancer and premature aging [16].

Flaxseed offers numerous health benefits, including Flaxseed oil (1000 mg/day, 12 weeks), which significantly reduced triglycerides and inflammation (hs-CRP) while increasing good cholesterol (HDL) in coronary artery disease patients [17]. Beneficial effects on glucose and lipid metabolism have also been demonstrated in adult patients with type 2 diabetes, in whom daily flaxseed intake significantly reduced fasting blood glucose, total cholesterol, and triglyceride levels over several months [18]. Given the widespread deficiency of omega-3 PUFAs in many industrialized nations, fortifying commonly consumed lipid-based foods like cheese with omega-3-rich ingredients presents a practical solution; experimental dairy fortification with flaxseed oil improved health-promoting fatty acid profiles (e.g., better n-6/n-3 ratios and bioactive lipid indices) in fortified milk products [19].

Scientific Hypothesis

The present study hypothesizes that the incorporation of varying concentrations of flaxseed oil into processed cheese spread will significantly affect its physicochemical, microbiological, and sensory characteristics over a 21-day refrigerated storage period. Specifically, it is assumed that increasing levels of flaxseed oil will alter moisture content, titratable acidity, pH, fat and protein composition, ash content, microbial load, and sensory attributes. These changes are expected to be measurable and statistically significant, with interactions between treatment levels and storage duration influencing the quality parameters of the cheese spread. This hypothesis is grounded in the premise that flaxseed oil's unique fatty acid profile and compositional attributes contribute to both nutritional enhancement and modifications in the product's shelf life and sensory acceptance. All proposed assumptions will be rigorously evaluated using appropriate statistical methods, including Two-Way ANOVA and Fisher's test, to determine the significance of observed effects.

Objectives

1. Studying Physiochemical properties of cheese spread (Primary Objective).
2. To check the better acceptance of cheese spread having the percentage of 25%,50%,75% 100% of Flaxseed oil incorporated into coagulate (Secondary Objective).

MATERIAL AND METHODS

Cheese spread samples were developed using coagulate, flaxseed oil, skimmed milk powder, emulsifying agent, salt, and stabilizer. Five treatments (T0–T4) were formulated with varying proportions of coagulate, flaxseed oil, and skimmed milk powder, while emulsifying agent (3%), salt (1%), and stabilizer (2%) remained constant in all treatments.

Table 1 The specific composition of each treatment per 100 g sample is presented below.

Ingredients	T0	T1	T2	T3	T4
Coagulate	85%	64%	48%	36%	27%
Oil	5%	7%	8%	10%	13%
Skimmed milk powder	4%	23%	38%	48%	54%
Emulsifying agent	3%	3%	3%	3%	3%
Salt	1%	1%	1%	1%	1%
Stabilizer	2%	2%	2%	2%	2%
TOTAL	100%	100%	100%	100%	100%

Samples

Samples description:

1 liter of skimmed milk for each treatment and 500 ml of Flaxseed oil as a whole was taken to prepare 4 treatments (T1–T4) with different percentages of Milk coagulate and Flaxseed oil. There was no addition of Flaxseed oil in T0.

Samples collection:

All essential raw materials were procured from the local market in Faisalabad, Punjab, Pakistan. Emulsifying agents and stabilizers were obtained from the University of Agriculture, Faisalabad, Pakistan. After preparation, cheese spread samples were filled into covered glass cups and stored at 5 ± 2 °C for 30 days. Samples were analysed after 7, 14, and 21 days of storage.

Samples preparation:

The cheese spread was prepared by heating the coagulate in a water bath at 65–75 °C for 25–30 minutes, adding skimmed milk powder to adjust the total solids, and stirring continuously. Flaxseed oil was heated to a mild temperature and mixed with soya lecithin as an emulsifying agent. The Salt (1%) was added to coagulate, followed by the gradual incorporation of flaxseed oil under continuous stirring at 65–75 °C. Stabilizer (2%) was added, and the mixture was blended for 3–4 minutes until uniform consistency was achieved. The hot cheese spread was manually poured into glass cups, cooled at room temperature, and stored in the refrigerator (5 ± 2 °C) until analysis. For analytical procedures, samples were homogenized prior to examination.

Food Process Flow Diagram

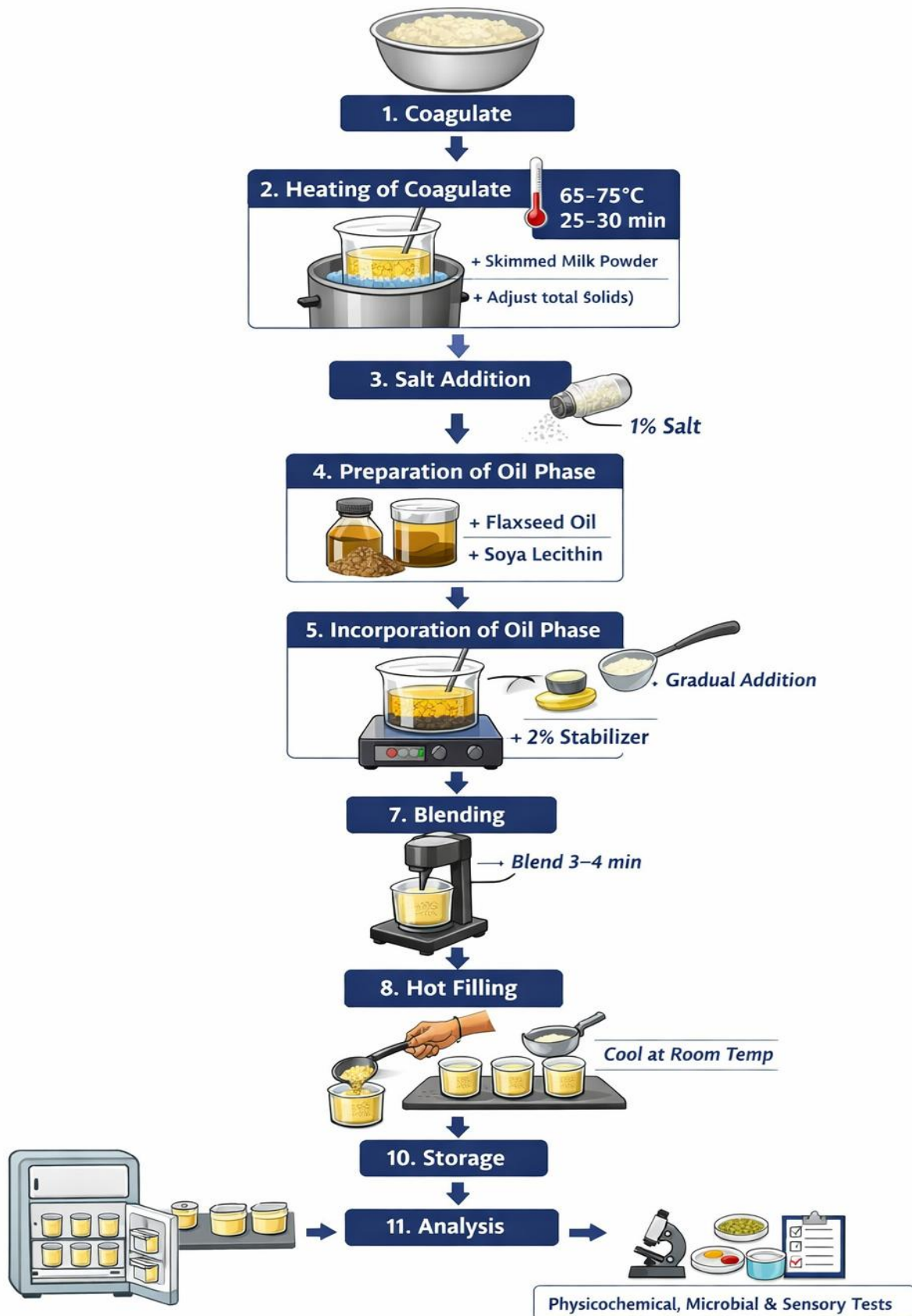


Figure 1 Cheese Spread production flow chart.

