Development of Phytosterol Ester-added Cheddar Cheese for Lowering Blood Cholesterol

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ABSTRACT : This study was carried out to investigate the effect of phytosterol ester addition on lowering blood cholesterol in cholesterol-reduced Cheddar cheese. For cholesterol removal, separated cream was treated with 10% β-cyclodextrin at 800 rpm, then blended with remaining skim milk and homogenized with 1,000 psi at 70°C. Experimental cheeses were manufactured by five different levels of phytosterol addition. After the cholesterol reduction process by β-cyclodextrin, the cholesterol removal rate was in the range of 91.0 to 92.1%. Amount of short-chain free fatty acid and free amino acids increased with an increase of phytosterol ester, and those were significantly different from that of control in all ripening periods. All rheological properties also increased with an increase of phytosterol ester during ripening period. In sensory analysis, the scores of rancid, bitterness Cheddar flavor and off-flavor intensities increased significantly, while texture was decreased during ripening in phytosterol ester-added groups. Total blood cholesterol was reduced by 18% when rats were fed Cheddar cheese treated with 8% phytosterol. The present study indicated that phytosterol ester addition resulted in a profound lowering effect of blood with cholesterol-reduced Cheddar cheese. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 2 : 267-276)***

Key Words : Phytosterol Ester, Blood Cholesterol, β-cyclodextrin, Cheddar Cheese

INTRODUCTION

The plasma cholesterol lowering properties of phytosterols has been known since the early 1950s, and there have been a plethora of studies reporting their hypocholesterolemic effects (Pollak, 1985; Ling and Jones, 1995). Increased cholesterol concentrations can be lowered by changing the fatty acid composition of diet, however, through consumption of products enriched with plant sterols (Expert panel on detection, 2001).

Phytosterols or plant sterols are natural components of the human diet, which are structurally related to cholesterol (Hepburn et al., 1999). They are minor constitutes of edible vegetable oils present in the unsaponifiable fraction and the most important dietary sources are vegetable oils and products based on vegetable oils like margarine. The most common phytosterols in nature are the 4-desmethylsterols, namely, β-sitosterols, campesterol and stigmasterols, and they occur in the free fatty acids, sugar moieties or phenolic acids (Hepburn et al., 1999). In northern European countries, phytosterol intake from normal food sources has been estimated to be about 200 mg/day (Morton et al., 1995).

In recent years, phytosterols have gained much attention as nutraceuticals for their blood cholesterol lowering efficacy (Ling and Jones, 1995; Leeson and Floter, 2002). This interest has now been translated into a range of health-promoting functional products, such as vegetable oil-based table spreads. Typically, the sterol content is between 6 and 10% to provide sufficient daily intake for the blood cholesterol lowering effect (Lesson and Floter, 2002). The plasma cholesterol lowering effect of a phytosterol ester-containing margarine has been confirmed in humans (Weststrate and Meijer, 1998), which have been re-esterified with unsaturated fatty acids to increase fat solubility.

In the other side, most consumers are concerned about the excessive intake of cholesterol (Grundy et al., 1982; Gurr, 1992), since a strong positive correlation exists between increased serum cholesterol concentrations and risk of coronary heart disease. A number of studies have been indicated that the cholesterol removal in food, including milk, cream and cheese, was effectively conducted by β-cyclodextrin (β-CD) (Oakenfull and Sidhu, 1991; Makoto et al., 1992; Ahn and Kwak, 1999; Lee et al., 1999; Kwak et al., 2001). Because β-CD is nontoxic, edible, non-hygroscopic, chemically stable and easy to separate (Mistry and Anderson, 1993), it has positive attributes when used for cholesterol removal from foods. Therefore, this study was designed to carry out the development of phytoesterol ester-added Cheddar cheese with reduced cholesterol for lowering blood cholesterol.

MATERIALS AND METHODS

Materials

Raw milk (milk fat: 3.5%) was obtained from Binggare Dairy Plant (Kyonggi-do, Korea). Phytosterol (53% β-sitosterol, 32% campesterol and 13% stigmasterol) was purchased from Wuhan Kaidi Fine Chemical Industrial Co.,

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Received September 22, 2004; Accepted October 28, 2004

Ltd (Wuhan, China). Commercial β-CD (purity 99.1%) and oleic acid were purchased from Nihon Shokuhin Kaku Co., Ltd (Osaka, Japan). Cholesterol, 5-α cholestane and other materials were purchased from Sigma Chemical (St Louis, MO, USA) and all solvents were gas chromatographic grade.

Esterification of phytosterol

Phytosterol was mixed with oleic acid in the ratio of 4 to 1 and dissolved in dichloromethane. Then 4-dimethylaminopyridine (DMAP) was added and stirred at 25°C. After complete dissolving, 1,3-dicyclohexyl-carbodiimide, an active material of carboxyl group, was added and stirred at 34°C for 3 h. The product was filtered and vacuum-evaporated, and oil phase was transferred to the tube and stirred vigorously in methanol at 40°C. Then cooled for 24 h and phytosterol ester was obtained as a wax-phase.

Milk treatment

The whole milk was not β-CD-treated and not homogenized for control. Experimental cheeses were manufactured as followed. Bulk raw milk was heated to 40°C to separate into cream and skim milk using a cream separator (CE elecrem, Vanves, France). The separated cream containing 35% milk fat was stirred with 10% β-CD at 800 rpm with a blender (Tops: Misung Co., Seoul, Korea) in a temperature-controlled water bath at 20°C for 30 min (Shim et al., 2004), then blended with remaining skim milk and homogenized with 1,000 psi at 70°C in a single stage homogenizer (HC 5,000, Microfluidics Corp., Newton, MA, USA) (Kwak et al., 2001). Each sample was centrifuged with 166×g for β-CD removal.

