

Effects of Temperature and Supplementation with Skim Milk Powder on Microbial and Proteolytic Properties During Storage of Cottage Cheese

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The aim of this study was to determine the effects of temperature and supplementation with skim milk powder (SMP) on the microbial and proteolytic properties during the storage of cottage cheese. Cottage cheese was manufactured using skim milk with 2% SMP and without SMP as the control, and then stored at 5°C or 12°C during 28 days. The chemical composition of the cottage cheese and the survival of the cheese microbiota containing starter lactic acid bacteria (SLAB) and non-starter culture lactic acid bacteria (NSLAB) were evaluated. In addition, changes in the concentration of lactose and lactic acid were analyzed, and proteolysis was evaluated through the measurement of acid soluble nitrogen (ASN) and non-protein nitrogen (NPN), as well as electrophoresis profile analysis. The counts of SLAB and NSLAB increased through the addition of SMP and with a higher storage temperature (12°C), which coincided with the results of the lactose decrease and lactic acid production. Collaborating with these microbial changes, of the end of storage for 28 days, the level of ASN in samples at 12°C was higher than those at 5°C. The NPN content was also progressively increased in all samples stored at 12°C. Taken together, the rate of SLAB and NSLAB proliferation during storage at 12°C was higher than at 5°C, and consequently it led to increased proteolysis in the cottage cheese during storage. However, it was relatively less affected by SMP fortification. These findings indicated that the storage temperature is the important factor for the quality of commercial cottage cheese.

Keywords: Cottage cheese, storage temperature, non-starter lactic acid bacteria, proteolysis

Introduction

Cottage cheese is a type of fresh soft cheese consisting of individual moist curds, which are variable in size and blended with a dressing [14]. It has been positioned as a diet food for decades owing to its high protein and low calorie content [22]. Cottage cheese curds can be manufactured by the acid coagulation of skim milk, typically through starter culture activity [6]. In the manufacture of cottage cheese, various factors have been found to influence the quality and yield of curd, specifically pH, agitation and heat treatment, milk composition, total solids, and casein content [22, 25]. Ideally, cottage cheese curds should be uniform and have a meaty texture but should not be too

firm, rubbery, or tough [3]. Green and Grandison [11] stated that the protein content in cheese positively correlated with the gel strength and yield for better control of moisture in the curd and improved quality of final products.

Milk fortified with protein powder has been used in the manufacture of various dairy products to obtain higher protein levels in the final products and higher overall productivity [14]. However, some problems have often arisen with cottage cheese, involving inconsistency in features of the product quality, such as the deterioration of flavor and texture, decreased curd-to-dressing ratio, and inconvenient packaging [6]. In addition, there has been a growing number of epidemic cases of foodborne diseases implicating *Listeria monocytogenes*, resulting from the increasing consumption

of various dairy products, particularly fresh cheeses [18].

Most problems related to the safety and quality of cottage cheese has occurred as a result of inadequate refrigerated storage conditions, particularly temperature. It has been recommended that cottage cheese be maintained at temperatures between 0°C and 5°C during its distribution and sale [26]. However, according to a survey in Korea, the temperature established in display stands for refrigerated foods range between 0°C and 7°C, and the surface temperatures of refrigerated foods on sale range between 4.6°C and 18.4°C [2].

Here, we investigated the effects of storage temperatures and supplementation with skim milk powder (SMP) on microbial changes and lactic acid profiles during the storage of cottage cheese, and then evaluated their proteolytic patterns. Hence, this study aims to establish which factor may have an impact on the quality for the development and distribution of commercial cottage cheese.

Materials and Methods

Cheese Manufacture

Cottage cheese was manufactured using conventional cheese-making methods in three separate trials. To determine the effects of adding SMP supplement to cottage cheese, samples were generated using skim milk in combination with SMP (2% (v/v)) and without SMP as the control. Skim milk and SMP were obtained from Seoul Dairy Cooperative (Korea). The SMP contained 35.3% protein, 46.7% lactose, and 1.2% fat. First, 20 kg of cheese milk was pasteurized at 68°C for 30 sec and cooled to 31°C in a temperature-controlled cheese vat.