Number of samples analysed:

Five treatments (T0–T4) were prepared. Samples were analysed at three storage intervals (7, 14, and 21 days), with three replications per treatment at each interval.

Chemicals

All chemicals used were of analytical grade.

Chemicals used in the Kjeldahl Method (Protein Analysis): concentrated sulfuric acid (H₂SO₄), catalyst mixture (potassium sulfate K₂SO₄ and copper sulfate CuSO₄), sodium hydroxide (NaOH, 40%), boric acid solution (H₃BO₃, 4%), mixed indicator (methyl red and bromocresol green), standard hydrochloric acid (HCl, 0.1 N) for titration.

Chemicals used in the Gerber Method (Fat Analysis): gerber sulfuric acid (specific gravity 1.820), amyl alcohol (isoamyl alcohol), distilled water.

Chemicals used for Titratable Acidity: sodium hydroxide (NaOH, 0.1 N), phenolphthalein indicator, distilled water.

Chemicals used in the Pour Plate Method (Microbial Analysis): Plate count Agar (PCA-BD Difco™ Plate Count Agar (BD Difco™, Becton, Dickinson and Company (BD), USA), sterile distilled water, peptone water (0.1%) for serial dilution.

Animals, Plants and Biological Materials

Flaxseed oil was obtained from *Linum usitatissimum*. No experimental animals were used in this study. Natural microflora of the cheese spread was evaluated; no specific microbial strains were inoculated.

Instruments

1. Kjeldahl digestion and distillation unit
2. Gerber centrifuge and butyrometer
3. Hot air oven (105 °C)
4. Muffle furnace (550 °C)
5. Digital pH meter (calibrated at pH 4.0 and 7.0)
6. Incubator (37 °C)
7. Analytical balance
8. Water bath
9. Autoclave

Laboratory Methods

Moisture Content: Moisture content was determined using the oven-drying gravimetric method at 105 °C until constant weight [20].

Titratable Acidity: Titratable acidity was determined in triplicate by titrating the sample with 0.1 N NaOH using phenolphthalein as an indicator [20].

Protein Content: Protein content was determined using the Kjeldahl method involving digestion with concentrated H₂SO₄ and catalyst mixture, distillation with NaOH, and titration with standard HCl. Nitrogen content was multiplied by the factor 6.38 for dairy products [20].

Fat Content: Fat content was determined using the Gerber method according to [20], using Gerber sulfuric acid and amyl alcohol, followed by centrifugation in a butyrometer.

Total Sugar Content: Total sugar content was calculated by difference:

$$100 - (\text{protein} + \text{fat} + \text{ash} + \text{moisture})$$

Results were expressed on a dry matter basis (g/100 g) [20].

pH: The pH was measured using a calibrated digital pH meter standardized at pH 4.0 and 7.0 [20].

Ash Content: Ash content was determined by incinerating samples in a muffle furnace at 550°C until light gray ash was obtained [20].

Microbial / Shelf-life Analysis: Shelf-life evaluation was conducted by determining Total Bacterial Count (TBC) using the pour plate method on Plate Count Agar. Serial dilutions were prepared in sterile 0.1% peptone water and incubated at 37 °C for 24–48 hours before colony counting [20].

Sensory Analysis: Sensory evaluation was conducted using a 20-point hedonic scale.

Sensory attributes evaluated included appearance, color, flavor, taste, texture, and overall acceptability [20].

Description of the Experiment

Study flow: The experiment was conducted in several sequential phases to evaluate the physicochemical, microbial, and sensory characteristics of flaxseed oil–fortified cheese spread during refrigerated storage.

In the first phase, raw materials were procured, and five treatments (T0–T4) of cheese spread were formulated with varying proportions of flaxseed oil, coagulant, and skimmed milk powder. All samples were prepared under controlled laboratory conditions and packaged in sterilized glass containers.

In the second phase, freshly prepared samples were stored at 5 ± 2 °C for 21 days. Analytical evaluations were performed at three storage intervals: day 7, day 14, and day 21.

In the third phase, physicochemical analyses were carried out, including the determination of moisture, protein, fat, ash, total sugar, pH, and titratable acidity. Microbial quality was assessed by total bacterial count using the pour plate method. Sensory evaluation was conducted using a 20-point scale to assess appearance, color, flavor, taste, texture, and overall acceptability.

In the final phase, all collected data were compiled and subjected to statistical analysis using Two-Way ANOVA to determine the effects of treatment and storage period. No major deviations from the planned study protocol occurred during the experimental period.

Quality Assurance

Number of repeated analyses:

All physicochemical and microbial analyses were performed in triplicate (three technical repetitions) for each treatment at each storage interval to ensure precision and repeatability.

Number of experiment replication:

The entire experiment was conducted with three independent replications, and each treatment (T0–T4) was evaluated at three storage intervals (7, 14, and 21 days).

Reference materials:

Standard analytical-grade chemicals were used for all laboratory analyses. Calibration standards for pH measurement (buffer solutions pH 4.0 and 7.0) were used to verify instrument accuracy.

Calibration:

- The digital pH meter was calibrated daily using standard buffer solutions (pH 4.0 and 7.0).
- The analytical balance was calibrated using certified standard weights.
- The Kjeldahl apparatus and Gerber centrifuge were checked for operational accuracy prior to analysis.
- The incubator temperature was verified using a calibrated laboratory thermometer.
- The muffle furnace temperature was monitored using an internal temperature control system.

Laboratory accreditation:

The experiments were conducted in the Food Science Laboratory of the University of Agriculture, Faisalabad. The laboratory is equipped for routine food analysis.

Data Access

Data supporting the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to institutional data management policies.

Statistical Analysis

Statistical analysis was performed using Statistix 8.1 (Analytical Software, Tallahassee, FL, USA).

A Two-Way Analysis of Variance (ANOVA) was applied to evaluate the effects of treatment (T0–T4), storage period (7, 14, and 21 days), and their interaction on physicochemical, microbial, and sensory parameters.

When significant differences ($p < 0.05$) were observed, means were compared using Fisher's Least Significant Difference (LSD) test.

The experimental design followed a completely randomized design (CRD) with factorial arrangement. Repeated measurements across storage intervals were accounted for in the factorial ANOVA model. Statistical significance was determined at a 95% confidence level.

Reporting and transparency statement

Samples were randomly allocated to experimental treatments. Sensory panelists include 20 randomly selected university students. No blinding was applied during laboratory or sensory analysis.

