

Cholesterol Removal and Flavor Development in Cheddar Cheese

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ABSTRACT : This study was carried out to find a cholesterol removal rate, flavor development and bitter amino acid productions in Cheddar cheese treated with β -cyclodextrin (CD): 1) Control (no homogenization, no β -CD), and 2) Milk treatment (1000 psi milk homogenization, 1% β -CD). The cholesterol removal of the cheese was 79.3%. The production of short-chain free fatty acids (FFA) increased with a ripening time in both control and milk treated cheese. The releasing quantity of short-chain FFA was higher in milk treated cheese than control at 5 and 7 mo ripening. Not much difference was found in neutral volatile compound production between samples. In bitter-tasted amino acids, milk treatment group produced much higher than control. In sensory analysis, texture score of control Cheddar cheese significantly increased with ripening time, however, that in cholesterol-reduced cheese decreased dramatically. Our results indicated that the cheese made by β -CD treated milk with low pressure homogenization showed an effective cholesterol reduction and a rapid cheese ripening, while no capture of flavor compounds by β -CD. (*Asian-Aust. J. Anim. Sci.* 2003. Vol 16, No. 3 : 409-416)

Key Words : 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 the excessive intake of cholesterol (Grundy et al., 1982; Gurr, 1992). Therefore, physical, chemical and biological methods to reduce cholesterol have been studied in foods including dairy products (Kwak et al., 2001; Ahn and Kwak, 1999; Lee et al., 1999; Szejtli, 1988).

A number of studies has been indicated that the cholesterol removal in milk, cream and Mozzarella cheese was effectively conducted by β -cyclodextrin (β -CD) (Kwak et al., 2001; Ahn and Kwak, 1999; Lee et al., 1999; Makoto et al., 1992; Oakenfull and Sihdu, 1991). Because β -CD is nontoxic, edible, non-hygroscopic, chemically stable and easy to separate (Nagamoto, 1985), it has positive attributes when used for the removal of cholesterol from foods. Milk must be homogenized prior to the cheese making process because of a low rate of cholesterol removal (30%) of unhomogenized milk (unpublished).

Adverse effects of homogenization on the process of cheese making, and physical and chemical properties of cheese needed to be considered (Mistry and Anderson, 1993; Metzger and Mistry, 1994; Metzger and Mistry, 1995), which gives cheese a creamy body. Several studies have indicated that homogenization in the manufacture of

cheese, leads to a slower drainage (Thakar, 1985), a reduced tension, a decreased elasticity of the curd (Emmons et al., 1980), and an increased rates of acid development (Jana and Upadhyay, 1993). However, there is a discrepancy in sensory aspects of Cheddar cheese (Thakar, 1985; Path et al., 1989), probably due to the difference in homogenizing conditions such as pressure, temperature and stage. Metzger and Mistry (1995) developed a new process for improving the body and texture of fat-reduced Cheddar cheese, which was that 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 in effects of β -CD treatment and homogenization for cholesterol removal, chemical, rheological and sensory characteristics (Arbige et al., 1986; Kwak et al., 1990). Therefore, our objectives of this study were to examine whether cholesterol was reduced by β -CD treatment of homogenized milk or not, and to find out the change of flavor development and other chemical, textural and sensory attributes in cholesterol-reduced Cheddar cheese.

MATERIALS AND METHODS

Materials

Raw milk was obtained from Binggare Dairy Plant (Kyonggi-do, Korea) and adjusted to 3.5% milk fat by 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, USA) and all solvents were gas chromatographic grade.

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Milk treatment

Bulk raw milk was homogenized at 1,000 psi (HC 5,000, Microfluidics Corp. Newton, MA, USA) and treated with 1.0% (w/v) β -cyclodextrin. The mixture was stirred with a blender (Tops: Misung Co., Seoul, Korea) in a temperature-controlled water bath at 4°C for 10 min. Each sample was centrifuged (HMR-220IV; Hanil Industrial Co., Seoul, Korea) with 166×g for 10 min (Lee et al., 1999). All treatments were run in triplicate. The whole milk was not treated with β -CD and not microfluidized and was used as the control. The cheese milk was pasteurized at 72°C for 17s prior to cheese making.

