JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Removal of Cholesterol from Cheddar Cheese by β-Cyclodextrin

H. S. KWAK,* C. S. JUNG, S. Y. SHIM, AND J. AHN

Department of Food Science and Technology, Sejong University, 98 Kunja-dong, Kwangjin-ku, Seoul, 143-747, Korea

This study was carried out to determine the cholesterol removal rate and resulting changes in flavor, fatty acid and bitter amino acid production in reduced-cholesterol Cheddar cheese, made by cream separation followed by 10% β-cyclodextrin (β-CD) treatment. The cholesterol removal from the cheese was 92.1%. The production of short-chain free fatty acids (FFAs) increased the ripening time in control and cream-treated cheeses. The quantity of short-chain FFAs released between treatments during ripening was different, while not much difference was found in the production of neutral volatile compounds in the samples. Reduced-cholesterol cheese produced much higher levels of bitter amino acids than the control. In sensory analysis, the texture score of control Cheddar cheese increased significantly with ripening time; however, that of the cream treatment group decreased dramatically with ripening time. On the basis of our results, we conclude that the cheese made from β-CD-treated cream had a higher rate of cholesterol removal and ripened rapidly.

KEYWORDS: Cholesterol removal; β-cyclodextrin; Cheddar cheese; homogenization

INTRODUCTION

Since a strong positive correlation exists between increased serum cholesterol concentrations and risk of coronary heart disease, most consumers are concerned about excessive intake of cholesterol (*1*, *2*). Therefore, physical, chemical, and biological methods to reduce cholesterol have been studied in foods, including dairy products (*3*−*6*).

A number of studies have indicated that cholesterol removal from milk, cream, and Mozzarella cheese was effectively achieved by treatment with β-cyclodextrin (β-CD) (*3*−*5*, *7*, *8*). Because β-CD is nontoxic, edible, nonhygroscopic, chemically stable, and easy to separate (*9*), it has positive attributes when used for the removal of cholesterol from foods.

Several studies (*10*−*12*) have indicated adverse effects of homogenization on the process of cheese making, such as slower drainage (*13*), reduced tension, decreased elasticity of the curd (*14*), and increased rates of acid development (*15*).

Metzger and Mistry (*12*) developed a new process for improving the body and texture of reduced-fat Cheddar cheese, in which the cream was homogenized separately and then blended with skim milk prior to cheese manufacture. It was suggested that homogenization of cream rather than milk was important to prevent adverse effects on milk proteins. However, little information is available on the effects of β-CD treatment and homogenization for cholesterol removal on chemical, rheological, and sensory characteristics (*16*, *19*). Therefore, our objectives in this study were to examine the short-chain fatty acid, neutral volatile compound, and free amino acid production in reduced-cholesterol Cheddar cheese and to find the differences in textural and sensory attributes.

* Corresponding author (fax +82-2-3408-3226, telephone +82-2-497-8931, E-mail kwakhs@sejong.ac.kr).

MATERIALS AND METHODS

Materials. Raw milk was obtained from Binggare Dairy Plant (Kyonggi-do, Korea) and adjusted to 3.5% milk fat with skim milk. Commercial β-CD (purity 99.1%) was purchased from Nihon Shokuhin Kaku Co. Ltd. (Osaka, Japan). Cholesterol and 5-α-cholestane were purchased from Sigma Chemical Co. (St. Louis, MO), and all solvents were gas chromatographic grade.

Milk Processing. Bulk raw milk (15 kg) was heated to 40 °C and separated into cream and skim milk using a cream separator (Elecrem, Vanves, France). The separated cream was stirred with 10% β-CD at 800 rpm in a blender (Tops, Misung Co., Seoul, Korea) in a temperature-controlled water bath at 20 °C for 30 min (*4*) and then blended with the remaining skim milk at 1000 psi at 70 °C in a single-stage homogenizer (HC 5000, Microfluidics Corp., Newton, MA) (*3*). Each sample was centrifuged at 166 x *g* for β-CD removal. All treatments were run in triplicate. The whole milk was not treated with β-CD and not microfluidized and was used as the control. Cheese milk were pasturized at 72 °C for 17 s prior to cheese making.

Manufacture of Cheddar Cheese. The cheese-making process was described by Metzger and Mistry (*11*). After manufacturing, pressed cheeses were weighed, vacuum packaged in a barrier bag, and ripened at 5 °C for 0, 1, 3, and 7 mo. The cheese sample stored in the refrigerator for 12 h was the 0 mo sample. The cheese-making experiment was carried out in triplicate on different days using different batches of treatments. Each batch of cheese making was done in triplicate.

