PHYSICOCHEMICAL CHANGES IN ZINC-FORTIFIED SOY CHEESE: A TWO-MONTH STORAGE STUDY FOR ENHANCED PRODUCTION AND SHELF LIFE OPTIMIZATION IN THE DAIRY INDUSTRY

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ABSTRACT

The objective of this study was to examine the physicochemical characteristics of soy cheese enriched with zinc sulfate throughout a two-month storage period. It was carried out at the Dairy Technology Laboratory, National Institute of Food Science and Technology, University of Agriculture, Faisalabad. The study concentrated on the effects of varying zinc sulfate fortification levels on important quality characteristics, including moisture, protein, fat, ash, pH, and acidity. The cream, milk, soybeans, and other raw materials were obtained, and the cheese spread was made according to accepted procedures. For 60 days of storage, samples were routinely examined in four treatment groups, each with a different degree of zinc sulfate fortification. The findings showed that the length of storage had a substantial effect on the moisture content, which gradually decreased with time. Although it varied between treatments, the protein content remained mostly constant while being stored. The amount of fat did not change between treatments or storage times. The concentration of minerals and moisture loss may have contributed to the increase in ash content over the course of storage. As the product was stored, the pH dropped and the acidity rose, indicating continued microbial activity. All things considered, this research offers insightful information about the physicochemical alterations that zinc-fortified soy cheese spreads during storage. The dairy industry may find the findings useful in maximizing the production and shelf life of comparable products. In order to ensure product quality and market viability, more investigation is required into sensory attributes and consumer acceptability.

Keywords: Soy cheese, Zinc sulfate fortification, Physicochemical analysis, Storage time, Moisture content

1. INTRODUCTION

In all animal species, milk a physiologically necessary secretion of the mammary glands of female mammals is vital to the upbringing of young. But humans have also taken advantage of it because of its high nutritional content, making it a staple of diets all across the world. Milk is a complete food supply since it is high in lactose, protein, fat, water, calcium, and phosphorus, among other micronutrients. Because of its high nutritional density, it is an essential food that benefits people's general health and wellbeing regardless of age or medical condition (Kubicová et al., 2019). Approximately 6 billion people worldwide consume milk and its derivative products, taking advantage of its nutritional advantages (Thorning et al., 2016). Beyond its inherent worth, milk is used as a raw material to make a range of dairy products, such as butter, cheese, yogurt, and more. According to Thorning et al. (2016), milk's high calcium content is essential for maintaining bone health and preventing diseases like osteoporosis. For generations, dairy products which come from various animal sources such as sheep, goats, and cows have been essential to human nourishment (Jo et al., 2018). Apart from meeting dietary needs, they are also culturally significant, representing regional culinary customs. The significance of dairy products and milk has led to a great deal of research on the effects these foods have on human health. These investigations offer important new understandings into the impacts of certain milk components and whole dairy products on human health (Visioli and Strata, 2014).

The dairy business is a significant economic force, and the number of domesticated animals has grown significantly. Productivity gains in this industry have been significant in recent years, thanks to initiatives including enhanced health coverage, cutting-edge animal breeding techniques, management procedures, artificial insemination services, specialized animal feed, and the prevention of livestock diseases (GOP, 2020). The tastes of consumers are changing, and there is an increasing need for dairy products with added benefits to health and immunity. Interest in value-added dairy products with particular geographic and functional qualities has increased as a result of this trend (Fortin et al., 2011). Cream cheese, renowned for its rich diacetyl flavor and silky texture, is one such notable dairy product. Cream cheese is usually made by mixing cream with whole milk or skim milk; the minimum amounts of fat and moisture content are specified by regulations. It can be used in a variety of culinary applications, such as spreads, baked dishes, and desserts (Almanza-Rubio et al., 2016).

The market value of the cheese production sector worldwide is expected to reach US$65 billion to US$68 billion by 2020, indicating its robust growth. Major players in this market are the USA and the EU-27, with Germany being the producer of the most cream cheese. Cream cheese is an essential product in the food industry due to its versatility in a variety of culinary scenarios (Pombo, 2021). Cheese has long been a staple of human diets, with several types called after their respective regions of origin (Josipović et al., 2015). Over time, the method of manufacturing cheese has changed dramatically, moving from cottage industry practices to a crucial area within the processed food industry. To obtain the correct texture and consistency, a number of complex procedures are involved in the creation of cheese spreads, including cream cheese spread. In order to create soluble sodium para-caseinate, an efficient emulsifier, from insoluble calcium para-caseinate, emulsifying salts are added (Guinee, 2009). Stabilizers and emulsifiers, as well as the amount of fat and moisture in the spread, all affect the final texture and qualities of cheese spreads (Walther et al., 2008). Beyond its taste, cheese is a nutritional powerhouse that contains proteins, bioactive peptides, vital amino acids, and other healthy ingredients (Boutrou and Gueguen, 2005). Cheese spread's smooth smoothness is largely attributed to the use of emulsifying salts in its manufacturing (Toro et al., 2016).

