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# Effect of Calcium Chloride and Trisodium Phosphate Fortification on Low Fat Buffalo Milk Cheeses

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**Abstract.** Cheese is a dairy product that is highly preferred by consumers. Cheese is a delectable and nutritious food item. Enhancing the quality of cheese necessitates innovation in cheese processing. The objective of the study was to determine the impact of calcium chloride and trisodium phosphate on the physical, chemical, microbiological, and rheological characteristics (namely firmness) of low-fat buffalo milk cheeses. This study had a fully randomized design, consisting of nine treatments and three replications. The cottage cheese, mozzarella, and cheddar were treated with varying quantities of calcium chloride and trisodium phosphate (10, 20, and 30 mM) and then stored for a period of 30 days. The cheeses supplemented with salt exhibited a noteworthy ($p \leq 0.05$) rise in pH, total nitrogen (TN), non-casein nitrogen (NCN), and non-protein nitrogen (NPN) for nearly all treatments. However, a subsequent decline was noted after storage. Similarly, the hardness (N) significantly improved (from 330.33 to 454) among treatments and reduced (from 427 to 276.33) after 30 days of storage, with a p-value of $\leq 0.05$. In addition, the total plate count (TPC) and total viable count (TVC) showed an upward trend during the ripening phase. In conclusion, it was observed that the physicochemical, rheological, and microbiological quality characteristics of cheeses can be effectively regulated with appropriate mineral fortification.

**Keywords:** calcium chloride, microbial traits, physicochemical attributes, trisodium phosphate

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## 1. Introduction

Cheese is widely consumed worldwide due to its abundant nutritional content. In response to consumer preference, cheese recipes have recently undergone different alterations. Cheese production serves as a means of preserving crucial nutrients found in milk, which are necessary for the growth and maintenance of the body [1]. Calcium chloride and phosphate have a vital part in the process of rennet coagulation of milk, as well as in buffering and organizing the structure of cheese. Calcium is added to neutralize the negatively charged casein residues, which leads to a gradual reduction in milk rennet coagulation time and an enhancement in micelles aggregation [2].

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The current worldwide situation necessitates a healthy and low-fat nutritional diet to prevent several lifestyle-related illnesses, including obesity, diabetes, coronary heart disease, and hypertension. Hence, it is imperative to create low-fat cheese alternatives that provide health benefits to customers. The daily consumption of total and saturated fats should not exceed 30% and 10% of the total energy intake, respectively, as advised [3]. This study aimed to evaluate the impact of different salts, namely calcium chloride and trisodium phosphate, on the quality of low-fat buffalo milk cheeses, including cottage, mozzarella, and cheddar. Furthermore, a range of cheeses underwent physicochemical, rheological, and microbiological analyses to evaluate their quality.

## 2. Materials and Methods

The dairy farm of the University of Agriculture, Faisalabad provided fresh buffalo milk. The milk was clarified using a cream separator. The media was acquired from Biokar Diagnostics, located in Pantin, France.

### 2.1. Analysis of Milk for Cheese Preparation

Following the collecting process, buffalo milk underwent a procedure to remove the cream in a hygienic environment and was subsequently stored at a temperature of 4°C. The milk's protein to fat ratio was normalized at 1.5% using Pearson's square technique. The acidity of milk samples was determined by titration using the method described by [4]. The fat content was quantified using the Gerber method, in accordance with the protocol described in reference [5]. The nitrogen concentration in milk was determined using technique [6]. The lactose content of milk was quantified using a lactoscope (Model No CIGMP-170), while the pH of milk was determined using a digital pH meter (Inolab WTW Series 720). A total of 9 distinct treatments were prepared during the cheese making process, with 3 duplicates for each treatment. These treatments are listed in Table 1.