Manufacture of Cheddar cheese

The cholesterol-reduced milk (15 Kg) was warmed up to 36°C. A frozen concentrated direct vat set mesophilic lactic acid starter culture designed for Cheddar cheese (R-703, Chr. Hansen's Lab., Denmark) was added into the milk. Cheese making process was same as described by Metzger and Mistry (1994). 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 refrigerator for 12 h was 0 mo sample. The cheese making experiment was triplicate on different days using different batches of treatments. Each batch of cheese making was triplicate.

Extraction and determination of cholesterol

Cholesterol was extracted from β -CD-treated cheese by method described by Adams et al. (1986) and stored at -20°C until analysis. Total cholesterol was determined on a silica fused capillary column (HP-5, 30 m×0.32 mm I.D.× 0.25 μ m thickness) using Hewlett-Packard 5,890A gas chromatography (Palo Alto, CA, USA) equipped with a flame ionization detector. The temperatures of 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 with a split ratio of 1/50. Quantitation of cholesterol was done by comparing the peak areas with a response of an internal standard.

The percentage of cholesterol reduction was calculated as followed: cholesterol reduction (%) = $100 - (\text{amount of cholesterol in } \beta\text{-CD-treated cheese} \times 100 / \text{amount of cholesterol in untreated cheese})$. Cholesterol determination for control was averaged with each batch of treatment.

Analysis of chemical composition and yield of cheese

Cheeses were analyzed for moisture, fat, protein and salt using the methods of Association of Official Analytical Chemists (AOAC, 1990). Cheese yield was determined as $\text{wt. cheese} \times 100 / \text{wt. milk}$.

Analysis of short-chain free fatty acid

Cheese samples (1g) were removed periodically from the cheeses ripened for 0, 1, 3, and 7 mo and extracted with diethylether and hexane for 2 h and eluted through a 10 mm i.d. glass column containing neutral alumina as described by Kwak et al. (1990). A Hewlett-Packard Model 5,880A GC equipped with a flame ionization detector was used. The preparation of FFA was achieved using a 15 m×0.53 mm i.d. Nukol fused-silica capillary column (Supleco Inc., Bellefonte, PA, USA). The GC was operated with helium carrier gas at 37 ml/min, hydrogen gas 37 ml/min, and air at 300 ml/min. The column oven was programmed as an initial holding for 1 min at 110°C and first level holding to 180°C at 5°C/min for 10 min and holding for 20 min. Both temperatures for injector and detector were 250°C. All quantitative analyses were done by relating each peak area of individual FFA to the peak area of tridecanoic acid as an internal standard. Each FFA was identified by the retention time of standard.

Analysis of neutral volatile compounds

Samples of cheeses (40 g) were removed periodically from the cheeses ripened for 0, 1, 3, and 7 mo and added with 10 ml distilled water. Two mL of distillate was used for headspace gas sample as described by Bassette and Ward (1975).

A Hewlett-Packard Model 5,880A GC equipped with a flame ionization detector was used. Headspace gas samples were analyzed on a capillary column (SupelcowaxTM10, 30m×0.32 mm I.D. Bellefonte, PA, USA). The column was operated with nitrogen carrier gas at a flow rate of 1.2 ml/min; hydrogen gas flow rate was 30.0 ml/min; air was 300.0 ml/min. Temperature for both injector port and detector was maintained at 230°C. The column oven was programmed at three temperature levels: initial holding for 5 min at 35°C/min and heating to 140°C at 15°C/min, holding for 30 min. The concentrations of volatile compounds were estimated by analyzing cheese samples that contained the known concentrations and those of containing no added standards. The difference between the two treatments was used for the estimation of 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. (2000). Samples were analyzed on a Waters HPLC system consisting of 600 pump, 486 tunable absorbance detector 254 nm, operated using Millennium software. The column used was a Waters PicoTag C₁₈ reversed-phase column maintained at 46 °C. For identification of amino acids (Sigma, St. Louis, MO, USA) was added an internal standard. A gradient with two solvents was used to run the sample: solution A comprised 70 mM sodium acetate adjusted to pH 6.55 with acetic acid and

added containing 2.5% acetonitrile and solution B was 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 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 at which the highest force during the first compression was hardness. The extent to which the sample returned to its original between the first and second compression was elasticity. The ratio of the area under the second compression was cohesiveness. Gumminess and chewiness were calculated by hardness × cohesiveness, and gumminess × elasticity, respectively.