A soft cheese variation known for its creamy texture, somewhat tart flavor, and endless culinary uses is cream cheese spread. Acidification, coagulation, curd scalding, whey drainage, heat treatment, shearing, and the addition of stabilizers and emulsifiers are some of the processes involved in its manufacturing (Schulz-Collins and Senge, 2004). Because of its many uses, cream cheese spread can be enhanced by adding different tastes, spices, and other additions to improve its sensory qualities (Shirashoji et al., 2006). According to Guinee and Hickey (2009), the commercial production of cheese spreads is an efficient process that minimizes cheese waste and produces products with significant nutritional and financial value. In order to preserve texture and quality, appropriate additives must be used in the preparation of low-fat cheese spread, which has also gained popularity (El-Shibiny et al., 2007). Soybeans have grown in importance in human diets due to their high protein content and nutritional value (Gulzar et al., 2015). Products made from soy, such as soy milk and cheese, are becoming more and more popular as substitutes for a variety of dietary needs, such as vegetarianism and lactose intolerance (Idowu-Adebayo et al., 2022). The incorporation of soy-based meals has exhibited possible health advantages, such as mitigating the likelihood of cancer, obesity, and osteoporosis, and managing gastrointestinal and kidney disorders (Li et al., 2013). Particularly soy milk has grown in popularity as a wholesome, vegan-friendly beverage (Jayarathna et al., 2021). Products made from soy are flexible and can be employed in a variety of culinary contexts (Agyei, 2015).

The primary objective is to create a zinc sulfate-fortified soy cheese spread by following exact scientific guidelines. This involves carefully combining zinc sulfate with soy-based ingredients to produce a new dairy substitute with improved nutritional qualities. A thorough scientific analysis of the physicochemical characteristics of the fortified soy cheese spread will be carried out in order to provide a full evaluation. This provides scientifically based insights into its structural and compositional features. It also offers in-depth assessments of its chemical composition, textural profile, and moisture content. Through the rigorous pursuit of these scientifically defined goals, this study seeks to provide solid empirical data about the origins, makeup, and prospective applications of the fortified soy cheese spread. This evidence-based methodology makes sure that the results are founded on strict scientific principles, which improves the validity and relevance of the study's conclusions. In brief, milk and dairy products that is, cream cheese and its soy-based substitutes are crucial to human nutrition and cooking. Due to their many uses and nutritional value, they are an essential part of diets all around the world. In order to give readers a thorough grasp of these dairy and soy-based products' roles in human nutrition and the food industry, this study will go deeper into their manufacturing, characteristics, and health consequences.

2. MATERIALS AND METHODS

The dairy Technology Laboratory, National Institute of Food Science and Technology (NIFSAT), Faculty of Food, Nutrition and Home Sciences (FFNHS), University of Agriculture, Faisalabad (UAF) is where the research for this thesis was conducted. All the details about the cheese spread's ingredients, production process, and flowchart have been provided. The years 2021–2022 saw the completion of the research trials.

2.1. Procurement of raw material

We bought the cream, soybeans, and milk needed for the experiment from the neighborhood market. We have used Chr. Hensen's freeze-dried mesophilic microbial culture for cheese and rennet. However, other components like stabilizers, emulsifiers, preservatives, and other reagents needed for analysis were bought from Sigma Aldrich's official distributor, New Chemical Centre (NCC), Lahore.

2.2. Ingredients

Milk, soybeans, cream, rennet, distilled monoglycerides, ascorbic acid, disodium phosphate, carrageenan, and locust bean gum are among the ingredients used in this situation. These components are frequently used in food processing, especially when making different dairy products and spreads. Their particular contributions to the final product's overall quality and features can take many different forms, including coagulation, emulsification, and texture enhancement. To get the intended results in food production, it's critical to measure and mix these ingredients precisely according with established recipes and formulations.

2.3. Preparation of soybean paste

After the beans were thoroughly cleaned of all impurities, they were soaked in water containing 0.3% sodium bicarbonate for ten to twelve hours in order to eliminate the bitterness. After 12 hours, the beans will have their husk removed and cleaned with fresh water. To make a paste, soaked soybeans were ground (Li et al., 2013).