**Table 1.** Treatment Plan for Cottage, Mozzarella, and Cheddar Cheeses

| Treatments | Description |
|---|---|
| $T_1$ | 10 mM each $CaCl_2$ and $Na_3PO_4$ enriched semi-skimmed cottage cheese |
| $T_2$ | 20 mM each $CaCl_2$ and $Na_3PO_4$ enriched semi-skimmed cottage cheese |
| $T_3$ | 30 mM each $CaCl_2$ and $Na_3PO_4$ enriched semi-skimmed cottage cheese |
| $T_4$ | 10 mM each $CaCl_2$ and $Na_3PO_4$ enriched semi-skimmed mozzarella cheese |
| $T_5$ | 20 mM each $CaCl_2$ and $Na_3PO_4$ enriched semi-skimmed mozzarella cheese |
| $T_6$ | 30 mM each $CaCl_2$ and $Na_3PO_4$ enriched semi-skimmed mozzarella cheese |
| $T_7$ | 10 mM each $CaCl_2$ and $Na_3PO_4$ enriched semi-skimmed cheddar cheese |
| $T_8$ | 20 mM each $CaCl_2$ and $Na_3PO_4$ enriched semi-skimmed cheddar cheese |
| $T_9$ | 30 mM each $CaCl_2$ and $Na_3PO_4$ enriched semi-skimmed cheddar cheese |

Note: Sample size n=36. EC: Electrical Conductivity; CEC: Cation Exchange Capacity

### 2.2. Preparation of Cottage, Mozzarella, and Cheddar Cheese

The calcium (Ca) and phosphorus (P) enriched cottage cheese was prepared by following the method of [7]. While mozzarella and cheddar cheeses were prepared by following the modified methods of [8] and [2] respectively. During heating process (65 °C), CaCl2 and Na3PO4 (10, 20 and 30 mM) were fortified because during cheese manufacturing mostly Ca and P present in colloidal phase.

### 2.3. Physicochemical, Rheological, and Microbial Analysis of Soft, Semi Hard, and Hard Cheese

A variety of soft, semi-hard, and hard cheeses were analyzed to evaluate their diverse physicochemical characteristics during a one-month aging period, with measurements recorded at intervals ranging from 0 to 30 days. In addition, the acidity, lactose, and nitrogen content were assessed using their respective methodologies [6]. The NCN content of soft, semi-hard, and hard cheese, which had been enriched with calcium and phosphorus, was determined using the method described in reference [9]. The NPN concentration of soft, semi-hard, and hard cheeses, which were enriched with calcium (Ca) and phosphorus (P), was determined using technique 20-4 as described in reference [10].The firmness of soft, semi hard, and hard cheeses was evaluated using the [11] technique. The total plate count (TPC) was determined following the parameters outlined in reference [12].

The total viable count (TVC) was determined using the methodology described in reference [13].

### 2.4. Statistical Analysis

The data that was gathered was examined utilizing statistical modeling, specifically by employing a completely randomized process. The effects of treatments on the examined parameters were analyzed using analysis of variance (ANOVA) with Statistical 8.1 software version.

## 3. Results and Discussion

The content of buffalo milk is variable and primarily influenced by genetic and environmental factors, lactation stage, health condition, milk production rate, and age of the animal [14]. An first assessment was conducted to determine the pH, acidity, total solids, lactose, moisture, fat, protein, and ash levels in the fresh milk. The pH was found to be 6.61 ± 0.33, acidity was 0.14 ± 0.02, total solids were 15.48 ± 0.77, lactose was 4.68± 0.23, moisture was 84.52± 2.53, fat was 6.35± 1.07, protein was 3.67 ± 0.18, and ash was 0.49 ± 0.02. Overall, as the days progressed, there was a noticeable decrease in pH from 5.38 ± 0.11 to 5.08 ± 0.13 between the $0^{th}$ and $30^{th}$ day.

Table 2 shows a significant decrease of pH from 5.40 ± 0.10 to 4.88 ± 0.03, 5.23 ± 0.12 to 5.04 ± 0.07 and 5.80 ± 0.28 to 5.06 ± 0.26 to 5.05 ± 0.16 in 30 mM mineral enriched cottage, mozzarella, and cheddar cheeses after 30 days of ripening. This result corresponds well with [15]. The authors demonstrated a notable decline in pH, from 5.20 to 4.75, in cheddar cheese over a period of 24 weeks of ripening. The storage revealed significant effect on TN% and maximum value was noted at 30$^{th}$ day 68.37 ± 1.32 for T9 while, T1 showed the lowest value of 31.09 ± 0.94% at 0 days of storage. This increase in TN could be due to low fat contents in the cheese milk [16] and depends on the preparation method used [17]. There was a significant rise in NCN contents from 2.91 ± 0.19 to 9.06 ± 0.36% between 0 days (T1) and 30 days (T9). Nevertheless, this pattern did not show statistical significance when comparing all treatments during the storage time. The number 18 is enclosed in square brackets. After analyzing an increase in non-casein nitrogen levels, it was determined that the decrease in intact casein might be linked to the probable impact of chymosin, protease, and peptides present in the initial culture. These factors contribute to the higher solubility of nitrogen. This event results in an elevation of the non-casein nitrogen (NCN) levels by decreasing the quantity of casein that is present. Moreover, the NPN content of several varieties of fully aged cheeses was significantly influenced ($p \leq 0.05$) by the different treatments (T1-T9).