Sensory analysis

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

Statistical analysis

Data from the determination of optimum conditions of cheese slurries, one-way ANOVA (SAS Institute Inc., Cary, NC, USA, 1985) was used. 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

Cholesterol removal rate and composition

This study was designed to find an optimum condition to remove cholesterol for Cheddar cheese making by using β -CD and to measure flavor, texture and sensory attribute of the Cheddar cheese during ripening. In past two decades, evidence has been 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

To find out whether the difference existed in cholesterol removal among treatments existed, cholesterol content was measured as shown in Table 1. The cholesterol content of control cheese was 102.3 mg/100g. The cholesterol reduction of experimental cheese reached 79.3% when cheese milk was homogenized at 1000psi, and treated with 1% β -CD. Our previous study (Kwak et al., 2001) showed only 63.9% of cholesterol reduction of Mozzarella cheese when homogenized milk was used.

In the composition of the Cheddar cheeses, moisture content of cheese was 41.7% and 34.0% fat. Homogenization of milk increased cheese moisture, which resulted from a slow curd drainage in reduced-fat Cheddar cheese (Metzger and Mistry, 1994). Lower fat content of experimental cheese than the control was expected since more amount of fat would be released in manufacture of experimental cheeses due to smaller size of fat globule resulted by homogenization. It may be explained by that fat globule size was too small to incorporate with casein or other protein compounds via fat-protein network.

Curd observation

The curd of the experimental cheese made by homogenized milk was softer and more brittle than that of control during cutting and cheddaring, which was similar to results from other reports (Metzger and Mistry, 1994; Emmons et al., 1980) for Cheddar cheese made from homogenized milk and β -CD treated cream. The coagulum observed in cheese with homogenized milk was more brittle and crumbly than that from the control milk, which could explain the loss of fines in the whey.

As expected, the soft curds of the experimental cheeses were found due to the influence of homogenization. The reason could be explained that a weak coagulum formed by homogenized milk is caused by the greater dispersion of the milk fat globules in the curd (Peters, 1956) and the reduced number of free casein available to form a strong network (Lemay et al., 1994), resulting in improper curd matting during cheese making (Green et al., 1983).

Table 1. Mean chemical composition of cholesterol-reduced Cheddar cheese¹

Component	Control	Milk treatment ²
Moisture, %	41.3 ^a	41.7 ^a
Fat, %	38.0 ^b	34.0 ^a
Protein, %	28.2 ^a	29.0 ^a
Cholesterol removal, %	0.0 ^a	79.3 ^b
Yield, %	10.5	12.5

¹ Means within a row with different superscript letter differ ($p < 0.05$).

² Means of triplicate.

low-cholesterol butter and cheese appeared to be indistinguishable from conventional products.

Table 2. Concentrations of short-chain fatty acids in cholesterol-reduced Cheddar cheese ripened at 5°C for 7 mo¹

Treatment	Ripening Period (mo)	FFA concentration (ppm) ²				
		C ₄	C ₆	C ₈	C ₁₀	Total
Control	0	17.8 ^a	16.8 ^a	20.1 ^a	19.8 ^a	74.5 ^a
	1	18.7 ^a	17.1 ^a	21.7 ^a	24.7 ^b	81.9 ^b
	3	18.5 ^a	17.5 ^a	21.5 ^a	26.0 ^{bc}	83.5 ^b
	7	19.1 ^{ab}	17.5 ^a	22.0 ^a	23.8 ^b	82.4 ^b
Milk ³ treatment	0	18.0 ^a	16.9 ^a	20.6 ^a	21.0 ^a	76.5 ^a
	1	19.2 ^{ab}	17.0 ^a	21.3 ^a	24.6 ^b	82.1 ^b
	3	21.8 ^{ab}	17.3 ^a	22.5 ^a	27.7 ^c	89.3 ^c
	7	22.8 ^b	17.6 ^a	23.2 ^{ab}	27.1 ^c	90.7 ^c

¹ Means within a column with different superscript letter differ ($p < 0.05$). Means of triplicate.

