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Chemical and Microbiological Quality, Capillary Electrophoresis Pattern, and Rennet Coagulation of UHT-treated and Irradiated Milk

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Abstract To see the possibility of irradiation as an alternative to ultra high temperature (UHT) sterilization, the quality characteristics of milk were analyzed. Milk treated by UHT (135°C for 4 sec) and irradiation at higher than 3 kGy showed no viable counts after 7 days of storage at 4°C. The contents of certain amino acids of milk, such as Arg, Asp, Glu, Ile, Leu, Lys, Pro, Ser, Thr, and Tyr, were lower in irradiated groups at 10 kGy than in UHT-treated one, but no difference was observed between irradiated milks at less than 5 kGy and UHT. The capillary electrophoresis (CE) patterns of the milk irradiated at 10 kGy showed a similar trend to the raw milk, low temperature long time (LTLT, 63°C for 30 min), and high temperature short time (HTST, 72°C for 15 sec) treated. However, the CE pattern of UHT-treated milk was different. Rennet coagulation test agreed with the CE results, showing that all milk samples were coagulated by rennet addition except for UHT-treated milk after 1 hr. These results suggest that irradiation of milk reduce the content of individual amino acids but it may not induce severe conformational change at a protein level when compared with UHT treatment.

Keywords: milk, irradiation, UHT, capillary electrophoresis

Introduction

It is well known that milk is a good food resource for human consumption because of its nutritional components and biologically active substances (1,2). Because of this reason it is also very important to control the contamination of microorganism during processing, packaging, and storage condition.

Conventional pasteurization methods have long been in place and with the advent of ultra high temperature (UHT) technology, the sterilization of fluid milk was achieved using higher temperature treatments for shorter periods (3). However, the shelf-stable milk has met with limited acceptability by consumer, especially in the United States, due in part to a high cooked flavor. Off-flavors in heated milk with high temperature often result from creation of organic sulfur compounds that arise during the decomposition of reactive protein sulfhydryl groups associated with the amino acids, methionine and cysteine. Often, their concentration has been correlated with the sensory detection of undesirable 'cooked' flavors in milk samples when exposed to high temperatures (4).

Abu-Tarboush *et al.* (5) and Kwon (6) reported that irradiation technology has positive effects in preventing the decay of food products by decontaminating the microorganisms as well as improving the safety and shelf-stability without compromising the nutritional or sensorial quality. However, Adeil Pietranera *et al.* (7) reported that irradiation of 6 and 9 kGy resulted in higher acid value and lower reducing sugar content in milk when compared with non-irradiated control. Schweigert (8) reported that milk is the most sensitive of all foods to the flavor and odor changes and lipid oxidation development that occur during irradiation. Therefore, Schweigert (8) developed a method to remove the irradiation-induced off-flavor but it has not been successfully employed in industry. Lee *et al.* (9) reported that irradiation of 32 kGy increased the viscosity of milk proteins which might be caused by cross-linking. However, lower viscosity values were detected after heating of the solutions irradiated with 32 kGy than after heating of the non-irradiated ones (10). Ciesla *et al.* (10) explained that creation of less stiff but better ordered gels after irradiation are probably from reorganization of aperiodic helical phase and β-sheets, in particular from increase of β-strands, which was confirmed by Fourier Transform Infrared (FTIR).

On the other hand, several reports have indicated that ionizing radiation can reduce the allergenicity of milk proteins by partial destruction of the human IgE-binding epitopes in food allergens (11-13). In fact, since the oxidative protein modifications during a heat treatment, especially UHT treatment, tend to increase the natural allergenicity of milk proteins (14), these findings may bring the consideration in the possibility of use of irradiation as an alternative to UHT-treatment.

In the present study, the quality characteristics of irradiated and UHT-treated milk were investigated for providing basic information on the possibility of the production of marketable milk by irradiation technology.

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Materials and Methods

Sample preparation Milk was obtained from an experimental farm of the National Institute of Animal Science

(NIAS), and partially skimmed by a cream separator (Westfalia, Germany). Heat treatments of the partly skimmed milk were carried out by low temperature long time (LTLT, 63°C for 30 min.), high temperature short time (HTST, 72°C for 15 sec), and UHT (135°C for 4 sec) in a pilot plant (APV, Hillerød, Denmark) of NIAS. The partially skimmed milk samples were exposed to irradiation doses of 1, 2, 3, 5, and 10 kGy (point source, AECL, IR-79; MDS Nordion, ONT, Canada) at the Korea Atomic Energy Research Institute (KAERI), Daejeon, Korea. The irradiation dose was validated by using 5-mm diameter alanine dosimeters (Bruker Instrument, Rheinstten, Germany). Free radicals were measured by using an EMS 104 EPR analyzer (Bruker Instrument). The actual doses were within ±2% of the target doses. Samples were turned 360° continuously during the irradiation process to achieve uniform target dose and the non-irradiated control was placed outside of the irradiation chamber to have the same temperature effect as the irradiating sample.

