The Effect of Evening Primrose Oil on Chemical and Blood Cholesterol Lowering Properties of Cheddar Cheese

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ABSTRACT : The present study was carried out to investigate the changes in chemical and sensory properties, and cholesterol lowering effect of evening primrose oil (EPO) addition in cholesterol-reduced Cheddar cheese. The cholesterol removal rate reached 92.07% by β-cyclodextrin in the cheese before EPO addition. The thiobarbituric acid (TBA) value of cholesterol-reduced and EPO-added cheese increased with both ripening time and amount of EPO addition. Addition of 5% EPO resulted in a significant difference in TBA value after 4-week ripening, compared with no addition of EPO. The production of short-chain free fatty acids (FFAs) increased with ripening period in all treatments. From 4 week of ripening, the amounts of short-chain FFA in 3 and 5% EPO-added groups were significantly higher than those in other groups. Among sensory characteristics, rancidity was mostly affected by EPO addition, however, the rancidity value of 1% EPO-added was not significantly different from that of EPO-free and cholesterol-reduced cheese. Also, Cheddar cheese flavor was not profoundly affected by 1% EPO addition in all ripening periods. Total blood cholesterol dramatically decreased from 184.0 to 137.1 mg/dL with 5% EPO-added and cholesterol-reduced cheese following 8 weeks of feeding. The present results indicated that 5% EPO addition resulted in a profound lowering effect on blood total cholesterol with some adverse effects on chemical and sensory properties. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 3 : 450-458)***

Key Words : Evening Primrose Oil, Total Blood Cholesterol, Cheddar Cheese, β-Cyclodextrin

INTRODUCTION

Public concern in cholesterol has increased due to the positive correlation of serum cholesterol concentration to the risk of developing coronary heart disease in addition to high dietary fat and low fiber (Grundy et al., 1982; Gurr, 1992; Law et al., 1994). Although the role of dietary cholesterol in human health has not been yet fully understood, factors to raise serum cholesterol, such as dietary cholesterol are generally considered to be unfavorable.

In addition, death from coronary heart disease is a major cause of mortality in many economically affluent societies like the USA, Australia and the UK (Chanock, 2000). With all its limitations, total plasma cholesterol remains the best single predictor of coronary heart disease risk in a population (Horrobin and Manku, 1983). The predictive value of the total cholesterol may be improved slightly by consideration of the ratio between low-density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol.

When the importance of cholesterol was first appreciated, a number of studies were carried out the effects of various diets on the blood cholesterol levels. Most organizations concerned with cardiovascular health have felt confident enough to recommend substantial increases in polyunsaturated fatty acids (PUFA) intake and decreases in saturated fat intake (Grundy et al., 1982; Expert panel on detection, 2001). The sustained campaign has led to large increases in PUFA intake in some countries, such as the USA, and such increases may in part be responsible for the recent fall in coronary disease mortality (Horrobin and Manku, 1983). Given the importance of the issue and the number of investigators involved, astonishingly little attention has been paid to the question of how PUFA lower plasma cholesterol.

Evening primrose (*Oenothera* spp., particularly *Oenothera* biennis) is of special interest because its seed contains an oil characterized by its content of γ-linolenic acid (all cis-6,9,12-octadecatrienoic acid) (Hudson, 1984). Particular interest attaches to the recent observation that γ-linolenic acid is present in human milk fat (Hudson, 1984). At present, evening primrose oil (EPO) is the most important source of γ-linolenic acid, which is in growing demand for its clinical and pharmaceutical applications as a very active essential fatty acid, and the precursor of prostaglandin E1 and its derivatives (Hudson, 1984). Although the evening primrose plant does not produce a high yield of seeds compared with the well-known commercial oil seeds, it is preferred to other sources of γ-linolenic acid because it is easy to produce and it dose not contain any α-linolenic acid.

Based on above information, we proposed that the effect of PUFA on lowering plasma cholesterol level could be more effective when added in cholesterol-reduced food products. Previously, several studies including our

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Received July 5, 2005; Accepted October 5, 2005

laboratory have indicated that the cholesterol in food including milk, cream and cheese was effectively reduced 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 (Nagatomo, 1985), it has positive attributes when used for cholesterol removal from foods. Therefore, this study was designed to examine the changes of chemical and sensory properties and blood cholesterol lowering effect of EPO-added and 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 with skim milk. Commercial β-CD (purity 99.1%) was purchased from Nihon Shokuhin Cako Co. Ltd. (Osaka, Japan). Evening primrose oil (7% γ-linolenic acid and 80% linoleic acid) was obtained from (Il-dong Pharmaceutical Co., Seoul, Korea). Cholesterol and 5α-cholestane were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and all solvents were gas-chromatographic grade.

