Flavor Compounds of Cholesterol-Reduced Cheddar Cheese Slurries

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ABSTRACT : This study was carried out to find the difference in flavor compounds between cholesterol-reduced Cheddar cheese slurries and control sample. The cheeses were made by 3 different treatments as followings: 1) Control (no homogenization, no β -CD), 2) Trt A (1,000 psi milk homogenization, 1% β -CD) and 3) Trt B (cream separation following by 10% β -CD, mixed with skim milk at 1,000 psi homogenization). The cholesterol removals of the cheeses were 79.30% (Trt A) and 91.22% (Trt B). The cheese slurries made by the cheeses were aged at 32°C for 3 wk. The production of short-chain fatty acids (SCFA) was significantly increased with storage time in all treatments. Total amount of SCFA was dramatically increased at 2 wk and maintained thereafter in control group. The amounts of acetone and acetaldehyde were slightly increased in control at 3 wk, however, no difference was found in others. Ethanol production was dramatically increased at 1 wk and decreased thereafter in all treatments. Based on our results, cheese slurries for Trt B showed a highest cholesterol removal rate. Although little difference was found in flavor production, lower amount of SCFA was found in Trts A and B in 2 and 3 wk. It may indicate that a certain amount of SCFA is decreased during β -CD treatment. (*Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 1 : 117-123*)

Key Words : Cholesterol Removal, β -Cyclodextrin, Cheddar Cheese Slurries, Homogenization

INTRODUCTION

Since it is well known that 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 food containing dairy products (Kwak et al., 2001; Ahn and Kwak, 1999; Lee et al., 1999).

A number of studies has been indicated that the removal of cholesterol 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). Beta-CD is a cyclic oligosaccaride composed of seven glucose units linked by -(1-4) bonds. It has a cavity at the center of the molecule which allows complex formation with cholesterol (Szejtli, 1982; Vollbrecht, 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. To apply this method to Cheddar cheese, milk must be homogenized prior to the cheese making process because of a low rate of cholesterol removal (30%) of unhomogenized milk by β-CD (unpublished).

It is well known that adverse effects of homogenization on the process of cheese making, and physical and chemical properties of cheese are various. Several studies have indicated that homogenization in the manufacture of dairy products including cheeses, leads to a slower drainage (Thakar, 1985), a reduced tension, a decreased elasticity of the curd (Emmons et al., 1980), and an increased rate of acid development (Jana and Upadhyay, 1993). However, there is a discrepancy in sensory aspects of Cheddar cheese (Thakar, 1985 and Path et al., 1989), probably due to the difference in homogenizing conditions such as pressure, temperature and stage.

Generally, the production of flavor and fatty acids are important for the acceleration of Cheddar flavor development (Arbige et al., 1986; Kwak et al., 1990). Of the various milk constituents, short-chain fatty acids were major components for Cheddar flavor development (Patton, 1963). However, little information is available in effects of β -CD treatment and homogenization on Cheddar cheese manufacture and flavor development. Therefore, our objective of this study was to examine the flavor and shortchain fatty acid productions in Cheddar cheese slurries made by 3 different treatments of cheese milk as a preliminary work for 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

Milks for Cheddar cheese manufacture were prepared from 3 different treatments as followed: 1) Control (no homogenization, no β -CD treatment), 2) Treatment A (Trt A: homogenized at 1,000 psi and treated with 1% β -CD), and 3) Treatment B (Trt B: after cream separation, cream was treated with 10% β -CD, and mixed with skim milk at 1,000 psi).

In detail, milk for Trt A was placed in a 2 L container, and 1.0% (w/v) β -cyclodextrin was added. The mixture was stirred at 800 rpm with a blender (Tops: Misung Co., Seoul, Korea) in a temperature-controlled water bath at 4°C for 10 min. The mixture was centrifuged (HMR-220 IV; Hanil Industiral Co., Seoul, Korea) with 166 × g for 10 min (Lee et al., 1999). For Trt B, raw milk was separated from cream to skim milk. The cream containing 35% milk fat was treated with 10% β -CD (Ahn and Kwak, 1999). Then homogenized in a single stage homogenizer (HC 5000, Microfluidics Corp., Newton, MA, USA) with 1,000 psi homogenization pressure at 70°C (Kwak et al., 2001), and mixed with remaining skim milk with 1,000 psi using homogenizer. Each sample was centrifuged with 166 × g for removal of β -CD. All treatments were run in triplicate.

