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Effects of varying concentrations of calcium chloride ($CaCl_2$) on the quality of Filipino white cheese (*kesong puti*) made from frozen-thawed cow's milk

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Abstract

Freezing raw milk is a process practiced by Filipino dairy farmers and cooperatives to manage fluctuations in milk production. Freezing can cause physicochemical changes in milk that may affect its processability into value-added products such as cheeses. However, complications in coagulation are often observed in frozen-thawed milk. Filipino fresh white cheese (*kesong puti*) is typically made from salted fresh milk curdled with rennet or acid (vinegar). Less typical is the use of calcium chloride which enhances curd formation in cheesemaking. Hence, this study determined the effects of varying concentrations of calcium chloride ($CaCl_2$) on the quality of *kesong puti* made from frozen-thawed cow's milk. *Kesong puti* treatments were produced by adding 0.02% w/v $CaCl_2$ (T_1), 0.05% w/v $CaCl_2$ (T_2), and 0.07% w/v $CaCl_2$ (T_3). Fat, protein, total solids, and pH were significantly higher in T_3 whereas moisture and titratable acidity were significantly higher in T_1. Aerobic bacteria, coliform, *Escherichia coli*, and yeast and mold counts did not significantly differ among treatments. Coliform and *Escherichia coli* counts in all treatments were acceptable based on the guidelines issued by the Food and Drug Administration. Sensory characteristics and product yield were not significantly different among treatments. Based on the results of this study, the concentration of 0.07% w/v $CaCl_2$ is the most effective in addressing problems in curd formation observed in frozen-thawed cow's milk.

Keywords - *calcium chloride, cow's milk, freezing, kesong puti, white cheese*

Introduction

In the Philippines, shortage or surplus of milk frequently occur due to fluctuating milk production throughout the year (Department of Agriculture, 2021). This can be attributed to types of climate, availability of forage and silage, and stage of lactation. During milk shortage or surplus, raw milk is temporarily refrigerated or frozen until a particular volume is attained for processing. Immediate refrigeration of raw milk at 4ºC after milking is a standard practice to lower microbial and enzymatic activity whereas freezing of raw milk at –18ºC is also practiced to injure or kill spoilage and pathogenic microorganisms (Pazzola et al., 2013; Wendorff, 2001). During freezing, the formation of intracellular and extracellular large ice crystals can puncture microbial cell walls and membranes while the development of a supersaturated solution can lower water activity hindering microbial mobility and growth (Alinovi et al., 2020; El-Kest & Marth, 1992; Smith et al., 2011). As freezable water forms into intracellular and extracellular large ice crystals, the unfrozen solution develops a concentration gradient leading to cryoprecipitation of milk proteins specifically casein micelles, one of the milk proteins which affect curd formation during cheesemaking and the rennetability of frozen-thawed milk (Fox et al., 2015). These occurrences can alter the structures, properties, and functionalities of milk components which may significantly affect its processability into value-added dairy products especially cheeses (Tribst et al., 2020).

Kesong puti or Filipino white cheese is an indigenous cheese commonly processed and marketed by dairy farmers and cooperatives. It is a fresh or unripened type of cheese made from salted

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fresh milk (carabao or cow) curdled with abomasal extract (rennet) or acid (vinegar) (Barraquio, 2006; Kisworo & Barraquio, 2003). It is very palatable and has a smooth texture and pleasant aroma. It is usually consumed right after it is processed. *Kesong puti* can be stored for two days at room temperature (28°C to 30°C) or for one week when refrigerated at 5°C to 10°C. Provinces such as Laguna, Cebu, Cavite, and Bulacan which specialize in processing *kesong puti* have different traditional methods. They differ in some parameters such as coagulant type, coagulant to milk ratio, salt content, whey draining process, and brining (Sanchez, 2008). The manufacture of *kesong puti* is considered to be one of the oldest cottage industries in the Philippines (Dulay, 1991). *Kesong puti* is either wrapped in banana leaves or packed in microwaveable plastic containers and marketed locally (Barraquio, 2006).