Treatment of milk

Bulk raw milk (15 kg) was heated to 40°C and separated into cream and skim milk using a cream separator (Elecrem, Navnes, France). The separated cream was stirred with 10% β-CD at 800 rpm in a blender (Tops, Misung Co., Seoul, Korea) in a temperature-controlled water bath at 20°C for 30 min (Ahn and Kwak, 1999) and then blended with the remaining skim milk at 1,000 psi at 70°C in a single-stage homogenizer (HC 5000, Microfluidics Corp., Newton, MA, USA) (Kwak et al., 2001). Each sample was centrifuged at 166 ×g for β-CD removal. All treatments were run in triplicate. The whole milk was not treated with β-CD, not microfluidized and was used as the control. Cheese milk was pasteurized at 72°C for 17 sec prior to cheese making.

Manufacture of Cheddar cheese

The cheese making process was described by Metzger and Mistry (1994). To manufacture cholesterol-reduced and EPO-added Cheddar cheese, different concentrations of EPO (0, 1, 3, and 5%) were added to cholesterol-reduced milk, and homogenized at 1,000 psi at 50°C in a single stage homogenizer (HC 5000, Microfluidics Corp., Newton, MA, USA). The milk was stored for 12 h in refrigerator as 0 day sample and all treatments were triplicate. After manufacturing, pressed cheeses were weighed, vacuum packaged in a barrier bag, and ripened at 5°C for 32 and 8 weeks in control and experimental cheeses, respectively, to study the changes in chemical, rheological and sensory aspects during ripening. The cheese making was carried out in triplicate on different days using different batches of treatments. Each batch of cheese making was done in triplicate.

Extraction and determination of cholesterol

For the extraction of cholesterol, 1 g of the EPO-added and cholesterol-reduced cheese was placed in a screw-capped glass tube (15 mm×180 mm), and 1 mL of 5α-cholestane (1 mg/mL) was added as an internal standard. The sample was saponified at 60°C for 30 min with 5 mL of 2 M ethanolic potassium hydroxide solution (Adams et al., 1986). After cooling to room temperature, cholesterol was extracted with 5 mL of hexane (Adams et al., 1986). The process was repeated four times. The hexane layers were transferred to a round-bottomed flask and dried under vacuum. The extract was redissolved in 1 mL of hexane and was 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 5890A 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 hold 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 treatments.

Analysis of chemical composition and yield of cheese

Cheese was analyzed for moisture, fat, salt, and protein using the methods of the Association of Official Analytical Chemists (AOAC, 1986). Cheese yield was determined as wt. cheese×100/wt. milk.

Thiobarbituric acid (TBA) test

The products of oxidation were analyzed spectrophotometrically using the TBA test (Hegenauer et al., 1979). The TBA reagent was prepared immediately before use by mixing equal volumes of freshly prepared 0.025 M TBA, which was neutralized with NaOH and 2 M H_3PO_4/2 M citric acid. Reactions of the TBA test were started with 1 g of cheese sample containing EPO into a glass centrifuge tube and mixed thoroughly with 2.5 mL of TBA reagent. The mixture was heated immediately in a boiling water bath

Table 1. Composition of modified AIN-76A purified rodent diet (g/kg)

Ingredient	High fat diet	Fat free diet
Casein, high nitrogen	200	200
Corn starch	150	150
Beef tallow	400	550
Sucrose	95	50
Cholesterol	50	-
Cellulose	50	50
Mineral mix[1]	35	35
Vitamin mix[2]	10	10
Cholic acid	5	-
DL-methionine	3	3
Choline bitartrate	2	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): thiamine·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.

for exactly 10 min and cooled on ice. Ten milliliters of cyclohexanone and 1 mL of 4 M ammonium sulfate were added and centrifuged at 2,490 ×g for 5 min at room temperature. The orange-red cyclohexanone supernatant was decanted and its absorbance at 532 nm measured spectrophotometrically in a 1-cm light path. All measurements were run in triplicate.

Short-chain free fatty acids (FFAs)

The treated cheese sample (1 g) was taken periodically, which was stored for 0, 2, 4, 6, and 8 weeks for expeirimental groups, and for 0, 8, 16, 24 and 32 weeks for control, 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 5880A GC equipped with a flame ionization detector was used. The quantitation of short-chain 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 the 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 short-chain FFA to the peak area of tridecanoic acid as an internal standard. Each FFA was identified by the retention time of standard.