Fermentation of cheese milk was performed with a 0.25% (v/v) mesophilic starter culture (Fresco 1000; Chr. Hansens A/S, Denmark). After incubation at 31°C for 30 min, diluted rennet was added at 0.004 ml/kg (CHY-MAX Plus; Chr. Hansens A/S). The cheese milk was incubated for 4 h until pH 4.70 had been reached. Then, the curds were cut into even cubes of about 10–12 mm and left to rest for 10–15 min. Cooking of the curd to 55–60°C was carried out slowly to reach the end point in 90–120 min. At this point, the whey was drained off and replaced by the same quantity of chilled water (4°C). This washing process was repeated in triplicate before the curd was finally drained, weighed, and stored at 5°C or 12°C. After each stage of storage, samples of cottage cheese were taken from each room and stored at -80°C until required for analysis.

Cheese Composition

Cheese samples were ground and analyzed in triplicate for moisture, ash, total fat, and total protein by the Association of Analytical Communities Official Methods [30], and for total protein analysis, an Automatic Kjeldahl System (Digestion System

K-431 and Distillation Unit K-350; BUCHI Labortechnik AG, Flawil, Switzerland) was used. The cheese composition was analyzed and compared at the beginning of storage and at the end of storage (after 28 days).

Survival of Bacteria in Cheeses

Viabilities of starter lactic acid bacteria (SLAB) and non-starter culture lactic acid bacteria (NSLAB) were assessed during storage periods of 28 days at 5°C or 12°C. During storage, pressed curd samples were collected after 7, 14, 21, and 28 days. Samples for enumeration were prepared according to Sheehan *et al.* [27]. Lactococci as SLAB were enumerated by plating on sterile BCP agar (Plate count agar with bromocresol purple; Eiken Chemical Co., Japan) under aerobic conditions for 3 days at 37°C. NSLAB during storage were enumerated on Rogosa Agar (MB Cell, USA) under anaerobic incubation at 30°C using an anaerobic jar (Mitsubishi Gas Chemical Co., Inc., Japan) for 3 days.

Determination of Lactose and Lactic Acid

The concentration of lactose was determined using a Waters Alliance HPLC System (Milford, USA) equipped with an RI-detector as per the analytical method of the Ministry of Food and Drug Safety [21]. Sample extraction of lactic acid in cottage cheese was performed according to the method of Kocaoglu-Vurma *et al.* [15]. A high-performance liquid chromatography (HPLC) system (Nanospace I; Shiseido, Japan) equipped with a UV-VIS detector monitored at 210 nm, and lactic acid was analyzed on an C18-column (Capcellpak C18 MG120, 4.6 × 150 mm, 5 μm; Shiseido) and kept at 35°C. The mobile phase used was 0.1% H₃PO₄ in 97.5:2.5 (v/v) distilled water:acetonitrile. The run time was 25 min at 1 ml/min and the injection volume was 7 μl. Peak identities were determined based on the retention time of standard compounds, and the concentration of individual lactic acid was quantified by using a standard curve relating the peak area to concentration.

Assessment of Proteolysis

Acid soluble nitrogen (ASN) at pH 4.6 and non-protein nitrogen (NPN) were prepared according to the method described by Guerra-Martínez *et al.* [12] with slight modifications. Ten grams of cottage cheese was homogenized in a Stomacher with 100 ml of 0.5 M tri-sodium citrate solution. For ASN analysis, the pH value of the suspension was adjusted to 4.6 by adding 2N HCl. After incubation for 20 min at 25°C, the suspension was centrifuged at 4,500 rpm for 15 min and filtered through Whatman paper No. 2. For NPN analysis, 10 ml of cheese suspension was prepared by blending 10 ml of 24% (w/v) trichloroacetic acid (TCA) to achieve a final concentration of 12%. After homogenization, it was incubated for 10 min at 25°C and filtered. All nitrogenous fractions were measured in triplicate with the Kjeldahl analysis developed by Ardö [1].

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

SDS-PAGE was carried out according to the stacking gel

procedure as described by Laemmli [16]. Protein concentration was quantified using a Synergy H1 plate reader (Bio-Tek Instruments, Inc., Winooski, US) with the Take3 microdrop addition. Each sample was redissolved in SDS-PAGE sample buffer (62.5 mM Tris-HCl, pH 6.8; 2% (w/v) SDS; 25% (v/v) glycerol; 5% (v/v) 2-mercaptoethanol; 0.01% (w/v) bromophenol blue) and denatured at 100°C for 5 min. Twenty micrograms of the samples was loaded on 10% Ready Gel Tris-HCl Gel (Bio-Rad, USA). The equipment employed was the Mini-PROTEAN Tetra Cell (Bio-Rad, USA). The gels were stained with Coomassie Brilliant Blue R-250 staining solution (Bio-Rad). Destaining was carried out with a Coomassie Brilliant Blue R-250 destaining solution (Bio-Rad).

Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA). Significant differences among means of replicates ($n = 3$) were determined by Duncan's multiple range test. The SAS statistical software package was used to perform all statistical tests (SAS Inst., 2010). Values of $p < 0.05$ were considered to indicate a significant difference.

Results and Discussion

Chemical Composition

The compositions of cheese samples during storage at different storage temperatures are compared in Table 1. As expected, the total solid content and total protein content in cottage cheese were significantly increased by the SMP supplement. Before storage, the total solid and total protein contents of all samples ranged from $15.91 \pm 0.18\%$ to $16.28 \pm 0.13\%$ and from $11.47 \pm 0.07\%$ to $13.94 \pm 0.06\%$, respectively. Similar results were observed in a previous study by Yun *et al.* [31]. They stated that the addition of milk protein to cheese increased the total nitrogen and decreased the cheese moisture. Moreover, they found that the addition of protein

powder contributed to a significant increase in yield, which can be attributed to increased water retention, with better curd size distribution in cottage cheese [14]. In addition, the total fat content in SMP-supplemented samples was augmented, at $0.55 \pm 0.01\%$, in comparison with $0.35 \pm 0.02\%$ in the control. However, no significant differences were observed in the compositional concentrations of all cheese samples during storage for 28 days, regardless of storage temperature and SMP supplement ($p < 0.05$).

Survival of SLAB and LSLAB During Different Storage Temperatures

The changes in viable counts of SLAB and NSLAB during storage at different conditions are presented in Table 2. At the beginning of storage, the population of SLAB ranged from 8.04 ± 0.12 to 8.14 ± 0.08 log CFU/ml, and higher counts were found in SMP-supplemented samples. Overall, the population of SLAB was higher when samples were stored at 12°C than at 5°C. However, the proliferation stopped and the SLAB population began to decrease after 21 days in both samples stored at 5°C and at 12°C. In a previous study, during the maturation of cheddar and many other cheeses, the starter culture population decreased and the initially small population of NSLAB became the dominant bacterial population in cheese [24, 28].

Typically, *L. plantarum*, *L. casei*, and *L. brevis* have been previously reported as representative NSLAB from cheeses [10]. This secondary cheese microbiota normally arises from adventitious microorganisms in milk that survive pasteurization [23]. In our study, the proliferation of NSLAB in cottage cheese was observed during storage. The counts of NSLAB from the cheese samples ranged from 1.85 ± 0.01 to 1.87 ± 0.03 log CFU/ml before storage.

Table 1. Compositional concentrations during storage of cottage cheese.

		5°C		12°C	
		Control	SMP	Control	SMP
Total solid (%)	0 day	15.91 ± 0.18	$16.28 \pm 0.13^*$	15.91 ± 0.18	16.28 ± 0.13
	28 days	15.31 ± 0.33	$16.59 \pm 0.24^*$	15.39 ± 1.48	16.49 ± 0.07
Total protein (%)	0 day	11.47 ± 0.07	$13.94 \pm 0.06^*$	11.47 ± 0.07	$13.94 \pm 0.06^*$
	28 days	11.45 ± 0.38	$13.79 \pm 0.09^*$	11.73 ± 0.23	$13.77 \pm 0.12^*$
Total fat (%)	0 day	0.35 ± 0.02	$0.55 \pm 0.01^*$	0.35 ± 0.02	$0.55 \pm 0.01^*$
	28 days	0.34 ± 0.01	$0.55 \pm 0.01^*$	0.34 ± 0.01	$0.55 \pm 0.02^*$
Ash (%)	0 day	0.86 ± 0.01	0.85 ± 0.02	0.86 ± 0.01	0.85 ± 0.02
	28 days	0.85 ± 0.03	0.84 ± 0.01	0.84 ± 0.01	0.82 ± 0.02

* $p < 0.05$, statistically significant compared with control values.

† $p < 0.05$, statistically significant between the values of 5°C and 12°C.

All data are presented as the mean \pm SD ($n = 3$).