**Table 2.** Characteristics of Soils Used for Isolating Purple Nonsulfur Bacteria in Thanh Phu - Ben Tre

| Parameter | $pH_{H2O}$ | $pH_{KCl}$ | EC | N total | $NH_4^+$ | Organic matter | $Al^{3+}$ | $Fe^{2+}$ | $Mn^{2+}$ | CEC |
|---|---|---|---|---|---|---|---|---|---|---|
| | - | - | mS $cm^{-1}$ | % | mg/kg | % | meq/ 100g | mg/kg | mg/kg | meq/ 100g |
| Maximum | 6.74 | 6.22 | 8.89 | 0.33 | 36.6 | 5.13 | 19.6 | 98.7 | 1456.3 | 13.5 |
| Mean | 5.75±0.75 | 5.39±0.41 | 2.91±2.26 | 0.21±0.05 | 23.1±6.47 | 3.38± 1.02 | 6.37±3.95 | 35.5±19.4 | 691.2±324.3 | 10.3±1.76 |
| Median | 6.05 | 5.28 | 1.99 | 0.21 | 22.4 | 3.31 | 5.15 | 27.6 | 587.9 | 10.0 |
| Minimum | 4.12 | 4.75 | 0.65 | 0.11 | 11.2 | 1.27 | 1.21 | 14.3 | 245.9 | 6.98 |

Note: Sample size n=36. EC: Electrical Conductivity; CEC: Cation Exchange Capacity

Isolation and purification resulted in 57 PNSB isolates from the salt-contaminated rice-shrimp system. Therein, there were 46 isolates originating from water samples, and 11 isolates isolated from soil samples, accounting for 80.7% and 19.3%, respectively. Additionally, morphological observation revealed that PNSB isolates from water samples were brown or brownish yellow, while isolates from soils were red or brownish yellow. All 57 PNSB isolates survived in BIM (pH=7.0) with $OD_{660} > 1.0$ under both MLC and ADC for 72 h. Therefore, isolates were chosen for evaluation in the following experiments. PNSB can accumulate energy from light under anaerobic conditions as well as from chemicals under aerobic conditions, because under both conditions, there are C sources from $CO_2$ or organic matters [36]. Moreover, PNSB exist in different phototrophic environments such as photoautotroph, photoheterotroph, and chemotroph

[37]-[40], so they are well adapted to harsh environments, such as acidic or saline soils. Thus, these bacteria should be further studied under saline conditions.

### 3.1. Selecting N-Fixing PNSB that Can Live In Saline Soil

*Selecting PNSB that can tolerate acidic saline conditions*: The PNSB isolates continued to be cultured in BIM (pH 5.0) for 72 h. For $OD_{660}$ > 1.0, there were 49 isolates. In particular, there were 3 isolates with $OD_{660}$ > 2.0 (W12, W31 and S69) under ADC. On the other hand, there were 46 isolates with $OD_{660}$ > 1.0 under MLC (Table 2). PNSB isolates that grew well in BIM (pH 5.0) continued to be cultured in BIM added with NaCl 5‰, and there were 24 isolates that could live under this condition. $OD_{660}$ values from 0.344 to 3.140 under MLC, while they were from 0.772 to 1.740 under ADC. However, the abilities of the PNSB isolates to tolerate salinity were equivalent among isolates (Table 2).