Rheological analysis

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

Sensory analysis

For the sensory test, cholesterol-reduced and EPO-added cheese was ripened at 4°C for 0, 2, 4, 6, and 8 weeks for experimental cheese and for 0, 8, 16, 24, and 32 weeks for control. A ten-trained panels evaluated randomly coded cheese. The intensities of flavor and texture were evaluated on a 7-point scale (1 = none, 4 = moderate and 7 = very strong). Overall preference was scored on a 7-point scale (1 = dislike extremely, 4 = neither like or dislike, and 7 = like extremely). A randomized, balanced, complete block design was used (Cochran and Cox, 1957) that resulted in two replications for all samples.

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 and the dark period from 0300 to 1500. 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 (Table 1). All animals were fed a 40% beef tallow modified rodent diet with 5% cholesterol and 0.5% cholic acid for 8 weeks, and normal rodent diet containing 2 different cheeses for 8 week *ad libitum*. Animals were given free access to tap water via a stainless steel delivery system.

Two different groups were as followed: 1) Cont: control group fed cheese containing no EPO, and 2) EPO: EPO-added group, fed a cholesterol-reduced cheese containing 5% EPO. 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. Total blood cholesterol, triglyceride, and HDL were measured by kit from Fuji Photo Film Co., LTD. (Kanagawa-ken, Japan).

Statistical analysis

Data from the determination of optimum conditions of cheeses, one-way ANOVA (SAS, 1985) was used. The

Table 2. Mean chemical composition of cholesterol-reduced and evening primrose oil (EPO)-added cheese[1]

Component	Cont[2]	Trt[3] A	Trt B	Trt C	Trt D
Moisture	38.3^{ab}	41.2^{a}	35.5^{b}	34.1^{b}	33.4^{c}
Fat	36.0^{b}	34.5^{b}	36.1^{b}	38.1^{a}	40.9^{a}
Protein	28.0^{b}	29.8^{a}	29.3^{a}	28.5^{a}	28.7^{a}
Cholesterol removal	0.0^{b}	91.2^{a}	91.8^{a}	92.1^{a}	93.2^{a}
Yield	10.5^{b}	12.5^{ab}	15.0^{a}	13.0^{a}	14.0^{a}

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

[2] Control: no treatment with β-CD and no EPO addition.

[3] Cream used was treated with 10% β-CD and mixed with skim milk. Trt A, B, C and D were added with 0, 1, 3, and 5% evening primrose oil, respectively.

significance of the results was analyzed by the least significant difference (LSD) test. Difference of $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Cholesterol removal rate and composition

The cholesterol content of control cheese was 102.3 mg/100 g and the cholesterol reduction reached 92.07% with 10% β-CD treatment. This result was in accordance to our previous study, indicating 92.1% cholesterol reduction rate in Cheddar cheese (Kwak et al., 2002). The efficient cholesterol removal over 90% by using β-CD has been proven in our laboratory (Lee et al., 1999; Kwak et al., 2001).

The composition of experimental cheeses are shown in Table 2. The moisture content was the highest in Trt A, which was treated with 10% β-CD and no EPO-added group as 41.2%. EPO-added groups showed lower moisture content, while higher fat content, compared with those in Cont and Trt A. These results indicated that moisture was substituted for fat in Cheddar cheese in EPO-added groups. Also, yield was slightly higher in all β-CD treated groups (Trt A-D), which may be due to moisture or fat substitution in those experimental cheeses.

TBA test during ripening

Polyunsaturated fatty acids like EPO are known to be susceptible to oxidation, resulting in rancidity with the development of an unpleasant odor and flavor. This potential of oxidized off-flavor and taste could be the main problem, which needed to be overcome in EPO-added cheese. Therefore, we determined the effect of EPO oxidation (as measured by the TBA test) in EPO-added cheese and control during 8 and 32 week ripening, respectively, as shown in Figure 1. The treatments were divided into five different groups as follows: (1) Cont: Cheddar cheese without β-CD treatment, (2) Trt A: cheese made by cream treated with 10% β-CD and added 0% EPO, (3) Trt B: cheese made by cream treated with 10% β-CD and added 1% EPO, (4) Trt C: cheese made by cream treated with 10% β-CD and added 3% EPO, and (5) Trt D: cheese made by cream treated with 10% β-CD and added 5% EPO.