General composition and microbiological analysis Protein, fat, lactose, total solid, and free fatty acid contents were measured by MilkoScan FT120 (Foss Electric, Hillerod, Denmark). For amino acid composition, approximately 1 g of a milk sample was weighed into a sample bottle, and 40 mL of 6 N HCl was added to the bottle while purging it with nitrogen gas. After a hydrolysis at 110°C for 24 hr, HCl was removed at 50°C by using a rotary evaporator (Eyela, Tokyo, Japan). The residues were washed with distilled water 3 times and evaporated, and then filtered through a filter paper (No. 5B; Toyo, Tokyo, Japan). The filtrate was messed up to 50 mL with distilled water, and analyzed by an amino acid analyzer (L-8500A; Hitachi, Tokyo Japan). Cysteine and methionine were changed to cysteic acid and methionine sulfone by adding 20 mL of a stabilizing solution (45 mL of 85% formic acid+5 mL of 30% H_2O_2) before the addition of HCl.

For microbiological analysis, decimal dilutions of a milk sample were prepared in a sterile phosphate buffer and plated in duplicate on an aerobic count plate and Coliforms Petrifilms (3 M Health Care, Berkshire, UK). The analysis was performed 4 samples per each treatment.

Sensory analysis A sensory test was performed to identify sensory difference among treatments. Ten semi-trained panelists, who had at least 1 year experience in milk processing and research, were employed to identify the sensory same-different between milks with LTLT-UHT, LTLT-3 kGy-irradiated, and UHT-3 kGy-irradiated by the triangle test as described by Rousseau *et al.* (15). The comments from sensory panelists were collected and reported.

Capillary electrophoresis Capillary electrophoresis (CE) was performed based on the method described by Revilla *et al.* (16) and De Jong *et al.* (17) by using a P/ACE™ system MDQ equipped with a DAD detector, temperature-controlled capillary compartment, and autosampler (Beckman Instruments, Fullerton, CA, USA). The reduction buffer was prepared by dissolving 73 mg of trisodium citrate dehydrate (Merck, Damstadt, Germany) and 38 mg of DL-dithiothreitol (DTT) (Sigma, St. Louis, MO, USA) in 37.5 mL of 8 M urea. The pH was adjusted to 8 with dilute sodium hydroxide solution and the volume was made up with water. Milk sample (0.5 mL) was diluted with 2.5 mL of the reduction buffer and incubated for 1 hr at room temperature. The resulting clear solution was used for CE analysis. Separations were performed using a fused-silica capillary column (eCap™, Beckman) (60 cm×50 μm i.d.; 50 cm to the detector window). The running buffer (50 mM) was prepared by mixing 14.7 M H_3PO_4 (847 μL) and 0.05% hydroxypropylmethylcellulose (HPMC) with 6 M of urea solution (250 mL). Sample solutions were injected for 5 sec at 0.5 psi. The separation was conducted at a constant voltage (25 kV) and temperature (20°C). Detection was performed at 214 nm. Before each injection, the capillary was washed with 0.1 M NaOH (5 min), deionized distilled water (5 min), and 1 M HCl (5 min) and equilibrated with the running buffer (5 min).

Rennet coagulation of the milk Milk samples (50 mL) were coagulated at 35°C with 10 mL of rennet which is the usual amount during cheese making in NIAS, Suwon, Korea. After 20, 40 and 60 min, the test tube was laid down to see coagulation and a picture was taken.

Statistical analysis Entire experimental procedure was replicated and statistical analysis was performed by one-way analysis of variance (SAS, release 8.01, Cary, NC, USA). When significance was defined the differences among mean values were calculated by Duncan's multiple range tests at $p<0.05$ level.

Results and Discussion

General composition and microbiological properties Protein, fat, lactose, and total solid content of the milk were 3.12-3.16, 1.63-1.69, 4.93-4.98, and 10.35-10.47%, respectively, without any difference among the treatment combination including the milk with raw state, LTLT-, HTST-, and UHT-treated, and irradiated at 1, 2, 3, 5, and 10 kGy ($p>0.05$, data not shown).