The sample size was determined based on similar previously published studies in dairy product development. The study included five treatments with three storage intervals and three replications per treatment.

All samples prepared were included in the analysis. No data was excluded. The study was experimental in nature and did not involve human clinical subjects or animal experimentation. All procedures were conducted following standard laboratory practices to ensure reproducibility and transparency.

RESULTS AND DISCUSSION

Moisture

The moisture content of cheese spread samples was significantly influenced by both treatment type and storage duration ($p < 0.05$), as indicated by the highly significant F-values in the ANOVA Table 3. Initial moisture content (fresh samples) ranged from 35.05% (T4) to 36.55% (T2). Over the 21-day storage period, all treatments exhibited a slight increase in moisture content. For instance, T0 increased from 36.33% to 36.51%, and T4 from 35.05% to 36.77% Table 2. On Day 7, T4 showed the highest moisture content at 36.77%. This value was consistently maintained by T4 at Day 21, and also by T3 at Day 14 and Day 21, representing the highest observed moisture levels.

Table 2 Effect of different treatments on moisture contents (%) of cheese spread per 100 g of sample.

Storage days	T0	T1	T2	T3	T4
Fresh	36.33 ± 0.01I	36.51 ± 0.03G	36.55 ± 0.03F	36.41 ± 0.03H	35.05 ± 0.03J
Day 7	36.51 ± 0.01G	36.62 ± 0.01E	36.72 ± 0.01C	36.75 ± 0.01B	36.77 ± 0.01A
Day 14	36.62 ± 0.02E	36.65 ± 0.01D	36.75 ± 0.03B	36.77 ± 0.01A	36.33 ± 0.01I
Day 21	36.51 ± 0.02G	36.62 ± 0.01E	36.72 ± 0.02C	36.75 ± 0.01B	36.77 ± 0.02A

Note: Values represent mean ± standard deviation moisture contents; T0 = control, T1 = 25% flaxseed oil, T2 = 50% flaxseed oil, T3 = 75%, and T4 = 100% at storage of 30 days.

Table 3 ANOVA table for moisture percentage.

SOV	DF	SS	MS	F-Value
Treatment	4	1.67736	0.419340	4193.40**
Storage	3	2.69680	0.898935	8989.35**
Treatment*Storage	12	3.76992	0.314160	3141.60**
Error	40	0.00400	0.000100	-
Total	59	8.14809	-	-

Note :non-significant, *=Significant, **= Highly significant ($p < 0.05$).

Titrateable Acidity

Titrateable acidity was significantly affected by treatment, storage time, and their interaction ($p < 0.05$) Table 5. Fresh samples exhibited titrateable acidity ranging from 0.55% (T4) to 0.65% (T0). A general increasing trend in titrateable acidity was observed across all treatments throughout the 21-day storage period Table 4. By Day 21, T3 showed the highest titrateable acidity at 1.17%, while T4 and T1 also exhibited high values at 1.12% and 1.11%, respectively.

Table 4 Effect of different treatments on Titrateable acidity of cheese spread.

Storage days	T0	T1	T2	T3	T4
Fresh	0.65 ± 0.01K	0.63 ± 0.03L	0.57 ± 0.02N	0.56 ± 0.02N	0.55 ± 0.03O
Day 7	0.68 ± 0.02J	0.67 ± 0.02J	0.65 ± 0.02K	0.63 ± 0.02L	0.61 ± 0.03M
Day 14	0.91 ± 0.02I	0.94 ± 0.03H	0.97 ± 0.02F	0.98 ± 0.02F	0.99 ± 0.03G
Day 21	1.15 ± 0.02C	1.11 ± 0.02D	1.03 ± 0.02E	1.17 ± 0.02B	1.12 ± 0.03A

Note: Values represent mean ± standard deviation Titrateable Acidity; T0 = control, T1 = 25% flaxseed oil, T2 = 50% flaxseed oil, T3 = 75% and T4 = 100% at storage of 30 days.

Table 5 ANOVA table for titrateable acidity.

SOV	DF	SS	MS	F-Value
Treatment	4	0.01082	0.002706	30.06**
Storage	3	2.95129	0.983762	10930.69**
Treatment*Storage	12	0.08756	0.007297	81.08**
Error	40	0.00360	0.000090	-
Total	59	3.05327	-	-

Note: non-significant, *=Significant, **= Highly significant ($p < 0.05$).

pH Analysis

The pH of the cheese spread was significantly impacted by treatment, storage duration, and their interaction ($p < 0.05$) Table 7 Fresh samples displayed pH values ranging from 6.16 (T0) to 6.31 (T4). A consistent decrease in pH was observed across all treatments over the 21-day storage period Table 6. By Day 21, T0 recorded the lowest pH at 5.57, while T4 maintained a comparatively higher pH of 5.92. Notably, an increase in flaxseed oil content in the formulation generally correlated with a higher initial pH. This pattern was also observed in samples stored at 5 ± 2 °C, where processed cheese containing 25% flaxseed oil had a pH similar to the control at both initiation and after 21 days of storage. Minimal differences in pH were observed between cheese samples containing 25% and 50% flaxseed oil after 21 days of cold storage. However, all processed cheese samples demonstrated noticeable pH variations compared to their fresh counterparts after prolonged storage.

Table 6 Effect of different treatments on pH of cheese spread.

Storage days	T0	T1	T2	T3	T4
Fresh	6.16±0.02D	6.17±0.03D	6.26±0.03B	6.28±0.02B	6.31±0.02A
Day 7	6.01±0.03G	6.09±0.02F	6.12±0.03E	6.18±0.02D	6.21±0.02C
Day 14	5.84±0.02K	5.88±0.03J	5.93±0.03H	5.95±0.02H	5.98±0.03G
Day 21	5.57±0.01O	5.68±0.02N	5.75±0.03M	5.78±0.02L	5.92±0.02I

Note: Values represent mean \pm standard deviation PH of Cheese spread; T0 = control, T1 = 25% flaxseed oil, T2 = 50% flaxseed oil, T3 = 75%, and T4 = 100% at storage of 30 days.

Table 7 ANOVA for PH analysis of cheese spread.

SOV	DF	SS	MS	F-Value
Treatment	4	0.32509	0.081273	251.36**
Storage	3	2.16625	0.722084	2233.25**
Treatment*Storage	12	0.04801	0.004001	12.37**
Error	40	0.01293	0.000323	-
Total	59	2.55229	-	-

Note: non-significant, *=Significant, **= Highly significant ($p < 0.05$).

Fat Contant

The fat percentage of the cheese spread samples showed a highly significant increase over the 21-day storage period across all treatments ($p < 0.05$), Table 8 and Table 9. Initial fat content (fresh samples) ranged from 6.01% (T0) to 15.5% (T4). By Day 21, T0's fat content increased to 8.45%, an absolute increase of 2.44 percentage points, while T4's increased to 18.45%, an increase of 2.95 percentage points. While all treatments showed an upward trend, the rate of increase varied; for example, T1 showed a more pronounced rise in fat content between Day 7 (8.45%) and Day 14 (9.45%) than between Day 14 and Day 21 (9.50%).

Table 8 Effect of different treatments on fat percentage of cheese spread.