**Table 2.** Effect of Treatments and Ripening on pH, Total Nitrogen, Non-Casein Nitrogen Non-Protein Nitrogen (%) of Various Developed Cheeses

| | pH | | TN (%) | | NCN (%) | | NPN (%) | |
|---|---|---|---|---|---|---|---|---|
| **Treatments** | **Days ($0^A$)** | **Days ($30^B$)** | **Days ($0^A$)** | **Days ($30^B$)** | **Days ($0^A$)** | **Days ($30^A$)** | **Days ($0^A$)** | **Days ($30^B$)** |
| **$T_1$** | 4.92±0.03$^{e}$ | 4.71±0.15$^{d}$ | 31.09±0.94$^{d}$ | 34.61±1.32$^{c}$ | 2.91±0.19 $^{cd}$ | 2.80±0.17$^{f}$ | 0.44±0.08$^{f}$ | 3.05±0.07$^{b}$ |
| **$T_2$** | 5.33±0.02$^{bcd}$ | 4.82±0.16$^{cd}$ | 41.30±1.16$^{b}$ | 42.33±1.16$^{bc}$ | 2.25 ±0.35$^{d}$ | 2.85±0.26$^{f}$ | 0.70±0.08$^{f}$ | 2.60±0.14$^{bc}$ |
| **$T_3$** | 5.40±0.10$^{bcd}$ | 4.88±0.03$^{cd}$ | 41.72±1.69$^{b}$ | 44.78±1.69$^{bc}$ | 8.26±0.29$^{ab}$ | 8.93±0.35$^{ab}$ | 0.77±0.04$^{ef}$ | 2.30±0.12$^{c}$ |
| **$T_4$** | 5.18± 0.07$^{d}$ | 4.93±0.09$^{cd}$ | 31.85±0.93$^{cd}$ | 36.06±1.47$^{c}$ | 6.82±0.14$^{b}$ | 6.84±0.19$^{cd}$ | 1.27±0.12$^{de}$ | 3.06±0.16$^{b}$ |
| **$T_5$** | 5.21± 0.03$^{d}$ | 5.05±0.11$^{cd}$ | 38.53±1.42$^{bcd}$ | 39.71±1.19$^{c}$ | 3.63±0.34$^{cd}$ | 4.48±0.34$^{e}$ | 0.83±0.16$^{ef}$ | 2.01±0.14$^{c}$ |
| **$T_6$** | 5.23±0.12$^{cd}$ | 5.04±0.07$^{bc}$ | 40.64±1.18$^{bc}$ | 42.58±1.68$^{bc}$ | 4.66±0.38$^{c}$ | 6.08±0.35$^{d}$ | 1.53±0.16$^{cd}$ | 2.58±0.1$^{bc}$ |
| **$T_7$** | 5.43±0.33$^{bc}$ | 5.18±0.3$^{a}$ | 54.86±1.53$^{a}$ | 50.78±1.69$^{b}$ | 7.17±0.2$^{ab}$ | 7.89±0.30$^{bc}$ | 2.06±0.16$^{bc}$ | 3.25±0.15$^{b}$ |
| **$T_8$** | 5.80±0.28$^{a}$ | 5.06±0.26$^{ab}$ | 55.43±1.35$^{a}$ | 64.47±1.16$^{a}$ | 7.90±0.36$^{ab}$ | 8.67±0.3$^{ab}$ | 2.56±0.10$^{b}$ | 4.11±0.13$^{a}$ |
| **$T_9$** | 5.53±0.23$^{b}$ | 5.05±0.16$^{ab}$ | 61.38±1.47$^{a}$ | 68.37±1.32$^{a}$ | 8.74±0.4$^{a}$ | 9.06±0.36$^{a}$ | 3.20±0.16$^{a}$ | 4.28±0.18$^{a}$ |

Note: All presented values are mean ± standard deviation of three replicates (n=3); Mean with different letters in the same column (Treatments) and rows (storage days) are significantly different ($p \leq 0.05$)