Extraction and Determination of Cholesterol. Cholesterol was extracted from β-CD-treated cheese by the method described by Adams et al. (*17*) and stored at −20 °C until analysis. Total cholesterol was

10.1021/jf020568m CCC: $22.00
Published on Web 11/07/2002

determined on a silica fused capillary column (HP-5, 30 m × 0.32 mm i.d. × 0.25 μm thickness) using a Hewlett-Packard 5890A gas chromatograph (Palo Alto, CA) equipped with a flame ionization detector. The temperatures of the injector and detector were 270 and 300 °C, respectively. The oven temperatures were programmed from 200 to 300 °C at 10 °C/min and held for 20 min. Nitrogen was used as a carrier gas at a flow rate of 2 mL/min, with a split ratio of 1/50. Quantitation of cholesterol was done by comparing the peak areas with the response of an internal standard.

The percentage of cholesterol reduction was calculated as follows: cholesterol reduction (%) = 100 − (amount of cholesterol in β-CD-treated cheese × 100/amount of cholesterol in untreated cheese). Cholesterol determination for the control was averaged with each batch of treatment.

Analysis of Chemical Composition and Yield of Cheese. Cheese was analyzed for moisture, fat, salt, and protein using the methods of the Association of Official Analytical Chemists (*18*). Cheese yield was determined as wt cheese × 100/wt milk.

Analysis of Short-Chain Free Fatty Acids. Cheese samples (1 g) were removed periodically from the cheeses ripened for 0, 1, 3, and 7 mo, extracted with diethyl ether and hexane for 2 h, and eluted through a 10 mm i.d. glass column containing neutral alumina, as described by Kwak et al. (*19*). A Hewlett-Packard model 5880A GC equipped with a flame ionization detector was used. The preparation of free fatty acids (FFAs) was achieved using a 15 m × 0.53 mm i.d. Nukol fused-silica capillary column (Supleco Inc., Bellefonte, PA). The GC was operated with helium carrier gas at 2 mL/min, hydrogen gas at 37 mL/min, and air at 300 mL/min. The column oven was programmed for an initial holding for 1 min at 110 °C, heating to 180 °C at 5 °C/min for 10 min, and holding for 20 min. The temperature for both the injector and detector was 250 °C. All quantitative analyses were done by relating each peak area of individual FFAs to the peak area of tridecanoic acid as an internal standard. Each FFA was identified by the retention time of a standard.

Analysis of Neutral Volatile Compounds. Cheese samples (40 g) were removed periodically from the cheeses ripened for 0, 1, 3, and 7 mo and added to 10 mL of distilled water. Two milliliters of each distillate was used as the headspace gas sample, as described by Bassette and Ward (*20*). A Hewlett-Packard model 5880A GC equipped with a flame ionization detector was used. Headspace gas samples were analyzed on a capillary column (Supelcowax 10, 30 m × 0.32 mm i.d., Supelco Inc.). The column was operated with nitrogen carrier gas at a flow rate of 1.2 mL/min, hydrogen gas at 30 mL/min, and air at 300 mL/min. The temperature for both the injector port and the detector was maintained at 230 °C. The column oven was programmed at three temperature levels: initial holding for 5 min at 35 °C, heating to 140 °C at 15 °C/min, and holding for 30 min. The concentrations of volatile compounds were estimated by analyzing cheese samples that contained known concentrations and those containing no added standards. The difference between the two treatments was used to estimate the concentrations of individual volatile compounds.

Analysis of Free Amino Acids. RP-HPLC analysis of the FAAs was performed according to the method of Izco et al. (*21*). Samples were analyzed on a Waters HPLC system consisting of a 600 pump and a 486 tunable absorbance detector at 254 nm, operated using Millennium software. The column used was a Waters PicoTag C_{18} reversed-phase column maintained at 46 °C. For identification of amino acids, methionine sulfone (Sigma) was added as an internal standard. A gradient with two solvents was used to run the sample: solution A, comprised of 70 mM sodium acetate adjusted to pH 6.55 with acetic acid and containing 2.5% acetonitrile; and solution B, containing 45% acetonitrile, 40% water, and 15% methanol. Before each injection, the column was equilibrated with solvent A for 2 min.

Rheological Analysis. Cylindrical samples (2 cm diameter × 2 cm height) were cut, and force−distance curves were obtained using a Sun Rheometer (CR-200D, Sun Scientific Co., Ltd., Tokyo, Japan) with a crosshead of 50 mm/min and chart speed of 200 mm/min. From these curves, the basic characteristics of the texture profile were determined, including hardness, elasticity, cohesiveness, gumminess, and chewiness. The point of highest force during the first compression was the hardness. The extent to which the sample returned to its original shape between the first and second compressions was the elasticity. The ratio of the area under the second compression curve was the cohesiveness. Gumminess and chewiness were calculated as hardness × cohesiveness, and gumminess × elasticity, respectively.