Experimental cheeses were subdivided into 5 differernt groups: Trt A; no addition of phytosterol ester, Trt B; 2% phytosterol ester added, Trt C; 4% phytosterol ester added, Trt D; 6% phytosterol ester added, and Trt E; 8% phytosterol ester added. Phytosterol ester was added into the middle of mixing the β-CD treated-cream and skim milk at 1,000 psi.

Manufacture of Cheddar cheese

Cheese making process was described by Metzger and Mistry (1994). After manufacturing, control and experimental cheeses (Trt A-E) were weighed, vacuum packaged in a barrier bag and ripened at 5°C for 0, 8, 16, 24 and 32 weeks, and for 0, 2, 4, 6 and 8 weeks, respectively. The cheese was stored in refrigerator for 12 hr as 0 week sample. The cheese making 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 (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 μm thickness) using Hewlett-Packard 5,890A gas chromatograpyh (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-(amount of cholesterol in β-CD-treated cheese×100/amount of cholesterol in control). Cholesterol determination for control was averaged with each batch of treatment.

Analysis of chemical composition and yield of cheese

Cheese was analyzed for moisture, fat and protein using the methods of Association of Official Analytical Chemists (AOAC). Cheese yield was determined as wt. cheese×100/wt. milk.

Analysis of short-chain free fatty acid

Cheese samples (1 g) were removed periodically from control cheese ripened for 0, 8, 16, 24 and 32 weeks and 0, 2, 4, 6 and 8 weeks for experimental cheeses 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 2 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 (30 g) were removed periodically from the control cheese ripened for 0, 8, 16, 24 and 32 weeks and the experimental cheese for 0, 2, 4, 6 and 8 weeks, added with 30 ml distilled water. Two ml of each distillate was used to take headspace gas sample as described by Bassette and Ward (1975).

Table 1. Composition of 40% beef tallow modified AIN-76A purified rodent diet with 5% cholesterol and 0.5% cholic acid

Ingredient	grams/kg
Casein, high nitrogen	200
Corn starch	150
Beef tallow	400
Sucrose	95
Cholesterol	50
Cellulose	50
Mineral mix[1]	35
Vitamin mix[2]	10
Cholic acid	5
DL-methionine	3
Choline bitartrate	2

[1] AIN-76 Mineral mix (g/kg): $CaHPO_4$ 500; NaCl 74; K citrate monohydrate 220; K_2SO_4 52; MgO, Mn carbohydrate 3.5; Fe citrate 6.0; Zn carbonate 1.6; Cu carbonate 0.3; KIO_3 0.01; $Na_2SeO_4 \cdot H_2O$ 0.01; $CrK(SO_4) \cdot 12H_2O$ 0.55; Sucrose 118.

[2] AIN-76 Vitamin mix (g/kg): thiamin·HCl 0.6; riboflavin 0.6; phydoxine·HCl 0.7; nicotinic acid 3; D-calcium pantothenate 1.6; folic acid 0.2; D-biotin 0.02; cyanocobalamin 0.001; retinyl palmitate 0.8; DL-α-tocopheryl acetate 20; cholecalciferol 0.00025; menaquinone 0.005.

A Hewlett-Packard Model 5,880A 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. 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 (FAA)

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 C_{18} reversed-phase column maintained at 46°C. An internal standard was added for identification of amino acids (Sigma, St. Louis, MO, USA). 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.

Table 2. Composition of fat free AIN-76A purified diet

Ingredient (%)	grams/kg
Casein	200
Corn starch	150
Sucrose	550
Cellulose	50
Salt mix[1]	35
Vitamin mix[2]	10
DL-methionine	3
Choline bitartrate	2

[1] AIN-76 Mineral mix (g/kg): $CaHPO_4$ 500; NaCl 74; K citrate monohydrate 220; K_2SO_4 52; MgO, Mn carbohydrate 3.5; Fe citrate 6.0; Zn carbonate 1.6; Cu carbonate 0.3; KIO_3 0.01; $Na_2SeO_4 \cdot H_2O$ 0.01; $CrK(SO_4) \cdot 12H_2O$ 0.55; Sucrose 118.

[2] AIN-76 Vitamin Mix (g/kg): thiamin·HCl 0.6; riboflavin 0.6; phydoxine·HCl 0.7; nicotinic acid 3; D-calcium pantothenate 1.6; folic acid 0.2; D-biotin 0.02; cyanocobalamin 0.001; retinyl palmitate 0.8, DL-α-tocopheryl acetate 20; cholecalciferol 0.00025; menaquinone 0.005.

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).

Animals and diets

Twenty male Sprague-Dawley rats obtained from the Jung-Ang Lab. Animal, Inc. (Seoul, Korea) weighing 60 to 75 g were placed individually in stainless-steel wire cages in a windowless room and were subjected to a light cycle with the light period from 1500 to 0300 h and the dark period from 0300 to 1500 h. The rats were acclimatized for 1 week and fed a commercial rat chow during this period. All diets were formulated as recommended by the American Institute of Nutrition (Tables 1 and 2). All animals were fed a 40% beef tallow modified rodent diet with 5% cholesterol and 0.5% cholic acid for 5 week, and fat free purified diet containing different cheesees for 6 week *ad libitum*.

Table 3. Mean chemical composition of phytosterol ester-added cholesterol-reduced Cheddar cheese[1]

Component	Control[2]	Trt A[3]	Trt B[4]	Trt C[5]	Trt D[6]	Trt E[7]
Moisture	38.3[a]	42.2[b]	39.6[a]	40.2[ab]	39.8[a]	39.5[a]
Fat	36.0[a]	34.5[a]	40.1[b]	39.7[b]	39.9[b]	39.5[b]
Protein	28.2[a]	30.8[a]	29.6[a]	29.1[a]	29.6[a]	29.8[a]
Cholesterol removal	0.0[b]	91.2[a]	92.0[a]	91[a]	92.0[a]	92.1[a]
Yield	10.5[a]	12.5[a]	11.2[a]	11.6[a]	10.4[a]	11.0[a]

[1] Means within column by the same capital letter are not significantly different ($p<0.05$).
[2] No β-CD treated and no phytosterol ester added.
[3] After cream separation, cream was treated with 10% β-CD and blended with skim milk at 1,000 psi.
[4] After cream separation, cream was treated with 10% β-CD and added 2% phytosterol ester.
[5] After cream separation, cream was treated with 10% β-CD and added 4% phytosterol ester.
[6] After cream separation, cream was treated with 10% β-CD and added 6% phytosterol ester.
[7] After cream separation, cream was treated with 10% β-CD and added 8% phytosterol ester.