**Table 3.** Effect of Treatments and Ripening on the Firmness, Total Plate Count, and Total Variable Count (%) Various Developed Cheeses

| | Firmness (N) | | TPC ($10^4$ CFU/g) | | TVC (104 CFU/g) | |
|---|---|---|---|---|---|---|
| **Treatments** | **Days ($0^A$)** | **Days ($30^B$)** | **Days ($0^A$)** | **Days ($30^A$)** | **Days ($0^A$)** | **Days ($30^B$)** |
| **$T_1$** | 330.33±0.03$^{e}$ | 276.33±0.41$^{f}$ | 9.16±0.24$^{bc}$ | 9.37±0.08$^{bc}$ | 2.16±0.04$^{b}$ | 2.8±0.51$^{a}$ |
| **$T_2$** | 342.0±0.11$^{cd}$ | 275.0±0.21$^{f}$ | 7.67±0.30$^{cde}$ | 7.44±0.14$^{cd}$ | 1.70±0.09$^{e}$ | 1.94±0.29$^{d}$ |
| **$T_3$** | 351.33±0.05$^{c}$ | 288.3±0.041$^{e}$ | 6.04±0.11$^{de}$ | 6.14±0.80$^{d}$ | 1.50±0.14$^{f}$ | 1.92±0.06$^{d}$ |
| **$T_4$** | 344.33±0.13$^{c}$ | 301.0±0.01$^{d}$ | 10.47±0.05$^{ab}$ | 10.78±0.74$^{ab}$ | 2.40±0.01$^{a}$ | 2.50±0.16$^{b}$ |
| **$T_5$** | 331.0±0.02$^{de}$ | 291.0±0.13$^{e}$ | 8.61±0.07$^{bcd}$ | 7.56±0.05$^{cd}$ | 1.83±0.43$^{d}$ | 1.97±0.32$^{d}$ |
| **$T_6$** | 347.0±0.21$^{c}$ | 301.0±0.75$^{d}$ | 7.75±0.19$^{cde}$ | 7.89±0.18$^{bcd}$ | 2.33±0.06$^{a}$ | 2.30±0.49$^{bc}$ |
| **$T_7$** | 440.0±0.32$^{b}$ | 404.0±0.63$^{c}$ | 12.50±0.25$^{a}$ | 12.43±0.51$^{a}$ | 1.88±0.31$^{d}$ | 2.50±0.79$^{b}$ |
| **$T_8$** | 448.0±0.03$^{ab}$ | 413.0±0.04$^{b}$ | 7.50±0.13$^{cde}$ | 7.87±0.62$^{bcd}$ | 1.92±0.45$^{cd}$ | 2.76±0.21$^{a}$ |
| **$T_9$** | 454.0±0.15$^{a}$ | 427.0±0.08$^{a}$ | 5.60±0.02$^{e}$ | 5.85±0.21$^{d}$ | 2.00±0.23$^{c}$ | 2.09±0.07$^{cd}$ |

Note: All presented values are mean ± standard deviation of three replicates (n=3); Mean with different letters in the same column (Treatments) and rows (storage days) are significantly different ($p \leq 0.05$).

Nevertheless, Table 3 depicts that firmness developed significantly ($p \leq 0.05$) among treatments and showed a decreasing trend during storage. The TPC and TVC showed decreasing trend among treatments as a function of increasing mineral level (10 to 30 mM) while during storage TVC contents increased significantly ($p \leq 0.05$) in some cases. In this context, [19] investigated the effect of Ca ions on the total viable count during storage. The study conducted by [19] identified the existence of a lipopolysaccharide layer in the structural arrangement of bacteria. This layer has a strong attraction to calcium ions and therefore plays a crucial function in stabilizing the microorganisms. When trisodium phosphate or tricalcium phosphate are stored, they are expected to have a comparable effect. In this effect, sodium or calcium, combined with phosphate, form powerful clusters of casein [7].

## 4. Conclusion and Recommendation

The cottage, mozzarella, and cheddar treatments supplemented with 30 mM CaCl2 and Na3PO4 shown higher performance in terms of pH, total nitrogen, non-casein nitrogen, non-protein nitrogen, and firmness when compared to the other treatments. The microbiological investigation showed a significant decrease in both the overall count of viable microorganisms and the total number of colonies for treatments T3, T6, and T9 when calcium chloride and trisodium phosphate were included. Overall, it was observed that all treatments of cheddar cheese exhibited greater stability and superior physicochemical and microbiological properties when compared to cottage and mozzarella cheese. Ultimately, the addition of minerals (CaCl2 and Na3PO4) to low-fat milk resulted in an improved cheese quality.

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