The single amino acid content (mg/100 g milk) is shown in Table 1. In contrast to the percentage ratio, it is noted a significant ($p<0.05$) decrease in the contents of amino acids including Arg, Asp, Glu, Ile, Leu, Lys, Pro, Ser, and Thr by 10 kGy-irradiation of milk when compared with UHT-treated. Diehl (18) reported that deamination and decarboxylation are the primary reactions of free radicals by irradiation in aliphatic amino acids. In general, the radiation-induced reactions in proteins are strongly influenced by their complex structure including the folding of peptide chains, disulfide linkages between the chains, secondary binding forces such as hydrogen bonds, hydrophobic bonds, ionic bonds, or those bonds holding several subunits together as a functional protein (19). Sulfur-containing and aromatic amino acids are known to the most sensitive to irradiation. The sulfur amino acids act as scavengers and react more readily with free radicals than the aliphatic amino acids, and thus provide a protective effect against ionizing radiation (20). However, the cysteine and methionine contents were not shown difference among treatments in this study.

Thermal treatment of milk is an essential operation

Table 1. Amino acid content (mg/100 g milk) of heat-treated and irradiated milk

Treatment		Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu
Raw milk		95.0	120.0[a2)]	233.0[a]	27.5	664.5[bc]	59.0	80.5	133.0[a]	287.5[a]
Heat[1)] treatment	LTLT	97.0	105.0[c]	231.5[a]	27.5	689.5[a]	59.5	79.5	132.0[a]	287.0[a]
	HTST	96.5	103.0[c]	231.0[a]	27.5	683.5[a]	59.5	80.5	131.0[a]	284.0[a]
	UHT	95.5	115.5[ab]	230.5[a]	27.5	688.0[a]	58.5	78.5	132.5[a]	283.5[a]
Irradiation dose (kGy)	1	96.0	117.5[ab]	230.0[a]	28.5	680.0[ab]	58.0	78.0	131.5[a]	284.0[a]
	2	96.5	117.5[ab]	232.0[a]	30.0	691.0[a]	59.0	79.0	132.5[a]	284.0[a]
	3	95.5	108.5[bc]	230.5[a]	29.0	686.5[a]	58.5	79.0	131.0[a]	283.0[a]
	5	95.0	108.0[bc]	228.0[a]	29.0	678.5[ab]	58.5	78.5	131.5[a]	283.0[a]
	10	93.0	110.0[bc]	221.5[b]	29.0	655.0[c]	56.5	76.0	126.5[b]	270.5[b]

Treatment		Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
Raw milk		242.5[a]	66.5	128.5	320.5[cd]	168.5[a]	132.5[a]	133.0	162.0
Heat[1)] treatment	LTLT	241.0[ab]	66.0	132.0	327.0[abc]	170.5[a]	133.5[a]	137.0	161.5
	HTST	238.0[ab]	64.5	129.0	323.5[bcd]	170.0[a]	133.0[a]	136.5	159.5
	UHT	234.5[bc]	63.5	128.0	330.5[ab]	169.0[a]	132.5[a]	133.5	162.0
Irradiation dose (kGy)	1	238.0[ab]	65.5	127.5	329.0[abc]	167.5[a]	132.0[a]	133.0	161.0
	2	237.0[ab]	67.0	126.0	332.5[a]	170.5[a]	133.0[a]	132.0	161.5
	3	236.0[ab]	66.5	128.0	330.5[ab]	169.5[a]	133.0[a]	136.0	161.0
	5	234.5[bc]	66.5	129.5	327.0[abc]	167.5[a]	130.5[a]	136.0	161.0
	10	228.0[c]	64.5	127.0	317.0[d]	162.0[b]	126.5[b]	130.5	156.5

[1)]LTLT (63°C for 30 min), HTST (72°C for 15 sec), and UHT (135°C for 4 sec).
[2)]Different letters within the same column differ significantly ($p<0.05$).

Table 2. Effect of heat and irradiation on the number of total aerobic bacteria and coliforms (CFU/mL)

Treatment		Same day		After 1 week at 4°C	
		Total aerobic bacteria	Coliforms	Total aerobic bacteria	Coliforms
Raw milk		3.33±0.01[1)]	2.92±0.03	7.13±0.05	6.84±0.02
Heat[2)]	LTLT	ND[3)]	ND	6.38 ± 0.02	6.38±0.04
	HTST	ND	ND	6.54 ± 0.02	6.27±0.01
	UHT	ND	ND	ND	ND
Irradiation dose (kGy)	1	ND	ND	4.11±0.07	2.06±0.02
	2	ND	ND	1.45±0.07	ND
	3	ND	ND	ND	ND
	5	ND	ND	ND	ND
	10	ND	ND	ND	ND

