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ARTICLE

Effect of Heat-Treat Methods on the Soluble Calcium Levels in the Commercial Milk Products

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Abstract

Milk is well known to be rich in some nutrients such as protein, calcium, phosphorus, and vitamins. In particular, absorption and bioavailability of calcium receive lots of attention because calcium is very little absorbed until it is changed to the ionized form in the intestine. In this study, concentration of the soluble calcium was determined in the commercial bovine milk products, which were processed by different heat-treatment methods for pasteurization. As for general constituents, lactose, fat, protein, and mineral were almost same in the liquid milk products by different processors. Ultrafiltration of the skimmed milk caused little change in the permeate as for lactose content but both fat and protein decreased. pH values ranges from 6.57-6.62 at room temperature and slightly increase after centrifugation, 10,000 *g*, 10 min. Rennet-coagulation activity was the lowest in the ultra high temperature (UHT-)milk compared to the low temperature long time (LTLT-) and high temperature short time (HTST-)milk products. Each bovine milk products contains 1056.5-1111.3 mg/kg of Ca. The content of sulfhydryl group was the lowest in raw milk compared to the commercial products tested. For the skimmed milks after ultrafiltration with a membrane (Mw cut-off, 3 Kd), soluble Ca in the raw milk was highest at 450.2 mg/kg, followed by LTLT-milk 336.4-345.1 mg/kg, HTST-milk 305.5-313.3 mg/kg, UHT-milk 370.3-380.2 mg/kg in the decreasing order. After secondary ultrafiltration with a membrane (Mw cut-off, 1 kD), total calcium in raw milk had a highest of 444.2 mg/kg, and those in the market milk products. As follow: UHT-milk, 371.3 to 378.2 mg/kg; LTLT-milk, 333.3 to 342.2 mg/kg; HTST-milk 301.9 to 311.2 mg/kg in a decreasing order.

Key words: bovine milk, heat-treat method, soluble calcium, sulfhydryl group, ultrafiltration (UF)

Introduction

As a natural food rich in nutrients such as protein, calcium, phosphorus and vitamins, milk has energy required for human activities and nutrients needed for building up the human body and metabolism (Chai, 1988). In particular, rich calcium content in milk prevents dietary fat from being absorbed and is dedicated to inhibit cholesterol absorption in collaboration with plant-origin phytosterols in the alimentary track. When the calcium intake from dietary source increases, it exerts to lower the serum cholesterol as well as fat level. However, when the calcium intake is insufficient, it adversely affects the bone growth and maintenance, resulting in osteoporosis or bone fracture and also vulnerability to various diseases such as high blood pressure and hypercholesterolemia (Kim, 1993; Lee *et al.* 1993; Lee and Kim, 2002; Park and Lee, 2002). Now that market milk contains about 1,000-1,200 mg of calcium per kg, and cheese and yogurt provide more calcium than market milk, the fermented dairy products are of great significance as an effective dietary source of calcium.

Calcium present in milk is actually divided into two types of the colloidal calcium and the soluble calcium. According to the earlier reports, about two thirds of the calcium is colloidal and the rest is soluble form, taking up approximately 10 percent. The calcium phosphates found in milk is saturated in terms of the phosphates and mostly insoluble (Holt and Jenness, 1984; Neville *et al*. 1994).

The raw bovine milk usually contains pathogenic microorganisms and also active enzymes, which frequently hamper digestion and absorption of milk. Accordingly, it becomes a common process that the raw milk goes through the heat-treatment as a safety measure (Lee, 1999). Once the raw milk has been heat- treated, it is able to destroy the inherent microorganisms and extend the shelf life after production. There are three types of heat-treatment process of raw milk for sterilization purpose: the low tem-

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perature-long time (LTLT, 30 min at 65°C), the high temperature-short time (HTST, 15 s at 72°C), and the ultrahigh temperature (UHT, 2-4 s at 135°C) methods (Woo and Maeng, 1998).