Animlas were given free access to tap water via a stainless steel delivery system.

By second week, the animals were fed high cholesterol-high fat diet (Table 1) for 6 weeks, and were assigned randomly to the following two groups: 1) control, fed a fat free diet (Table 2) containing 0.5 g/day of commercial cheese, and 2) phytosterol ester-added group (Phyto), fed a fat free diet containing 0.5 g/day of 8% phytosterol ester-added cholesterol-reduced cheese. To examine blood analysis, animals were fasted for 12 h and 1.5 ml blood sample was withdrawn from a tail and centrifuged at 3,000 rpm for 10 min, and stored at -20°C until analysis.

Statistical analysis

Data from the determination of optimum conditions of cheeses, one-way ANOVA (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

The cholesterol content of control cheese was 102.3 mg/100 g. The cholesterol removal rate of five different experimental cheeses (phytosterol ester content was 0, 2, 4, 6 and 8% added) reached 91.0 to 92.1%. The composition of control and experimental Cheddar cheeses were compared in Table 3. Moisture content was in the range of 39.5 to 42.2% and fat was 34.5 to 40.1%. Trt A containing no phytosterol ester, contained high amount of moisture, which has generally shown in reduced-fat Cheddar cheese due to a slow curd drainage (Metzger amd Mistry, 1994), while others showed lower amount of moisture probably due to addition of phytosterol ester.

Table 4. The production of short-chain free fatty acids in phytosterol ester-added cholesterol-reduced Cheddar cheese ripened at 7°C for 8 weeks[1]

Treatment	Ripening (week)	FFA concentration (ppm)				
		C_4	C_6	C_8	C_{10}	Total
C[2]	0	18.2[a]	17.1[a]	20.4[a]	18.5[a]	74.2[a]
	8	19.1[a]	17.2[a]	21.7[a]	25.9[a]	83.9[a]
	16	18.7[a]	17.8[a]	21.8[a]	29.2[a]	87.5[a]
	24	18.9[a]	17.7[a]	22.1[a]	23.6[a]	82.3[a]
	32	19.2[a]	17.7[a]	22.7[a]	24.5[a]	84.1[a]
Trt A[3]	0	19.3[a]	16.9[a]	21.9[a]	20.4[a]	78.5[a]
	2	22.7[a]	17.3[a]	22.1[a]	24.9[a]	87.0[a]
	4	22.9[a]	18.6[a]	22.9[a]	25.7[a]	90.1[ab]
	6	23.6[a]	18.2[a]	24.2[a]	26.3[a]	92.3[ab]
	8	27.8[b]	19.6[a]	26.3[ab]	29.3[a]	103.3[b]
Trt B[4]	0	20.6[a]	18.3[a]	22.3[a]	21.4[a]	82.6[a]
	2	21.3[a]	18.7[a]	22.9[a]	21.9[a]	84.8[a]
	4	22.9[a]	19.3[a]	24.6a	23.6[a]	90.4[ab]
	6	25.8[b]	20.4[a]	25.7[ab]	22.7[a]	94.6[ab]
	8	28.1[c]	22.4[ab]	27.6[ab]	26.3[a]	104.4[b]
Trt C[5]	0	21.3[a]	20.7[a]	23.4[a]	24.7[a]	90.1[a]
	2	22.6[a]	21.6[a]	24.9[a]	25.9[a]	95.0[a]
	4	22.3[a]	21.4[a]	26.7[b]	26.3[a]	96.7[a]
	6	24.9[ab]	23.1[a]	27.0[b]	28.3[b]	103.3[a]
	8	26.3[b]	24.0[a]	29.6b[b]	33.5[c]	113.4[a]
Trt D[6]	0	22.9[a]	20.4[a]	22.4[a]	23.9[a]	89.6[a]
	2	22.1[a]	21.6[a]	23.6[a]	25.1[a]	92.4[a]
	4	23.6[a]	22.4[a]	25.8[ab]	25.9[a]	97.7[a]
	6	24.8[a]	22.2[a]	27.3[b]	27.1[a]	101.4[ab]
	8	26.7[b]	25.9[b]	30.1[b]	29.9[b]	112.6[b]
Trt E[7]	0	19.6[a]	18.2[a]	22.5[a]	23.4[a]	83.7[a]
	2	20.8[a]	19.6[a]	24.6 [ab]	24.7[a]	89.7[a]
	4	22.6[a]	20.7[a]	24.8 [ab]	25.0[a]	93.1[a]
	6	24.9[ab]	23.9[a]	28.0[b]	26.8[a]	103.6[ab]
	8	27.7[b]	25.5[b]	30.5[b]	30.1[ab]	113.8[b]

[1] Means within column by the same capital letter are not significantly different ($p<0.05$).
[2] No β-CD treated and no phytosterol ester added.
[3] After cream separation, cream was treated with 10% β-CD and blended with skim milk at 1,000 psi.
[4] After cream separation, cream was treated with 10% β-CD and added 2% phytosterol ester.
[5] After cream separation, cream was treated with 10% β-CD and added 4% phytosterol ester.
[6] After cream separation, cream was treated with 10% β-CD and added 6% phytosterol ester.
[7] After cream separation, cream was treated with 10% β-CD and added 8% phytosterol ester.