In normal milk, a casein micelle is composed of sub-micelles ($\alpha1_{S1}$, $\alpha1_{S2}$, β, and κ) held together by calcium phosphate nanoclusters. During cheesemaking, rennet coagulation of milk undergoes a two-stage process. At the first stage, κ-caseins positioned on the surface of casein micelles for stabilization are enzymatically hydrolyzed into caseinomacropeptide (CMP) and para-κ-casein by rennet. At the second stage, para-κ-caseins start to aggregate when approximately 70% of the κ-caseins have been hydrolyzed (Walstra et al., 2006).

The addition of calcium salts (i.e., calcium chloride) significantly influences the rate of CMP release. The release of CMP neutralizes the electrostatic repulsion between casein micelles which enables protein aggregation leading to rennet coagulation (Sørensen et al., 2019). Calcium reduces the overall ζ-potential of casein micelles, increasing calcium-protein interactions either via cross-linking with the phosphate moiety of the micellar calcium phosphate or direct binding with the carboxyl group of aspartate and glutamate of the casein micelles (Wolfschoon-Pombo, 1997; Sandra et al., 2012).

In processing cheeses, typically hard cheeses, the inclusion of $CaCl_2$ is considered as a standard practice. The usual level of concentration is 0.02% w/v. However, it can be increased in moderation without drastically affecting quality (Wolfschoon-Pombo, 1997). $CaCl_2$ can adjust the seasonal variations in milk composition and correct the effect of freezing on the dissociation of calcium and phosphate in milk (Lawrence et al., 2004; Fox et al., 2015). Soluble calcium in milk can also be insolubilized by freezing leading to the increase in hard-to-exchange calcium (Yamauchi & Yoneda, 1977). This can affect the enzymatic activity of rennet during the coagulation of milk (Tamime et al., 2006). Rennet-induced coagulation of milk is dependent on the availability of sufficient colloidal and soluble calcium. A continuous gel network is produced through an increase in colloidal calcium solubility resulting in the structure rearrangement and fusion of casein micelles (Salvatore et al., 2011).

The addition of calcium chloride into cheese milk significantly reduces pH which increases the bridging of casein micelles during rennet coagulation and decreases clotting time (Amenu & Deeth, 2007; Lin et al., 2018; Tarapata et al., 2020). Cheese added with calcium chloride is generally firmer due to lower moisture or water holding capacity and higher syneresis or whey expulsion (Bille et al., 2001; Fagan et al., 2007; Landfeld et al., 2002). Some properties of calcium chloride also include an increase in curd yield and retention of nutrients such as fats, proteins, and minerals (Sandra et al., 2012; Wolfschoon-Pombo, 1997). Complications in coagulation are often observed when using frozen-thawed milk due to freezing-induced physicochemical changes (Fox et al., 2015; Tribst et al., 2020). Although calcium chloride can enhance curd formation in cheesemaking, it is not typically used in processing *kesong puti* (Aquino et al., 2011). Therefore, this study determined the effects of varying concentrations of calcium chloride ($CaCl_2$) on the quality of *kesong puti* made from frozen-thawed cow's milk.

Methodology

Experimental Design

Six liters of raw cow's milk were subjected to freezing then thawing. Each *kesong puti* treatment used two liters of frozen-thawed raw cow's milk added with calcium chloride ($CaCl_2$). The treatments were as follows: T_1: 0.02% w/v $CaCl_2$, T_2: 0.05% w/v $CaCl_2$, and T_3: 0.07% w/v $CaCl_2$. In a preliminary experiment, it was observed that frozen-thawed cheese milk without the inclusion of $CaCl_2$ (0.0% $CaCl_2$) did not coagulate and was not successfully processed into *kesong puti*. The study had five replications. Each replication had sample duplicates in every analysis.