These pasteurization and sterilization processes affect in various ways on the sensory and nutritional quality of milk (In and Jung, 2001). Sandhu (1973) reported that the total calcium content in the heat-treated milk products was not changed, while Burton (1984) claimed that heating processes of milk influenced the balance of calcium salts in its distributions, resulting the reduced soluble calcium contents because the ionic calcium combined with the phosphates or the denatured proteins turns into the colloidal calcium form while the calcium moves to the inside of casein micelles, reduced the content of the ultrafiltrable calcium accordingly. Depending on the heat-treatment processes, the soluble calcium and the ionic calcium are shifted into the casein micelles along with the structural changes of milk proteins, and consequently the total soluble calcium content decreases (Woo and Maeng, 1998). Pouliot *et al.* (1989) reported that soluble calcium reduced when raised the temperature up to 90°C. There are several papers that the relative ratios between the ionic calcium and the soluble calcium content is changed by heat treatments, resulted that physicochemical properties of milk change. But it still remains uncertain about calcium distributions according to the heat-treatment methods applied to the processing milk.

Therefore, this study was performed in order to compare the soluble calcium content and physicochemical properties as a function of heat-treatment on the commercial liquid milk products produced by such methods as LTLT-, HTST- and UHT- methods currently adopted by the domestic milk processors.

Materials and Methods

Commercial milk products used

The liquid milk products used in this experiment were purchased at superstores and marketing agencies in Korea. Conditions for pasteurization of each product are listed in Table 1. Non-homogenized raw milk was supplied by Dairyzen Inc. (Wonju) and raw farm milk was transferred to this laboratory within an hour. To remove milk fat, the centrifugal separation (Supra 28K, Hanil, Korea) was conducted for 10 min at 10,000 rpm before experiments.

Ultrafiltration of skimmed milk

Ultrafiltration of skimmed milk was conducted to measure the content of the soluble calcium. The ultrafiltration module (Model 3622, Vision Scientific, Korea) mounted with a cellulose membrane was used and the ultrafiltration was carried to collect the permeates, which were subjected to analysis. As for ultrafiltration membranes (MA 01821, Millipore, USA), two kinds of membranes, high (Mw cut-off 30 kD) and low molecular (Mw cut-off 10 kD), were used, depending on the molecular weight of proteins to be removed.

Table 1. Heat-treatments methods on raw milk and some commercial bovine milk products

Methods of heat-treatment	Symbol*	Pasteurization/Sterilization	
		Temperature (°C)	Time
None (Raw milk)	R	-	-
Low temperature long time (LTLT)	A	63	30 min
	B	63	30 min
	C	63	30 min
Hight temperature short time (HTST)	D	72	15 s
	E	72	15 s
Ultra high temperature (UHT)	F	130	2 s
	G	130	2 s
	H	130	2 s

*R: Raw milk, A: Ildongfoodis, B: Konkuk, C: Pasteur, D: Sangha, E: Denmark, F: Seoul milk, G: Maeil, H: Namyang

Determination of crude protein content

This was carried out according to the official AOAC method (1995) and the content of crude protein was calculated by the formula below with the use of the conversion factor (6.38).

$$\text{Crude protein (\%)} = \frac{(V_1 - V_2) \times F \times \text{Conversion Faxtor}}{E} \times 100$$

E = sample volume (mg)
F = titre of 0.1 N HCl
V_1 = Volume (mL) of 0.1 N HCl consumed for the samples
V_2 = Volume (mL) of 0.1 N HCl consumed for the blank test

Determinantion of fat concentration

Fat was determined using the Röse-Gottlieb method (AOAC, 1995).

Determination of lactose concentration

HPLC (Waters Alliance System 2690, USA) was used in the quantitative analysis of lactose as the operational conditions shown in Table 2. Each of 5 mL sample was taken and mixed well with 30 mL of deionized water to mess up to 50 mL with acetonitrile, and then centrifugated (J2-

Table 2. Operating conditions of HPLC for lactose analysis in the commercial milk products

Items	Conditions
Instruments	Waters Alliance System 2690 (Waters, USA)
Column	Carbohydrate (Waters, USA)
Detector	RI
Flow rate	1.4 mL/min
Injection volume	250 μg
Mobile phase	Pure water 25%, acetonitrile 75%

21M/E, Beckman, USA) at 10,000 rpm at 35°C for 10 min. The supernatant went through the membrane filter (a 0.45 μm PVDF filter, Gelman Lab, USA), which was used for lactose analysis. Sugar standard solution (Sigma, USA) is prepared to be 0.1-0.5% (w/w), which was adjusted to 100 mL of final volume by adding acetonitrile.

Ash content

Five gram of milk sample was incinerated at 550°C and the ash content was calculated according to the AOAC method (1995).