Production of short-chain free fatty acids (FFA)

It is well known that short-chain free fatty acids (FFA, C_4 through C_{10}) 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. Our previous study (Kwak et al., 2002) indicated that cheese by the mixture of β-CD-treated cream and skim milk was ripened rapidly with the high production of short-chain FFA, compared with control.

Table 5. The production of neutral volatile flavor compounds in phytosterol ester-added in cholesterol-reduced Cheddar cheese ripened at 7°C for 8 weeks[1]

Treat-ment	Ripening (week)	Acet-aldehyde	Acetone	2-Butanone	Ethanol	2-Heptanone
		(ppm)				
C[2]	0	0.05[a]	7.90[a]	1.18[a]	5.61[a]	2.81[a]
	8	0.15[a]	6.80[a]	1.19[a]	13.36[a]	2.66[a]
	16	0.39[ab]	6.87[a]	1.22[a]	63.41[b]	2.68[a]
	24	0.65[b]	6.85[a]	1.19[a]	73.22[b]	2.56[a]
	32	0.78[b]	6.79[a]	1.17[a]	103.43[c]	2.67[a]
Trt A[3]	0	0.14[a]	6.93[a]	1.12[a]	4.65[a]	2.41[a]
	2	0.26[a]	7.23[a]	1.16[a]	15.36[a]	2.63[a]
	4	0.29[ab]	7.25[a]	1.18[a]	50.11[a]	2.53[ab]
	6	0.35[ab]	7.32[a]	1.26[a]	87.70[b]	2.75[ab]
	8	0.52[ab]	7.41[a]	1.41[ab]	104.13[c]	2.68[b]
Trt B[4]	0	0.09[a]	6.95[a]	1.04[a]	12.52[a]	2.13[a]
	2	0.17[a]	7.23[a]	1.17[a]	23.45[a]	2.44[a]
	4	0.31[ab]	7.56[a]	1.26[a]	30.67[ab]	2.48[a]
	6	0.36[ab]	7.24[a]	2.54[b]	59.21[b]	2.53[a]
	8	0.40[ab]	7.87[a]	2.36[b]	63.39[b]	2.98[a]
Trt C[5]	0	0.11[a]	7.11[a]	1.11[a]	5.02[a]	2.03[a]
	2	0.16[a]	6.39[a]	1.20[a]	19.58[a]	2.45[a]
	4	0.27[a]	6.98[a]	1.19[b]	36.17[a]	2.58[a]
	6	0.35[ab]	7.20[a]	1.36[b]	49.11[b]	2.98[a]
	8	0.39[b]	7.24[a]	1.59[b]	50.57[c]	3.47[a]
Trt D[6]	0	0.12[a]	7.12[a]	1.16[a]	16.99[a]	1.19[a]
	2	0.23[a]	7.03[a]	1.24[a]	27.15[a]	2.41[a]
	4	0.35[ab]	7.56[a]	1.34[a]	29.03[a]	2.87[a]
	6	0.36[ab]	7.47[a]	2.31[b]	48.77[ab]	3.95[ab]
	8	0.45[a]	7.59[a]	2.04[b]	68.65[b]	2.86[a]
Trt E[7]	0	0.09[a]	7.14[a]	1.17[a]	14.02[a]	2.68[a]
	2	0.17[a]	7.16[a]	1.24[a]	25.73[a]	2.92[a]
	4	0.33[a]	7.26[a]	1.33[a]	29.65[ab]	3.45[a]
	6	0.36[ab]	7.28[a]	1.59[a]	75.32[b]	2.76[a]
	8	0.52[ab]	7.30[b]	2.36[b]	85.37[b]	2.98[a]

[1] Means within column by the same capital letter are not significantly different ($p<0.05$).

[2] No β-CD treated and no phytosterol ester added.

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

[4] After cream separation, cream was treated with 10% β-CD and added 2% phytosterol ester.

[5] After cream separation, cream was treated with 10% β-CD and added 4% phytosterol ester.

[6] After cream separation, cream was treated with 10% β-CD and added 6% phytosterol ester.

[7] After cream separation, cream was treated with 10% β-CD and added 8% phytosterol ester.

Short-chain FFA in control and experimental cheeses ripened for 32 and 8 weeks at 5°C, respectively, are shown in Table 4. Total amount of short-chain FFA was higher in cheeses made by β-CD-treated cream than control group ($p<0.05$). More amount of phytosterol ester-added group developed more amount of total short-chain FFA. Not much difference was found during 32 week ripening in control, however, FFA production in experimental cheeses (Trt A–E) increased significantly at 8 week ripening ($p<0.05$). During 8 week ripening, the total production of short-chain FFA in high phytosterol ester-added groups, especially Trt D and E, was higher than those of control and Trt A-C.

In control, the amount of total short-chain FFA increased from 74.2 to 84.1 ppm during 32 weeks. In phytosterol ester-added cheeses, 78.5 to 90.1 ppm of total short-chain FFA were found at 0 week, and 103.3 to 113.8 ppm were produced at 8 weeks. The present results indicated that the cheese made by phtytosterol ester addition produced more short-chain FFA than control. Also, the experimental cheeses were ripened much faster than control, which were already reported in our previous study (Kwak et al., 2002).

Production of neutral volatile flavor compounds

The production of neutral volatile compounds was examined whether phytosterol ester addition influenced or not (Table 5). In all groups, almost no acetaldehyde was found at 0 week and increased steadily up to 0.78 ppm at 32 weeks in control, while 0.39 to 0.52 ppm at 8 weeks in phytosterol ester-added groups.

Ethanol production was the highest among flavor compounds measured and showed a similar trend in all samples. After 0 week, the ethanol productions in control and Trt A increased dramatically upto 32 and 8 weeks as 103.43 and 104.13 ppm, respectively, while the production of ethanol in other groups (Trt B–E) reached the lowest amount 50.57 ppm in Trt C and the highest amount 85.37 ppm in Trt E.