[1)]Mean ± SD (n = 8).
[2)]LTLT (63°C for 30 min), HTST (72°C for 15 sec), and UHT (135°C for 4 sec).
[3)]Viable colonies were not detected with a detection limit at 10 CFU/mL.

during the commercial dairy processes in order to provide an acceptable safety and shelf-life. UHT-treated milk kept sterility after 7 days of storage at 4°C and the same effect was observed in irradiated milk at more than 3 kGy (Table 2). Imm *et al.* (1) reported that UHT-treated milk kept sterility in 7 days of storage at 4°C but did not in 14 days.

The sensory triangle test was performed to differentiate among LTLT, UHT, and 3 kGy-irradiated milk (Table 3) because 3 kGy irradiation had the same microbiological quality with UHT treatment. All panelists were able to differentiate between LTLT- or UHT- and irradiation-treated milk at 3 kGy. The panelists were also possible to differentiate between LTLT- and UHT-treated milk. The comments from the panelists indicated that odor and flavor changes were considerable which may be an identification factor. A slight cooked odor was commented from UHT-treated milk when compared with LTLT-treated one. Irradiation of 3 kGy resulted into characteristic metalic odor which can be detectable by sensory panelists. Jo and Ahn (21) found that irradiation produced new volatile compounds from oil emulsion containing Leu, Val, Ile, Phe, Met, and Cys. They suggested that radiolysis of protein or amino acid may play an important role in characteristic irradiation off-odor because the irradiation off-odor was different from oxidized one (21). Abdel Baky *et al.* (22) reported that an oxidized flavour in the fresh and 1-month-old irradiated milk cheese disappeared during ripening. At the end of ripening, an irradiated cheese had a better consistency and

Table 3. The same-different test (triangle test) of low temperature long time (LTLT)-, ultra high temperature (UHT)-treated, and 3 kGy-irradiated milk[1,2]

	Number of panelist		
	LTLT-3 kGy	LTLT-UHT	UHT-3 kGy
Correct answer	10	8	10
Incorrect answer	0	2	0

[1]Triangle test was performed to choose 1 different sample among 3 provided samples.
[2]LTLT (63°C for 30 min) and UHT (135°C for 4 sec).

increased flavour compared with cheese made from heat pasteurization (22).

Capillary electrophoresis (CE) of the milk proteins In milk samples, electrophoresis is widely used for showing the milk proteins but differences in concentration between caseins and whey proteins make the results unsatisfactory. A capillary electrophoresis is the first high-resolution analytical technique that requires simple preparation and is capable of determining whey proteins and caseins simultaneously (16).

Figure 1 shows the CE electropherograms which was pre-tested by standard materials including α-lactalbumin (LA), β-lactoglobulin (LG), α-casein, κ-casein, and β-casein and comparative results of Miralles *et al.* (23). All the CE patterns of milk samples were similar except for the sample treated with UHT. The CE electropherograms of UHT sample showed interactions among whey proteins, α-casein, and κ-casein, but not for β-casein. The CE electropherograms of irradiated milk samples in this study demonstrated less changes in κ-casein, suggesting that irradiation of milk proteins affect much less on rennet coagulation than UHT treatment.