Table 1. Mean Chemical Composition of Reduced-Cholesterol Cheddar Cheese[a]

component	no treatment	cream treatment[b]
moisture, %	41.3a	43.2a
fat, %	38.0b	35.5a
protein, %	28.2a	30.8a
cholesterol removal, %	0a	92.1c
yield, %	10.5	12.5

[a] Means within a row with different letters differ significantly ($p < 0.05$). Means of triplicate analysis. [b] After cream separation, cream was treated with 10% β-CD and blended with skim milk at 1000 psi.

Sensory Analysis. Seven trained sensory panelists evaluated randomly coded cheeses. Texture and overall flavor were evaluated on a nine-point scale (1 = poor and 9 = excellent). Typical Cheddar cheese flavor intensity, acidity, and bitterness were also scored on a nine-point scale (1 = low intensity to 9 = high intensity).

Statistical Analysis. Data from the determination of optimum conditions of cheese slurries were analyzed by one-way ANOVA (*22*). The significance of the results was analyzed by the least significant difference (LSD) test. Difference of $p < 0.05$ were considered to be significant.

RESULTS

Curd Observation. The curd of the experimental cheese made from β-CD-treated cream was softer and more brittle than that of the control during cutting and cheddaring, which was similar to results from other reports (*11*, *14*) for Cheddar cheese made from homogenized milk. The coagulum observed in cheese with cream treatment was more brittle and crumbly than that from the control milk, which could be explained by the loss of fines in the whey of treated cream.

Cholesterol Removal Rate and Composition. To find out whether a difference existed in cholesterol removal between treatments, the cholesterol content was measured as shown in **Table 1**. The cholesterol content of the control cheese was 102.3 mg/100 g. The cholesterol reduction of the cream treatment group reached 92.1% when separated cream was treated with 10% β-CD and mixed with skim milk. Our previous study (*3*) showed only 63.9% cholesterol reduction of Mozzarella cheese when homogenized milk was used. The cholesterol removal rate improved for the cheese made from β-CD-treated cream rather than homogenized milk (79.3%, unpublished data).

In the Cheddar cheeses, the moisture content was 43.2%, and the fat content was 35.5%. Homogenization increased cheese moisture, which resulted from slow curd drainage in reduced-fat Cheddar cheese (*11*). A lower fat content of the experimental cheese than the control was expected since more fat would be released in the manufacture of experimental cheese due to the smaller size of fat globules, resulting from homogenization. It may be that the fat globule was too small to incorporate with casein or other protein compounds via a fat−protein network.

Production of Short-Chain Free Fatty Acids. It is well known that short-chain free fatty acids (C4 through C10) are primarily responsible for Cheddar flavor (*23*). Therefore, the production of short-chain FFA profiles was considered to be an important aspect of this study. The production of short-chain FFAs in control and experimental cheese ripened for 7 mo at 5 °C is shown in **Table 2**. The total amount of short-chain FFAs was higher in cheese made from β-CD-treated cream than in

Table 2. Concentrations of Short-Chain Fatty Acids in Reduced-Cholesterol Cheddar Cheese Ripened at 5 °C for 7 mo[a]

		FFA concentration (ppm)				
treatment	ripening period (mo)	C4	C6	C8	C10	total
control	0	17.8a	16.8a	20.1a	19.8a	74.5a
	1	18.7a	17.1a	21.4a	24.7b	81.9b
	3	18.5a	17.5a	21.5a	26.0bc	83.5b
	7	19.1ab	17.5a	22.0a	23.8b	82.4b
cream	0	18.3a	17.3a	21.4a	23.8b	80.8b
treatment[b]	1	19.7ab	16.9a	20.9a	24.3b	81.8b
	3	22.2b	18.2ab	22.3a	25.7bc	88.4c
	7	25.3c	18.2ab	24.3ab	30.3d	98.1d

[a] Means within a column with different letters differ significantly ($p < 0.05$). Means of triplicate analysis. [b] After cream separation, cream was treated with 10% β-CD and then mixed and homogenized at 1000 psi.