Milk Collection and Preparation

For every replication during the experiment, fresh raw cow's milk was provided by a dairy enterprise in Batangas, Philippines. The period of lactation, storage temperature, and storage duration were mid-lactation, 4°C, and <4 h, respectively. Freezing at –18°C for 16 h then thawing at 4°C for 22 h of raw cow's milk were done using an upright refrigerator and freezer. The chemical composition of frozen-thawed cow's milk was as follows: 4.07% fat, 2.97% protein, 8.60% solids-not-fat, 87.33% moisture, 12.67% total solids, and 0.154% lactic acid. The density was 1.03 $kg \cdot L^{-1}$, and the pH was 6.58.

Processing of *Kesong Puti* (Filipino White Cheese)

The method of Emata and Yukit (2017) was followed in processing *kesong puti*. Each *kesong puti* treatment used two liters of frozen-thawed raw cow's milk. Table salt (2.5% w/v) was added to the milk and stirred until completely dissolved. Milk was pasteurized at 72°C for 15 s using a double boiler then cooled down to 40-42°C. Varying concentrations of food grade CaCl2 was added per treatment (T_1: 0.02% w/v $CaCl_2$, T_2: 0.05% w/v $CaCl_2$, and T_3: 0.07% w/v $CaCl_2$) and stirred until completely dissolved. Diluted rennet (5 g rennet 100 L^{-1} milk) in distilled water was also gradually added to the milk while stirring. The milk was left undisturbed to allow coagulation for 20 to 30 min. The milk coagulum was cut into 1 in^2 when a clean separation of the cut coagulum was observed. The cut coagulum or curd was left uninterrupted for 5 to 10 min before gently stirring it for another 10 to 15 min to induce whey separation. After stirring, the curd was left undisturbed for 3 min before draining one-third of the whey. The curd was poured into a perforated tray lined with sanitized cheesecloth and further drained overnight in the refrigerator (4°C). After draining, the white cheese was cut into blocks, packed aseptically in containers, and stored at 4°C for subsequent analyses.

Chemical Analysis

The treatment samples were tested for fat, protein, moisture and total solids, and titratable acidity (% lactic acid) using Gerber, Kjeldahl, oven, and titration methods, respectively (AOAC, 2006). All samples were tested for pH using a pH meter.

Microbial Analysis

Serial dilutions from each treatment sample were prepared in sterile phosphate buffer saline diluents (pH 7.2) (HiMedia®, Mumbai, India). The samples were tested for counts of aerobic bacteria, coliform, *Escherichia coli*, and yeast and mold. All microbial counts were determined using a specific plate (3M™ Petrifilm™ Aerobic Count Plate, *Escherichia coli*/Coliform Count Plate, and Yeast and Mold Count Plate, 3M™, Canada). Sample preparation, incubation temperature, and incubation period were followed according to the manufacturer's instructions. Positive colonies from *Escherichia coli*/Coliform plates were confirmed by biochemical tests (Feng et al., 2018).

Sensory Analysis

Five sensory evaluation sessions were held during the experiment, and each session was treated as a replicate. For each session, ten grams of coded *kesong puti* treatment samples along with drinking water were served to 15 trained panelists from the Institute of Animal Science (IAS), the Dairy Training and Research Institute (DTRI), and the College of Agriculture and Food Science (CAFS), all at the University of the Philippines Los Baños (UPLB), College, Laguna, Philippines. The panelists evaluated the samples for color, aroma, firmness, texture, flavor, saltiness, whey separation, and general acceptability using a linear scale of 0 to 100 (Mabesa, 1986).