2.4. Preparation of soy cheese spread fortified with zinc Sulphate

It started with standard full cream milk (4.6 percent fat) and 34% fat cream to make the zinc-fortified soy cheese spread. One could achieve a 13% fat content by mixing 1400 milliliters of milk with 600 milliliters of cream. After that, the mixture was homogenized to guarantee even distribution of fat. The milk was homogenized and then pasteurized for 20 minutes at 70°C. After the milk was homogenized, soybean paste was added and the temperature was lowered to 30°C. After adding 0.78g/kg of freeze-dried mesophilic culture, the mixture was incubated for 30 minutes. After adding rennet, the incubation process was continued until curds formed. After the mixture had curdled, it was sliced into smaller blocks and scalded for ten minutes at 40°C. Fresh cream cheese was produced by draining the whey that was produced during curding for at least 45 minutes using cheesecloth. The curd was mixed, sheared, and heated in order to make the cheese spread. After heating the cheese to 70°C, shearing and mixing were done for ten minutes with a hand stirrer. Emulsifiers and stabilizers were added in this step, making up 2% of the initial weight of the sample. Zinc sulfate was also added in accordance with the planned treatment strategy.

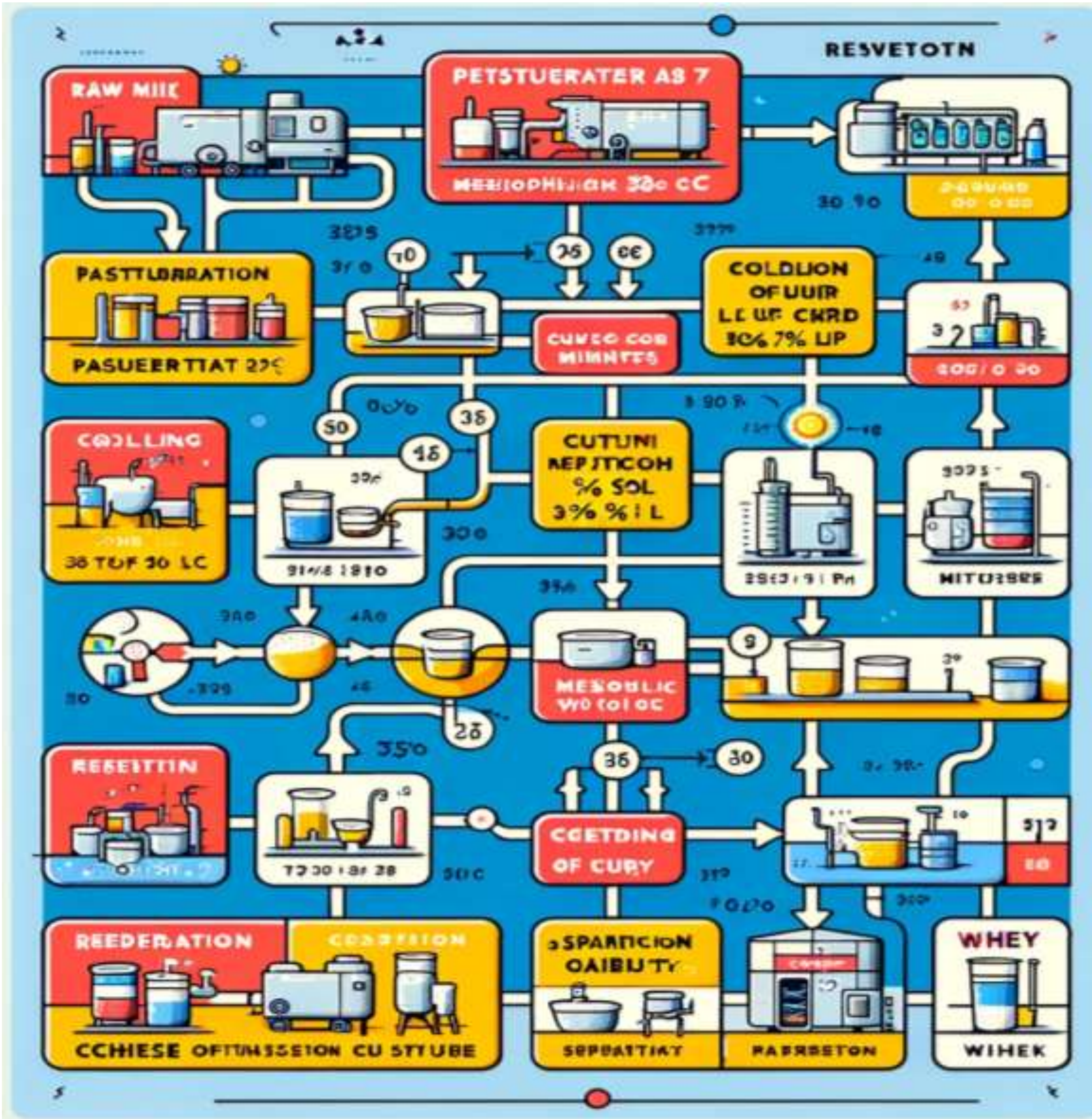

Figure 1: Flowchart for cheese spread processing

Table1. Treatment plan and doses

Treatment (S)	Soybeans Paste (g)	Zinc Sulphate (mg)
T_0	-	-
T_1	40	6
T_2	40	9
T_3	40	12

2.5.Storage study

Storage study of cheese spread will be performed for 2 months, and analysis will be done after every 15 days.

Table 2. Study storage of cheese spread

Study	Storage days
1	0
2	15
3	30
4	45
5	60

2.6.Proximate Analysis of Zinc-Fortified Soy Cheese Spread

2.6.1. Moisture Analysis of Zinc-Fortified Soy Cheese Spread

It used the conventional AOAC (2016) method to ascertain the moisture content of the zinc-fortified soy cheese spread. We used a digital balance to weigh the soy cheese spread that had been supplemented with zinc sulfate. A 3g sample was dried in a hot air oven for 24 hours at 100±5°C. The sample was taken out of the oven once it had dried completely, allowed to cool in a desiccator to avoid moisture reabsorption, and then weighed again. The following formula was used to get the moisture content:

Moisture (%) = (Initial sample weight - Weight of residue after drying) × 100 / Initial sample weight

2.6.2. Protein Determination of Zinc-Fortified Soy Cheese Spread