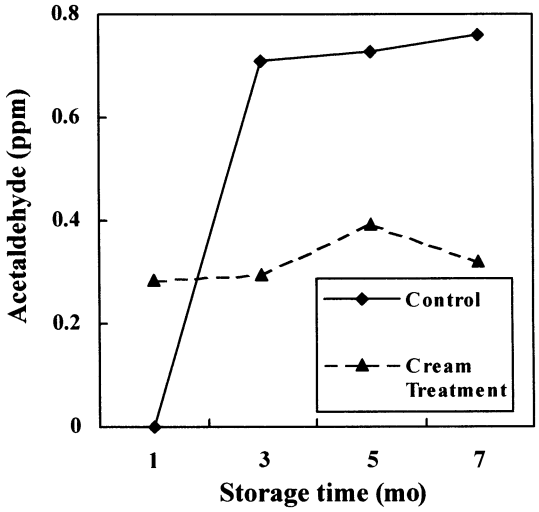

Figure 1. Change of acetaldehyde production in reduced-cholesterol Cheddar cheese ripened at 5 °C for 7 mo.

the control group. During the 7 mo ripening period, the total release of short-chain FFAs from the cream treatment cheese was not much different from those of the control at 0 and 1 mo ripening. However, from 3 mo, the total amount of short-chain FFAs in the cream treatment cheese was significantly different from that in the control.

The release of butyric acid (C4) and capric acid (C10) at 7 mo ripening contributed to the increase in the total amount of FFAs. In the control, the amount of total short-chain FFAs increased from 74.5 to 83.5 ppm during 7 mo, while it increased from 80.8 to 98.1 ppm for the cream treatment group during the same time. The above results indicate that the cheese made from β-CD-treated cream ripened faster than that with no treatment (control).

Production of Neutral Volatile Flavor Compounds. The production of neutral volatile compounds was observed when β-CD treatment of cream influenced the cheese making and ripening, as shown in **Figures 1** and **2**. In the control group, almost no acetaldehyde was found at 0 mo, and it increased steadily to 0.78 ppm at 7 mo (**Figure 1**). In comparison, cheese made from β-CD-treated cream showed a high amount of acetaldehyde production, even at an early stage of ripening (0 mo), 0.23 ppm, and it steadily increased to 0.68 ppm at 7 mo.

Ethanol production was the highest among flavor compounds measured and showed a similar trend in both samples (**Figure 2**). After 0 mo, the ethanol production increased dramatically to 7 mo in the control; however, the highest production of

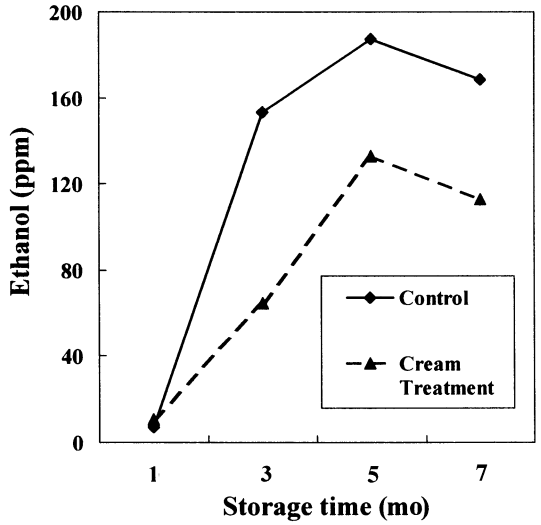

Figure 2. Change of ethanol production in reduced-cholesterol Cheddar cheese ripened at 5 °C for 7 mo.

ethanol at 7 mo was observed in the cheese made from β-CD-treated cream.

Other neutral flavor compounds listed in **Table 3** were dimethyl sulfide, acetone, ethyl acetate, butanone, pentanone, and heptanone. During 3 mo of ripening, the production of these compounds was not significantly different, neither in terms of ripening periods nor within groups. This study indicated that neutral volatile flavor compounds in reduced-cholesterol Cheddar cheese were not different from those in the control Cheddar cheese.

Production of Free Amino Acids. The production of total free amino acids during 7 mo of ripening is shown in **Table 4**. The cheeses made from β-CD-treated cream produced much higher amounts of individual FAAs than the control in all periods. Total amount of FAA was 120.8 μmol/g of cheese in the control and 231.6 μmol/g of cheese in the cream treatment group after the 7 mo ripening period. In terms of concentration of individual amino acids produced, glutamic acid, valine, phenylalanine, leucine, and lysine dominated in all samples. The concentration of lysine was high at the end of ripening in the cheese treament group, and the concentrations of glutamic acid and leucine were high in the control samples.

The production of bitter amino acids during ripening in β-CD-treated and homogenized cheeses is shown in **Figure 3**. The total amount of bitter amino acids was significantly greater in cheese made from β-CD-treated cream than in the control, and the highest concentration was found in cheese made from β-CD-treated cream, 110.3 μmol/g of cheese after 7 mo of ripening. These results suggest that the cheese made from β-CD-treated cream ripened more rapidly than other samples in terms of FAA production.