Rennet coagulation test

The method described by McMahon *et al*. (1984) was slightly modified for rennet coagulation test. Briefly, each of 5 mL milk sample was poured into the plastic test tube, added the rennet enzyme (Christian Hansen, Denmark) up to 5% (v/v) of the final concentration and incubated at 35°C. After 30, 60, and 90 min, it was tilted at a fixed angle on the flat surface and took photos of apparent viscosity of the clotted milk in the test tube.

Measurements of sulfhydryl group (SH)

Sulfhydryl groups in the milk proteins were measured by a modification method reported previously (Torovazquez and Regenstein, 1989). 0.05 mL of the skimmed milk was dissolved in 2 mL of Tris-glycine buffer solution (dissolved 20 g Tris, 13.5 g glycine, and 6 g EDTA into 1 L distilled water and adjusted to pH 8.0) and 2.5 mL of 5 M guanidine hydrochloride, adjusted the final volume to 5 mL with distilled water.

Analysis of soluble calcium

An Inductively Coupled Plasma analysis system (Optima 3300XL, Perkin Elmer, USA) was utilized for quantitation. The pre-treatment was conducted according to the official AOAC method (1995). About 2 g of sample was taken into the melting vessel, incinerated in the electric furnace at about 550°C, diluted at the appropriate level with distilled water and the diluted solution of HCl, which was used as a sample solution. Standard solution was prepared for calcium at the concentrations of 1, 2.5, 5.0, 10.0 mg/100 mL (Table 3).

Table 3. Optimum operational conditions for determination of calcium by ICP

Items	Conditions
Wave length	317.93 nm
Nebulisation pressure	1.0 bar
Entrance slit	20
Exit slit	80
Increment	0.004 nm
Generator power	1,000 W
sample gas flow rate	0.7 L/min
Plasma gas flow rate	12 L/min
Auxilary gas flow rate	0 L min
Nebulisation flow rate	0.02 L/min

Statistical analysis

Data of the experiments were statistically treated with the use of Statistic Analysis System (SAS Ver. 9.2 Program) and verified with Duncan's multiple range test ($p<0.05$).

Results and Discussion

General ingredients

Table 4 shows the analysis data of general ingredients after removing fat from the milk samples. Protein concentration was ranged 2.98 to 3.03%, fat 0.99 to 1.05%, lactose 4.69 to 4.78% and ash 0.62 to 0.66%. Judging from the data of little differences between the measured values for each ingredient, it appears that the heating time and temperature to the milk do not affect the content of respective general ingredients. After ultrafiltration of the skimmed milk samples above, the results of general ingredients in the samples prepared by using the high molecular membrane (Mw cut-off 30 kD) and the low molecular membrane (Mw cut-off 10 kD) as shown in Table 5 and 6. Protein levels of the permeates passed through the primary ultrafiltration was 0.49 to 0.57% and fat 0.45 to 0.52%, showing a greater reduction in both ingredients than those of non-ultrafiltrated samples. The contents of lactose and ash, on the other hand, showed no major changes as expected. The permeates through the secondary ultrafiltration were 0.28 to 0.33% for protein and 0.17 to 0.22% for fat, which substantially decreased compared to those in the permeates through the primary ultrafiltration only, but the contents of lactose and ash had no major change, as expected. High molecular weight components fail to penetrate the membrane in the process of ultrafiltration, measured at a very low level in the permeates, whereas low-

Table 4. General compositions of raw milk and some commercial milk products

Method	Symbol*	Amount (%)			
		Protein	Fat	Lactose	Ash
Raw milk	R	3.01±0.03^{a}	0.99±0.01^{d}	4.71±0.06^{a}	0.66±0.01^{a}
Low temperature long time (LTLT)	A	3.02±0.02^{a}	1.01±0.01^{bcd}	4.77±0.05^{a}	0.62±0.0^{c}
	B	3.03±0.04^{a}	0.99±0.01^{d}	4.77±0.06^{a}	0.63±0.02^{bc}
	C	3.03±0.04^{a}	1.00±0.01^{bcd}	4.69±0.03^{a}	0.64±0.02^{bc}
Hight temperature short time (HTST)	D	3.03±0.06^{a}	0.99±0.01^{d}	4.76±0.04^{a}	0.66±0.01^{a}
	E	3.01±0.06^{a}	1.02±0.01^{bc}	4.78±0.05^{a}	0.62±0.02^{c}
Ultra high temperature (UHT)	F	2.99±0.04^{a}	1.03±0.02^{ba}	4.69±0.04^{a}	0.63±0.0^{bc}
	G	3.02±0.02^{a}	1.02±0.02^{bc}	4.69±0.03^{a}	0.62±0.02^{a}
	H	2.98±0.09^{a}	1.05±0.02^{a}	4.75±0.04^{a}	0.65±0.02^{a}