Table 2. Starter lactic acid bacteria (SLAB) and non-starter lactic acid bacteria (NSLAB) populations of cottage cheese at different storage conditions.

		5°C		12°C	
		Control	SMP	Control	SMP
SLAB (log CFU/ml)	0 day	8.04 ± 0.12 ^c	8.14 ± 0.08 ^c	8.04 ± 0.12 ^d	8.14 ± 0.08 ^e
	7 days	8.05 ± 0.10 ^c	8.49 ± 0.09 ^{b†}	8.32 ± 0.10 ^{c†}	8.98 ± 0.03 ^{b*†}
	14 days	8.44 ± 0.05 ^b	8.76 ± 0.03 ^{a†}	9.30 ± 0.07 ^{a†}	9.14 ± 0.02 ^{a*†}
	21 days	8.70 ± 0.02 ^a	8.74 ± 0.04 ^a	9.12 ± 0.05 ^{ab†}	8.82 ± 0.02 ^{c*†}
	28 days	8.22 ± 0.09 ^{bc}	8.46 ± 0.11 ^b	8.88 ± 0.11 ^{b†}	8.55 ± 0.04 ^{d*}
NSLAB (log CFU/ml)	0 day	1.85 ± 0.01 ^d	1.87 ± 0.03 ^e	1.85 ± 0.01 ^d	1.87 ± 0.03 ^e
	7 days	2.09 ± 0.04 ^c	2.22 ± 0.05 ^{d*}	3.10 ± 0.02 ^{c†}	4.66 ± 0.01 ^{d*†}
	14 days	2.13 ± 0.04 ^c	2.37 ± 0.04 ^{c*}	5.20 ± 0.06 ^{b†}	5.26 ± 0.02 ^{c†}
	21 days	2.94 ± 0.02 ^b	3.33 ± 0.06 ^{b*}	5.90 ± 0.02 ^{a†}	5.32 ± 0.01 ^{b*†}
	28 days	3.27 ± 0.05 ^a	3.92 ± 0.01 ^{a*}	5.98 ± 0.03 ^{a†}	6.60 ± 0.03 ^{a*†}

Values within columns not sharing a common superscript differ significantly ($p < 0.05$).

* $p < 0.05$, statistically significant compared with control values.

† $p < 0.05$, statistically significant between the values of 5°C and 12°C.

All data are presented as the mean ± SD ($n = 3$).

Interestingly, although minor increases were noted in the counts of NSLAB from samples at 5°C until 14 days, after this point, they intensively increased until the end of storage. In contrast, the NSLAB counts in samples stored at 12°C increased dramatically during storage (Table 2). Finally, proliferation of NSLAB was observed in all samples after 28 days, ranging from 3.27 ± 0.05 to 6.60 ± 0.03 log CFU/ml. In particular, the numbers of NSLAB from samples stored at 12°C were significantly higher than from samples stored at 5°C. Additionally, the highest populations of NSLAB were found in SMP-supplemented samples at 12°C and the lowest in controls at 5°C.

According to a study by Crow *et al.* [5], NSLAB had no significant effect on quality when the cheese was made under hygienic conditions and stored at low temperature and for a short time. However, with longer ripening periods and higher storage temperatures, NSLAB contributed to the texture and flavor of cheese. Moreover, Ong and Shah [23] reported that the initial level of NSLAB was 3.10 log CFU/g; this increased to 5.39 log CFU/g and 6.09 log CFU/g after 24 weeks of ripening at 4°C and 8°C, respectively. Cromie *et al.* [4] also found that the NSLAB counts from stored cheddar cheese samples at above 8°C were rapidly increased during the first 4 weeks of ripening. Thus, it is considered that the storage temperature plays an important role in safety in terms of controlling the bacterial flora of cheese to prevent quality defects, especially during the early stage of storage.