Storage days	T0	T1	T2	T3	T4
Fresh	6.01 \pm 0.01O	8.08 \pm 0.01L	10.50 \pm 0.02I	13.50 \pm 0.02F	15.5 \pm 0.02D
Day 7	7.41 \pm 0.02M	8.45 \pm 0.03k	10.45 \pm 0.03I	13.53 \pm 0.02F	16.45 \pm 0.03C
Day 14	7.21 \pm 0.02N	9.45 \pm 0.03J	11.50 \pm 0.02H	14.45 \pm 0.03E	17.45 \pm 0.03B
Day 21	8.45 \pm 0.03K	9.50 \pm 0.02J	12.50 \pm 0.02G	14.50 \pm 0.02E	18.45 \pm 0.03A

Note: Values represent mean \pm standard deviation. Fat percentage; T0 = control, T1 = 25% flaxseed oil, T2 = 50% flaxseed oil, T3 = 75%, and T4 = 100% at storage of 30 days.

The inclusion of flaxseed oil significantly altered the fatty acid composition. Processed cheese samples containing flaxseed oil exhibited lower total saturated fatty acid (SFA) levels and higher total unsaturated fatty acid (USFA) levels than the control (T0). Flaxseed oil itself contains a high percentage of USFA (90%), with a notable presence of C18:3 n3 (alpha-linolenic acid, 58.9%) and C18:2 n6 (linoleic acid, 12.7%).

Table 9 ANOVA table for Fat analysis.

SOV	DF	SS	MS	F-Value
Treatment	4	727.518	181.879	185591.27**
Storage	3	33.358	11.119	11346.36**
Treatment*Storage	12	6.129	0.511	521.19**
Error	40	0.039	0.001	-
Total	59	767.044	-	-

Note: non-significant, *=Significant, **= Highly significant (p < 0.05).

Protein Content

The crude protein content of the cheese spread was significantly affected by the treatment type (p < 0.05) and storage duration (p < 0.05), with a significant interaction between the two factors (p < 0.05) (Table 11). Initial protein content was highest in the control (T0) at 21.39% and progressively decreased with increasing flaxseed oil substitution: 16.06% for T1, 12.03% for T2, 9.04% for T3, and 6.73% for T4 (Table 10). Over the 21-day storage period, all treatments showed a slight, consistent increase in protein percentage. For instance, T0's protein content increased from 21.39% (fresh) to 21.49% (Day 21), a modest 0.1 percentage point difference. Similar slight increases of approximately 0.1 percentage point were observed across all other treatments (T1, T2, T3, T4).

Table 10 Effect of different treatments on Protein Percentage of cheese spread.

Storage days	To	T1	T2	T3	T4
Fresh	21.39 ± 0.01C	16.06 ± 0.03F	12.03 ± 0.03I	9.04 ± 0.02L	6.73 ± 0.03O
Day 7	21.42 ± 0.02B	16.09 ± 0.02E	12.06 ± 0.03H	9.07 ± 0.02K	6.76 ± 0.02N
Day 14	21.45 ± 0.02B	16.12 ± 0.02E	12.09 ± 0.02H	9.10 ± 0.02K	6.79 ± 0.03N
Day 21	21.49 ± 0.03A	16.16 ± 0.03D	12.13 ± 0.02G	9.14 ± 0.02J	6.83 ± 0.03M

Note: Values represent mean ± standard deviation crude protein analysis; T0 = control, T1 = 25% flaxseed oil, T2 = 50% flaxseed oil, T3 = 75% and T4 = 100% at storage of 30 days.

Table 11 ANOVA table for protein analysis.

SOV	DF	SS	MS	F-Value
Treatment	4	1627.74	406.934	668932.91**
Storage	3	0.09	0.030	48.55**
Treatment*Storage	12	0.00	0.000	0.07*
Error	40	0.02	0.001	-
Total	59	1627.85	-	-

Note: non-significant, *=Significant, **= Highly significant (p < 0.05).

Ash Content

The ash content of cheese spread was significantly influenced by both treatment type and storage duration (p < 0.05), with a significant interaction between them Table 13. Initial ash content (fresh samples) ranged from 2.69% (T0) to 2.83% (T4). Treatments with higher flaxseed oil inclusion (T4) initially presented higher ash content due to the mineral richness of flaxseed oil.

Table 12 Effect of different treatments on Ash Analysis of cheese spread.

Storage days	To	T1	T2	T3	T4
Fresh	2.69 ± 0.01L	2.71 ± 0.02L	2.73 ± 0.02K	2.78 ± 0.02H	2.83 ± 0.03F
Day 7	2.73 ± 0.03K	2.73 ± 0.02K	2.75 ± 0.03J	2.83 ± 0.03F	2.86 ± 0.02D
Day 14	2.76 ± 0.03I	2.78 ± 0.02H	2.80 ± 0.02G	2.88 ± 0.02C	2.92 ± 0.03B
Day 21	2.82 ± 0.02F	2.85 ± 0.03D	2.84 ± 0.02E	2.93 ± 0.03B	2.96 ± 0.02A

Note: Values represent mean ± standard deviation Ash analysis; T0 = control, T1 = 25% flaxseed oil, T2 = 50% flaxseed oil, T3 = 75%, and T4 = 100% at storage of 30 days.

Over the 21-day storage period, a slight increase in ash content was observed across all treatments Table 12. For example, T0 increased from 2.69% (fresh) to 2.82% (Day 21), and T4 increased from 2.83% (fresh) to 2.96% (Day 21). On Day 7, T4 had the highest ash content at 2.86%, which further increased to 2.92% on Day 14 and 2.96% on Day 21, remaining the highest value.

Table 13 ANOVA table for Ash Analysis in the cheese spread.

SOV	DF	SS	MS	F-Value
Treatment	4	0.177573	0.044393	543.59**
Storage	3	0.151738	0.050579	619.34**
Treatment*Storage	12	0.003187	0.000266	3.25**
Error	40	0.003267	0.000082	-
Total	59	0.335765	-	-

Note: non-significant, *=Significant, **= Highly significant ($p < 0.05$).

Total Sugar Content

The total sugar content in the cheese spread was significantly affected by treatment type and storage duration, and their interaction also showed a highly significant effect ($p < 0.05$) Table 15. Initial total sugar content (fresh samples) varied considerably among treatments, ranging from 1.87% (T4) to 5.1% (T0) Table 14. As the proportion of flaxseed oil increased (from T0 to T4), the initial total sugar content generally decreased.

Table 14 Effect of different treatments on Total sugar contents of cheese spread.

Storage days	T0	T1	T2	T3	T4
Fresh	5.1 ± 0.01B	3.77 ± 0.02C	2.84 ± 0.03E	2.5 ± 0.03H	1.87 ± 0.03I
Day 7	5.18 ± 0.02A	3.72 ± 0.02D	2.83 ± 0.02F	2.57 ± 0.03G	1.88 ± 0.02I
Day 14	5.16 ± 0.01A	3.76 ± 0.03C	2.86 ± 0.02E	2.55 ± 0.02G	1.89 ± 0.02I
Day 21	5.18 ± 0.03A	3.76 ± 0.02C	2.88 ± 0.02E	2.57 ± 0.02G	1.86 ± 0.03I

Note: Values represent mean ± standard deviation Total sugar contents; T0 = control, T1 = 25% flaxseed oil, T2 = 50% flaxseed oil, T3 = 75% and T4 = 100% at storage of 30 days.