The Kjeldahl apparatus was used to measure the crude protein content of the zinc-fortified soy cheese spread in accordance with AOAC (2016) guidelines. The Kjeldahl flask was filled with a 1g sample of cheese spread and covered with butter paper. The Kjeldahl flask also received one digestion tablet and thirty milliliters of concentrated H_2SO_4. The mixture was allowed to break down for three to four hours, during which time it took on a light green hue. Using the Kjeldahl method, the protein content was determined following dilution and distillation, and a factor of 6.38 was applied:

Protein (%) = (Nitrogen (%) × 6.38)

2.6.3. Fat Determination of Zinc-Fortified Soy Cheese Spread

The fat content of soy cheese spread fortified with zinc was measured using the methodology outlined in AOAC (2016). 16 milliliters of distilled water were used to dissolve a 6-gram sample, which was then shaken until it was fully dissolved. After that, the butyrometer was filled with 10 milliliters of H_2SO_4, 1 milliliter of isoamyl alcohol, and the sample slurry. After giving the sample a good shake to dissolve it, the butyrometer was centrifuged. The butyrometer's scale was used to calculate the fat content.

Crude Fat (%) = (Initial sample weight (g) - Final sample weight (g)) × 100 / Initial sample weight

2.6.4. Ash Contents of Zinc-Fortified Soy Cheese Spread

Using the AOAC (2016) method, the ash content of soy cheese spread fortified with zinc was determined. A 5g sample that was devoid of moisture was gathered in a china dish and burned in order to eliminate volatile substances. After that, the sample was burned at 550°C in a muffle furnace until it turned into a powdered residue that was gray in color. The ash was represented by the remaining residue, and its percentage was computed using the following formula:

Ash % = (Weight of crucible and ash - Weight of crucible) × 100 / (Weight of crucible and sample - Weight of crucible).

2.6.5. PH Measurement:

Using a Hanna Instruments pH meter, the pH of the soy cheese spread fortified with zinc sulfate was measured in compliance with AOAC guidelines (2012). The pH meter was calibrated using buffer solutions with values of 4 and 7. We took a sample of the soy cheese spread and dilute it with purified water. After cleaning the electrode of the pH meter with an appropriate solution, the electrode was submerged in the sample, and the pH reading was seen on the meter's display screen.

2.6.6. Acidity Determination:

A slurry was made by combining 1g of the sample with 9ml of distilled water in order to measure the soy cheese spread's acidity. Using phenolphthalein as an indicator, a 0.1N NaOH solution was used to titrate this slurry. The burette's initial and final readings were noted, and the following formula was used to determine acidity:

Acidity = (Volume of 0.1N NaOH used × 0.009) / 10 ml × 100%

3.7. Statistical Analysis:

Minitab software was used to perform one-way ANOVA under a completely randomized design (CRD) for data analysis. According to Montgomery (2017), Tukey's test was used to compare means.