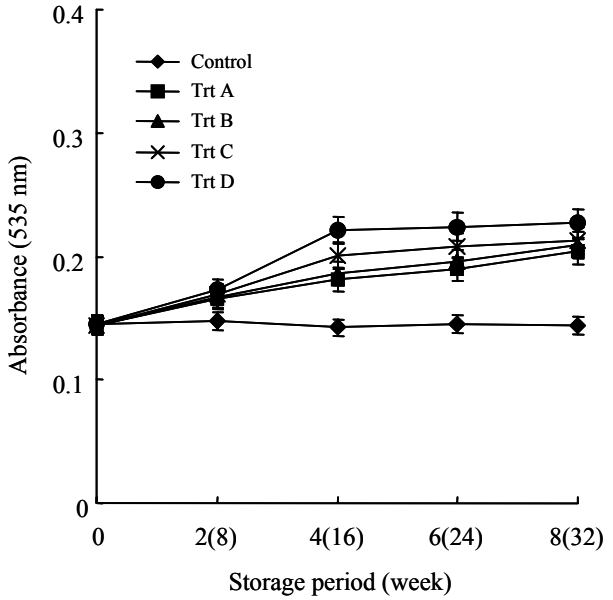

Figure 1. Changes of thiobarbituric acid (TBA) values for evening primrose oil-added and cholesterol-reduced Cheddar cheese ripened at 4°C for 8 weeks. Control, not β-CD treated and EPO added, ripened for 32 weeks; Trt A, cream was treated with 10% β-CD; Trt B-D, 1, 3, and 5% EPO was added in cholesterol-reduced Cheddar cheese, respectively.

In all groups, TBA absorbance increased proportionally to ripening period and amount of EPO addition except for Cont group. TBA absorbance was significantly different between the EPO-added (Trt B-D) and EPO-unadded groups (Cont and Trt A) in all ripening periods. In Trt D (β-CD-treated and 5% EPO-added), TBA absorbance increased slowly from 0.14 to 0.165 for initial 2 weeks, but showed a dramatic increase up to 0.21 and thereafter. Even though 5% EPO was added, the TBA value reached at most 0.21 during 8 week ripening when the TBA was 0.14 in Cont. Therefore, this result indicated that lipid oxidation proceeded more highly in EPO-added cheeses than in EPO-unadded cheeses regardless of β-CD treatment, however, that value may not cause a significantly adverse effect on the quality of Cheddar cheese.

Production of short-chain free fatty acids (FFAs)

The production of short-chain FFAs in control and experimental cheeses ripened for 32 and 8 weeks, respectively, at 5°C is shown in Table 3. Among individual short-chain FFAs, C_4 was lower and C_8 was higher in Cont and Trt A in all ripening periods, respectively, compared with those in other groups with EPO addition ($p<0.05$). Within EPO-added groups in each ripening periods, there

Table 3. Concentrations of short-chain free fatty acids in EPO-added and cholesterol-reduced Cheddar cheese ripened at 7°C[1]

Ripening period (week)	Treatment	Short chain FFAs concentration (ppm)			
		C_4	C_6	C_8	C_{10}
0	Cont[2]	18.2[b]	17.1[a]	20.4[a]	15.5[ab]
	Trt[3] A	19.3[b]	16.9[a]	21.9[a]	20.4[a]
	Trt B	28.3[a]	16.5[a]	11.7[b]	19.1[a]
	Trt C	28.7[a]	16.8[a]	12.1[b]	18.5[a]
	Trt D	27.2[a]	19.7[a]	13.3[b]	20.4[a]
$2^3(8^2)$	Cont	19.1[b]	17.2[a]	21.7[a]	25.9[a]
	Trt A	22.7[b]	17.3[a]	22.1[a]	24.9[a]
	Trt B	30.6[a]	18.5[a]	13.4[b]	21.6[a]
	Trt C	31.4[a]	18.1[a]	13.4[b]	23.5[a]
	Trt D	31.4[a]	20.2[a]	15.2[b]	23.3[a]
4(16)	Cont	18.7[b]	17.8[b]	21.8[a]	29.2[a]
	Trt A	22.9[b]	18.6[ab]	22.9[a]	25.7[a]
	Trt B	31.2[a]	19.1[a]	14.1[b]	22.5[b]
	Trt C	33.7[a]	19.5[a]	14.8[b]	26.7[a]
	Trt D	35.5[a]	23.5[a]	18.2[a]	27.4[a]
6(24)	Cont	19.8[b]	18.1[b]	23.5[a]	25.9[ab]
	Trt A	23.6[b]	18.2[b]	24.2[a]	26.3[a]
	Trt B	32.5[a]	20.5	15.7[b]	21.8[b]
	Trt C	36.6[a]	21.3[ab]	15.8[b]	30.9[a]
	Trt D	35.9[a]	26.3[a]	19.6[ab]	28.8[a]
8(32)	Cont	21.5[b]	18.7[b]	25.3[a]	27.0[a]
	Trt A	27.8[b]	19.6[b]	26.4[a]	29.3[a]
	Trt B	37.9[a]	22.8[b]	16.7[b]	28.3[a]
	Trt C	39.6[a]	24.9[a]	16.6[b]	29.6[a]
	Trt D	40.2[a]	28.7[a]	18.9[b]	30.5[a]