*R: Raw milk, A: Ildongfoodis, B: Konkuk, C: Pasteur, D: Sangha, E: Denmark, F: Seoul , G: Maeil, H: Namyang
$^{a\sim e}$Means with the different letter in same column are significantly different by Duncan's multiple range test (p<0.05).

Table 5. General compositions of permeates obtained by ultrafiltration(Mw cut-off 30 kD membrane)

Methods	Symbol*	Content (%)			
		Protein	Fat	Lactose	Ash
Raw milk	R	0.57±0.01^{a}	0.47±0.01^{d}	4.68±0.07^{a}	0.65±0.02^{a}
Low temperature long time (LTLT)	A	0.50±0.00^{b}	0.51±0.01^{a}	4.65±0.06^{a}	0.61±0.01^{b}
	B	0.49±0.01^{b}	0.52±0.02^{a}	4.59±0.11^{a}	0.60±0.01^{b}
	C	0.56±0.02^{a}	0.48±0.0^{dc}	4.59±0.11^{a}	0.62±0.0^{ba}
Hight temperature short time (HTST)	D	0.49±0.02^{b}	0.47±0.0^{d}	4.59±0.08^{a}	0.65±0.02^{a}
	E	0.50±0.01^{b}	0.45±0.02^{e}	4.61±0.02^{a}	0.62±0.02^{ba}
Ultra high temperature (UHT)	F	0.49±0.02^{b}	0.49±0.0^{bc}	4.65±0.05^{a}	0.62±0.0^{ba}
	G	0.56±0.01^{a}	0.51±0.01^{a}	4.66±0.10^{a}	0.62±0.02^{ba}
	H	0.57±0.02^{a}	0.52±0.0^{a}	4.65±0.07^{a}	0.62±0.02^{ba}

*R: Raw milk, A: Ildongfoodis, B: Konkuk, C: Pasteur, D: Sangha, E: Denmark, F: Seoul, G: Maeil, H: Namyang
$^{a\sim e}$Means with the different letter in same column are significantly different by Duncan's multiple range test (p<0.05).

Table 6. General compositions of permeate obtained by ultrafiltration (Mw cut-off 10 kD membrane)

Methods	Symbol*	Content (%)			
		Protein	Fat	Lactose	Ash
Raw milk	R	0.32±0.0^{a}	0.22±0.0^{a}	4.45±0.07^{a}	0.64±0.0^{a}
Low temperature long time (LTLT)	A	0.33±0.01^{a}	0.21±0.01^{ba}	4.49±0.05^{a}	0.61±0.0^{ba}
	B	0.28±0.01^{c}	0.20±0.01^{bc}	4.47±0.07^{a}	0.60±0.0^{b}
	C	0.28±0.0^{c}	0.19±0.0^{dc}	4.49±0.05^{a}	0.62±0.0^{ba}
Hight temperature short time (HTST)	D	0.29±0.0^{bc}	0.18±0.0^{de}	4.46±0.05^{a}	0.63±0.0^{ba}
	E	0.28±0.01^{c}	0.17±0.01^{e}	4.47±0.07^{a}	0.62±0.0^{ba}
Ultra high temperature (UHT)	F	0.31±0.0^{ba}	0.20±0.0^{c}	4.49±0.08^{a}	0.61±0.0^{ba}
	G	0.31±0.0^{ba}	0.21±0.01^{ba}	4.45±0.02^{a}	0.62±0.0^{ba}
	H	0.32±0.01^{a}	0.20±0.01^{bc}	4.49±0.02^{a}	0.61±0.0^{ba}

*R: Raw milk, A: Ildongfoodis, B: Konkuk, C: Pasteur, D: Sangha, E: Denmark, F: Seoul, G: Maeil, H: Namyang
$^{a\sim e}$Means with the different letter in same column are significantly different by Duncan's multiple range test (p<0.05).