The production of other neutral flavor compounds such as acetone, 2-butanone and 2-heptanone, were not significantly different from neither ripening periods nor groups. This result indicated that neutral volatile flavor compounds from phytosterol ester-added Cheddar cheese were not significantly different from that of the control Cheddar cheese, except for the ethanol production.

Production of free amino acids (FAA)

The production of free amino acids during ripening period is shown in Table 6. The phytosterol ester-added cheeses produced much higher amounts of individual FAA than the control in all periods. Total FAA amounts were 107.6 at 32 weeks in control and about 240.0 μmol/g cheese in phytosterol ester-added group at 8 week ripening period (data not shown). In all samples, certain amino acids such as glutamic acid, valine, phenylalanine, isoleucine, leucine and lysine, were dominated during ripening periods The data in Table 6 showed that lysine was high in experimental cheese groups, and glutamic acid and leucine were still higher than the those of control sample at the end of ripening.

Table 6. The production of free amino acid in phytosterol ester-added cholesterol-reduced Cheddar cheese ripened at 7°C for 8 weeks[1]

Treat -ment	Ripening (week)	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Tyr	Met	Val	Phe	Ile	Leu	Lys
		(μmol/g cheese)														
C[2]	0	1.7	3.0	0.8	-	0.4	1.2	0.1	1.5	0.5	0.2	1.4	2.2	0.5	2.3	3.0
	8	3.5	12.4	1.6	-	2.4	1.5	2.5	2.6	1.0	1.2	7.9	7.4	2.4	4.7	5.8
	16	4.6	16.5	1.7	0.2	2.9	1.8	2.8	4.0	1.0	1.6	2.4	9.3	2.9	16.2	3.5
	24	4.8	15.4	2.4	0.5	3.5	2.0	2.8	4.5	1.0	1.7	16.8	10.6	3.6	15.3	3.6
	32	4.3	15.9	2.6	0.6	3.0	2.3	2.7	5.4	0.9	2.3	16.7	12.7	3.5	26.7	8.0
Trt A[3]	0	1.9	3.2	0.5	-	0.2	1.3	-	1.3	0.5	0.3	1.4	2.3	0.4	2.7	2.6
	2	3.0	10.7	1.3	0.1	2.9	2.9	2.6	4.7	1.0	1.4	12.3	14.7	16.7	24.1	16.5
	4	4.8	25.0	3.6	0.6	3.5	3.5	2.7	7.4	1.0	2.6	14.7	18.9	26.4	25.3	25.0
	6	5.2	30.9	5.7	0.7	3.9	3.9	3.6	10.9	1.0	3.9	1.9	21.6	28.5	25.6	32.7
	8	9.3	41.5	7.7	0.7	4.8	4.8	4.0	11.4	1.0	4.2	21.7	25.7	29.3	38.5	36.8
Trt B[4]	0	1.6	4.2	0.4	-	0.1	1.4	0.2	1.6	0.3	0.2	1.9	2.6	0.1	1.9	2.1
	2	2.5	25.1	1.7	-	1.6	3.0	2.9	4.7	0.4	1.1	16.4	5.7	13.4	21.4	22.5
	4	3.7	26.8	2.5	0.4	2.9	6.7	3.6	5.9	0.9	2.4	17.3	9.8	15.7	26.7	26.8
	6	4.8	34.7	5.7	0.5	3.8	6.7	4.0	12.4	0.9	2.7	19.5	16.7	20.4	25.9	35.6
	8	8.1	49.5	8.9	0.8	4.0	7.9	3.9	12.9	1.2	5.3	22.1	25.0	26.9	30.5	40.1
Trt C[5]	0	1.4	3.5	0.6	0.6	-	0.3	1.6	-	0.5	0.5	1.4	1.9	0.5	2.1	3.0
	2	2.6	16.5	1.2	1.2	0.2	1.3	3.5	0.2	1.2	1.1	12.6	1.5	16.3	23.5	19.5
	4	3.5	23.9	2.0	2.0	0.6	1.2	4.0	3.6	1.0	1.5	13.4	18.4	14.5	25.1	20.6
	6	3.9	32.4	4.5	4.5	0.6	2.4	4.8	3.4	1.0	2.6	12.8	23.5	25.7	29.8	28.9
	8	7.6	49.5	8.2	8.2	0.9	4.9	8.8	4.4	1.3	6.8	16.4	26.7	29.6	33.0	34.5
Trt D[6]	0	1.7	3.5	0.5	0.5	0.1	0.2	1.4	0.1	0.6	02	1.8	1.8	0.6	2.5	2.6
	2	3.2	12.5	1.5	1.5	0.5	1.0	3.7	2.1	1.5	1.3	16.7	16.5	18.7	23.5	11.5
	4	4.0	22.7	3.6	3.6	0.6	1.9	5.2	3.9	1.4	1.9	17.9	15.8	28.9	26.1	25.9
	6	5.8	36.8	4.0	4.0	0.8	2.6	6.9	4.2	1.0	2.4	26.4	25.7	30.6	35.6	27.9
	8	6.9	47.5	4.9	4.9	0.7	4.7	7.8	3.8	1.6	2.6	26.4	26.8	32.5	40.0	45.2
Trt [7]	0	1.7	3.1	0.7	0.7	-	0.3	1.2	0.3	0.4	0.6	1.4	2.6	0.4	0.9	2.8
	2	3.3	15.7	1.5	1.5	0.4	3.6	2.6	2.5	1.0	1.5	12.5	15.8	23.5	6.4	23.5
	4	4.1	26.5	2.7	2.7	0.6	4.5	4.7	3.4	4.0	1.7	10.3	23.5	24.7	19.8	34.5
	6	5.3	33.8	3.5	3.5	0.6	5.5	5.0	4.7	1.6	2.6	21.4	24.8	33.6	25.7	33.7
	8	6.7	46.2	4.9	4.9	0.8	6.8	6.8	4.8	1.5	4.9	24.6	25.7	32.5	29.1	41.0

[1] Means within column by the same capital letter are not significantly different ($p<0.05$).