Over the 21-day storage period, the total sugar content remained relatively stable across most treatments, with only slight fluctuations observed. For instance, T0's total sugar content ranged from 5.1% (fresh) to 5.18% (Day 21). Similarly, T4's total sugar content remained around 1.87% to 1.88%. While some minor changes were noted (e.g., T0 and T2 showed slight increases on Day 21), these were not substantial, indicating that the overall sugar content did not undergo significant alterations during storage.

Table 15 ANOVA table for Total sugar contents in the cheese spread.

SOV	DF	SS	MS	F-Value
Treatment	4	77.2326	19.3082	30810.88**
Storage	3	0.0084	0.0028	4.49**
Treatment*Storage	12	0.0228	0.0019	3.03**
Error	40	0.0251	0.0006	-
Total	59	77.2889	-	-

Note: non-significant, *=Significant, **= Highly significant ($p < 0.05$).

Microbial/Shelf-Life Analysis

The total bacterial count (TBC) of the cheese spread was significantly influenced by treatment type, storage duration, and their interaction ($p < 0.05$) Table 17. Across all treatments, the TBC increased over the 21-day storage period, indicating microbial growth Table 16. Fresh samples had initial TBC values ranging from 83 CFU/g (T0) to 91 CFU/g (T4). By Day 21, the TBC for T0 rose to 101 CFU/g, while T4 showed a more pronounced increase, reaching 116 CFU/g. This suggests that cheese spreads with higher flaxseed oil content (T4) experienced a greater increase in TBC over time.

Table 16 Effect of different treatments on Microbial analysis of cheese spread cheese spread.

Sr.no	Treatments periods	T0	T1	T2	T3	T4
1	Fresh	83 ± 0.01P	85 ± 0.03O	88 ± 0.01N	90 ± 0.03L	91 ± 0.02L
2	Day 7	89 ± 0.02M	95 ± 0.02K	95 ± 0.03J	98 ± 0.03H	98 ± 0.03G
3	Day 14	94 ± 0.03I	96 ± 0.02K	99 ± 0.03G	104 ± 0.02E	108 ± 0.03D
4	Day 21	101 ± 0.02F	108 ± 0.02D	110 ± 0.03C	112 ± 0.02B	116 ± 0.02A

Note: Values represent mean ± standard deviation Microbial analysis; T0 = control, T1 = 25% flaxseed oil, T2 = 50% flaxseed oil, T3 = 75% and T4 =100% at storage of 30 days.

Table 17 ANOVA table Microbial analysis in cheese spread.

SOV	DF	SS	MS	F-Value
Treatment	4	966.14	241.53	1189.17**
Storage	3	3859.28	1286.43	6333.59**
Treatment*Storage	12	102.58	8.55	42.09**
Error	40	8.12	0.20	-
Total	59	4936.12	-	-

Note: non-significant, *=Significant, **= Highly significant (p < 0.05).

Sensory Analysis

Sensory attributes, including appearance, color, aroma, taste, and overall acceptability, were significantly impacted by both treatment type and storage duration (p < 0.05), with significant interactions observed for all attributes. Generally, a decreasing trend in sensory scores was observed across all attributes and treatments over the 21-day storage period, indicating a decline in sensory quality.

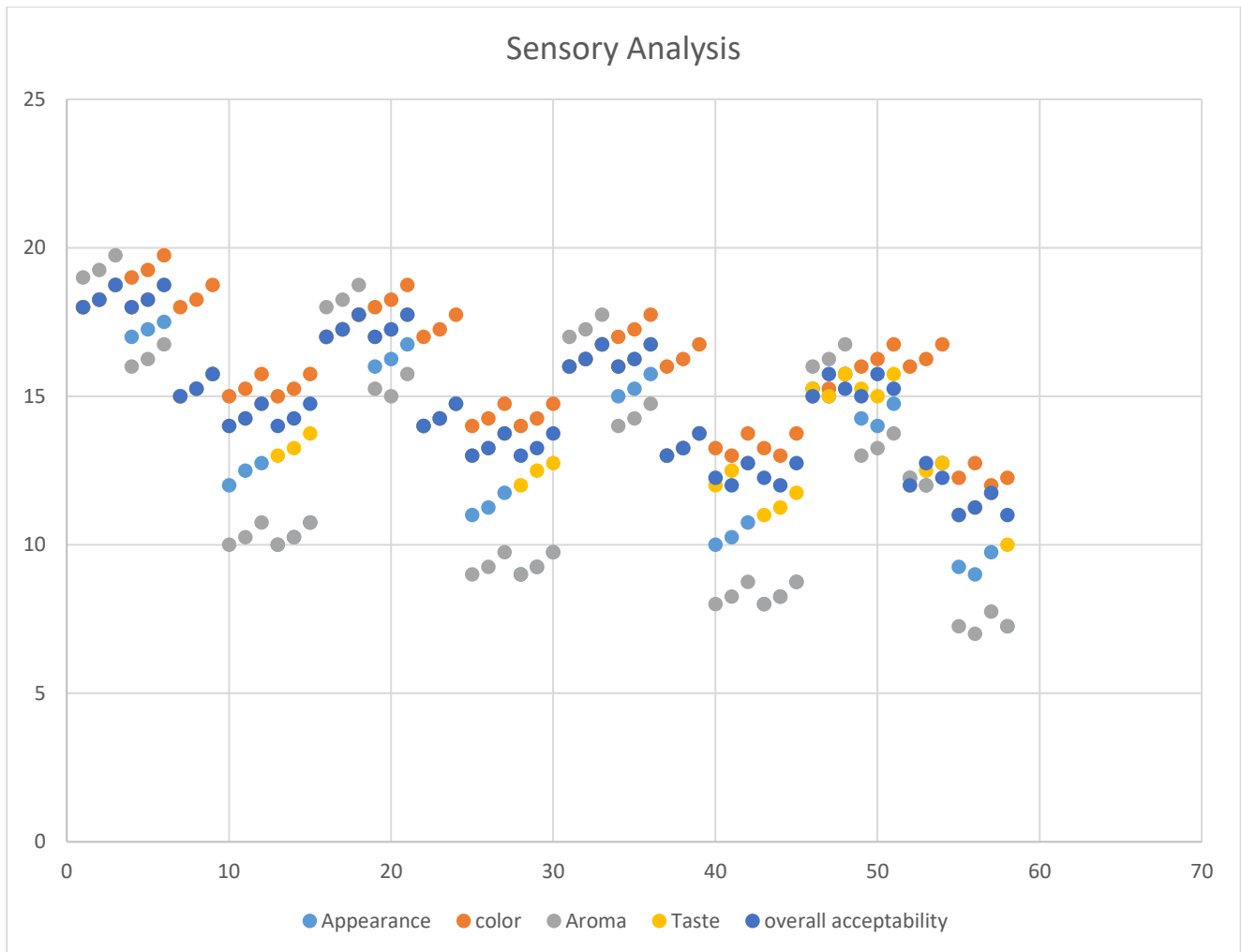


Figure 2 Sensory Analysis of cheese spread over a period of 21-day storage.