Manufacture of Cheddar cheese

The cheese milk was standarized for 3.5% milk fat. pasturized at 72°C for 17 s, and homogenized with different treatments as mentioned. The milk was then cooled to 10°C, mixed with β-CD, stirred and centrifuged as mentioned above. The cholesterol-reduced milk (15 kg) was warmed up to 36°C. A frozen concentrated direct vat set mesophilic lactic starter culture designed for Cheddar cheese (R-703, Chr. Hansen's Lab., Denmark) was added to the milk with the percentage of 0.004, followed by CaCl₂ (10% Ca) with 0.03%. After 40 min of ripening, rennet (Standard Plus 900, Chr. Hansen's Lab., Denmark) was added with 0.019%. Curd was formed in 40 to 50 min and cut with 1.2 cm wire knife and allowed to heal for 15 min. It was cooked at 34°C for 40 min and developed whey acidity with 0.18 and 0.19%. Whey was then drained at 0.22-0.24% whey acidity. Curds were piled in the both sides of the vat. Curds were cheddared to 0.5-0.6%. Whey was then drained and cheddaring time was ranged from 1.5 to 2.0 h. The cheddared curds were milled and salted at the rate of 2.0%. The cheddared curds were hooped in 0.3 kg blocks and pressed overnight. Pressed cheeses were weighed, and vacuum packaged in a barrier bag at 4°C for 1 wk. The cheese making experiment was triplicated on different days using different batches of treatments. Each batch of cheese making was triplicated.

Preparation of cheese slurries

Cheese slurries were prepared with the Cheddar cheese according to the modified method of Kwak et al. (1989). The cheese slurries were incubated at 32°C for 0, 1, 2, and 3 wk for the acceleration of aged Cheddar flavor development. The cheese slurry sample storaged in refrigerator for 12 h was 0 wk sample.

Analysis of chemical composition

Cheese curds used for slurries were analyzed for moisture, fat, protein and salt using the methods of Association of Official Analytical Chemists (AOAC, 1990).

Analysis of free fatty acids

Cheese slurries were removed periodically at 0, 1, 2, and 3 wk and extracted with diethylether and hexane for 2 hr and eluted through a 10 mm i.d. glass column containing neutral alumina as described by Kwak et al. (1990). The column containing alumina with adsorbed free fatty acids (FFA) was dried under vacuum and transferred to a stoppered glass tube. One ml isopropyl ether containing 6% formic acid was added and mixed with the alumina. The tube was centrifuged at 2,000 rpm for 5 min at room temperature and 1ul aliquot of the supernatant was injected into gas chromatography (GC). A Hewlett-Packard Model 5880A GC equipped with a flame ionization detector was used for all analyses. The preparation of FFA was achieved using a 15 m \times 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.

Sample preparation, steam distillation, and headspace gas sampling

Samples of cheese slurry were removed periodically (0, 1, 2, and 3 wk) and added with 10 ml distilled water. Fifty ml of the cheese slurry were transferred into the distilled flask of a Kemmer-Hellet type micro-Kjeldahl distillation unit. The slurry was steam-distilled while ice-water was circulated through the condenser by a submersible-type pump. The steam generator (water flask) was heated in a constant manner to collect the first drop of distillate in 2 min after boiling, and 5 ml of distillate were collected in 3 min. Two ml of each distillate was used to take headspace gas sample as described by Bassette and Ward (1975).

Gas chromatography and quantitation of neutral volatile compounds

A Hewlett-Packard Model 5880A GC equipped with a flame ionization detector was used for all analyses. Headspace gas samples were analyzed on capillary column (Supelcowax[™]10, 30 m × 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 slurry 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.

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 AND DISCUSSION

Cheese curd characteristics

The curds of Trts A and B were softer and more elastic than that of control during cheddaring, which was similar to results from other reports (Metzger and Mistry, 1994; Emmonds et al., 1980) for full- and reduced-fat Cheddar cheeses made from homogenized milk. Homogenization of milk normally caused curd shattering and improper curd matting during cheese making (Green et al., 1983), which was shown in our result.

Composition and cholesterol removal

The composition of the Cheddar cheese was presented in table 1. Moisture content of cheese was ranged from 41.3 to 44.2% and fat from 31.5 to 38.0%. Homogenization of milk increased cheese moisture and similar effect on cheese moisture have been reported for reduced-fat Cheddar cheese made from homogenized milk (Metzger and Mistry, 1994). Peters (1956) reported that homogenization of milk leaded slow curd drainage and high cheese moisture.