² C₄: butyric acid, C₆: caproic acid, C₈: caprylic acid, C₁₀: capric acid.

³ Homogenized at 1,000 psi and treated with 1% β -CD.

Production of short-chain free fatty acids (FFA)

It is well known that short-chain free fatty acids (FFA, C₄ through C₁₀) constitute the backbone of Cheddar flavor (Lin and Jeon, 1987). Therefore, the production of short-chain FFA profiles was considered to be an important aspect in this study. The productions of short-chain FFA in control and experimental cheese ripened for 7 mo at 5°C are shown in Table 2. Between treatment and control, total amount of short-chain FFA in cheese made by homogenized milk was higher than control. During 7 mo ripening period, the total release of short-chain FFA production was not much different at 0 and 1 mo ripening in both groups. However, from 3 mo ripening, total amount of short-chain FFA of experimental cheese was significantly different from the control. The release of butyric acid (C₄) and capric acid (C₁₀) at 7 mo ripening contributed to the increase of total amount of FFA. In control, the amount of total short-chain FFA was increased

from 74.5 to 82.4 ppm during 7 mo, while 76.5 to 90.7 ppm for milk treatment cheese. Above results indicated that the cheese made from milk treatment was ripened faster than control.

In the case of free fatty acid production, we may assume that small particles of fat by homogenization can be easily released from cheese curds in the process of cheese manufacture. Fat globule separation was easily observed in the process of Cheddar cheese making, therefore, thin and opaque layer was found in upper portion and it was aggregated after a while. This phenomenon was proven by low content of fat in the experimental cheese. However, the production was higher in the experimental cheese than in control, which we may suggest an increase of lipolysis in experimental cheese. It is well accepted that lipase in milk is mostly linked with casein, and it is activated by mechanical process such as homogenization (Walstra et al., 1999). Thus, homogenized milk that contains lipase strongly enhances lipolysis and so accelerates the development of the characteristic taste. This should be explained by lipase being capable of penetrating the membrane formed by homogenization.

Since cheese flavors, constituted by short-chain free fatty acids (FFA) may be generally considered as a major aspect, we need to look at whether an adverse effect of homogenization or β -CD treatment on the production of short-chain FFA profiles in the present study. In this study, total amount of FFA was higher in cheese made by homogenized milk than in control cheese. These results indicated that β -CD treatment did not capture or remove the short-chain fatty acids.

Production of neutral volatile flavor compounds

The production of neutral volatile compounds was observed if β -CD treatment and/or homogenization of milk influence on the cheese making and ripening as shown in Figs. 1 and 2. In control group, almost no acetaldehyde was found at 0 mo and increased steadily up to 0.78 ppm at 7 mo (Figure. 1). In comparison, cheese from homogenized milk