Contents of Lactose and Lactic Acid During Different Storage Temperatures

Before storage, the lactose content of cottage cheese ranged from 1.32 ± 0.01 to 1.60 ± 0.03 g/100 g sample. After storage for 28 days, the levels of lactose were significantly decreased in samples stored at 12°C compared with those stored at 5°C; the values ranged from 0.50 ± 0.01 to 0.57 ± 0.03 g/100 g sample at 12°C and 1.08 ± 0.02 to 1.37 ± 0.00 g/100 g sample at 5°C (Table 3). In the comparison of protein supplementation versus no supplementation, the rate of decrease in lactose concentrations in the supplemented samples was higher than that of controls. NSLAB use residual lactose from glycomacropeptide in casein, glycoprotein in the milk-fat globule membrane, and autolytic and proteolytic products formed during ripening [24]. In addition, the concentration of lactic acid in all samples increased during storage, particularly in samples stored at 12°C after 28 days. Moreover, compared with controls, SMP-supplemented samples produced higher concentrations of lactic acid ($p < 0.05$). In particular, the highest rate of increase in lactic acid content was found in SMP-supplemented samples at 12°C, from 1.07 ± 0.05 to 6.19 ± 0.10 mg/g. This result coincided with the changes in NSLAB counts, which indicated that lower storage temperature and supplementation with SMP were positively correlated with the proliferation of NSLAB in the manufacture of cottage cheese. Guerra-Martínez *et al.* [12] reported that all the nitrogen fractions seemed to be related to organic acid production, especially to lactic acid

Table 3. Changes of lactic acid and lactose during storage at different temperature conditions.

		5°C		12°C	
		Control	SMP	Control	SMP
Lactose (g/100 g)	0 day	1.32 ± 0.01 ^a	1.60 ± 0.03 ^{ba*}	1.32 ± 0.01 ^a	1.60 ± 0.03 ^{ba*}
	7 days	1.16 ± 0.01 ^b	1.48 ± 0.02 ^{ba*}	1.07 ± 0.02 ^{b†}	1.32 ± 0.01 ^{ba†}
	14 days	1.12 ± 0.01 ^b	1.42 ± 0.01 ^{ca*}	0.93 ± 0.01 ^{c†}	1.02 ± 0.01 ^{ca†}
	21 days	1.09 ± 0.00 ^c	1.41 ± 0.02 ^{ca*}	0.61 ± 0.01 ^{d†}	0.69 ± 0.01 ^{dca†}
	28 days	1.08 ± 0.02 ^c	1.37 ± 0.00 ^{ca*}	0.50 ± 0.01 ^{d†}	0.57 ± 0.03 ^{ca†}
Lactic acid (mg/g)	0 day	1.28 ± 0.08 ^d	1.07 ± 0.05 ^d	1.28 ± 0.08 ^c	1.07 ± 0.05 ^c
	7 days	1.69 ± 0.13 ^c	1.94 ± 0.13 ^c	3.36 ± 0.27 ^{b†}	2.80 ± 0.12 ^{d†}
	14 days	2.08 ± 0.01 ^b	2.12 ± 0.10 ^c	3.47 ± 0.08 ^{b†}	4.75 ± 0.12 ^{ca†}
	21 days	2.54 ± 0.09 ^a	2.63 ± 0.06 ^b	4.03 ± 0.06 ^{a†}	5.25 ± 0.11 ^{ba†}
	28 days	2.58 ± 0.09 ^a	2.65 ± 0.10 ^a	5.44 ± 0.04 ^{a†}	6.19 ± 0.10 ^{ba†}

Values within columns not sharing a common superscript differ significantly ($p < 0.05$).

* $p < 0.05$, statistically significant compared with control values.

† $p < 0.05$, statistically significant between the values of 5°C and 12°C.

All data are presented as the mean ± SD ($n = 3$).

and propionic acid. Proteolysis contributes to textural changes of the cheese matrix by breaking down the protein network, and contributes directly to the flavor and off-flavor of cheese through the formation of peptides and free amino acids [28].

Assessment of Proteolysis During Different Storage Temperatures

ASN, NPN, and the electrophoresis profile were investigated to evaluate the proteolysis of cottage cheese during storage. ASN is a parameter used to evaluate primary proteolysis in cottage cheese and to determine the most abundant proteolytic fraction containing the mixture of high and low molecular weight peptides. On the other hand, NPN contains low molecular weight peptides and free amino acids [19]. The ASN index (% ASN/TN) in all samples before storage ranged from $5.91 \pm 0.03\%$ to $7.00 \pm 0.24\%$ (Fig. 1). The ASN content increased gradually, especially in the samples stored at 12°C. At the end of storage for 28 days, the level of ASN in samples at 12°C was higher than those at 5°C. The NPN content was also progressively increased in all samples stored at 12°C, and in particular, did so more intensively after 7 days compared with those stored at 5°C (Fig. 2). This result was in accordance with previous studies, which reported that the casein content in cheddar and mozzarella cheeses decreased intensively with significant increases in the ASN content when the ripening temperature was elevated [8, 9, 13].