[2] No β-CD treated and no phytosterol ester added.

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

[4] After cream separation, cream was treated with 10% β-CD and added 2% phytosterol ester.

[5] After cream separation, cream was treated with 10% β-CD and added 4% phytosterol ester.

[6] After cream separation, cream was treated with 10% β-CD and added 6% phytosterol ester.

[7] After cream separation, cream was treated with 10% β-CD and added 8% phytosterol ester.

Rheological characteristics

The effect of phytosterol ester addition for textural properties on cholesterol-reduced Cheddar cheese was shown in Table 7. Hardness was significantly different between control and phytosterol-added groups during ripening period. Hardness increased with an increased amount of phytosterol ester added (from Trt A to E samples) during ripening period. Elasticity was higher in experimental cheese, compared with that of control in all periods. Similar trend was found in cohesiveness and gumminess. The gumminess was significantly higher in Trt D and E, which were added high amount of phytosterol ester even in 0 week ripening. The present results indicated that the phytosterol ester-added cheeses showed a rapid ripening process in textural properties, which was similar to properties ripened for long period in control.

Sensory evaluation

The sensory attributes of phytosterol ester-added Cheddar cheese at 0 week were shown in Table 8. At 0 week, rancid score was significantly higher in phytosterol ester-added groups than those of control and no phytosterol ester-added group (Trt A). At 0 week ripening. β-CD-treated cheeses, regardless of phytosterol ester addition, produced a very strong bitterness. Cheddar cheese flavor was developed in experimental cheeses with sterol ester-added groups along with strong off-flavor in Trt A-E. Texture score was high in all cheese, while overall preference was significantly lower in phytosterol ester-added cholesterol-reduced cheese.

During 4 week ripening, similar trend was shown in rancid aspect (Table 9). However, bitterness increased significantly in control, which was ripened for 16 weeks,

Table 7. Textural properties in phytosterol ester-added cholesterol-reduced Cheddar cheese ripened at 7°C for 8 weeks[1]

Treatment	Ripening (week)	Hardness	Elasticity	Cohe-siveness	Gummi-ness	Chewi-ness
C[2]	0	804.6	60.7	53.5	201.6	122.4
	8	1,387.1	75.8	62.6	1181.6	849.0
	16	1,041.9	79.6	64.1	781.2	622.2
	24	5,328.2	78.1	65.7	576.3	402.6
	32	5,542.1	76.7	66.6	439.5	337.2
Trt A[3]	0	1,194.5	76.2	91.4	307.3	296.0
	2	1,993.0	76.3	93.2	416.8	401.5
	4	1,251.2	86.3	106.9	547.6	421.6
	6	1,220.6	84.5	103.5	577.6	433.0
	8	1,352.5	85.3	91.6	623.4	398.8
Trt B[4]	0	1,542.6	85.3	77.2	385.7	306.4
	2	2,123.4	86.2	75.9	421.6	526.9
	4	1,663.2	86.4	91.5	495.3	455.2
	6	2,125.3	96.9	89.5	512.3	421.7
	8	1,688.6	92.7	88.6	577.2	332.9
Trt C[5]	0	1,168.5	85.1	72.6	416.9	316.9
	2	1,352.8	73.2	78.5	421.0	402.6
	4	1,745.9	95.9	82.6	573.6	421.6
	6	1,885.2	98.0	97.8	663.5	398.5
	8	1,685.3	92.9	90.6	642.1	348.9
Trt D[6]	0	1,274.6	79.2	74.9	400.2	434.8
	2	1,523.6	82.6	78.5	413.6	528.6
	4	2,485.6	94.7	97.3	530.4	622.4
	6	1,963.4	98.9	80.6	529.6	751.4
	8	2,639.5	93.7	82.4	556.8	559.3
Trt E[7]	0	1,469.3	74.2	75.8	321.6	430.6
	2	2,785.6	78.5	72.3	385.9	411.8
	4	2,239.5	98.6	82.4	452.6	596.3
	6	2,856.4	90.3	85.1	493.6	520.4
	8	2,983.6	91.9	79.5	602.3	502.8

[1] Means within column by the same capital letter are not significantly different ($p<0.05$).
[2] No β-CD treated and no phytosterol ester added.
[3] After cream separation, cream was treated with 10% β-CD and blended with skim milk at 1,000 psi.
[4] After cream separation, cream was treated with 10% β-CD and added 2% phytosterol ester.
[5] After cream separation, cream was treated with 10% β-CD and added 4% phytosterol ester.
[6] After cream separation, cream was treated with 10% β-CD and added 6% phytosterol ester.
[7] After cream separation, cream was treated with 10% β-CD and added 8% phytosterol ester.

and reached the scores of β-CD-treated cheeses (Trt A-E). No difference was found among groups in Cheddar flavor intensity, while off-flavor scores were higher in phytosterol-added groups (Trt C-E).