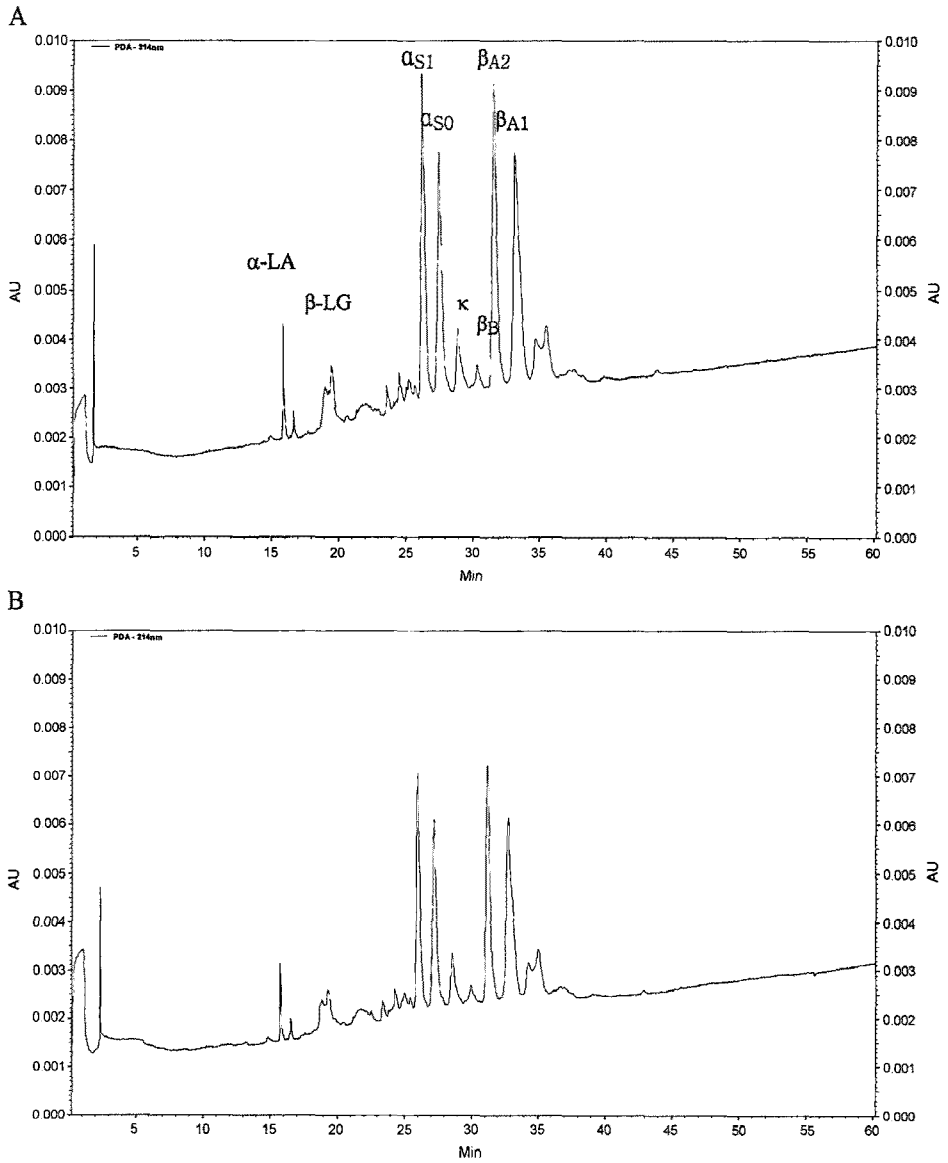


Fig. 1. Capillary electrophoresis patterns of heat- and irradiation-treated milk protein.
A, Raw milk; B-D, LTLT-(63°C for 30 min), HTST-(72°C for 15 sec), and UHT-(135°C for 4 sec) treated milk; E-I, irradiation-treated milk with 1, 2, 3, 5, and 10 kGy. Abbreviation: α-lactalbumin (α-LA), β-lactoglobulin (β-LG), αs_0-, αs_1-, β_{A1}-, β_{A2}-, β_B-, and κ-casein.