Product Yield

The product yield of each treatment was computed after every processing of *kesong puti*. The weight of the used frozen-thawed raw cow's milk (kg) was obtained by multiplying the determined milk density ($kg \cdot L^{-1}$) and total milk volume used (L). The weight of the produced *kesong puti* (kg) was also obtained. The following formula was used to determine the product yield (%):

$$\text{Product Yield (\%)} = \frac{\text{produced } \textit{kesong puti} \text{ (kg)}}{\text{used frozen-thawed raw cow' s milk (kg)}} \times 100\%$$

Statistical Analysis

The data on chemical, microbial, sensory, and product yield analyses of all samples were analyzed using Analysis of Variance (ANOVA) in a Completely Randomized Design (CRD). For each treatment, means were compared using Pairwise Mean Comparison in Least Significant Difference (LSD). All statistical analyses were carried out using the SAS® University Edition software version SAS Studio 3.8 and SAS 9.4M6 (SAS Institute Inc., USA).

Results and Discussion

Chemical Composition of *Kesong Puti* (Filipino White Cheese)

The chemical compositions of *kesong puti* treatments with varying concentrations of calcium chloride ($CaCl_2$) are presented in Table 1. Fat, protein, total solids, and pH were significantly higher in T_3 whereas moisture and titratable acidity (% lactic acid) were significantly higher in T_1.

The highest recovery of fat, protein, and total solids was observed in T_3. This may be attributed to its highest concentration level of $CaCl_2$ (0.07% w/v). High calcium content in milk promotes more calcium-protein and protein-protein interactions during coagulation. This makes the curd compact, increasing syneresis or whey expulsion which intensifies moisture loss and recovery of milk solids. The addition of $CaCl_2$ creates finer pore sizes in the curd, entrapping more fat globules (Wolfschoon-Pombo, 1997). The formation of calcium paracaseinate in the curd leads to higher casein retention and protein recovery in the curd instead of losing it in whey (Kosikowski, 1977; Kessler, 1981). The same observations were reported by Bille et al. (2011) wherein fat, protein, and total solids were significantly higher in cheese with $CaCl_2$.

T_3 had the lowest titratable acidity and highest pH among the treatments due to its highest $CaCl_2$ concentration among the treatments. When added to milk, $CaCl_2$ ionizes into calcium (Ca^+) and chloride (Cl^-) ions. Calcium ions induce the release of hydroxyl (^-OH) ions from the curd binding with hydrogen (H^+) ions from the system which produces water molecules (H_2O) that will be expelled together with whey. This occurrence leads to moisture loss, reducing the potential of the curd to develop acidity after curd formation. Calcium ions also stimulate cross binding of proteins, lowering the water-binding capacity of the curd through the reduction of binding sites where water molecules would typically attach. The addition of $CaCl_2$ lowers pH and increases the rate of enzymatic reaction (Lin et al., 2018; Schulz-Collins and Senge, 2004). Lower pH decreases clotting time and increases the curd firming rate during cheesemaking (Daviau et al., 2000; Lucey & Fox, 1993). On the other hand, cheese without $CaCl_2$ retained more moisture leading to higher acidity, faster maturation, shorter shelf life, and undesirable flavor change (Bille et al., 2001)

Microbial Counts of *Kesong Puti* (Filipino White Cheese)

The microbial counts of *kesong puti* treatments with varying concentration of calcium chloride ($CaCl_2$) are presented in Table 2. No significant differences in aerobic bacteria, coliform, *Escherichia coli*, and yeast and mold counts were observed among the treatments. Results indicate that coliform and *Escherichia coli* counts from all treatments were acceptable based on the standards set by FDA for soft and semi-soft cheeses (Food and Drug Administration [FDA], 2013). Aerobic bacteria and yeast and mold counts were also relatively low for all treatments.

A possible explanation for the low microbial counts is that the addition of $CaCl_2$ significantly reduces the capacity of the curd to hold moisture. This will improve syneresis and lessen acidity development that imparts a harsh taste during storage or ripening (Tarapata et al., 2020). Furthermore, a decrease in water activity transpires inhibiting microbial growth and prolonging shelf life

Table 1. Chemical compositions of *kesong puti* (Filipino white cheese) treatments with varying concentrations of calcium chloride ($CaCl_2$).