3. RESULTS AND DISCUSSION

The major goal of this research was to prepare a soy cheese spread and evaluate its qualityparameters to deduct our conclusion. To reach this target, soybean paste was prepared in the first step. After that, in the second step, a base of cream cheese and soybean paste was used to prepare the soy cheese spread. Four treatments of soy cheese spread were developed by adding different concentrations of zinc sulphate for experimentation and others are keptconstant. To evaluate the overall quality of soybean cheese spread, several analyses were conducted such as physicochemical analysis. All these analyses were conducted in the Dairy Technology Laboratory as well as other labs of the National Instituteof Food Science and Technology (NIFSAT). Following were the treatments of soy cheese spread which was prepared: T_0 sample fortified with 0mg of zinc sulphate (ZnS) T_1 sample fortified with 6mg of zinc sulphate (ZnS) T_2 sample fortified with 9mg of zinc sulphate (ZnS) T_3 sample fortified with 12mg of zinc sulphate (ZnS). The undergoing treatments were subjected to Physicochemical analysis and statistical analysis with the time interval of 0, 15, 30, 45 and 60 days.

3.1. Physicochemical analysis of soy

cheese spread fortified with zinc sulphate

3.1.1. Moisture analysis of soy cheese spread fortified with zinc sulphate

Moisture is a significant component of cheeses used for classification since it adds softness, quality attributes, and acts as a reactant in many reactions. In addition to being important for product standards, moisture assessment impacts cheese's textural characteristics, shelf life, and functional qualities (Loudiyi *et al*., 2019). Moisture is bounded with protein to retain cheese elasticity and improve pliability and meltability, as evidenced by improved flow-ability (Hubbard *et al*., 2013). Table 3 shows the results of statistical analysis of variance which indicated that the impact of treatment on the moisture content is non-significant ($p>0.05$) whereas the effect of storage was highly significant ($p<0.01$). Meanwhile, the interaction effect of treatment and storage was also found to be non-significant ($p>0.05$). The mean values of moisture ofsoy cheese spread are shown in table 3. The table shows that the range of mean values of moisture content of different treatment of cheese spread is 57.52 to 58.53%. On further inspection, the minimum reading of the moisture content was 57.545% of treatment T0 while the maximum reading was 58.518% of treatment T3.

The moisture content for the cheese spread documented by Gámbaro *et al*. (2017) range from 53%-56.2% and the study of Kondyli *et al.* (2016) suggest the moisture content of cheese spread ranges from 54%-57.4%. Results from both Gambaro and Kondyli are comparable to the current study.

Table 3 Impact of storage interval on the moisture content

Treatment	Storage (Days)					Mean
	0	15	30	45	60	
T0	58.53± 0.4	58.02± 0.3	57.54±0.5	57.54±0.5	57.54±0.5	58.03^{b}
T1	58.51 ±0.5	57.99± 0.6	57.54± 0.6	57.54±0.4	57.53±0.6	58.02^{b}
T2	58.52 ±0.5	57.98± 0.8	57.55± 0.4	57.52±0.3	57.54±0.4	58.03^{ab}
T3	58.51±0.8	57.99± 0.9	57.54± 0.4	57.54±0.7	57.53±0.4	58.03^{b}

Means that share the similar letter in a column or row are not significantly ($p>0.05$) different from one another. T0 = sample fortified with 0mg of zinc sulphate (ZnS) T1 = sample fortified with 6mg of zinc sulphate (ZnS) T2 = sample fortified with 9mg of zinc sulphate (ZnS) T3 = sample fortified with 12mg of zinc sulphate (ZnS)

3.1.2. Protein determination of soy cheese spread fortified with zinc sulphate

Cheese is typically a great source of protein primarily casein. Crude protein is considered an important quality parameter of cheese and cheese products like cheese spread because cheese is a concentrated protein gel. Gelation of cheese milk, dehydration of the gel toproduce a curd, and treatment of the curd (including texturization, stirring, molding, salting,and pressing etc.) are all steps in the manufacturing process (Korakhashvili, 2016). The final cheese quality and its acceptance are determined by the total protein content and biochemical alterations that occur throughout production and ripening of the cheese. Whilecasein protein present in milk which mainly contributes during ripening and processing of milk. It improves the melting and stretching properties of cheese spreads (Guinee, 2009). Table 4 shows the results of statistical analysis of variance, which indicated that the p- value for the treatments was highly significant ($p<0.01$) which can be easily verified fromthe mean values of the samples. On the other hand, the storage time and their interaction with treatment showed no significant impact ($p>0.05$) on the protein content. Table 4 shown the mean values of protein content. The minimum value of protein content was atT_0 with 10.04% at 0-day storage and maximum value was at T_3 with 17.19% at 30-daystorage. The protein content for the cheese spread documented by Santos *et al*. (2016) have same values according to the current study. High protein contents of the milk yield high yield ofcheese spread.