At 8 week ripening, rancid score was significantly higher in phytosterol ester-added groups than that in control. However, no change was found in bitterness and Cheddar flavor. Along with rancid, off-flavor was also significantly higher in phytosterol ester groups. The present results indicated that phytosterol ester addition may adversely affect in certain cheese sensory characteristics, especially

Table 8. Sensory characteristics in phytosterol ester-added cholesterol-reduced Cheddar cheese ripened at 7°C for 0 week[1]

Sensory description	Treatment				
	Control[2]	Trt A[3]	Trt C[4]	Trt D[5]	Trt E[6]
Rancid	1.0[a]	1.2[a]	2.8[ab]	3.5[b]	4.8[b]
Bitter	1.0[a]	2.8[b]	3.0[b]	3.3[b]	3.2[b]
Cheddar flavor intensity	1.3[a]	2.3[ab]	3.2[b]	3.0[b]	3.0[b]
Off-flavor intensity	1.0[a]	1.5[a]	3.5[b]	3.3[b]	4.2[bc]
Texture	5.0[b]	3.5[ab]	3.0[a]	4.8[b]	4.8[b]
Overall	5.0[b]	4.5[b]	3.2[ab]	2.8[a]	2.8[a]

[1] Means within column by the same capital letter are not significantly different ($p<0.05$). The scale of bitter, off-taste, off-flavor, texture: 1=none, 3=moderate, 5= very strong. The scale of overall scores: 1=dislike very much, 3=neither like nor dislike, 5=like very much.
[2] No β-CD treated and no phytosterol ester added.
[3] After cream separation, cream was treated with 10% β-CD and blended with skimmilk at 1,000 psi.
[4] After cream separation, cream was treated with 10% β-CD and added 4% phytosterol ester.
[5] After cream separation, cream was treated with 10% β-CD and added 6% phytosterol ester.
[6] After cream separation, cream was treated with 10% β-CD and added 8% phytosterol ester.

Table 9. Sensory characteristics of phytosterol ester-added cholesterol-reduced Cheddar cheese ripened at 7°C for 4 weeks[1]

Sensory description	Treatment				
	Control[2]	Trt A[3]	Trt C[4]	Trt D[5]	Trt E[6]
Rancid	1.2[a]	1.5[a]	4.8[b]	4.8[b]	4.2[b]
Bitter	4.2[a]	4.5[a]	4.2[a]	4.2[a]	4.2[a]
Cheddar flavor intensity	4.5[a]	4.5[a]	4.2[a]	4.2[a]	4.2[a]
Off-flavor intensity	1.3[a]	2.8[b]	4.5[c]	4.2[c]	3.2[bc]
Texture	4.2[b]	2.8[a]	4.2[b]	4.2[b]	4.8[b]
Overall	4.2[b]	3.2[ab]	2.8[a]	4.2[b]	2.7[a]

[1] Means within column by the same capital letter are not significantly different ($p<0.05$). The scale of bitter, off-taste, off-flavor, texture: 1=none, 3=moderate, 5=very strong. The scale of overall scores: 1=dislike very much, 3=neither like nor dislike, 5=like very much.
[2] No β-CD treated and no phytosterol ester added.
[3] After cream separation, cream was treated with 10% β-CD and blended with skimmilk at 1,000 psi.
[4] After cream separation, cream was treated with 10% β-CD and added 4% phytosterol ester.
[5] After cream separation, cream was treated with 10% β-CD and added 6% phytosterol ester.
[6] After cream separation, cream was treated with 10% β-CD and added 8% phytosterol ester.

rancid and off-flavor intensity, while texture was not affected profoundly

Animal study

After 5 weeks of 40% beef tallow and 5% cholesterol containing diet, the average food intake was 26 and 27 g/day during next 6 weeks. Body weight gain was not significantly different between control and phytosterol

Table 10. Sensory characteristics of phytosterol ester-added cholesterolreduced Cheddar cheese ripened at 7°C for 8 weeks[1]

Sensory description	Treatment				
	Control[2]	Trt A[3]	Trt C[4]	Trt D[5]	Trt E[6]
Rancid	1.3[a]	2.2[ab]	4.2[c]	4.5[c]	3.3[bc]
Bitter	4.2[a]	4.2[a]	4.8[a]	4.5[a]	4.8[a]
Cheddar flavor Intensity	4.5[a]	4.5[a]	4.2[a]	3.5[a]	4.2[a]
Off-flavor intensity	1.2[a]	2.8[a]	4.2[b]	4.5[b]	3.5[ab]
Texture	2.8[ab]	1.8[a]	3.5[b]	3.3[b]	3.1[ab]
Overall	3.5[b]	2.8[ab]	2.3[a]	2.2[a]	2.0[a]

[1] Means within column by the same capital letter are not significantly different ($p<0.05$). The scale of bitter, off-taste, off-flavor, texture: 1=none, 3=moderate, 5=very strong. The scale of overall scores: 1=dislike very much, 3=neither like nor dislike, 5=like very much.
[2] No β-CD treated and no phytosterol ester added.
[3] After cream separation, cream was treated with 10% β-CD and blended with skimmilk at 1,000 psi
[4] After cream separation, cream was treated with 10% β-CD and added 4% phytosterol ester.
[5] After cream separation, cream was treated with 10% β-CD and added 6% phytosterol ester.
[6] After cream separation, cream was treated with 10% β-CD and added 8% phytosterol ester.

ester-added group as 66.5 and 67.9 g for 6 week period.

In blood analysis, after 5 weeks of high cholesterol, high fat diet feeding, the average total serum cholesterol was 153.4 and 161.9 mg/dl in control and phytosterol ester-added group, respectively (Table 12). During 6 weeks of experimental cheese feeding period, the serum cholesterol fed 8% phytosterol ester-added cheeses decreased significantly from 161.9 to 132.9 mg/dl. Comparatively, blood cholesterol in control slightly increased from 153.4 to 165.8 mg/dl, which was not significantly different. The serum high density lipoprotein (HDL) did not show any significant change during treatment. Serum triacylglycerol (TG) kept increasing from the initial to final week.

DISCUSSION

In past 2 decades, evidence has being gathered to suggest that an excess of cholesterol might be deleterious. The blood cholesterol lowering properties of phytosterols has been known about since the early 1950s, and since then there have been a plethora of studies reporting their hypocholesterolemic effects (Hepburn et al., 1999). Phytosterols lower serum cholesterol by inhibiting the absorption of cholesterol in the small intestine and the principal mechanism is considered to be competition between cholesterol and phytosterols for micellar solubilization (Hepburn et al., 1999). Phytosterols and in particular, their fatty acids-derived esters, have gained much attention in recent years as nutraceutricals for their blood cholesterol lowering efficacy (Ling and Jones, 1995; Leeson and Floter, 2002). This interest has been translated into a range of health-promoting products, such as vegetable oil-based table spreads.