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

[2] Control: no treatment with β-CD and no EPO addition.

[3] Cream used was treated with 10% β-CD and mixed with skim milk. Trt A, B, C, and D were added with 0, 1, 3, and 5% evening primrose oil, respectively.

was no difference in production of short-chain FFAs. The release of butyric acid (C_4) in every periods and caproic acid (C_6) at 8 week ripening contributed to the increase in the total amount of FFAs.

The production of short-chain FFAs increased with longer period of ripening. During 8 weeks, the total release of short-chain FFAs in high amount of EPO-added cheeses (Trt C and D) was not much different from those of other groups at 0 and 2 week ripening. However, from 4 weeks, the total amounts of short-chain FFAs in Trt C and D were significantly higher than those in others. In Cont, the amount of total short-chain FFAs increased from 71.2 to 92.5 ppm during 8 weeks. In 3 and 5% EPO-added cheeses (Trt C and D, respectively), 76.1 and 80.6 ppm of total short-chain FFAs were found at 0 week, and 110.7 to 118.3 ppm were produced at 8 weeks, respectively. The present results indicated that the EPO-added cheeses, especially the high amount above 3%, produced more short-chain FFAs than the EPO-unaddd groups, regardless of β-CD treatment. Another aspects was that even in Cont and Trt A, the total

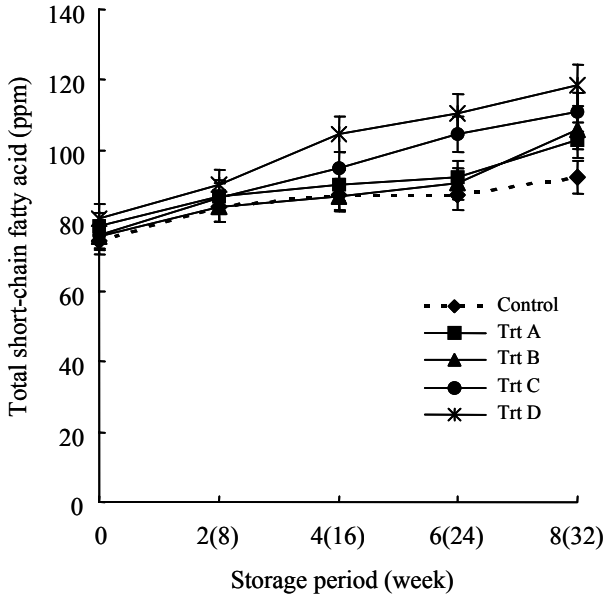

Figure 2. Production of total free fatty acids in evening primrose oil-added and cholesterol-reduced Cheddar cheese ripened at 4°C for 8 weeks. Control, not β-CD treated and EPO added, ripened for 32 weeks; Trt A, cream was treated with 10% β-CD; Trt B-D, 1, 3, and 5% EPO was added in cholesterol-reduced Cheddar cheese, respectively.

FFAs increased with ripening, which indicated that the production of FFAs may be enhanced during storage regardless of amount of EPO addition. The above results indicated that the cheeses made from β-CD-treated cream and EPO-added ripened faster than that with no treatment (control).

The amount of γ-linolenic acid in experimental cheeses decreased for 8 week ripening (data not shown). In Trt B which was added 1% EPO, γ-linolenic acid decreased from 19.02 to 8.59 ppm from initial to 8 week. Similar trend was found in Trt C and D, with a decrease to half of initial amounts.