Components (%[1])	Treatment[2]			*p*-value
	T_1: 0.02% $CaCl_2$	T_2: 0.05% $CaCl_2$	T_3: 0.07% $CaCl_2$	
Fat	10.71 ± 0.55^c	10.78 ± 0.53^b	10.84 ± 0.55^a	0.0486
Protein	9.34 ± 0.88^b	10.48 ± 1.31^{ab}	11.25 ± 1.56^a	0.0090
Moisture	64.97 ± 1.13^a	63.37 ± 0.92^b	60.97 ± 1.16^c	0.0000
Total solids	35.03 ± 1.13^c	36.63 ± 0.92^b	39.03 ± 1.16^a	0.0000
Titratable acidity (% lactic acid)	0.1680 ± 0.01^a	0.1580 ± 0.01^b	0.1480 ± 0.01^c	0.0000
pH	5.67 ± 0.09^c	5.77 ± 0.09^b	5.87 ± 0.09^a	0.0001

[1]Except for pH values
[2(a,b,c)]Means within rows having different superscripts are significally different ($p < 0.05$)

Table 2. Microbial counts of *kesong puti* (Filipino white cheese) treatments with varying concentrations of calcium chloride ($CaCl_2$).

Counts ($CFU \cdot g^{-1*}$)	Treatment			*p*-value
	T_1: 0.02% $CaCl_2$	T_2: 0.05% $CaCl_2$	T_3: 0.07% $CaCl_2$	
Aerobic bacteria	21.60 ± 12.76	22.80 ± 14.52	27.60 ± 38.35	0.9218
Coliform	19.60 ± 31.55	24.80 ± 34.91	37.20 ± 32.97	0.6974
Escherichia coli, $MPN \cdot g^{-1*}$	0.00	0.00	0.00	-
Yeast and mold	19.20 ± 23.83	8.80 ± 7.33	13.80 ± 16.44	0.6449

*Except for *Escherichia coli* counts

(Bille et al., 2001). On the contrary, high moisture content can promote rapid proteolysis and lipolysis, hastening unfavorable maturation (Lucey & Fox, 1993).

Sensory Characteristics of *Kesong Puti* (Filipino White Cheese)

The sensory characteristics of *kesong puti* treatments with varying concentrations of calcium chloride ($CaCl_2$) are presented in Table 3. The scores for color, aroma, firmness, flavor, saltiness, whey separation, and general acceptability did not significantly differ among the treatments. The same findings were reported by Makhal et al. (2013) wherein $CaCl_2$ addition had no significant influences on color, appearance, flavor, body, and texture. However, among the sensory characteristics, adding $CaCl_2$ in milk extensively affects firmness as well as whey separation during cheesemaking and consumption. Although statistically insignificant, T_3 had the highest firmness and lowest whey separation scores during the sensory evaluation sessions.

The inclusion of $CaCl_2$ in the milk increases total calcium content and accelerates coagulation rate, resulting in a firmer and rigid texture of the curd (Tarapata et al., 2020). The increased curd firmness may be due to a more developed protein network through calcium bridging between casein micelles (Sandra et al., 2012). This produces a curd with a denser and homogenous structure having smaller pore sizes. Finer pores shrink the curd but can retain more milk solids and can lessen shatter susceptibility (Zhang et al., 2017; Zhang et al., 2019).

Table 3. Sensory characteristics of *kesong puti* (Filipino white cheese) treatments with varying concentrations of calcium chloride ($CaCl_2$).