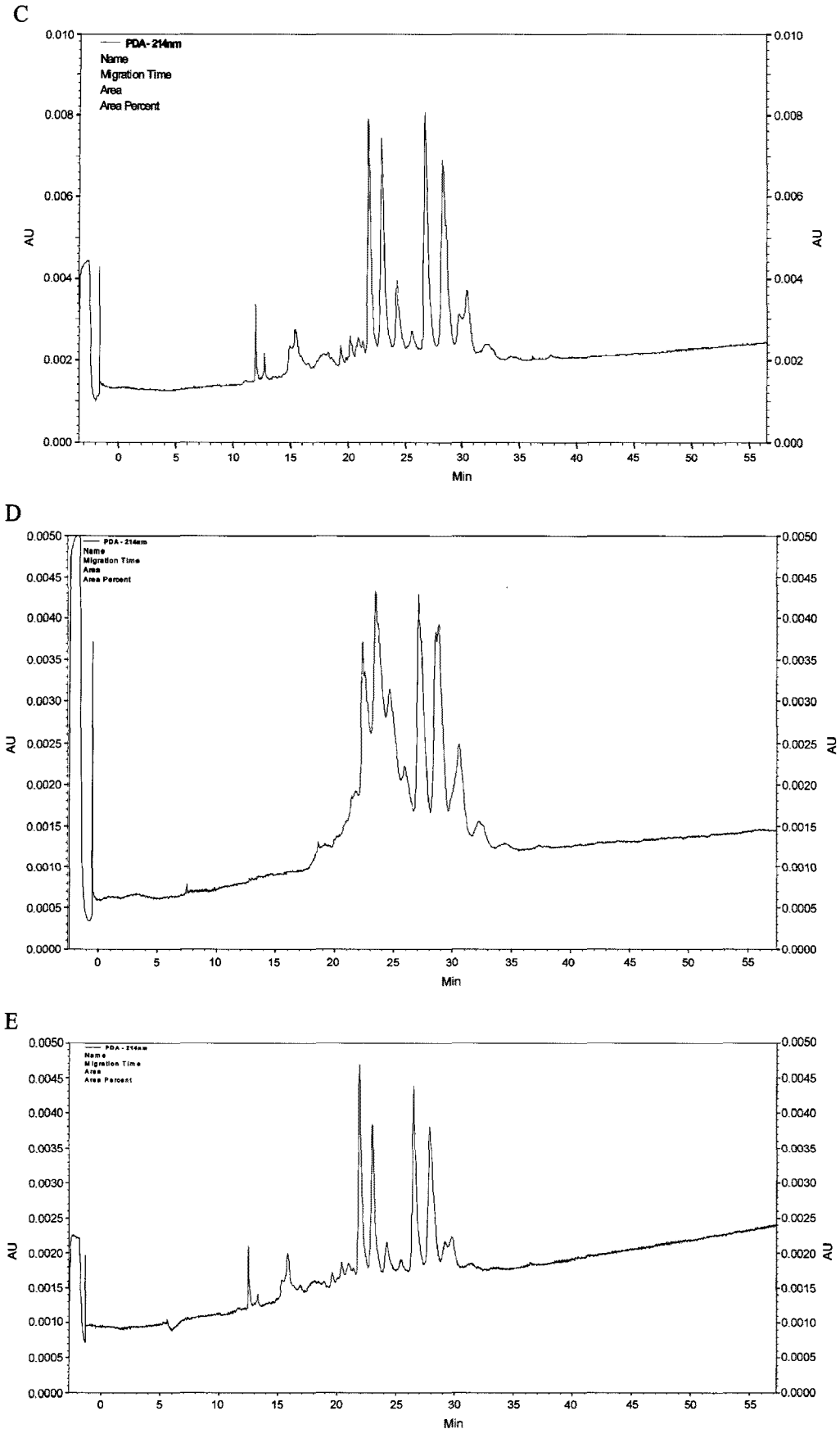
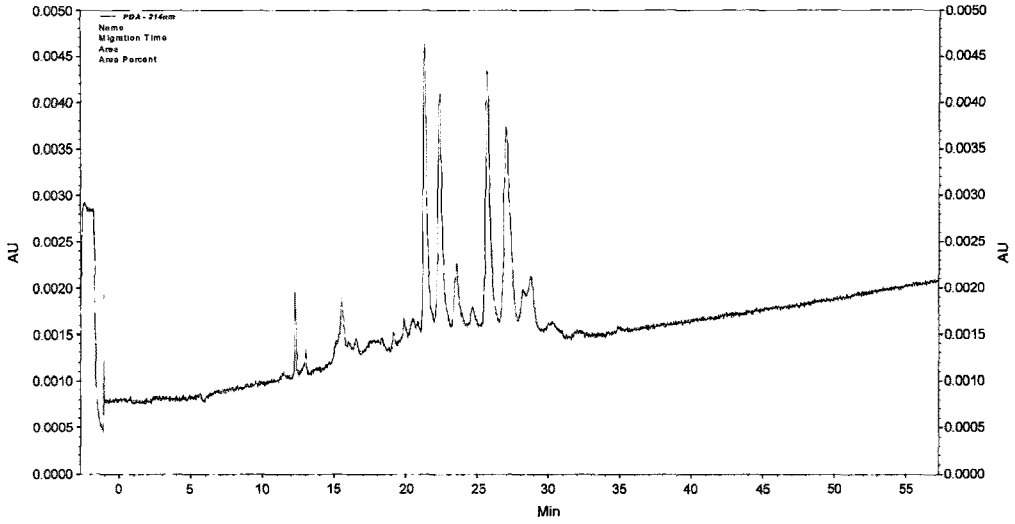
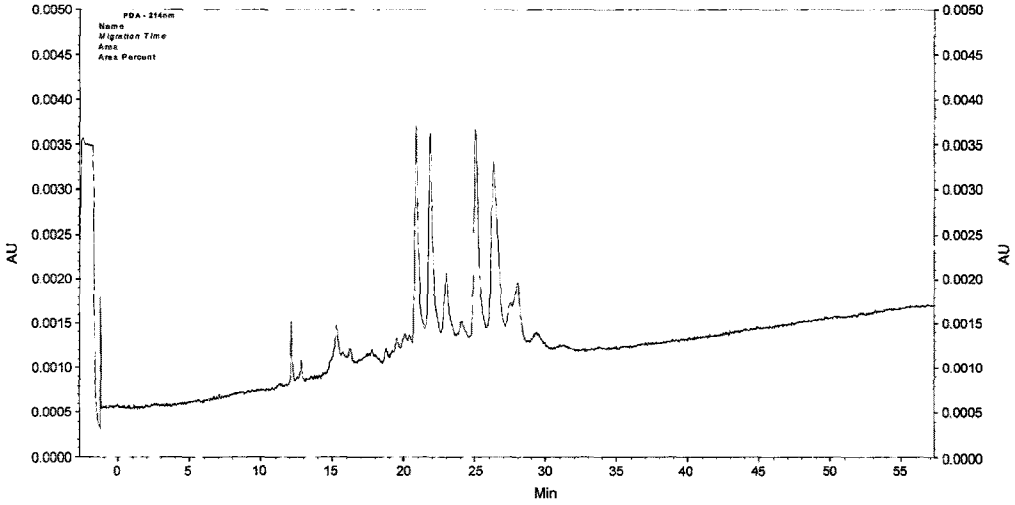