Table 4. Impact of storage interval on the crude protein

Treatment	Storage (Days)					Mean
	0	15	30	45	60	
T0	10.04 ±0.7	10.03 ±0.7	10.05 ± 0.9	10.05 ± 0.9	10.06 ± 0.9	10.04^{e}
T1	15.52 ±0.9	15.48 ±0.8	15.53±0.04	15.46±0.04	15.49±0.3	15.49^{d}
T2	16.34±0.9	16.32 ±0.3	16.35 ±0.8	16.33±0.8	16.30 ±0.5	16.32^{c}
T3	17.18 ±0.7	17.17 ±0.6	17.19±0.09	17.18±0.9	17.16± 0.4	15.17^{b}

Means that share the similar letter in a column or row are not significantly ($p>0.05$) different from one another. T0 = sample fortified with 0mg of zinc sulphate (ZnS) T1 = sample fortified with 6mg of zinc sulphate (ZnS) T2 = sample fortified with 9mg of zinc sulphate (ZnS) T3 = sample fortified with 12mg of zinc sulphate (ZnS)

3.1.3. Fat determination of soy cheese spread fortified with zinc sulphate

Fat is the basic content of milk and mainly present in cheese spreads. It contributes to color,taste, flavor, appearance, and texture by changing the interstitial space of minerals structureand protein network. It enhances the firmness, spreadable texture and melting properties of product. Cheese spread is the conversion of fat and protein content of the milk. Emulsifierslike CMC and lecithin are used to replace the fat contents in cheese spreads. It also improves the flavor and better mouth feel properties of cheese spreads. Determination of fat content is essential for cream cheese spread to qualify it to be called cream cheese according to laws and regulations (Frye, 2015). The table 5 is giving the result of the statistical analysis which shows that the effect oftreatment on the fat content of the soy cheese spread samples was non-significant (p>0.05). Similarly, the outcome of storage time on the samples was also non-significant (p>0.05). Likewise, the interaction effect of treatment and storage time was also found to be non- significant (p>0.05). If we examine the table 5 closely, the table shows that the highest fat content was 30.151% of treatment T2 at 0-day storage and the lowest mean value was 29.950% in treatment T3 at 15 days of storage. The fat content for the cheese spread documented by Hayaloglu *et al*. (2013) range from 20%-33% and the study of Hamad and Ismail (2012) suggest the fat content is significantly high. Results from both Hayaloglu and Hamad are comparable to the current study. Kondyli*et al*. (2016) reported slightly decreased results as he prepared soft cheese by using thermophilic culture which may be the reason of deviation of results.

Table 5 Impact of storage interval on fat content

Treatment	Storage (Days)					Mean
	0	15	30	45	60	
T0	30.15 ±0.7	30.02±0.5	29.98± 0.7	29.98± 0.7	29.98±0.7	29.98[a]
T1	30.15 ± 0.8	30.04±0.6	30.05± 0.5	30.05± 0.5	30.05±0.5	30.08[a]
T2	29.97 ± 0.5	30.17±0.7	30.07± 0.4	30.07± 0.4	30.07±0.1	30.07[a]
T3	30.07 ± 0.4	29.95±0.4	30.04± 0.8	30.04± 0.8	30.04±0.8	30.02[a]

Means that share the similar letter in a column or row are not significantly (p>0.05) different from one another. T0 = sample fortified with 0mg of zinc sulphate (ZnS) T1 = sample fortified with 6mg of zinc sulphate (ZnS) T2 = sample fortified with 9mg of zinc sulphate (ZnS) T3 = sample fortified with 12mg of zinc sulphate (ZnS)