Rheological characteristics

Effects of EPO addition and β-CD treatment on textural properties of cholesterol-reduced Cheddar cheese are shown in Table 4. In Cont, hardness increased from 16 week ripening, while it kept unchanging in experimental cheeses throughout 8 week ripening. Other properties including elasticity, cohesiveness, and gumminess were not different between Cont and treated groups regardless of ripening periods. Chewiness was lower in control than those in treated cheeses at every ripening period.

Sensory evaluation

The sensory attributes of the EPO-added Cheddar cheese were shown in Table 5. Texture scores were slightly higher in EPO-added cheese than in control in all ripening

Table 4. Textural properties in evening primrose oil-added and cholesterol-reduced Cheddar cheese ripened at 7°C[1]

Ripening period (week)	Treatment	Cont[2]	Trt A[3]	Trt B	Trt C	Trt D
0	Hardness	804.6	1,194.5	1,846.8	2,112.0	1,977.9
	Elasticity	60.7	76.2	90.7	90.5	91.2
	Cohesiveness	53.5	91.4	78.0	86.9	84.5
	Gumminess	201.6	307.3	800.3	889.4	602.6
	Chewiness	122.4	296.0	726.5	730.5	819.7
$2^3(8^2)$	Hardness	1,387.1	1,993.0	2,601.6	2,250.7	2,260.4
	Elasticity	75.8	76.3	95.2	95.1	91.9
	Cohesiveness	62.6	93.2	78.8	87.8	88.5
	Gumminess	1,181.6	416.8	831.3	804.8	752.5
	Chewiness	849.0	401.5	935.1	761.4	889.9
4(16)	Hardness	1041.9	1,251.2	2,031.6	2,453.0	2,177.8
	Elasticity	79.6	86.3	94.0	93.0	92.8
	Cohesiveness	64.1	106.9	81.2	88.9	87.2
	Gumminess	781.2	547.6	1,233.4	676.0	795.4
	Chewiness	622.2	421.6	1,160.1	636.9	733.3
6(24)	Hardness	5,328.2	1,220.6	3797.6	2,362.3	2,057.5
	Elasticity	78.1	84.5	93.9	93.2	97.7
	Cohesiveness	65.7	103.5	80.1	87.2	91.1
	Gumminess	576.3	577.6	1,167.3	795.4	682.1
	Chewiness	402.6	433.0	1,223.4	733.3	666.6
8(32)	Hardness	5,542.2	1,352.5	3479.7	1,994.2	2,426.2
	Elasticity	76.7	85.3	93.8	92.8	93.6
	Cohesiveness	66.6	91.6	81.6	87.3	87.6
	Gumminess	439.5	623.4	1,488.3	974.1	857.2
	Chewiness	337.2	398.8	1,342.8	899.9	714.0

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

[2] Control: no treatment with β-CD and no EPO addition.

[3] Cream used was treated with 10% β-CD and mixed with skim milk.

Trt A, B, C, and D were added with 0, 1, 3, and 5% evening primrose oil, respectively.

periods. The rancidity was not changed much during ripening in all treatments. The rancidity was lower in Trt A and B, compared with those in Trt C and D. There was no difference in rancidity scores in cheese flavor between Trt A and B groups at 0 and 2 weeks, however, those were significantly different from those of Trt C and D in every ripening periods. Especially, Trt C and D, which were added 3 and 5% EPO, respectively, showed a significantly high rancidity score. Bitterness score was significantly higher in all treated groups with longer period of ripening, especially 4 and 6 week periods, however the amount of EPO did not affect. Typical Cheddar cheese flavor increased dramatically in Trt A and B with ripening, while increased slightly, not significantly in Trt C and D. Accordingly, off-flavor increased with the amount of EPO addition, regardless of ripening period.

The significant high scores of rancidity and off-flavor probably resulted from the EPO itself or derived from the fat oxidation. These adverse effects would be more intense with higher amounts of EPO addition and longer ripening period, however, 1% EPO addition did not show a significant difference from Cont group.

Interestingly, the texture score of the treatment groups increased up to 8 weeks, especially in groups containing high amount of EPO. This result indicated that β-CD treatment and EPO addition may improve cheese texture characteristics. Generally, body and texture improved for Cheddar cheeses during ripening as shown in our results. The improved body and texture of cheese may be due to the increased fat globule surface area produced by homogenization.

The overall quality was highly affected by 3 and 5% EPO additions. However, no difference was found between control and 1% EPO added group in every stage of ripening. The higher amount of EPO addition (3 and 5%) resulted in a profound adverse effect on overall quality of cheese throughout the 8 week ripening. Based on those results, small amount of EPO (1%) could be added in Cheddar cheese without any adverse effect on sensory characteristics.