Appearance

Initial appearance scores (fresh samples) were highest for T0 (18.45) and lowest for T4 (10.11), indicating that increasing flaxseed oil content negatively impacted initial appearance Table 18. By Day 21, all treatments showed a decrease in appearance scores, with T0 dropping to 15.45 and T4 to 7.11.

Table 18 Effect of different treatments on the appearance of cheese spread.

Sr.no	Treatments periods	T0	T1	T2	T3	T4
1	Fresh	18.45 ± 0.03A	17.11 ± 0.02B	15.45 ± 0.03D	12.11 ± 0.02H	10.11 ± 0.02J
2	Day 7	17.21 ± 0.02B	16.53 ± 0.02C	14.45 ± 0.03E	11.21 ± 0.02I	9.11 ± 0.02K
3	Day 14	16.45 ± 0.03C	15.31 ± 0.02D	13.11 ± 0.02F	10.21 ± 0.02J	8.11 ± 0.02L
4	Day 21	15.45 ± 0.03D	14.33 ± 0.02E	12.41 ± 0.02G	9.11 ± 0.02K	7.11 ± 0.02M

Note: Values represent mean ± standard deviation Appearance; T0 = control, T1 = 25% flaxseed oil, T2 = 50% flaxseed oil, T3 = 75% and T4 = 100% at storage of 30 days.

Table 19 ANOVA table appearance of cheese spread.

SOV	DF	SS	MS	F-Value
Treatment	4	575.929	143.982	18164.28**
Storage	3	79.948	26.649	3361.98**
Treatment*Storage	12	0.815	0.068	8.57**
Error	40	0.317	0.008	-
Total	59	657.008	-	-

Note: non-significant, *=Significant, **= Highly significant (p < 0.05).

Color

For color, fresh T1 samples received the highest score (19.11), while T0 and T2 also had high initial scores (18.45 each). T3 and T4 had lower initial color scores (15.45 and 15.11, respectively), suggesting an impact of flaxseed oil on initial color, Table 20. Over 21 days, color scores generally decreased across all treatments, with T0 falling to 15.45 and T4 to 12.11.

Table 20 Effect of different treatments on the Color of cheese spread.

Sr.no	Treatments periods	T0	T1	T2	T3	T4
1	Fresh	18.45 ± 0.03B	19.11 ± 0.02A	18.45 ± 0.03B	15.45 ± 0.03J	15.11 ± 0.02K
2	Day 7	17.45 ± 0.03C	18.45 ± 0.03B	17.11 ± 0.02E	14.45 ± 0.02L	14.11 ± 0.02M
3	Day 14	16.21 ± 0.02H	17.31 ± 0.02D	16.45 ± 0.02F	13.11 ± 0.02O	13.45 ± 0.03N
4	Day 21	15.45 ± 0.03J	16.33 ± 0.02G	16.11 ± 0.02I	12.21 ± 0.02P	12.11 ± 0.02Q

Note: Values represent mean ± standard deviation color; T0 = control, T1 = 25% flaxseed oil, T2 = 50% flaxseed oil, T3 = 75% and T4 = 100% at storage of 30 days.

Table 21 ANOVA table color of cheese spread.

SOV	DF	SS	MS	F-Value
Treatment	4	181.317	45.3292	55961.94**
Storage	3	69.587	23.1958	28636.76**
Treatment*Storage	12	2.244	0.1870	230.84**
Error	40	0.032	0.0008	-
Total	59	253.180	-	-

Note: non-significant, *=Significant, **= Highly significant (p < 0.05).

Aroma

Aroma scores were highest for T0 (19.45) in fresh samples and lowest for T3 and T4 (10.11 each), indicating a negative effect of higher flaxseed oil concentration on initial aroma Table 22. By Day 21, aroma scores had declined for all treatments, with T0 at 16.21 and T3/T4 at 7.11/7.45.

Table 22 Effect of different treatments on the Aroma of cheese spread.

Sr.no	Treatments periods	T0	T1	T2	T3	T4
1	Fresh	19.45 ± 0.03A	16.53 ± 0.02D	15.11 ± 0.03G	10.11 ± 0.02M	10.11 ± 0.02M
2	Day 7	18.46 ± 0.02B	15.21 ± 0.03F	14.65 ± 0.03H	9.11 ± 0.02O	9.45 ± 0.03N
3	Day 14	17.11 ± 0.03C	14.21 ± 0.03I	13.65 ± 0.02J	8.11 ± 0.02P	8.11 ± 0.02P
4	Day 21	16.21 ± 0.02E	13.11 ± 0.02K	12.45 ± 0.02L	7.11 ± 0.02R	7.45 ± 0.03Q

Note: Values represent mean ± standard deviation Aroma; T0 = control, T1 = 25% flaxseed oil, T2 = 50% flaxseed oil, T3 = 75% and T4 = 100% at storage of 30 days.

Table 23 ANOVA table Aroma of cheese spread.

SOV	DF	SS	MS	F-Value
Treatment	4	768.589	192.147	288220.98**
Storage	3	76.976	25.659	38488.19**
Treatment*Storage	12	1.360	0.113	170.04**
Error	40	0.027	0.001	-
Total	59	846.953	-	-

Note: non-significant, *=Significant, **= Highly significant (p < 0.05).

Taste

Fresh taste scores were highest for T0 and T1 (18.45 each) and lowest for T4 (13.45) Table 24. A consistent decline in taste scores was observed over the storage period, with T0 dropping to 15.45 and T4 to 10.45 by Day 21.

Table 24 Effect of different treatments on the Taste of cheese spread.

Sr.no	Treatments periods	T0	T1	T2	T3	T4
1	Fresh	18.45 ± 0.03A	18.45 ± 0.03A	15.45 ± 0.03F	14.45 ± 0.02H	13.45 ± 0.03J
2	Day 7	17.21 ± 0.02B	17.11 ± 0.02C	14.65 ± 0.03G	13.45 ± 0.02J	12.11 ± 0.02L
3	Day 14	16.65 ± 0.03D	16.11 ± 0.02E	13.65 ± 0.02I	12.45 ± 0.02K	11.53 ± 0.02M
4	Day 21	15.45 ± 0.03F	15.45 ± 0.03F	12.45 ± 0.02K	11.11 ± 0.02N	10.45 ± 0.02O

Note: Values represent mean ± standard deviation Taste; T0 = control, T1 = 25% flaxseed oil, T2 = 50% flaxseed oil, T3 = 75% and T4 = 100% at storage of 30 days.

Table 25 ANOVA table Taste of cheese spread.

SOV	DF	SS	MS	F-Value
Treatment	4	250.493	62.6232	66976.64**
Storage	3	75.839	25.2795	27036.90**
Treatment*Storage	12	1.097	0.0914	97.74**
Error	40	0.037	0.0009	-
Total	59	327.465	-	-

Note: non-significant, *=Significant, **= Highly significant (p < 0.05).

Overall Acceptability

Overall acceptability also followed a similar trend, with fresh T0 (18.45) and T1 (18.21) being most preferred, and T3/T4 (14.11 each) being least preferred initially, Table 26. By Day 21, overall acceptability scores had decreased for all treatments, with T0 at 15.45 and T4 at 11.65.