Fig. 1. Continued

F



G



H

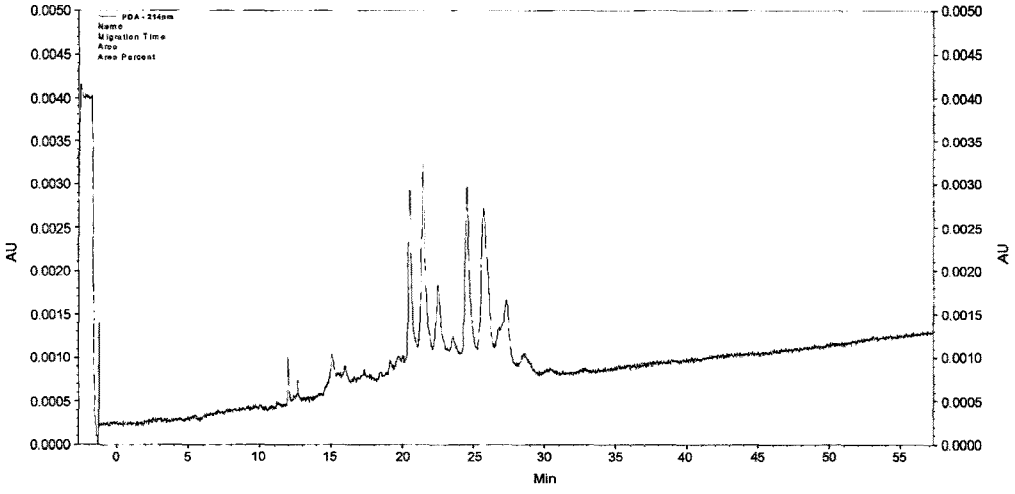


Fig. 1. Continued

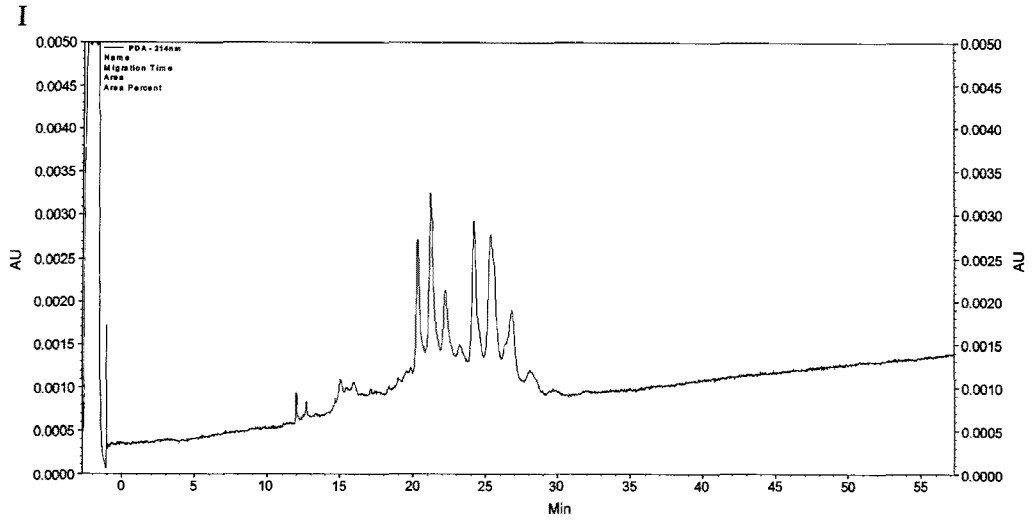


Fig. 1. Continued

The native 3-dimensional structure of proteins is maintained by a variety of non-covalent interactions between amino acid residues within the polypeptide chain and between residues and solvent molecules. Any change in the environment of the protein molecule, which can influence the non-covalent interactions, results in an alteration of the secondary and tertiary structures. Of the environmental conditions that can lead to changes in protein structure, temperature represents the most important factor. As the temperature is increased from 15°C, the strength of hydrophobic bonds increase with increasing temperature up to 60°C but decrease at higher temperatures and they are probably not existed above 100°C. Disulfide bonds can undergo sulfhydryl-disulfide interchange or become oxidized by heating (24). Protein structure undergoes temperature-dependent changes over a very broad temperature range. The changes which occur at low temperatures (0-60°C) are usually reversible, but exposure to high temperatures (>60°C) frequently causes irreversible denaturation. Following denaturation, proteins often interact either with themselves or other molecules to form aggregates, precipitates or gels which are virtually irreversible. Considerable research has been carried out on the effects of heat on casein using model systems, consisting of mixtures of purified proteins in simple buffer solutions under a variety of conditions (19). In milk, a protein modification and the formation of complexes between the κ-casein and β-lactoglobulin and between the casein and lactose were found (25).

Rennet coagulation of the milk The complete coagulation was achieved by 40 min for raw milk and by 60 min for irradiation-treated at 10 kGy (Fig. 2) but the milk treated by UHT did not. This result demonstrates that a gamma irradiation induces much less conformational changes of milk proteins than UHT treatment. Similarly, irradiation of 5 kGy slightly increased the acid and peroxide values of milk fat, as well as the time required for complete coagulation of milk (22).