3.1.4. Ash contents of soy cheese spread fortified with zinc sulphate

Ash in any food is an inorganic residue that remained after combustion of the organic material of that food. Higher amount of ash indicates higher amount of minerals in the sample. The main purpose of measuring the ash content is to measure the mineral matter present in the food product. Due to possible loss from volatilization, the ash produced maynot always have the same composition as the mineral matter found in the original food. The composition of cheese, in terms of minerals, is rather variable and depends on such factorsas the initial composition of the milk and cheese making procedures which are different fordifferent cheese types (Stocco et al., 2021). Inorganic residues improve the stability and melting properties of cheese spreads. Ash contents in milk imparts ionic strength and gives gel formation and is also rich in calcium (Fox *et al.*, 2017). Table 6 expresses the influence of storage time on the ash content of cream cheese spread samples and it was non-significant ($p>0.05$) whereas the impact of treatment on the samples were highly significant ($p<0.01$). Meanwhile, the interaction effect of both treatment and storage time on the samples was also non-significant ($p>0.05$). The table 6 indicates the overall mean values of the measured ash content according to the treatments and storage time. On closer inspection of the data, the maximum value was found to be 1.253% in T_3 at 15 days of storage and the minimum value was 1.010% in T0 at 0 days of storage.

The ash content for the cheese spread documented by Santos *et al*. (2016) range from 1.3%-1.5% which is significantly high. Results from Santos are comparable to the current study.According to the findings of the cheese spread brining study, the increase in ash contents may be linked to the increase in salt contents. When salt is added to cheese spread, a rind forms, and moisture is lost over time which leads to an increase in salt content that is the cause of the rising ash contents. Since salt is a mineral that was left in the sample after burning in a muffle furnace its presence caused the ash contents to increase (El-Shibiny *et al*, 2007).

Table 6. Impact of storage interval on ash content

Treatment	Storage (Days)					Mean
	0	15	30	45	60	
T0	1.05 ± 0.6	1.04 ± 0.8	1.04 ± 0.5	1.05 ±0.6	1.04 ± 0.5	1.044[a]
T1	1.04 ± 0.9	1.03 ± 0.4	1.03±0.09	1.03±0.7	1.04±0.09	1.034[b]
T2	1.05±0.09	1.05±0.10	1.05±0.11	1.05±0.11	1.05±0.11	1.05[c]
T3	1.04 ± 0.4	1.04±0.11	1.04 ± 0.4	1.04 ± 0.4	1.04 ± 0.4	1.04[d]

Means that share the similar letter in a column or row are not significantly ($p>0.05$) different from one another. T0 = sample fortified with 0mg of zinc sulphate (ZnS) T1 = sample fortified with 6mg of zinc sulphate (ZnS) T2 = sample fortified with 9mg of zinc sulphate (ZnS) T3 = sample fortified with 12mg of zinc sulphate (ZnS)

3.1.5. PH of soy cheese spread fortified with zinc sulphate

PH is the most crucial parameter to evaluate food products quality and especially in the case of cheese and cheese products like cheese spread. The impact of pH on the quality of cheese spread and other dairy products can be enormous. Fluctuation in the pH causes the change in the sensory and textural properties of the food product. Low pH gives acidic flavor and high pH gives creamy flavor. Similarly, a decrease in pH changes the texture to become grainy and firm. On the other hand, an increase in pH causes it to become moist, soft, and elastic texture. The pH and acidity have an inversely proportional relationship with each other, and pH decreases due to the increase in the acidity. The lowering of pH and increasing acidity might be due to the slow activity of the residual bacteria left which was added during the acidification stage of manufacturing, producing lactic acid in small quantities (Snyder et al., 2021).

Effects of treatments and storage interval implied in the research are presented in the table7. The ANOVA table for the pH of the cheese spread samples exhibited that the influence of treatments on the pH of samples remains non-significant ($p>0.05$). Meanwhile,the impact of storage intervals on cheese spread was highly significant ($p<0.01$), whereas the impact of interaction between treatment and storage was non-significant ($p>0.05$). Means of different treated cheese spread samples have been exhibited in the table 7 which shows that the maximum mean value of pH was 5.02 in T0 and the minimum mean value 4.73 was found in T4. The pH content for the cheese spread documented by Mozuraityte (2019) range from 5.7%-5.9%which is significantly high. Results from Mozuraityte are comparable to the current study indicates that increasing the storage period resulted in a reduction in the pH of the cheese spread (Bulut *et al*., 2019).