Table 26 Effect of different treatments on the Overall Acceptability of cheese spread.

Sr.no	Treatments periods	T0	T1	T2	T3	T4
1	Fresh	18.45 ± 0.03A	18.21 ± 0.02B	15.11 ± 0.02G	14.11 ± 0.02I	14.11 ± 0.02I
2	Day 7	17.65 ± 0.03C	17.33 ± 0.02D	14.45 ± 0.03H	13.11 ± 0.02K	13.53 ± 0.03J
3	Day 14	16.21 ± 0.02E	16.11 ± 0.02E	13.45 ± 0.03J	12.45 ± 0.03L	12.45 ± 0.02L
4	Day 21	15.45 ± 0.03F	15.21 ± 0.02G	12.11 ± 0.02M	11.45 ± 0.03O	11.65 ± 0.03N

Note: Values represent mean ± standard deviation overall acceptability; T0 = control, T1 = 25% flaxseed oil, T2 = 50% flaxseed oil, T3 = 75%, and T4 = 100% at storage of 30 days.

Table 27 ANOVA table Overall Acceptability of cheese spread.

SOV	DF	SS	MS	F-Value
Treatment	4	201.980	50.4949	6303.99**
Storage	3	69.604	23.2012	2896.53**
Treatment*Storage	12	1.110	0.0925	11.55**
Error	40	0.320	0.0080	-
Total	59	273.014	-	-

Note: non-significant, *=Significant, **= Highly significant (p < 0.05).



Figure 3 Cheese spread fortified with flaxseed oil.

Despite the general decline in sensory attributes over time, the T0 and T1 treatments consistently exhibited the most desired sensory qualities across all parameters. The addition of flaxseed oil impacted appearance (leading to brownish-yellow hues in higher concentrations) and aroma (with increased flaxseed smell as its proportion grew). While T1 showed a favorable scent and pleasant taste, the sensory panel noted alterations in flavor as the flaxseed oil percentage increased. However, within each treatment, no significant variation was observed in the aforementioned parameters after storage, aligning with previous studies that found flaxseed oil did not significantly affect appearance or color and, in some cases, even improved aroma, taste, and overall acceptability. The findings generally suggest a trade-off between the nutritional benefits of flaxseed oil and its impact on the cheese spread's sensory profile, particularly at higher concentrations.

Discussion

This study investigated the physicochemical, microbiological, and sensory properties of cheese spread supplemented with varying concentrations of flaxseed oil (T0: 100% Milk Coagulate, T1: 25% Flaxseed Oil, T2: 50% Flaxseed Oil, T3: 75% Flaxseed Oil, T4: 100% Flaxseed Oil) over a 21-day refrigerated storage period. The findings provide comprehensive insights into how the incorporation of flaxseed oil and storage time influence key quality attributes of cheese spread, offering valuable information for product development and shelf-life optimization.

Moisture Content

The results indicated that moisture content was significantly affected by both treatment and storage, showing a slight increase across all samples over time. Higher flaxseed oil concentrations (T3 and T4) generally showed higher moisture values compared to control (T0), which may be attributed to the water-holding capacity of the emulsion matrix, as the addition of oil emulsion promotes protein-mediated water association within the cheese matrix. These findings are consistent with [21], who reported significant moisture variation in omega-3 fortified processed cheese spread and attributed the increased moisture to the addition of oil emulsion, and with [22], who similarly confirmed that both treatment and storage had a highly significant ($p \leq 0.05$) effect on moisture content in processed cheese spread. Furthermore, [23] demonstrated through NMR analysis that refrigerated storage promotes moisture migration into the casein protein matrix, while [24] reported a clear increase in moisture content during storage of oil-fortified white cheese, attributing it to the high protein content favoring water-holding capacity within the emulsion matrix. While higher moisture levels are associated with better spreadability and smoother texture, they also pose a risk for microbial growth, making effective moisture control crucial for ensuring product safety and quality.

Titratable Acidity and pH

Titratable acidity and pH were significantly affected by treatment, storage time, and their interaction ($p < 0.05$), showing a consistent inverse relationship throughout storage, with titratable acidity increasing progressively while pH declined across all treatments. The higher initial pH observed in higher flaxseed oil treatments (T4 = 6.31 vs T0 = 6.16) can be attributed to the dilution effect of the oil phase on the aqueous protein matrix, which reduces buffering capacity and elevates initial pH; however, all treatments still underwent significant acidification during storage driven by lipolysis of unsaturated fatty acids releasing free fatty acids into the aqueous phase and residual microbial metabolic activity producing organic acids under refrigerated conditions. These findings are consistent with [25] and [21], who reported similar increases in titratable acidity alongside pH decreases in flaxseed oil-fortified processed cheese spread during storage, attributing these changes to progressive lipolysis and organic acid accumulation, while [26] and [27] similarly confirmed pH reduction in flaxseed oil-incorporated cheese systems during storage. While moderate acidification enhances flavor development and microbial safety, excessive pH decline may compromise the emulsion stability of processed cheese spread, warranting careful control of flaxseed oil levels and storage conditions.

Fat Content

The fat content of the cheese spread significantly increased over the storage period across all treatments, with higher initial fat percentages correlating with higher flaxseed oil inclusion. This increase in fat content over time is likely an artifact of moisture loss, which progressively concentrates the fat within the remaining sample matrix rather than representing an absolute increase in total fat mass. Since total fat was determined using the Gerber method in this study, individual fatty acid composition of the finished cheese spread was not directly quantified. However, based on the well-documented composition of flaxseed oil as a raw ingredient — which contains approximately 90% unsaturated fatty acids, with α -linolenic acid (omega-3) comprising the dominant fraction [28] it is reasonable to expect that increasing levels of flaxseed oil substitution would result in a progressively

more favorable fatty acid profile in the finished product, with a higher proportion of unsaturated fatty acids relative to saturated fatty acids compared to the control. This expected compositional improvement, if confirmed through gas chromatography or equivalent fatty acid profiling in future work, would represent a meaningful contribution to the nutritional value of the cheese spread. While polyunsaturated fatty acids are susceptible to oxidation during storage, potentially leading to decreases in their levels [28], the overall formulation strategy of incorporating flaxseed oil supports the goal of producing a nutritionally enhanced product. Maintaining an optimum fat percentage is also critical for textural attributes and sensory quality of the cheese spread, as fat content directly influences spreadability, mouthfeel, and the overall structural integrity of the product [29].

Protein Content

The crude protein content of the cheese spread was significantly affected by both treatment type and storage duration, as confirmed by the highly significant F-values reported in the ANOVA Table 11. The initial protein content decreased progressively with increasing flaxseed oil substitution, from 21.39% in the control (T0) to 6.73% in T4, which is a direct and expected consequence of the proportional reduction of coagulate and addition of skimmed milk powder in order to maintain the solids not fat of cheese spread. Over the 21-day storage period, all treatments exhibited a slight but statistically significant increase in protein percentage of approximately 0.1 percentage point, as reflected by the significant storage effect in Table 11. This slight increase during storage is likely attributable to minor physicochemical rearrangements within the cheese matrix under refrigerated conditions rather than any absolute gain in protein mass, and is consistent with the generally stable behavior of the protein network reported in analogous processed cheese spread formulations [25]. Regardless of substitution level, protein content remained the primary determinant of the textural and rheological characteristics of the cheese spread across all treatments, as a decrease in protein content is well established to produce less viscous, less consistent, and more spreadable products in processed cheese spread systems [30]. The progressive reduction in protein with increasing flaxseed oil inclusion therefore represents a formulation-driven trade-off between nutritional protein delivery and the textural and functional properties desired in the finished spread.