The rennet coagulation of milk may be divided into primary (proteolysis), secondary (aggregation), and possibly tertiary (syneresis and structural rearrangement) stages. During the primary stage, κ-casein is cleaved by rennet (chymosin, E.C.3.4.23.4, or chymosin substitute) at Phe_{105}-Met_{106} bond. Severe heat treatment of milk impairs its renneting properties, and it has been established that when heated, β-lactoglobulin and κ-casein form a complex by sulfhydryl-disulfide interchange (26). Van Hooydonk *et al.* (27) reported that complex formation reduced both the initial rate of κ-casein hydrolysis and the amount of hydrolyzable κ-casein in milk when heated at temperatures up to 120°C for 5 min. From the results, the individual amino acid content in proteins was affected by irradiation at 10 kGy but the bond cleavage at Phe_{105}-Met_{106}, which is responsible for rennet coagulation, was not occurred.

In summary, the microbiological quality of milk irradiated at higher than 3 kGy was similar to UHT treatment. Irradiation of milk at 10 kGy reduced the amount of indi-

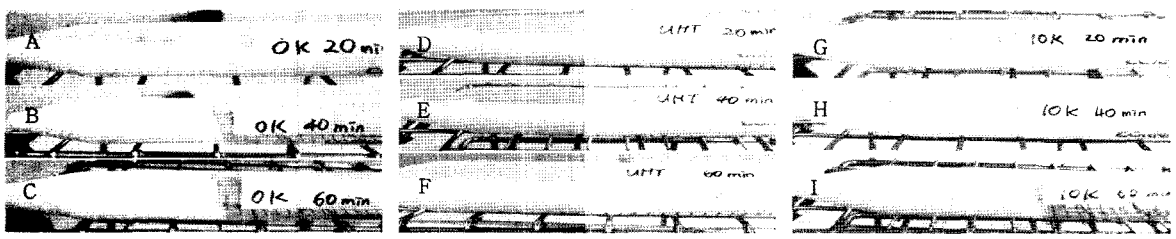


Fig. 2. Presentation of rennet coagulation of control (raw milk), UHT (135°C for 4 sec)- and 10 kGy-irradiation-treated milk during reaction for 20, 40, and 60 min. A-C, Raw milk; D-F, UHT-treated milk; G-I, irradiation-treated milk at 10 kGy.

vidual amino acid content compared with UHT treatment, but it may not induce the severe conformational change at a protein level as UHT treatment does. However, the characteristic irradiation odor of milk, which can be detected even at 3 kGy, should be solved before application of irradiation technology to industry.

Acknowledgments

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