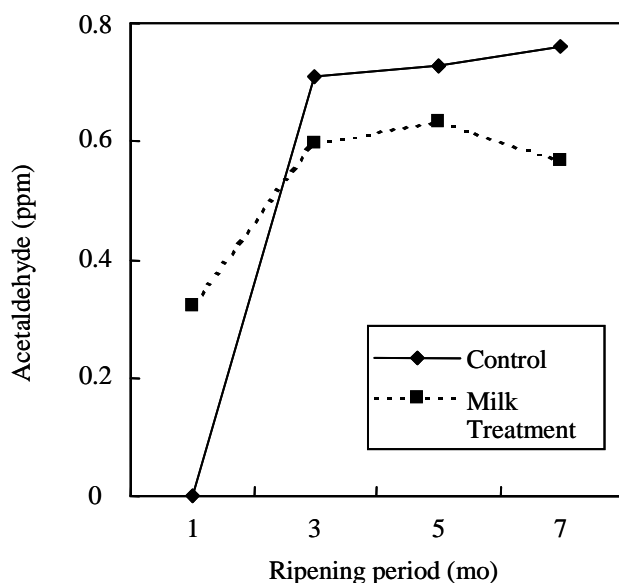


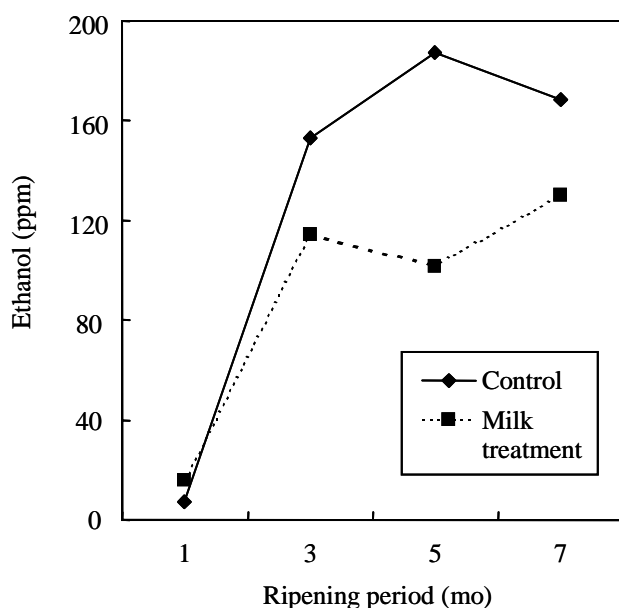
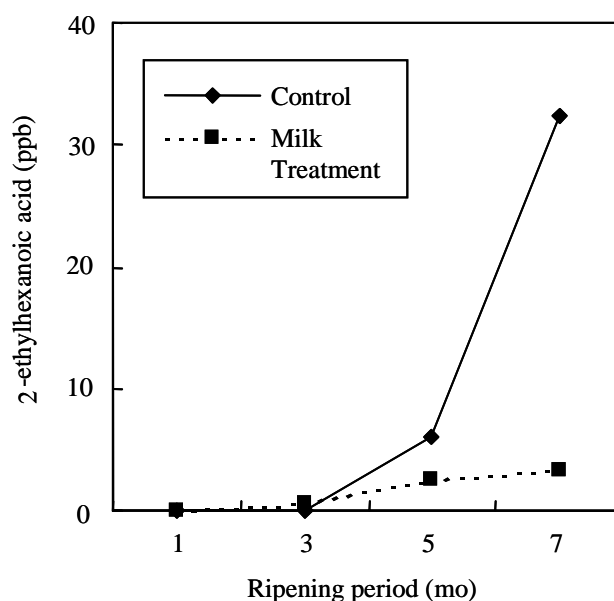
Figure 1. Change of acetaldehyde production in cholesterol-reduced Cheddar cheese ripened at 5°C for 7 mo.

Table 3. The production of neutral volatile compounds in cholesterol-reduced Cheddar cheese ripened at 5°C for 7 mo¹

Treatment	Ripening period (mo)	Dimethyl-sulfide	Acetone	Ethyl-acetate (ppm)	Butanone	Pentanone	Heptanone
Control	0	5.30 ^a	7.90 ^a	4.10 ^b	-	5.97 ^a	2.81 ^a
	1	5.31 ^a	6.80 ^a	3.02 ^a	1.19 ^a	5.67 ^a	2.66 ^a
	3	5.00 ^a	6.87 ^a	3.09 ^a	1.22 ^a	5.70 ^a	2.68 ^a
	7	4.71 ^a	7.79 ^{ab}	3.02 ^{ab}	1.17 ^a	5.64 ^a	2.67 ^a
Milk ² treatment	0	5.43 ^a	6.70 ^{ab}	3.31 ^a	1.21 ^a	5.69 ^a	2.69
	1	5.28 ^a	7.12 ^a	3.44 ^a	1.22 ^a	6.30 ^b	2.87 ^a
	3	5.65 ^a	7.10 ^a	4.23 ^b	1.25 ^a	5.68 ^a	3.62 ^a
	7	4.72 ^a	6.94 ^a	3.11 ^a	1.18 ^a	5.64 ^a	2.66 ^a

¹ Means within a column with different superscript letter differ ($p < 0.05$). Means of triplicate.

² Homogenized at 1,000 psi and treated with 1% β -CD.