molecular weight lactose goes easily through the membrane, resulted in the similar content each other. After ultrafiltration, the contents of fat, protein and non-fat milk solids of the permeates were in the very low level, whereas lactose was the similar results to the previous report by Chon *et al.* (2012) that lactose drew no big difference in the contents between raw milk, concentrated milk, and UF-permeate. Based on the results that there was a greater reduction in content of protein in the ultrafiltered milk than that of general skim milk, colloidal calcium were thought to be almost removed.

pH Measurement

Table 7 indicates the pH values of the skim milk differently heat-treated and their permeates through primary ultrafiltration membrane (Mw cut-off 30 kD) and secondary ultrafiltration membrane (Mw cut-off 10 kD). The raw milk prior to ultrafiltration and the pH value of pasteur-

Table 7. pH values in raw milk and some commercial milk products

Methods	Symbol*	pH		
		Skimmed milk	Ultrafiltration (30 kD)	Ultrafiltration (10 kD)
Raw milk	R	6.62±0.01[a]	6.69±0.01[a]	6.69±0.02[a]
Low temperature long time (LTLT)	A	6.62±0.02[a]	6.67±0.01[b]	6.67±0.0[bc]
	B	6.62±0.0[a]	6.67±0.01[b]	6.68±0.0[ba]
	C	6.62±0.0[a]	6.66±0.01[cb]	6.66±0.0[bc]
Hight temperature short time (HTST)	D	6.62±0.0[a]	6.69±0.01[a]	6.69±0.02[a]
	E	6.62±0.01[a]	6.67±0.01[b]	6.67±0.0[bc]
Ultra high temperature (UHT)	F	6.57±0.01[c]	6.64±0.01[d]	6.65±0.02[c]
	G	6.58±0.0[cb]	6.65±0.01[cd]	6.65±0.01[c]
	H	6.59±0.0[b]	6.65±0.01[cd]	6.66±0.0[bc]

*R: Raw milk, A: Ildongfoodis, B: Konkuk, C: Pasteur, D: Sangha, E: Denmark, F: Seoul, G: Maeil, H: Namyang
[a-e]Means with the different letter in same column are significantly different by Duncan's multiple range test (p<0.05).

ized milk products (HTST- and LTLT-milk) was 6.62, showing no difference in pH values. The UHT milk, however, was 6.57 to 6.59 of a relatively lower pH. The pH of all treatments through primary and secondary ultrafiltration returned 6.64 to 6.69, resulted in slightly higher pH than non-ultrafiltrated samples. The reason that the pH slightly increased following the ultrafiltration is explained mainly due to the fact that part of minerals slipped out into the permeate. This result is similar to the one form the previous study (On-Nom *et al*., 2010) that pH value slightly goes up when milk is ultrafiltrated.

Rennet coagulation test

Fig. 1 shows the results of milk coagulation experiment after adding rennet to each sample and incubated for 30, 60, 90 min, respectively. After every 30 min following addition of rennet, milk coagulation was measured, resulted that raw milk was confirmed to have the most effective compared to other samples, and additional incubation of 90 min has little change on the apparent coagulation state of HTST- and UHT-treated milk used. The UHT-treat milk, on the other hand, was considerably reduced in coagulation activity, compared to other samples where the coagulation state of the UHT-treated milk after 90 min incubation significantly dropped in rennet coagulation activity than those of raw milk, HTST-, and LTLT-treated milk. These results hold a similar tendency to other's work (Ham *et al*., 2008) that coagulation activity of raw milk was the most effective at 60 min after adding rennet to the UHT-treated milk and raw milk was far more effective than UHT-treated milk in terms of milk coagulation.

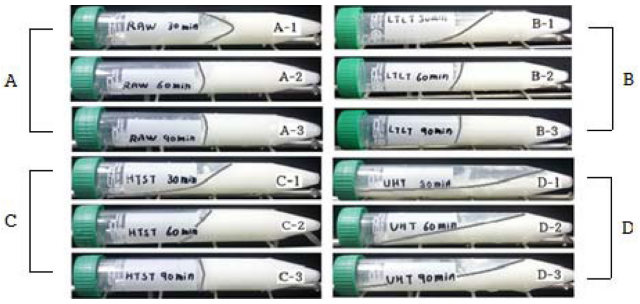

Fig. 1. Results of the rennet coagulation test on raw milk and some commercial milk products after incubation for 30(1), 60(2), and 90(3) min, respectively. A1-A3: Raw milk, B1-B3: LTLT (Low temperature long time) treated milk, C1-C3: HTST (Hight temperature short time) treated milk, D1-D3: UHT (Ultra high temperature) treated milk.