The cholesterol content of the control was 102.3 mg/100 g. The cholesterol reduction reached 79.30% when cheese milk was homogenized at 1,000 psi, and treated with 1% β -CD (Trt A), while 91.2% when separated

 Table 1. Mean chemical composition of cholesterolreduced Cheddar cheese¹

Component	Control	Trt A ²	Trt B ³
Moisture, %	41.3 ^a	43.7 ^a	44.2 ^a
Fat, %	38.0 ^b	34.0 ^a	31.5 ^a
Protein, %	28.2 ^a	31.0 ^a	30.8 ^a
Cholesterol	0^{a}	79.3 ^b	92.1 ^c
removal, %			

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

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

 3 After cream separation, cream was treated with 10% β -CD and mixed with skim milk at 1,000 psi.

cream was homogenized at 1,000 psi and treated with 10% β -CD (Trt B) (table 1).

Production of short-chain free fatty acids (FFA)

It is well known that free fatty acids (FFA) constitute the backbone of Cheddar flavor (Lin and Jeon, 1987). Among FFAs, short-chain fatty acids (C4 through C10) are considered to be particularly important. Therefore, the production of short-chain FFA profiles identical to those in aged cheese was investigated by a number of researchers for the acceleration of Cheddar flavor development (Arbige et al., 1986; Kwak et al., 1990). In this study, it was investigated whether short-chain free fatty acids differ in the sample cheesetreated with β -CD and homogenization or not.

The productions of short-chain FFA (C4, C6, C8 and C10) in control and experimental cheese slurries ripened for 3 wk at 32°C are shown in table 2. Since little amount of fat was expected to be released in manufacture of experimental cheeses due to smaller size of fat globules, the releasing quantity of short-chain FFA was not much different. At 0 wk, capric acid (C10) was at the highest concentration in all groups, while caprylic acid (C8), caproic acid (C6), and butyric acid (C4) were the next in decreasing order. Similar trends of the releasing pattern of FFA were shown at other storage periods. The concentrations of four short-chain FFAs were not significantly different at 0 wk among three different groups. These results meant that β -CD treatment on the milk or cream may not capture the SCFAs as expected. Butyric acid concentration was increased dramatically at 2 wk in control from 19.5 to 89.1 ppm, while increasing trends of other two groups were mostly proportional to the storage period. Therefore, during 3 wk storage, significantly higher amount of butyric acid was released in control than those in others. Higher amount of butyric acid was released in Trt B than that in Trt A.

The release of caproic acid (C6), caprylic acid (C8), and capric acid (C10) was dramatic at 2 wk and increased thereafter in control. Similar trend was found in Trt A and

Treatment	Time (wk) —	FFA concentration (ppm)					
		C4	C6	C8	C10	Total	
Control	0	19.5 ^a	24.8^{a}	25.5 ^a	35.9 ^a	105.7 ^b	
	1	19.9 ^a	27.1 ^{ab}	25.2^{a}	36.0^{a}	108.2^{b}	
	2	89.1 ^c	59.0 ^b	65.6 ^b	104.8 ^c	318.5 ^d	
	3	82.1 ^c	115.4 ^c	119.7 ^c	174.9 ^d	492.1 ^c	
Trt A ²	0	18.7^{a}	19.4 ^a	21.6 ^a	25.2 ^a	85.2 ^a	
	1	19.5 ^a	30.0 ^{ab}	31.3 ^a	50.3 ^b	131.1 ^c	
	2	27.4 ^{ab}	33.5 ^{ab}	32.3 ^a	54.3 ^b	147.5 ^c	
	3	34.4 ^b	60.4 ^b	92.2 ^c	95.0 ^c	282.0^{d}	
Trt B ³	0	18.0^{a}	$18.4^{\rm a}$	21.2^{a}	24.1 ^a	81.7^{a}	
	1	26.9 ^{ab}	19.7^{a}	32.4 ^a	30.4 ^a	109.4 ^b	
	2	30.1 ^{ab}	29.0 ^{ab}	43.0 ^a	50.2 ^{ab}	152.3 ^c	
	3	44.3 ^b	42.5 ^b	53.3 ^{ab}	73.3 ^{bc}	213.4 ^{ed}	

Table 2. Concentrations of short-chain fatty acids of different homogenization processes in cholesterol-reduced Cheddar cheese slurries ripened at 32°C for 3 wk¹

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.

³ After cream separation, cream was treated with 10% β-CD, and mixed and homogenized at 1,000 psi.

B, however, the amount was less than half of that in control at 2 and 3 wk. The reason why the short-chain FFA release was lower in experimental cheese slurries could be partially due to a lower fat content in Trt A and Trt B as 34.0% and 31.5% of milk fat, respectively, than in control (38.0%).