Proteolysis directly influences flavor through the short peptides and amino acids produced in this process. Some

of them are flavored through free amino acids, which are substrates for a series of catabolic reactions, and generate various important flavors [17, 20]. Basically, proteinases and peptidases, which catalyze proteolysis during cheese ripening, originate from primary sources such as coagulants, milk, SLAB, or NSLAB.

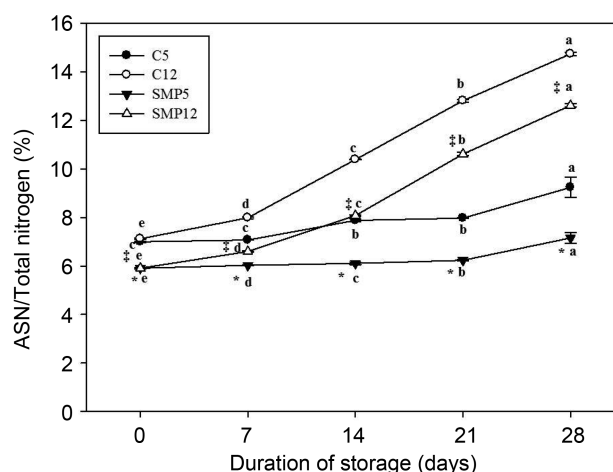


Fig. 1. Percentages of acid soluble nitrogen (ASN) of cottage cheese during storage.

^aDifferent letters indicate statistically significant differences ($p < 0.05$),

^{b*} $p < 0.05$ vs control 5°C. ^{c†} $p < 0.05$ vs SMP-supplemented samples at

5°C. ^dValues are expressed as the mean ± SD ($n = 3$). C5, Cottage cheese

control at 5°C; C12, Cottage cheese control at 12°C; SMP5, Cottage cheese

supplemented with SMP at 5°C; SMP12, Cottage cheese supplemented

with SMP at 12°C.

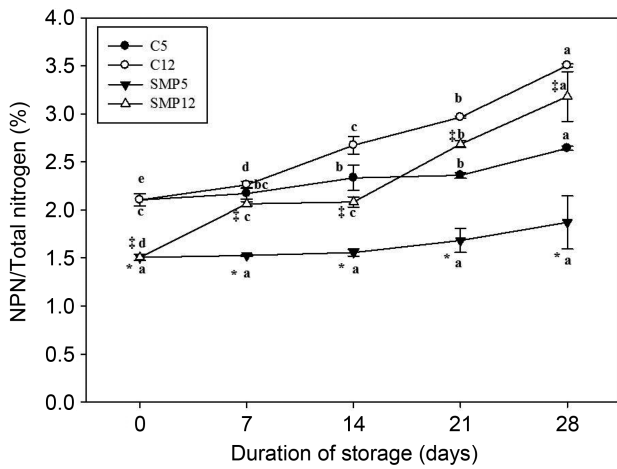


Fig. 2. Percentages of non-protein nitrogen (NPN) of cottage cheese during storage.

^aDifferent letters indicate statistically significant differences ($p < 0.05$).

^{b*} $p < 0.05$ vs control 5°C. ^{c†} $p < 0.05$ vs SMP-supplemented samples at 5°C.

^dValues are expressed as the mean \pm SD ($n = 3$). C5, Cottage cheese control at 5°C; C12, Cottage cheese control at 12°C; SMP5, Cottage cheese supplemented with SMP at 5°C; SMP12, Cottage cheese supplemented with SMP at 12°C.

In addition, according to the comparison results regarding supplemented protein, the levels of ASN and NPN in the control were slightly higher than in the supplemented group. Similar results from previous studies showed that the levels of ASN were higher in control cheddar cheese

(without milk protein concentrate; MPC) than in MPC-supplemented cheddar cheeses (1% and 2%) throughout ripening. They suggested that the use of MPC probably could reduce specific activities of plasmin or residual chymosin in MPC-supplemented cheddar cheese [29].