**Figure 2.** Change of ethanol production in cholesterol-reduced Cheddar cheese ripened at 5°C for 7 mo**Figure 3.** Change of total amount of bitter amino acids¹ in cholesterol-reduced Cheddar cheese ripened at 5°C for 7 mo. Bitter amino acids were Asp, His, Arg, Tyr, Val, Phe, Ile and Leu summarized from Table 4.

showed a high amount of acetaldehyde production even at an early stage of ripening (0 mo) as 0.23 ppm and steadily increased upto 0.59 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 upto 7 mo in both cheeses (168 ppm in control and 133 ppm in milk treatment).

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

Production of free amino acids

The production of total free amino acids (FAA) during 7 mo ripening is shown in Table 4. The cheese made by homogenized milk produced much higher amounts of individual FAA than the control in all periods. Total amounts of FAA were 120.8 in control, and 212.3 $\mu\text{mol/g}$ cheese in experimental cheese at 7 mo ripening period. In concentration of individual amino acids produced, glutamic acid, valine, phenylalanine, leucine and lysine were dominated in both samples. The concentration of lysine was high in milk treated group and those of glutamic acid and leucine were high in control sample.

The production of bitter amino acids during ripening of the cheese made by β -CD treated and homogenized milk was shown in Figure. 3. Total amount of bitter amino acids were significantly greater in cheese made by β -CD treated and homogenized milk than in control. These results suggested

that the cheese made by treated milk showed a more rapid ripening than control in FAA production.

Rheological characteristics

The effect of homogenization and β -CD treatment on textural properties on cholesterol-reduced Cheddar cheese was shown in Table 5. The cheese made by homogenized milk and treated with 1% β -CD exhibited a significantly lower hardness, compared with those in control.

In control, cohesiveness increased to 67.8 at 3 mo ripening and plateaued thereafter. However, at 0 and 1 mo ripening, the cohesiveness value was almost same as control at 3 and 7 mo ripening in homogenized milk group. Similar trend was found in elasticity. The highest gumminess was found at 1 mo in control. The present results indicated that the cheese made by homogenized milk showed a rapid ripening process in textural properties.

Sensory evaluation

The sensory attributes of cholesterol-reduced Cheddar cheese were shown in Table 6. Interestingly, texture score of control Cheddar cheese significantly increased up to 7 mo in control, however, that in homogenized milk group decreased dramatically from 0 to 7 mo. This result indicated that homogenization may affect adversely

cheese texture mo in control, however, that in homogenized milk group decreased dramatically from 0 to 7 mo. This result indicated that homogenization may affect adversely cheese texture characteristics. Generally, body and texture improved for Cheddar cheeses during ripening as shown in our result. Cheddar cheese from homogenized milk had higher body and texture scores than control cheese in early stage of ripening. Since homogenization of milk increased 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 overall flavor, three treatments showed a similar trend, which was a steadily increase. Also, flavor intensity showed a similar result 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 (1956) reported that homogenization did not affect flavor in full fat Cheddar cheese. Rao et al. (1985) also found similar results but reported that mean flavor scores decreased as homogenization pressure increased.

Another interesting phenomenon was acid and bitterness scores. During ripening, the values increased in homogenized

Table 4. Production of free amino acids in cholesterol-reduced Cheddar cheese ripened at 5°C for 7 mo

Amno acids	Ripening period							
	0 mo		1 mo		3 mo		7 mo	
	Control ¹	Milk ²	Control	Milk ²	Control	Milk ²	Control	Milk ²
	$\mu\text{mol} / \text{g cheese}$							
Asp	0.69	0.96	1.49	2.13	3.08	4.10	3.80	6.58
Glu	3.02	5.89	11.01	13.0	21.43	34.24	23.51	40.20
Ser	0.43	0.53	0.59	0.95	1.10	4.35	1.64	5.49
His	-	-	-	0.45	0.53	1.20	1.21	3.18
Gly	0.29	0.91	1.60	2.33	3.14	4.77	3.96	6.59
Thr	1.20	1.33	1.26	1.42	1.93	4.21	2.39	6.19
Arg	0.13	1.27	2.48	2.63	2.74	2.67	2.81	3.11
Ala	1.29	2.90	2.94	3.96	3.72	7.57	5.70	10.93
Tyr	0.48	0.61	1.06	1.31	1.04	1.38	1.08	1.53
Met	0.33	0.57	0.56	1.04	1.27	3.15	2.13	4.34
Val	1.38	2.90	4.65	6.63	11.35	15.34	13.03	19.01
Phe	0.95	3.42	5.58	7.89	11.83	14.33	12.62	18.72
Ile	0.54	0.82	1.22	1.74	4.21	6.36	3.60	8.53
Leu	2.49	5.63	9.41	14.98	23.60	27.38	28.04	36.90
Lys	2.12	3.71	4.49	5.91	10.33	37.95	15.56	40.95

¹ Control, ² Homogenized at 1,000 psi and treated with 1% β -CD.