Rheological Characteristics. The effect of homogenization and β-CD treatment on textural properties of reduced-cholesterol Cheddar cheese is shown in **Table 5**. In the control and cream treatment groups, hardness reached the highest value after 1 mo of ripening and decreased thereafter. In the control, cohesiveness increased to 67.8 after 3 mo of ripening and plateaued thereafter. However, at 0 and 1 mo of ripening, the cohesiveness value was even higher in the cream treatment cheese. A similar trend was found in elasticity. The highest gumminess was found at 1 mo in both the control and cream treatment groups. Notably, very high gumminess was found at 0 mo in the cream treatment group. The present results indicated

Table 3. Production of Neutral Volatile Compounds in Reduced-Cholesterol Cheddar Cheese Ripened at 5 °C for 7 mo[a]

		neutral volatile compounds produced (ppm)					
treatment	ripening period (mo)	dimethyl sulfide	acetone	ethyl acetate	butanone	pentanone	heptanone
control	0	5.30a	7.90a	4.10b		5.97a	2.81a
	1	5.31a	6.80a	3.02a	1.19a	5.67a	2.66a
	3	5.00a	6.87a	3.09a	1.22a	5.70a	2.68a
	7	4.71a	7.79ab	3.02ab	1.17a	5.64a	2.67a
cream	0	4.84a	6.68a	3.01a	1.19a	5.73a	2.59a
treatment[b]	1	5.23a	7.37a	3.64ab	1.49b	5.93a	2.69a
	3	4.73a	6.76a	3.20a	1.35b	5.80a	2.86a
	7	4.90a	7.00a	3.84ab	1.22b	5.23a	2.66a

[a] Means within a column with different letters differ significantly ($p < 0.05$). Means of triplicate analysis. [b] Homogenized at 1000 psi and treated with 1% β-CD. [c] After cream separation, cream was treated with 10% β-CD and then mixed and homogenized at 1000 psi.

Table 4. Production of Free Amino Acids in Reduced-Cholesterol Cheddar Cheese Ripened at 5 °C for 7 mo

	amino acid production after the given ripening period (μmol/g of cheese)							
amino	0 mo		1 mo		3 mo		7 mo	
acid	control	cream[a]	control	cream	control	cream	control	cream
Asp	0.69	1.22	1.49	2.37	3.08	6.45	3.80	7.89
Glu	3.02	6.52	11.01	21.68	21.43	43.00	23.51	42.48
Ser	0.43	0.79	0.59	3.20	1.10	6.98	1.64	6.24
His				0.79	0.53	1.45	1.21	5.67
Gly	0.29	1.86	1.60	2.68	3.14	5.29	3.96	5.63
Thr	1.20	2.09	1.26	2.71	1.93	6.73	2.39	7.18
Arg	0.13	1.38	2.48	2.72	2.74	3.52	2.81	3.57
Ala	1.29	3.73	2.94	5.15	3.72	10.44	5.70	10.95
Tyr	0.48	0.67	1.06	1.52	1.04	1.60	1.08	1.52
Met	0.33	0.74	0.56	1.50	1.27	3.77	2.13	4.96
Val	1.38	4.02	4.65	8.23	11.35	20.52	13.03	21.64
Phe	0.95	4.12	5.58	9.89	11.83	16.15	12.62	19.80
Ile	0.54	1.58	1.22	3.64	4.21	7.52	3.60	8.94
Leu	2.49	7.63	9.41	18.31	23.60	35.63	28.04	41.22
Lys	2.12	4.95	4.49	13.48	10.33	39.98	15.56	43.93

[a] After cream separation, cream was treated with 10% β-CD and then mixed and homogenized at 1000 psi.

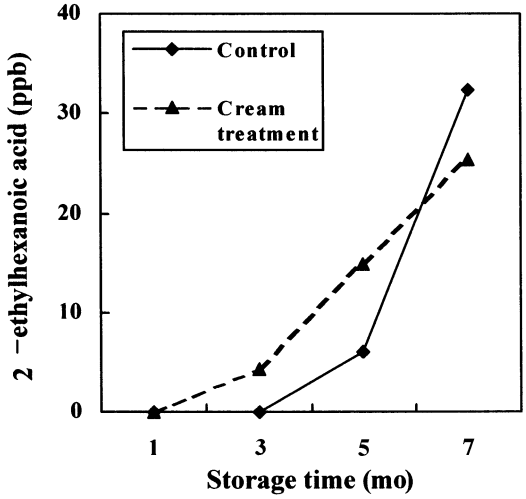

Figure 3. Change of total amount of bitter amino acids in reduced-cholesterol Cheddar cheese ripened at 5 °C for 7 mo. Bitter amino acids were Asp, His, Arg, Tyr, Val, Phe, Ile, and Leu (summaried in **Table 4**).

that the cheese made from β-CD-treated cream showed rapid ripening in terms of textural properties, which were similar to those in the control.