Sensory characteristics*	Treatment			*p*-value
	T_1: 0.02% $CaCl_2$	T_2: 0.05% $CaCl_2$	T_3: 0.07% $CaCl_2$	
Color	69.65 ± 1.81	70.91 ± 3.01	72.07 ± 3.14	0.4008
Aroma	54.62 ± 5.30	50.85 ± 2.84	52.65 ± 3.79	0.3794
Firmness	48.36 ± 3.97	52.27 ± 7.77	58.29 ± 7.77	0.1038
Flavor	60.25 ± 6.11	61.21 ± 4.94	61.01 ± 9.42	0.9749
Saltiness	60.44 ± 5.18	64.48 ± 3.60	59.80 ± 8.88	0.4662
Whey separation	49.32 ± 10.70	46.68 ± 9.63	40.95 ± 11.76	0.4740
General acceptability	59.28 ± 5.94	60.29 ± 6.49	60.37 ± 10.02	0.9692

*Color: 0 (off-white) to 100 (white); Aroma: 0 (absence of aroma) to 100 (extremely aromatic); Firmness: 0 (extremely soft) to 100 (extremely firm); Flavor: 0 (absence of flavor) to 100 (extremely flavorful); Saltiness: 0 (absence of saltiness) to 100 (extremely salty); Whey Separation: 0 (absence of whey) to 100 (presence of whey); and General Acceptability: 0 (extremely unacceptable) to 100 (extremely acceptable)

The microscopic consequences of adding $CaCl_2$ are increased protein interaction and gel permeability which improves syneresis (Fagan et al., 2007). The volume of whey expelled is crucial in cheesemaking since moisture greatly influences microbial and sensory qualities. High moisture content generally develops undesirable flavor and texture characteristics during storage (Lawrence & Gilles, 1980; Lucey & Fox, 1993). On the other hand, separation or presence of whey during consumption is an undesirable sensory characteristic due to incomplete syneresis during cheesemaking.

Product Yields of *Kesong Puti* (Filipino White Cheese)

The product yield (%) of *kesong puti* increased with increasing concentration of $CaCl_2$ (21.72 ± 2.88 for T_1, 22.35 ± 1.56 for T_2, and 23.20 ± 2.59 for T_3), but the differences were not significant ($p = 0.5624$). Cheese yield is an important profit indicator and can be greatly influenced by milk composition and quality as well as curd firmness at cutting (Amenu & Deeth, 2007). A loss of 1% in product yield can result in an economic loss considered unbearable by dairy processors (Lacroix et al., 1991). Adding $CaCl_2$ in milk increases product yield due to more retention and recovery of milk solids (Sandra et al., 2012). High calcium content stimulates the formation of numerous protein networks which creates finer pores entrapping more fat and protein in the curd (Tarapata et al., 2020; Wolfschoon-Pombo, 1997). It was observed that the number of curd fines lost during syneresis was very minimal resulting in a higher yield of *kesong puti*.

Conclusion

This study reported that varying concentrations of calcium chloride ($CaCl_2$) in *kesong puti* made from frozen-thawed cow's milk had significant effects on some quality parameters. Fat, protein, total solids, and pH were significantly higher in T_3 (0.07% w/v $CaCl_2$) while moisture and titratable acidity were significantly higher in T_1 (0.02% w/v $CaCl_2$). Microbial counts (aerobic, coliform, *Escherichia coli*, and yeast and mold) did not significantly differ among treatments. In all treatments, coliform and *Escherichia coli* counts were acceptable based on the guidelines issued by the Philippines' Food and Drug Administration for soft and semi-soft cheeses. Sensory characteristics (color, aroma, firmness, flavor, saltiness, whey separation, and general acceptability) and product yield were not significantly different among treatments. Overall, the concentration of 0.07% w/v $CaCl_2$ can be considered as the most effective in resolving the complications in curd formation observed in frozen-thawed cow's milk. The general objective of the study was limited to the effects of $CaCl_2$ on the quality (chemical, microbial, and sensory) and yield of *kesong puti* made from frozen-thawed cow's milk. Other quality parameters such as rennet clotting time, texture profile, and dynamic rheology can be determined in future studies. The effects of $CaCl_2$ can also be investigated in various local cheeses made from different milk sources.

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Disclosure Statement

No potential conflict of interest was declared by the authors.

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