The extent of proteolysis of cottage cheese during storage at different temperatures was measured by SDS-PAGE (Fig. 3). Protein bands were identified by molecular weight and compared with standards of casein, specifically α -, β -, and κ -casein. The SDS-PAGE patterns were substantially different between the beginning and end of storage, but no significant difference was found between controls and SMP-supplemented samples. When samples were stored at 12°C, α - and β -casein degraded gradually during storage, although their levels were almost constant at 5°C. The electrophoresis results indicated that increasing storage temperature could lead to an increase in the rate of degradation of α - and β -casein, especially after storage for 14 days. Overall, the trends observed with SDS-PAGE were consistent with the results in the nitrogen fractions, which showed that proteolysis was significantly affected by storage temperature. However, it was relatively less affected by protein fortification. Feeney *et al.* [7] found that increasing the ripening temperature for mozzarella cheese from 0°C to 15°C resulted in a higher level of degradation of α_{s1} -casein.

It is suggested that adequate cold storage temperature is a considerable factor in the proteolysis of cottage cheese, as suggested from the increase in NSLAB at 12°C. Moreover, the low molecular weight peptides and free amino acids

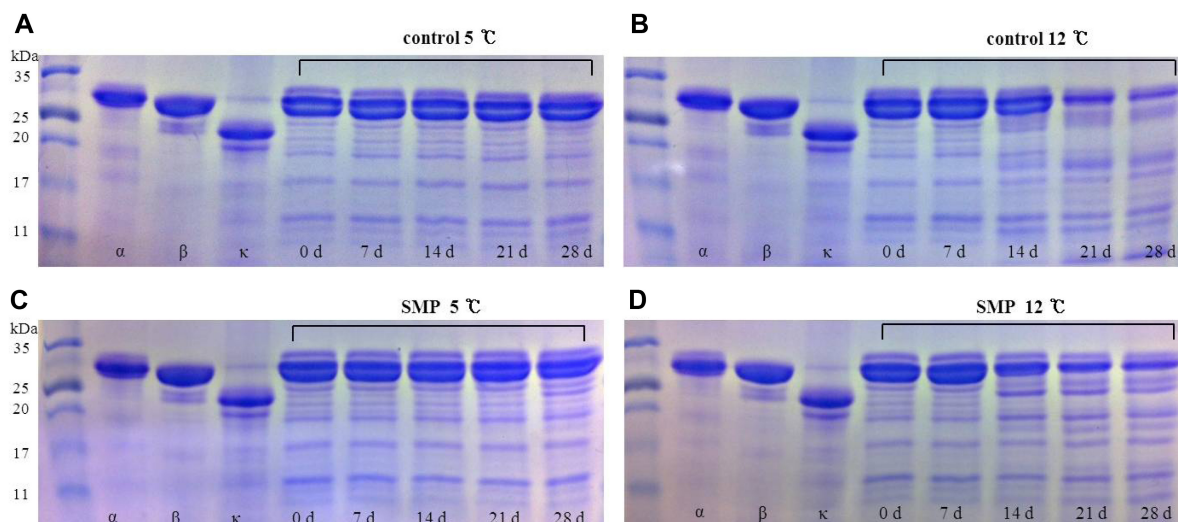


Fig. 3. SDS-PAGE analysis of cottage cheese at different storage temperatures.

(A) Cottage cheese control at 5°C; (B) Cottage cheese control at 12°C; (C) Cottage cheese supplemented with SMP at 5°C; (D) Cottage cheese supplemented with SMP at 12°C.

form rapidly during improper cold storage for more than 14 days, which could have an influence on the quality of cottage cheese.

In conclusion, in this study, we evaluated the effects of storage temperature and SMP supplementation on the characteristics of cottage cheese. Total solid, total protein, and total fat contents in the cheese increased through supplementation with SMP, whereas the ash content did not. However, during storage, no significant difference was observed in the samples at different storage temperatures. In addition, the populations of SLAB and NSLAB at the end of storage for 28 days were higher when SMP was added and the samples were stored at 12°C than without SMP at 5°C. It was also found that proteolysis was significantly affected by storage temperature. The ASN and NPN contents increased significantly, especially in the samples stored at 12°C. Therefore, appropriate quality management in several aspects such as storage time, temperature, and fortified compounds is needed to control proteolysis as well as to regulate the composition of SLAB and NSLAB in the manufacturing and cold chain process. In particular, the storage temperature plays an important role in the distribution of commercial cottage cheese.

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