Ash Content

Ash content was significantly influenced by both treatment type and storage duration, with treatments containing higher flaxseed oil substitution consistently presenting higher ash values throughout storage. This treatment-related increase is attributable to the proportional rise in skimmed milk powder content across treatments, which is a well-established concentrated source of minerals including calcium, phosphorus, and magnesium [31], while flaxseed oil as a refined lipid fraction contributes negligible ash; a parallel trend was similarly observed in walnut-fortified processed cheese, where ash increases were attributed to the mineral-rich ingredient rather than the lipid component [32]. A slight but statistically significant increase in ash content was observed across all treatments over the storage period, consistent with minor physicochemical changes within the cheese matrix during refrigerated storage. These findings align with previous studies on flaxseed oil-enriched cheese products confirming that the mineral profile remains stable and detectable throughout storage [27], and with flaxseed oil-fortified processed cheese spreads where chemical composition parameters remained traceable across treatments during extended refrigerated storage [21].

Microbial/Shelf-Life Analysis

Total bacterial count increased significantly across all treatments over the refrigerated storage period, yet all recorded TBC values remained substantially below the internationally accepted microbiological safety limit for processed cheese spread established by Codex Alimentarius standards, confirming that the product remained microbiologically safe and of excellent quality throughout the entire storage period even in the absence of chemical preservatives [25]. The trend of marginally higher TBC values in treatments with greater flaxseed oil substitution is consistent with the slightly elevated moisture content observed in those treatments, as higher available moisture within the cheese matrix can support a marginally greater degree of residual microbial activity under refrigeration a relationship similarly documented in processed cheese spread systems, where moisture content was shown to directly influence total viable bacterial counts during cold storage [33]. These treatment-dependent microbial dynamics are further contextualised by [34], who reported a measurable increase in lactic acid microflora in hard rennet cheese containing flax seeds during early storage, and [35], who observed elevated microbial loads in 5-20% flaxseed-fortified Cheddar under similar conditions, another study by [27], who confirmed that microbial counts in flaxseed oil-fortified soft white cheese remained within acceptable ranges throughout refrigerated storage, although both matrices differ from processed cheese spread in pH, water activity, heat treatment history, and emulsification. The progressive but controlled increase in TBC observed across all treatments also aligns with the findings of [36], who reported a gradual rise in microbial counts in raw milk

stretched cheese during refrigerated storage while confirming that such increases are a normal and expected phenomenon in dairy matrices held under cold conditions. Conversely, some studies like [37] found delayed non-starter lactic growth in flax-related dairy processing, highlighting formulation-specific effects. The observed microbial growth underscores the necessity of stringent hygiene and appropriate storage conditions to ensure product safety and extend shelf life.

Sensory Analysis

Sensory evaluation showed all attributes — appearance, colour, aroma, taste, and overall acceptability — declined significantly across all treatments over the storage period, with higher flaxseed oil substitution levels yielding lower initial scores attributable to the oil's distinct colour and characteristic flavour profile. While this decline reflects the inherently perishable nature of cheese spread and the susceptibility of polyunsaturated fatty acids to progressive oxidation during storage, T0 and T1 maintained superior acceptability throughout, consistent with [25], who reported that substitution up to 25% was sensory-nearest to the control with no significant flavour differences, and with [26], who similarly observed that sensory scores in flaxseed oil-fortified queso blanco declined more markedly at higher oil concentrations. However, the designation of T1 as the optimal treatment must be considered critically across all quality dimensions rather than on sensory grounds alone: T1 also demonstrated favourable microbiological counts, compositionally moderate moisture and ash values, and lower oxidative risk relative to higher-substitution treatments, making it the most balanced formulation when all quality indicators are considered together. From a product-development standpoint, these findings collectively indicate that a flaxseed oil substitution threshold of approximately 25% represents the upper practical limit for maintaining consumer acceptability without compromising overall quality, and that future formulation work should prioritise the inclusion of natural antioxidants such as rosemary or vitamin E to suppress lipid oxidation in higher-substitution variants, as lipid oxidation during storage has been identified as the primary driver of sensory deterioration in omega-3-fortified dairy products [38]. Furthermore, emulsification optimisation as demonstrated by the beneficial role of soya-lecithin in improving sensory stability in flax-fortified cheese systems and adoption of oxygen-barrier or vacuum packaging would be necessary to extend shelf life beyond the 21-day window demonstrated here, given that packaging type has been shown to significantly influence the oxidative and sensory stability of omega-3-enriched food products [38]; the use of oil-in-water emulsion systems has also been shown to substantially delay off-flavour development in processed cheese fortified with unsaturated oils [39]. Although emulsifiers such as soya lecithin mitigated the impacts, as in [40] flax-fortified cheeses. The general decline in sensory attributes over time highlights the perishable nature of cheese spread and the importance of appropriate storage to maintain consumer satisfaction.

Limitations

This study was conducted under controlled laboratory conditions with a storage period limited to 21 days, which may not fully represent commercial processing or extended market storage conditions, and extrapolation to large-scale industrial production should be made with caution. Oxidative stability assessment was incomplete, as detailed fatty acid profiling via GC analysis and indicators such as anisidine value or Rancimat induction time were not included. Microbial acceptability thresholds were not clearly defined for this product matrix, and rheological and textural properties were not systematically reported as quantitative results, restricting objective comparison across formulations and storage intervals. Finally, sensory evaluation relied on a limited panel, which reduces the statistical robustness and generalizability of the hedonic assessments.

CONCLUSION

In conclusion, the incorporation of flaxseed oil into cheese spread significantly influenced its physicochemical, microbiological, and sensory properties. While flaxseed oil positively impacted the fatty acid profile by increasing unsaturated fatty acids, its higher concentrations generally led to a decrease in initial sensory scores (T3, T4) due to oil's distinct colour and flavor profile. T0 (control) and T1 (25% oil) maintained superior acceptability. A greater increase in total bacterial count over time as fresh samples had initial TBC values ranging from 8.3 log₁₀ CFU/g (T0) to 9.1 log₁₀ CFU/g (T4). By Day 21, the TBC for T0 rose to 10.1 log₁₀ CFU/g, while T4 showed a more pronounced increase, reaching 11.6 log₁₀ CFU/g. Moisture, titratable acidity, pH, and ash content also showed significant changes influenced by both flaxseed oil levels and storage duration. The T1 treatment (25% flaxseed oil) consistently demonstrated a balance between nutritional enrichment and maintaining desirable sensory attributes as compared to T2, T3 and T4. Future research should focus on optimizing flaxseed oil incorporation levels, exploring different emulsifiers or processing techniques, and investigating the specific microbial changes to further enhance the shelf life and overall quality of flaxseed oil-fortified cheese spreads.

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