When another study examined the effect of EPO on sensory properties in yogurt (Lee et al., 2006), the most affected properties were rancidity and off-taste, which was accordance with the present study. With EPO addition, the most profound change was found in sensory properties, especially higher amount of EPO (3 and 5%). Most of sensory properties were impaired highly with higher amount of EPO addition, also the difference was significant in every ripening period ($p<0.05$). This may be mainly due

Table 5. Sensory properties in evening primrose oil-added and cholesterol-reduced Cheddar cheese ripened at 7°C[1]

Ripening period (week)	Sensory description	Treatment			
		Cont[2]	Trt[3] B	Trt C	Trt D
0	Rancidity	3.9[b]	4.3[b]	5.0[a]	5.3[a]
	Bitterness	3.9[b]	4.3[a]	4.5[a]	4.1[ab]
	Cheddar flavor	4.0[a]	3.6[ab]	3.4[b]	3.3[b]
	Off-flavor	3.9[c]	4.1[bc]	4.5[b]	5.1[a]
	Texture	4.1[b]	4.2[b]	4.5[a]	4.6[a]
	Overall	6.3[a]	5.3[b]	3.0[c]	3.2[c]
$2^3(8^2)$	Rancidity	3.7[b]	4.4[b]	5.2[a]	5.4[a]
	Bitterness	3.8[a]	4.4[a]	4.3[a]	4.2[a]
	Cheddar flavor	4.2[a]	3.7[ab]	3.5[b]	3.4[b]
	Off-flavor	4.0[b]	4.1[b]	4.6[a]	5.0[a]
	Texture	3.7[b]	4.3[a]	4.4[a]	4.5[a]
	Overall	6.5[a]	5.7[b]	3.4[b]	2.7[b]
4(16)	Rancidity	4.0[c]	4.5[b]	5.2[a]	5.5[a]
	Bitterness	4.3[b]	5.0[a]	4.4[ab]	4.6[a]
	Cheddar flavor	4.3[a]	4.0[a]	3.5[b]	3.4[b]
	Off-flavor	4.0[a]	4.1[a]	3.7[a]	3.5[b]
	Texture	3.5[b]	4.2[a]	4.3[a]	4.5[a]
	Overall	6.2[a]	5.8[bc]	4.3[b]	1.4[c]
6(24)	Rancidity	4.0[c]	4.6[b]	5.2[a]	5.5[a]
	Bitterness	4.2[b]	5.3[a]	4.8[a]	5.1[a]
	Cheddar flavor	4.8[ab]	4.3[a]	3.7[b]	3.6[b]
	Off-flavor	4.0[b]	4.1[b]	4.2[b]	4.6[a]
	Texture	3.4[b]	4.2[ab]	4.2[ab]	4.6[a]
	Overall	6.7[a]	5.7[b]	3.4[b]	2.7[b]
8(32)	Rancidity	4.0[c]	4.7[b]	5.3[ab]	5.7[a]
	Bitterness	4.2[bc]	5.7[a]	4.0[c]	4.6[b]
	Cheddar flavor	5.3[a]	4.6[a]	3.8[ab]	3.6[b]
	Off-flavor	4.0[b]	4.3[b]	4.7[ab]	5.3[a]
	Texture	3.4[b]	4.11[a]	4.2[a]	4.4[a]
	Overall	6.3[a]	5.7[c]	3.4[b]	2.7[c]

[1] Means within raw by the same letter are not significantly different ($p<0.05$). The scale of sensory score: 1 = very slight, 4 = moderate and 5 = very strong. The scale of overall score: 1 = dislike extremely, 4 = neither like nor dislike, and 7 = like extremely.

[2] Control: no treatment with β-CD and no EPO addition. [3] Cream was treated with 10% β-CD and mixed with skim millk. Trt B, C, and D were added with 1, 3, and 5% evening primrose oil, respectively.

Table 6. Effects of experimental diets on food intake and body weight gain[1]

Treatment	Food intake (g/day)	Body weight gain (g/ 8 week)
Cont[2]	26.0[a]	66.5[a]
EPO[3]	26.1[a]	77.2[a]

[1] Rats were fed for 8 weeks. Means within column by the same letter are not significantly different ($p<0.05$).

[2] Control: no treatment with β-CD and no EPO addition.