As for the mechanism addressing milk coagulation, phenylalanine (105)-methionine (106) of κ-casein is sensitive to proteolytic enzyme rennet. Accordingly, stability of casein micelle decreases and gets coagulated when this peptide bond is digested. If heated at high temperature, however, the sensitivity of κ-casein to rennet reduce in coagulation strength because enzyme action is hindered as a result of the complex between β-lactoglobulin and κ-casein (Lucey, 1995). Sterilization process of raw milk is known to have a critical effect on the balance of calcium phosphate and the balance of colloidal calcium phosphate-k-casein complex. When the ratio between soluble calcium and colloidal calcium increases, clotting time by rennet extends. Therefore, when soluble calcium is added, it was suggested that the clotting time of milk can be shortened by complementing the amount of calcium ion lost by heat treatment (Kim *et al*., 2011).

Measurement of sulfhydryl group

The sulfhydryl group in intact milk proteins becomes activated and concomitant changes of its tertiary structure during heat treatment. When heat is applied to milk, volatile sulfide compounds are formed to generate the cooked flavor as a off-flavor as some sulfur-containing amino acids liberate the sulfhydry groups (Jaddou and Pavey, 1978). When milk proteins are contacted to heat, it tends to cause

Table 8. Contents of the sulfhydryl group of raw milk and some commercial milk products

Methods	Symbol*	Sulfhydryl group (μM/g protein)
Raw milk	R	2.71±0.09^{c}
Low temperature long time (LTLT)	A	3.22±0.10^{ba}
	B	3.30±0.08^{ba}
	C	3.23±0.05^{ba}
Hight temperature short time (HTST)	D	3.33±0.09^{ba}
	E	3.39±0.11^{a}
Ultra high temperature (UHT)	F	3.38±0.14^{a}
	G	3.35±0.09^{a}
	H	3.29±0.13^{ba}

*R: Raw milk, A: Ildongfoodis, B: Konkuk, C: Pasteur, D: Sangha, E: Denmark,F: Seoul, G: Maeil, H: Namyang
$^{a\sim e}$Means with the different letter in same column are significantly different by Duncan's multiple range test (p<0.05).

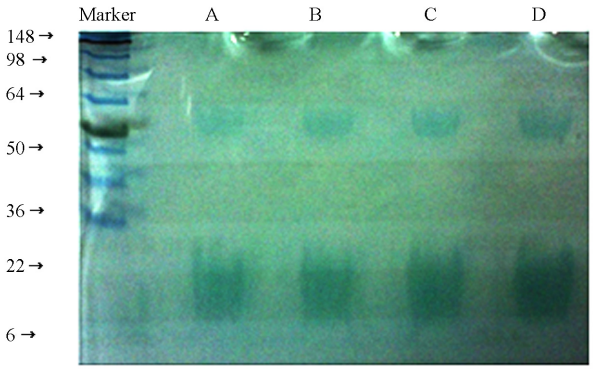

Fig. 2. Non-denaturation polyacrylamide gel electrophorosis (ND-PAGE) of defatted raw milk and some commercial milk products defatted. A: Raw milk, B: LTLT (Low temperature long time) treated milk, C: HTST (Hight temperature short time) treated milk, D: UHT (Ultra high temperature) treated milk.

a structural change, exposed the inside SH groups to the outside, therefore the amounts of SH group is consequently influenced by the time and the temperature applied, composition of raw milk, initial acidity, oxygen content, and storage conditions (Klostermeyer, 1976). As shown in Table 8, the values for SH groups in the commercial milk products (symbol A to H) were measured to be 3.22-3.39, while the value of raw milk was 2.71, which is significantly lower than in the heat-treated samples. This result indicates the structural change in milk proteins probably took place even by means of pasteurization or sterilization process. To confirm the structural changes in casein according to heat treatment, non-denaturation polyacrylamide gel electrophorosis (ND-PAGE) was performed. However, the banding pattern was all identical to those of the denatured samples on polyacrylamide gel electrophoresis (Fig. 2).