Table 5. The textural properties in cholesterol-reduced Cheddar cheese ripened at 5°C for 7 mo¹

Treatment	Ripening period (mo)	Hardness	Elasticity	Cohesiveness	Gumminess	Chewiness
Control	0	559,938	64.8	47.5	341.5	227.0
	1	1,083,846	76.2	60.6	1,145.3	872.2
	3	575,631	76.4	67.8	824.8	646.1
	7	546,116	76.9	67.3	451.0	347.1
Milk ² treatment	0	552,151	77.7	67.9	538.1	238.9
	1	627,590	79.1	69.2	615.9	487.4
	3	895,676	70.9	56.3	717.3	508.9
	7	7,886,133	45.5	34.5	397.5	188.2

¹ Means within a column with different superscript letter differ (p<0.05). Means of triplicate.² Homogenized at 1,000 psi and treated with 1% β -CD.**Table 6.** The sensory characteristics in cholesterol-reduced Cheddar cheese ripened at 5°C for 7 mo¹

Treatment	Ripening period (mo)	Texture	Overall flavor	Flavor intensity	Acid	Bitterness
control	0	1.0 ^a	1.1 ^a	1.0 ^a	1.1 ^a	1.0 ^a
	1	2.6 ^{ab}	2.3 ^a	2.1 ^a	1.4 ^a	2.0 ^a
	3	3.3 ^a	3.1 ^a	3.0 ^a	3.1 ^{ab}	2.6 ^a
	7	5.6 ^b	4.6 ^{ab}	4.7 ^{ab}	2.9 ^a	5.3 ^b
Milk ² treatment	0	7.7 ^b	2.4 ^a	3.0 ^a	2.3 ^a	4.9 ^b
	1	2.0 ^a	3.4 ^a	3.4 ^a	2.5 ^a	4.7 ^b
	3	1.3 ^a	3.7 ^a	4.1 ^{ab}	4.3 ^b	5.0 ^b
	7	1.6 ^a	5.0 ^a	5.9 ^b	6.0 ^c	6.7 ^c

¹ Means within a column with different superscript letter differ (p<0.05). Means of triplicate.² Homogenized at 1,000 psi and treated with 1% β -CD.

milk group during 7 mo ripening but not in control.

This result was expected from data in bitter amino acid production. In addition, it was indicated that the cheese made

from homogenized milk was effective on cholesterol removal and acceleration of cheese ripening.

Another aspect we found in this study was an increase of bitterness score in experimental cheese from 1 mo ripening and thereafter. This was probably due to a significant difference of amino acid production in the experimental cheeses. Therefore, we may suggest that homogenization or β -CD treatment to the experimental cheese resulted in an 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 a ripening period may reflect the higher peptidase activity in the experimental cheeses than in control. Proteolysis in cheese during ripening results in an increase in peptides, which is directly involved in bitterness (Fernandez-Espla et al., 1998; Smit et al., 2000). In conclusion, the present study suggested that the treatment of β -CD was an effective process for cholesterol removal in Cheddar cheese making and for acceleration of the cheese ripening.

CONCLUSION

In the present study, the Cheddar cheese made by milk with homogenization and β -CD treatment showed 79.3% cholesterol removal rate. Compared with control, cholesterol-reduced Cheddar cheese had an impaired texture. However, the productions of the flavor, short-chain fatty acids, neutral volatile compounds and bitter amino acids were not significantly influenced during ripening. Therefore, the present study suggested that the treatment of β -CD was an effective process for cholesterol removal in Cheddar cheese making, and did not remove flavor compounds, and was effect on rapid ripening of cheese.

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