Table 11. Effects of experimental diets on food intake and body weight gain[1]

Treatment	Food intake (g/day)	Body weight gain (g/6 week)
Control[2]	26[a]	66.5[a]
Phyto[3]	27[a]	67.9[a]

[1] Rats were fed for 6 weeks (n=10). Means within column by the same capital letter are not significantly different ($p<0.05$).
[2] Cholesterol-reduced Cheddar cheese with no phytosterol ester addition.
[3] 8% phytosterol ester-added cholesterol-reduced Cheddar cheese.

Table 12. Effects of experimental diets on the change of blood triacylglycerol, total cholesterol and high-density lipoprotein in rats fed 6 weeks[1]

Treatment	TG		Total CH		HDL	
	Initial	Final	Initial	Final	Initial	Final
	(mg/dl)					
Control[2]	64.5[a]	63.5[a]	153.4[a]	165.8[a]	32.3[a]	31.4[a]
Phyto[3]	58.9[a]	61.4[a]	161.9[a]	132.9[b]	39.5[a]	41.0[a]

[1] Rats were fed for 6 weeks (n=10). Means within column by the same capital letter are not significantly different ($p<0.05$).
[2] Cholesterol-reduced Cheddar cheese with no phytosterol ester addition.
[3] 8% phytosterol ester-added cholesterol-reduced Cheddar cheese.

The plasma cholesterol lowering effect of a phytosterol ester-containing margarine has been confirmed in humans (Weststrate and Meijer, 1998). More recently margarine products, supplemented with plant sterols and plant stanols, have started to appear on the market. Although the hypocholesterolemic effects of plant stanol esters incorporated into oil-based products, such as margarines, shortenings and mayonnaise, have been examined (Law, 2000; Mensick et al., 2002), little information is available in applying into relatively high-fat and cholesterol containing dairy products. Therefore, this study was designed to examine the hypercholesterolemic effect of phytosterol ester addition in cholesterol-reduced cheese and to find out whether the chemical, physical, and sensory characteristics were changed by cholesterol reducing process and phytosterol ester addition or not.

In addition, we suggested if phytosterol ester was added to cholesterol-reduced cheese, which was successfully manufactured in our laboratory, that products may help lowering blood cholesterol more effectively. To make cholesterol-reduced cheese, cholesterol must be removed from milk using a β-CD treatment and the resulting low cholesterol cheese appeared to be indistinguishable from conventional products, except for weak texture properties (Ahn and Kwak, 1999; Lee et al., 1999; Kwak et al., 2002). However, over 90% of cholesterol was removed in commercial milk at refrigerated temperature with 1% β-CD (Lee et al., 1999).

As expected, the soft curds of the phytosterol ester-added cholesterol-reduced cheese was found due to the

influence of β-CD treatment. The reason could be explained that a weak coagulum 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). However, one thing we found in the present study was that the textural characteristic of β-CD-treated cheese at early-stage ripening (0 or 2 weeks) showed similar aspects in control ripened for 24 weeks or over. This indicated that β-CD treatment in cream revealed a rapid-ripening effect on Cheddar cheese manufacture.

Since cheese flavors, which 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 β-CD treatment or phytosterol ester addition on the production of short-chain FFA profiles. Total amount of short-chain FFA was higher in cheese made by phytosterol addition and β-CD treated cream than in control cheese. Neutral volatile flavor compounds were not significantly different among treatments ($p>0.05$). These above results indicated that phytosterol ester addition and β-CD treatments resulted in more short-chain FFA production even in early stage of ripening period, and did not show any adverse effects such as capture or removal the short-chain fatty acids.

Another aspect we found in this study was an increase of bitterness score in phytosterol ester-added and β-CD treated cheese from 0 week ripening and thereafter. This was probably due to a significant difference of amino acid production resulted from β-CD treatment rather than phytosterol ester addition. The larger increase in total and individual amino acids including bitter amino acids observed through a ripening period may reflect the enhanced proteolysis 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 and Fox, 1998; Smit et al., 2000).

With phytosterol ester addition, the most profound change was found in rancid score. The rancid and off-flavor scores increased dramatically with phytosterol ester addition, also the difference was significant ($p<0.05$). This may be mainly due to phytosterol ester, which is susceptible to lipid oxidation. Even though phytosterol ester addition showed a profound adverse effect on rancid and off-flavor, low amount of phytosterol ester like 4% addition was not significantly different in most of sensory characteristics, even rancid and off-flavor. Therefore, this study showed the possibility of phytosterol ester addition into Cheddar cheese.

It is well known that the average consumption of the spread per day (approximately 20 g in Western Europe) supplemented with between 8-10% plant sterol lowers serum total cholesterol and LDL cholesterol by 8-13% (Weststrate and Meijer, 1998; Hendricks et al., 1999). The blood cholesterol-lowering effect of plant sterols has been investigated in a large number of clinical trials on over 1,800 people, with up to 25 g/d, and durations up to three years. No significant adverse effects have been observed in the decades of medically supervised clinical efficacy testing and the general clinical use of plant sterols.

Since the hypocholesterolemic effect of phytosterol ester which has been reported in experimental animals and man for many years, we need to examine whether the 8% phytosterol ester-added cholesterol-reduced cheese is effective in lowering blood cholesterol in rat. Our present data indicated that total blood cholesterol level was reduced by 18% without difference in HDL cholesterol when rats were fed Cheddar cheese treated with 8% phytosterol (400 mg/day). Based on above results from this study, we suggest that phytosterol ester, which showed the hypocholesterolemic effect, could be added into cholesterol–reduced Cheddar cheese without any profound adverse effect. Thus, this can be used to manufacture more effective dairy products in lowering blood cholesterol.

ACKNOWLEDGEMENTS

This study was supported by Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

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