Sensory Evaluation. The sensory attributes of reduced-cholesterol Cheddar cheese are shown in **Table 6**. Interestingly, the texture score of the control Cheddar cheese significantly increased up to 7 mo in the control; however, that in the cream treatment group decreased dramatically from 0 to 7 mo. This result indicated that β-CD treatment may adversely affect cheese texture characteristics. Generally, body and texture improved for Cheddar cheeses during ripening, as shown in our results. Cheddar cheese made from homogenized milk had higher body and texture scores than control cheeses in early stages of ripening. Homogenization of milk and cream increases cheese moisture, which may account for part of the improvement in body and texture, such as smoothness. The improved body and texture of cheese may be due to the increased fat globule surface area produced by homogenization.

In terms of overall flavor, three treatments showed a similar trend, which was a steady increase. Also, flavor intensity showed a result similar to overall flavor, as expected. Previous research indicated that, as moisture content in reduced-fat Cheddar cheese increases, the flavor of the cheese becomes poorer, which was not found in the present study. Peters (*24*) reported that homogenization did not affect flavor in full fat Cheddar cheese. Rao et al. (*25*) also found similar results but reported that mean flavor scores decreased as homogenization pressure increased.

Another interesting phenomenon was the trend of acidity and bitterness scores. During ripening, the values increased in all treatments. The increase was dramatic in the cream treatment group during 7 mo of ripening but not in the control. This result was expected from the data on bitter amino acid production. In addition, it was indicated that the cheese made from β-CD-

Table 5. Textural Properties in Reduced-Cholesterol Cheddar Cheese Ripened at 5 °C for 7 mo[a]

treatment	ripening period (mo)	hardness	elasticity	cohesiveness	gumminess	chewiness
control	0	559938	64.8	47.5	341.5	227.0
	1	1083846	76.2	60.6	1145.3	872.2
	3	575631	76.4	67.8	824.8	646.1
	7	546116	76.9	67.3	451.0	347.1
cream	0	873522	82.7	75.4	876.1	415.1
treatment[b]	1	1074503	85.1	72.3	926.7	788.7
	3	634579	73.2	52.9	714.4	526.9
	7	672730	40.4	53.3	454.7	202.0

[a] Means of triplicate analysis. [b] After cream separation, cream was treated with 10% β-CD and then mixed and homogenized at 1000 psi.

Table 6. Sensory Characteristics in Reduced-Cholesterol Cheddar Cheese Ripened at 5 °C for 7 mo[a]

treatment	ripening period (mo)	texture	overall flavor	flavor intensity	acidity	bitterness
control	0	1.0	1.1a	1.0	1.1	1.0a
	1	2.6a	2.3a	2.1a	1.4a	2.0a
	3	3.3a	3.1a	3.0a	2.1	2.6a
	7	5.6a	4.6ab	4.7ab	2.9a	5.3a
cream	0	5.0a	3.3a	4.0a	3.1	5.4a
treatment[b]	1	1.7a	4.3a	4.1ab	3.3a	5.4a
	3	1.1a	3.9a	4.1ab	4.9a	6.9a
	7	1.6a	5.0a	5.6ab	6.0a	6.3a

[a] Means within a column with different letters differ significantly ($p < 0.05$). Means of triplicate analysis. [b] After cream separation, cream was treated with 10% β-CD and then mixed and homogenized at 1000 psi.

treated cream and reconstituted with skim milk demonstrated effective cholesterol removal and acceleration of cheese ripening.

DISCUSSION

This study was designed to find an optimum condition to remove cholesterol for Cheddar cheese making by using β-CD. In the past two decades, evidence has being gathered to suggest that an excess of cholesterol might be deleterious. Therefore, cholesterol has been removed from milk and dairy products by a β-CD-based process, and the resulting low-cholesterol butter and cheese appear to be indistinguishable from conventional products.

A number of studies have indicated that the removal of cholesterol from milk and cream was effectively conducted by treatment with β-CD (*4*, *5*). In our laboratory, over 90% of the cholesterol was removed from commercial milk at refrigerated temperature with 1% β-CD (*5*). To apply this method to cheese manufacture, milk must be homogenized prior to the cheese-making process because the rate of cholesterol removal from unhomogenized milk by β-CD is low (30%, unpublished). In our previous study we achieved 64% reduction of the cholesterol in Mozzarella cheese with 1000 psi homogenization pressure and 1% β-CD treatment (*3*). The present study was focused on finding a better process to improve the cholesterol removal rate without any adverse effects. As a result, cholesterol reduction of experimental cheese reached 92% when separated cream was treated with 10% β-CD and mixed with skim milk.