It is well accepted that homogenization of milk significantly reduced the fat globule size (Lelievere et al., 1990). Based on above, we assumed that small portions of fat (the amount of fat decreased) was separated from emulsion state of milk, therefore, it was easily released from cheese curds in the process of cheese manufacture. Especially, in Trt B, which cream was separated firstly from milk and secondly cream was treated with 10% β-CD, and thirdly skim milk and treated cream were mixed and homogenized at 1,000 psi, fat globule separation was easily detected in manufacture process in Cheddar cheese. Thin and opaque layer was found in upper portion and it was aggregated with time. However, additional major unknown factor may involve in the productions of the FFAs in the experimental slurries in connection with homogenization and/or β-CD.

Other study using reduced fat Cheddar cheese (RFCC) showed relatively little Cheddar flavor from mixtures of alkanoic acids with carbon chains C2 to C10 because of low fat content (Metzger and Mistry, 1994). Gilles and Lawrence (1987) reported that the stretch characteristics of Mozzarella cheese were impaired and significantly reduced melting and stringiness were observed with ripening when recombined milk was used.

Above problems in cheese from recombined milk could arise from the homogenization step used to emulsify the fat. Homogenization is known to limit the stretch and melt of Mozzarella cheese (Kosikowski, 1997), hence this process could influence on the quality of cheese. The surface of the fat in recombined milk is not bound by the fat globule membrane present in fresh milk and this may be significant. In addition, when homogenized under conventional conditions, the fat particles predominantly contain casein at the fat-water interface and therefore, participate in the formation of the casein matrix in the cheese (Walstra and van Vliet, 1986). Neogi and Jude (1978) observed decreased fat contents in Cheddar cheese prepared from homogenized milk, which the effect was marked with increasing homogenization pressure.

Production of neutral volatile flavor compounds

In this study, it was observed that whether the cholesterol-reduced Cheddar cheese slurries treated by β -CD differed from control sample in neutral volatile flavors. The acetaldehyde production is shown in figure 1. In control group, no acetaldehyde was found at 0 wk, increased dramatically up to 0.71 ppm at 1 wk and plateaued thereafter (0.76 ppm). In comparison, those from milk (Trt A) and cream (Trt B) homogenization groups, high amount of acelaldehyde production was observed at 0 wk (0.53 ppm) and plateaued until 3 wk (0.60 ppm). In Trt A, small amount was produced at 1 wk (0.13 ppm) and increased slowly up to 0.39 ppm.

Ethanol production was the highest among all flavor compounds. Little difference was found among 3 different groups (figure 2). At 0 wk, ethanol production was the lowest in control (6.95 ppm), compared other two treatments (16.05 ppm for Trt A and 10.78 ppm for Trt B). After 0 wk, the ethanol production increased dramatically, especially in control. In Trt B, the highest production was found at 3 wk.

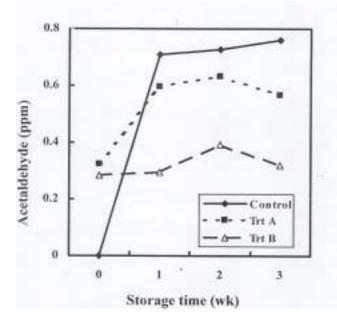


Figure 1. Change of acetaldehyde production in cholesterol-reduced Cheddar cheese slurries ripened at 32°C for 3 wk

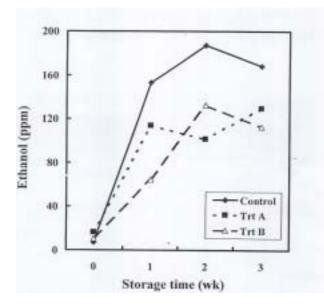


Figure 2. Change of ethanol production in cholesterolreduced Cheddar cheese slurries ripened at 32°C for 3 wk

The production of 2-ethylhexanoic acid showed a different trend (figure 3). Until 1 wk, 2-ethylhexanoic acid was not produced in all groups. In control, none of that was produced at 0 and 1 wk, and increased slowly and reached to the highest amount of production at 3 wk with 32.44 ppb. In milk group (Trt A), slow increase was found at reached 3.39 ppb at 3 wk. However, in cream (Trt B), higher amount of 2-ethylhaxanoic acid was produced than that in milk, and the increase rate was dramatic. Most of minor

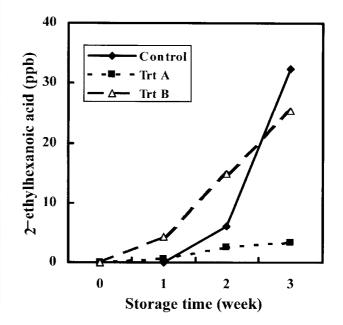


Figure 3. Change of 2-ethylhexanoic acid production in cholesterol-reduced Cheddar cheese slurries ripened at 32°C for 3 wk

neutral flavor compounds listed in table 3 were dimethylsulfide, acetone, ethyl acetate, butanone, pentanone, and heptanone. During 3 wk, no significant difference was found among neither time periods nor groups in these compounds.