[3] 5% EPO-added and cholesterol-reduced Cheddar cheese (0.5 g/day).

to EPO itself or susceptibility to lipid oxidation of milk fat. Even though EPO addition showed a profound adverse effect, low amount of EPO like 1% addition was not significantly different in most of sensory characteristics. Therefore, the present study showed the possibility of EPO addition into Cheddar cheese.

Animal study

After 8 week feeding of 40% beef tallow and 5% cholesterol containing diet, the average food intake was 26.0 g/day in both groups during next 8 weeks. There was no difference in body weight gain between Cont (66.5 g) and EPO group (77.2 g) for 8 week period (Table 6).

In blood analysis, after 8 weeks of high cholesterol and high fat diet feeding, the average total serum cholesterol was 153.4 and 184.0 mg/dL in Cont and EPO-added group, respectively (Table 7). During 8 weeks of experimental cheese feeding period, the blood cholesterol fed 5% EPO-added cheese decreased dramatically from 184.0 to 137.1 mg/dL. Comparatively, a slight increase was found in Cont. from 153.4 to 165.8 mg/dL. These results were accordance with those of similar study, which investigated the lowering effect of EPO addition in yogurt (Lee et al., 2005). In that study, 10% EPO group resulted in a lower increase rate in total blood cholesterol without any effect on HDL level. Also, another study (Hwang et al., 2005) indicated that the difference of blood total cholesterol level from initial to final time during 8 weeks, was significantly higher in 10% EPO-added milk. Even though there was no consistent data

Table 7. Effects of evening primrose oil-added and cholesterol-reduced cheddar cheese on the changes of blood total cholesterol, triglyceride, and high-density lipoprotein in rats fed for 8 weeks[1] (mg/dL)

Treatment	Total cholesterol		Triglyceride		High-density lipoprotein	
	Initial	Final	Initial	Final	Initial	Final
Cont[2]	153.4[a]±17.9	165.8[a]±20.7	64.5[a]±8.2	63.5[a]±7.5	32.3[a]±7.0	31.4[a]±9.1
EPO[3]	184.0[a]±18.3	137.1[b]±15.9	53.9[b]±5.0	60.8[a]±7.8	37.4[a]±8.5	38.8[a]±7.9

[1] Means in a column with the same letter are not significantly different ($p<0.05$).
[2] Control: no treatment with β-CD and no EPO addition.
[3] 5% EPO-added and cholesterol-reduced Cheddar cheese (0.5 g/day).

about the effect of EPO addition in various dairy products, these results may indicate the lowering trend of total blood cholesterol.

In triglyceride (TG) data, no difference was found in Cont, while slight increase in EPO-added rats. Also, there was no difference in blood high density lipoprotein (HDL) between groups. The present data indicated that EPO addition showed a total cholesterol lowering effect in rat, which has been proposed by several studies (Horrobin and Manku, 1983; Sugano et al., 1986; Lee et al., 2006).

Several studies have investigated the effect of γ-linolenic acid on lowering cholesterol property in human and animal. Sugano et al. (1986) have indicated a significant hypocholesterolemic efficacy of γ-linolenic acid in rat. Horrobin and Manku (1983) found that human γ-linolenic acid, as EPO has an approximately 170 times greater cholesterol-lowering ability than linoleic acid, indicating that linoleic acid is converted to γ-linolenic acid to exert its hypocholesterolemic effects. Huang et al. (1984) fed high-cholesterol diets to essential fatty acid-deficient rats and found that plasma cholesterol levels rose sharply when the dietary fat was safflower oil, but not at all with EPO.

CONCLUSIONS

The present study designed to examine the effect of EPO addition and β-CD treatment on total blood cholesterol, and the changes of chemical and sensory properties during Cheddar cheese ripening. As expected, TBA value increased with higher amount of EPO addition in cholesterol-reduced Cheddar cheese. During ripening period, the production of short-chain FFAs increased significantly in 3 and 5% EPO-added groups compared with those of others. Most of sensory characteristics were not significantly different between control and 1% EPO-added group, while high amount of EPO addition (3 and 5%) resulted adverse effects on cholesterol-reduced Cheddar cheese. EPO addition (5%) showed a lowering effect on blood total cholesterol, not on triglyceride and high-density lipoprotein. Therefore, the present study indicated that EPO addition may result in lowering effect on total blood cholesterol level, even though sensory properties were affected adversely with high amount of EPO. Therefore, it may be considered as the first evidence that provides the possibility of EPO-added and cholesterol-reduced Cheddar cheese.

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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