As expected, the curds of the experimental cheeses were found to be soft due to the influence of homogenization. The reason could be that a weak coagulum formed by homogenized milk is caused by the greater dispersion of the milk fat globules in the curd (*24*) and the reduced amount of free casein available to form a strong network (*26*), resulting in improper curd matting during cheese making (*27*). However, one thing we found in this study was that the textural characteristics at early-stage ripening (0 or 1 mo) of the cheese made by the cream treatment process showed aspects similar to those of the control cheese ripened for 3 mo or more. This is the first evidence that β-CD treatment of cream has a rapid-ripening effect on Cheddar cheese manufacture.

With regard to free fatty acid production, we may assume that small particles of fat due to homogenization can be easily released from cheese curds in the process of cheese manufacture. In Cheddar cheese made from β-CD-treated cream in the present study, in which cream was first separated from milk and then treated with 10% β-CD, and then skim milk and the treated cream were mixed, fat globule separation was observed to occur easily. A thin and opaque layer was found in the upper portion, which aggregated after a while. This phenomenon was evidenced by the low content of fat in the experimental cheeses. However, the FFA production was higher in the experimental cheeses than in the control, which we may attribute to an increase of lipolysis in experimental cheeses, especially in cheese made from β-CD-treated cream.

It is well accepted that lipase in milk is mostly linked with casein, and it is activated by mechanical processes such as homogenization (*28*). Thus, homogenizing milk that contains lipase strongly enhances lipolysis and so accelerates the development of the characteristic taste. This is explained by the fact that lipase is capable of penetrating the membrane formed by homogenization.

Since cheese flavors, which are constituted by short-chain free fatty acids, may be generally considered as a major aspect, we need to look at whether there is an adverse effect of homogenization or β-CD treatment on the production of short-chain FFAs in the present study. Among three treatments, the total amount of FFA was highest in cheese made from β-CD-treated cream, while the cheese made from homogenized milk and the control cheese were next in decreasing order. Neutral volatile flavor compounds were not significantly different among

treatments ($p > 0.05$). These results indicated that β-CD treatments did not capture or remove the short-chain fatty acids.

Another aspect we found in this study was an increase of bitterness score in experimental cheese after 1 mo of ripening and thereafter. This was probably due to a significant difference of amino acid production in the experimental cheese. Therefore, we may suggest that β-CD treatment in the case of the experimental cheese resulted in enhanced proteolysis, which could be one reason among other unknown factors. The larger increase in total and individual amino acids, including bitter amino acids, observed through the ripening period may reflect the higher peptidase activity in the experimental cheese than in the control. Proteolysis in cheese during ripening results in an increase in peptides, which is directly related to bitterness (*29*, *30*). In conclusion, the present study shows that β-CD treatment of separated cream was an effective process for cholesterol removal in Cheddar cheese making. In addition, the ripening of Cheddar cheese was accelerated.

LITERATURE CITED

(1) Grundy, S. M.; Brheimer, D.; Blackburn, H.; Brown, W. V.; Kwiterovich, P. O.; Mattson, F.; Schonfeld, G.; Weidman W. H. Rationale of the diet−heart statement of the Americal Heart Association Report of the Nutrition Committee. *Circulation* **1982**, *65*, 839A−854A.

(2) Gurr, M. I. Dietary lipids and coronary disease: old evidence, new perspectives and progress. *Lipid Res.* **1992**, *31*, 195−243.

(3) Kwak, H. S.; Nam, C. G.; Ahn, J. Low cholesterol Mozzarella cheese obtained from homogenized and β-cyclodextrin-treated milk. *Asian-Aust. J. Anim. Sci.* **2001**, *14* (2), 268−275.

(4) Ahn, J.; Kwak, H. S. Optimizing cholesterol removal in cream using β-cyclodextrin and response surface methodology. *J. Food Sci.* **1999**, *64*, 629−632.

(5) Lee, D. K.; Ahn J.; Kwak, H. S. Cholesterol removal from homogenized milk with β-cyclodextrin. *J. Dairy Sci.* **1999**, *82*, 2327−2330.

(6) Szejtli, J. Cyclodextrin inclusion complexes. In C*yclodextrin technology*; Szejtli, J., Ed.; Kluwer Akademic Publishers: Dordrecht, The Netherlands, 1988; Chapter 2, pp 79−170.

(7) Makoto, K.; Akio, O.; Reijiro S. (Honnen Ltd.). Cholesterol removal from animal with cyclodextrin by inclusion. Japan Patent 4,168,198, 1992.

(8) Oakenfull, D. G.; Sihdu, G. S. (Commonwealth scientific and industrial research organization). Cholesterol reduction. International Patent 91/11114, 1991.

(9) Nagamoto. S. Cyclodextrin−expanding the development of their functions and applications. *Chem. Econ. Eng. Rev.* **1985**, *17*, 28−34.

(10) Mistry, V. V.; Anderson, D. L. Composition and microstructure of commercial full-fat and low-fat cheeses. *Food Struct.* **1993**, *12*, 259−262.