Among flavor compounds, 2-butanone, major evennumbered methyl ketone, was a desirable component for Cheddar flavor. Lin and Jeon (1987) indicated that the production of 2-butanone was accelerated in lipase-treated cheese. However, odd-numbered methyl ketones (2pentanone, 2-heptanone, and 2-nonqnone) may be a minor contributor for Cheddar flavor (Walker and Keen, 1974). These methyl ketones were increased consistently up to 2 wk and increased inconsistently at 3 wk in the study on Cheddar chese slurries (Kwak et al., 1990).

The production of aldehyde for all samples was rapidly increased up to 1 wk and declined thereafter (Kwak et al., 1990). This decline may be due to the reduction of acetaldehyde to ethanol (Lees and Jago, 1976). The concentrations of ethanol were the highest among neutral volatile compounds, the production of ethanol for all samples was rapidly increased up to 1 wk and declined thereafter. However, in calf lipase-treated slurry, it peaked at 2 wk (Kwak et al., 1990). Ethanol may contribute to cheese flavor (Anders and Jago, 1970), but the high concentrations of ethanol tended to be associated with high concentrations of esters and fruity defect (McGugan et al., 1975). Their results indicated that lipase additions may not influence the production of ethanol in cheese slurries. 2ethylhexanoic acid was in trace amount during ripening

Treatment	Time (wk)	Dimethyl- sulfide	acetone	Ethyl- acetate (ppm)	butanone	pentanone	heptanone
Control	0	-	$6.90^{\rm a}$	-	-	-	3.40 ^a
	1	4.71 ^a	6.83 ^a	2.96 ^a	1.21 ^a	5.63 ^a	2.83 ^a
	2	4.72 ^a	6.80^{a}	3.03 ^a	1.24 ^a	5.63 ^a	3.20 ^a
	3	4.73 ^a	7.90^{ab}	3.71 ^{ab}	1.30 ^a	5.64 ^a	2.88^{a}
Trt A ²	0	4.82^{a}	7.30 ^{ab}	3.10 ^a	1.76^{ab}	5.66 ^a	-
	1	4.37 ^a	7.01 ^a	3.06 ^a	2.78^{b}	5.72 ^a	2.69 ^a
	2	4.75 ^a	6.83 ^a	3.02 ^a	2.72 ^b	5.66 ^a	2.67^{a}
	3	4.75 ^a	6.94 ^a	3.28^{a}	2.88^{b}	5.72 ^a	$2.72^{\rm a}$
Trt B ³	0	4.75 ^a	6.94 ^a	2.97^{a}	1.30 ^a	-	2.65^{a}
	1	4.74 ^a	6.76^{a}	3.00^{a}	1.85^{ab}	5.65 ^a	2.68^{a}
	2	4.75 ^a	6.72^{a}	3.00 ^a	1.63 ^{ab}	5.65 ^a	2.67^{a}
	3	4.75 ^a	6.84 ^a	3.17 ^a	2.02^{ab}	5.71 ^a	2.70^{a}

Table 3. The effect of different homogenization processes on the production of neutral volatile compounds in cholesterolreduced Cheddar cheese slurries ripened at 32° C for 3 wk¹

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.

 3 After cream separation , cream was treated with 10% β -CD, and homogenized at 1,000 psi.

period. Other study indicated that ethylacetate production was increased up to 1 wk and decreased thereafter, while ethylbutyrate for all samples was produced linearly increased during the ripening time (Kwak et al., 1990). This study showed that cholesterol-removed Cheddar cheese slurries do not influence on the neutral volatile flavors.

CONCLUSION

This study indicated that cholesterol-reduced Cheddar cheese slurries homogenized and treated with β -CD did not influence initially on the production of the flavor compounds, but they produced the low rate of short-chain fatty acids, and the same rate of neutral volatile compounds during ripening periods.

ACKNOWLEDGEMENT

This research was supported by the Brain Korea 21 Project, Seoul, Korea.

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