Table 7. Impact of storage time on the pH

Treatment	Storage (Days)					Mean
	0	15	30	45	60	
T0	5.02± 0.4	4.80±0.02	4.75±0.3	4.76±0.3	4.74±0.3	4.92[a]
T1	5.01 ± 0.6	4.81 ± 0.2	4.81 ± 0.5	4.75 ± 0.4	4.75± 0.3	4.93[a]
T2	5.00 ± 1.0	4.80 ± 0.4	4.81±0.06	4.76±0.0.6	4.74± 0.05	4.94[a]
T3	5.01± 0.7	4.80±0.06	4.80±0.1	4.74 ± 0.2	4.73 ± 0.4	4.91[a]

Means that share the similar letter in a column or row are not significantly ($p>0.05$) different from one another. T0 = sample fortified with 0mg of zinc sulphate (ZnS) T1 = sample fortified with 6mg of zinc sulphate (ZnS) T2 = sample fortified with 9mg of zinc sulphate (ZnS) T3 = sample fortified with 12mg of zinc sulphate (ZnS)

3.1.6. Determination of acidity of soy cheese spread fortified with zinc sulphate

A regulated synthesis of lactic acid from lactose by LAB is a crucial stage in the manufacturing process of cheese spread because it is a fermented dairy product. The acidityis started by the LAB added to milk during cheese making. To determine a food's overall acid content, titratable acidity is computed (Łepecka *et al.*, 2022). This value is the result of an extensive titration of intrinsic acids with a reference base. Titratable acidity, rather than pH, is a good indicator of acid's effect on flavor. There is an inverse relation betweenpH and acidity. When acid production starts due to the activity of lactic acid bacteria, the titratable acidity increases meanwhile the pH decreases (Rusmana *et al.*, 2020). To determine the quality of the cheese spread, acidity is an important parameter because acidity effects the texture and sensory properties of the food (Cais et al., 2021).

Table 8 shows the results of statistical analysis of variance for acidity in which the impact of storage was observed highly significant ($p>0.01$) and the treatment impression on acidity was non-significant ($p>0.05$). However, the interaction of treatment and storage and its effects on acidity remains non-significant. Mean values of percentage acidity of various treatments are given in table 8. The highest value of acidity was 0.089% observed in treatment T3 at 60 day of storage study and the lowest value of acidity was 0.443% in T3 at day 0, which shows that there is significant impact of storage on the acidityof soy cheese spread. The rising acidity may be a result of the storage temperature activating the milk's natural microflora and causing lactose fermentation which led to the developmentof acidity. The main reason to produce acid is due to the fermentation of Lactose present in the milk by mesophilic or thermophilic lactic acid bacteria. The results are comparable to the study conducted by Harper et al., (2022) in whichthe effect of storage on the sensory and physicochemical characteristics of Sudanese whitecheese, soymilk cheese, and their mixture was examined. The results indicated that the storage period had a substantial ($p<0.001$) impact on the titratable acidity, and it was found that the highest levels of acidity were attained at the end of the storage period.

Table 8. Impact of storage time on acidity percentage

Treatment	Storage (Days)					Mean
	0	15	30	45	60	
T0	0.44 ± 0.1	0.58 ± 0.4	0.79 ± 0.7	0.82 ± 0.7	0.87 ± 0.7	0.61[a]
T1	0.44 ± 0.2	0.57 + 0.5	0.78 ± 0.8	0.84 ± 0.1	0.87 ± 0.8	0.60[a]
T2	0.44 ± 0.2	0.58 ± 0.3	0.78 ± 0.5	0.84 ± 0.3	0.88 ± 0.5	0.61[b]
T3	0.43 ± 0.3	0.59 ± 0.1	0.79 ± 0.1	0.85 ± 0.1	0.89 ± 0.1	0.60[b]

Means that share the similar letter in a column or row are not significantly ($p>0.05$) different from one another. T0 = sample fortified with 0mg of zinc sulphate (ZnS) T1 = sample fortified with 6mg of zinc sulphate (ZnS) T2 = sample fortified with 9mg of zinc sulphate (ZnS) T3 = sample fortified with 12mg of zinc sulphate (ZnS)

CONCLUSION

The research focused on creating a soy cheese spread enriched with zinc sulfate, a nutritious and versatile product. Zinc is essential for our health. This study was conducted at NIFSAT, University of Agriculture, Faisalabad. The process involved several steps, including making soybean paste milk, standardizing cream, pasteurization, acidification, coagulation, removing whey, and adding zinc sulfate, emulsifier, stabilizer, and salt. Different treatments were tested, and after a 60-day storage period, it was observed that storage time had an impact on pH, moisture content, acidity, and plate count. The treatments affected the protein and ash content, while the fat content remained constant. Zinc levels increased with higher treatment concentrations, although they slightly decreased over time but remained higher than the control group. The panel of judges noticed taste differences due to the natural beany flavor of soybeans. Overall, Treatment T3 was found to be the most sensory acceptable. In summary, this study successfully produced a zinc-fortified soy cheese spread that not only provides essential nutrition but also offers a pleasing taste.

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