Determination of total calcium and soluble calcium

As shown in Table 9, content of the total calcium in the market milk products and raw milk. have shown that the raw milk contained the highest value of 1,111 mg/kg, and product H (UHT- treated) was the lowest value of 1,056 mg/kg. No significant difference (p<0.05) was found in each sample as for the total calcium content. A similar tendency was found in the earlier report, claimed that heat treatment has no influence on the total calcium content in milk (Sandhu, 1973). For determination of soluble calcium, ultrafiltration was conducted with the primary (Mw cut-off 30 Kd) and the secondary membrane (Mw cut-off 10 kD) in order were carried out, resulted that the total calcium content was between 27.4 and 41%. This result was very similar to the values previously reported by Miguel *et al*. (2004) that the soluble calcium of UHT milk accounts for 24 to 37% of the total calcium content. Total calcium contents in the market milk products were as follows: UHT-milk was 370.3 to 380.2 mg/kg; LTLT-milk, 336.4 to 345.1 mg/kg; HTST-milk, 305.5 to 313.3 mg/kg. After secondary ultrafiltration, total calcium in raw

Table 9. Concentrations of total and soluble calcium in raw milk and some commercial milk products

Methods	Symbol*	Total calcium (mg/mL)	Soluble calcium (mg/mL)	
			by UF membrane (30 kD)	by UF membrane (10 kD)
Raw milk	R	1111.3±21.3^{a}	450.2±3.5^{a}	442.2±6.7^{a}
Low temperature long time (LTLT)	A	1087.2±17.8^{a}	336.4±2.4^{e}	333.3±7.8^{c}
	B	1091.1±21.9^{a}	337.2±3.4^{ed}	334.1±8.2^{c}
	C	1099.5±8.2^{a}	345.1±4.0^{d}	342.2±4.9^{c}
Hight temperature short time (HTST)	D	1100.4±40.8^{a}	305.5±1.8^{f}	301.9±7.2^{d}
	E	1101.2±31.3^{a}	313.3±6.5^{f}	311.2±4.9^{d}
Ultra high temperature (UHT)	F	1086.4±8.2^{a}	375.4±4.1^{cb}	372.7±5.8^{b}
	G	1104.3±40.8^{a}	370.3±4.7^{c}	371.3±3.9^{b}
	H	1056.5±24.5^{a}	380.2±4.1^{b}	378.2±1.6^{b}

*R: Raw milk, A: Ildongfoodis, B: Konkuk, C: Pasteur, D: Sangha, E: Denmark, F: Seoul, G: Maeil, H: Namyang
$^{a\sim e}$Means with the different letter in same column are significantly different by Duncan's multiple range test (p<0.05).

milk had a highest of 444.2 mg/kg, and those in the market milk products: UHT- milk, 371.3 to 378.2 mg/kg; LTLT-milk, 333.3 to 342.2 mg/kg; HTST-milk 301.9 to 311.2 mg/kg in a decreasing order. It appears that the content of soluble calcium is high as UHT-milk is contacted with heat in a short period of time (2-3 s) though the high temperature above 130°C was applied for sterilization. While LTLT-, HTST-milk products went through a longer time in heat treatment stage yet at a low temperature than that of UHT- milk, which might be responsible to a comparatively low content of soluble calcium.

According to the study by On-Nom *et al*. (2010), the content of ionic calcium as a part of the soluble calcium decreases as the heat-treatment temperature rises. A similar tendency was drawn to a previous study reported that the ionic calcium content was higher in the milk sterilized for 2 s at 120°C than in the one heat-treated for 15 s at 85°C. It is said that the amount of calcium is cut down during the course of heating, sterilizing and treating milk, and that ultrafiltrable calcium content is reduced after milk is combined with the phosphoric acid or the denatured proteins, and then transferred to casein micelle and in turn to the colloidal calcium. As seen above, temperature and time for heat treatment turned out to have no particular influence on the total calcium content of milk, whereas the content of soluble calcium was confirmed to turn into colloidal calcium according to the temperature and time by heat treatment before being reduced. According to a publication (In and Jung, 2001), the decrease in the amount of soluble calcium by such a heat treatment of milk or change in its form of existence is reported to have a small impact on the bioavailability but there is still huge uncertainty. A further study needs in the future on the bioavailability of the insoluble calcium and soluble calcium.

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