(11) Metzger, L. E.; Mistry, V. V. A new approach using homogenization of cream in the manufacture of reduced fat Cheddar cheese. 1. Manufacture, composition, and yield. *J. Dairy Sci.* **1994**, *77*, 3506−3515.

(12) Metzger, L. E.; Mistry, V. V. A new approach using homogenization of cream in the manufacture of reduced fat Cheddar cheese. 2. Microstructure, fat globule distribution and free oil. *J. Dairy Sci.* **1995**, *78*, 1883−1895.

(13) Thakar, P. N. Evaluation of selected treatments for accelerating ripening of buffalo milk Cheddar cheese. Ph.D. Thesis, Gujarat Agricultural University, Anand, India, 1985.

(14) Emmons, D. B.; Kalab, M.; Larmond, E.; Lowrie, R. J. Milk gel structure. X. Texture and microstructure in Cheddar cheese made from whole milk and from homogenized low-fat milk. *J. Texture Stud.* **1980**, *11*, 15−34.

(15) Jana, A. H.; Upadhyay, K. G. Homogenization of milk for cheese making A review. *Aust. J. Dairy Technol.* **1993**, *47*, 72−79.

(16) Arbige, M. V.; Freund, P. R.; Silver, S. C.; Zelko, Z. T. Novel lipase for Cheddar cheese flavor development. *Food Technol.* **1986**, *40* (4), 91−96.

(17) Adams, M. L.; Sullivan, D. M.; Smith, R. L.; Richer, E. F. Evaluation of direct saponification method in determination of cholesterol in meats. *J. Assoc. Anal. Chem.* **1986**, *69*, 844−846.

(18) AOAC. *Official methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Arlington, VA, 1990; Method 948.12, pp 840−844.

(19) Kwak, H. S.; Jeon, I. J.; Park, J. Effects of food grade porcine pancreatic lipase on the production of short-chain fatty acids and its contribution. *Korean J. Food Sci. Technol.* **1990**, *22* (3), 248−254.

(20) Basette, R.; Ward G. Measuing part per billion of volatile materials in milk. *J. Dairy Sci.* **1975**, *58*, 428−430.

(21) Izco, J. M.; Irigoyen, A.; Torre, P.; Barcina Y. Effect of the activity levels of the added proteolytic enzyme mixture on free amino acids in ripening Ossau-Iraty cheese. *J. Chromatogr.* **2000**, *881*, 69−79.

(22) *SAS Users Guide, Statistics*, Version 5; SAS Institute, Inc.: Cary, NC, 1985.

(23) Lin, J. C. C.; Jeon, I. J. Effects of commercial food grade enzymes on fatty acid profiles in granular cheese. *J. Food Sci.* **1987**, *52*, 78−82.

(24) Peters, I. I. Cheddar cheese made from pasteurized milk homogenized at various pressures. *J. Dairy Sci.* **1956**, *39*, 1083−1088.

(25) Rao, A.; Spurgeon, K. P.; Parsons, J. G.; Torrey, G. S.; Baer, R. J.; Seas S. W. Effects of homogenization of milk on yield and quality of Cheddar cheese. *J. Dairy Sci.* **1985**, *68* (Suppl. 1), 58.

(26) Lemay, A.; Paquin, P.; Lacroix, C. Influence of microfluidization of milk on Cheddar cheese composition, color, texture and yield. *J. Dairy Sci.* **1994**, *77*, 2870−2879.

(27) Green, M. L.; Marshall R. J.; Glover, F. J. Influence of homogenization of concentrated milks on the structure and properties of rennet curds. *J. Dairy Res.* **1983**, *50*, 341−348.

(28) Walstra, P.; Geurts, T. J.; Noomen, A.; Jellema, A.; van Boekel, M. A. J. S. Homogenation. In *Dairy Technology*; Walstra, P., et al., Eds.; Marcel Dekker Inc.: New York, 1999; Chapter 8, pp 260−265.

(29) Fernandez-Espla, M. D.; Fox, P. F. Effect of adding propiobacterium shermanii NCDO 853 or *Lactobacillus casei* ssp. *casei* IFPL 731 on proteolysis and flavor development of Cheddar cheese. *J. Agric. Food Chem.* **1998**, *46*, 1228−1234.

(30) Smit, G.; Verheul, A.; van Kranenburg, R.; Ayad, E.; Siezen, R.; Engels, W. Cheese flavour development by enzymatic conversions of peptides and amino acids. *Food Res. Int.* **2000**, *33*, 153−160.

Received for review May 15, 2002. Revised manuscript received September 13, 2002. Accepted September 13, 2002. This research was supported by the Brain Korea 21 Project, Seoul, Korea.

JF020568M