Properties of Cholesterol-reduced Butter and Effect of Gamma Linolenic Acid Added Butter on Blood Cholesterol

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ABSTRACT : The present study was carried out to develop cholesterol-reduced and gamma linolenic acid (GLA)-added butter and to examine the changes in chemical and sensory properties, and cholesterol lowering effect of GLA addition. The cholesterol removal rate reached 93.2% by β-cyclodextrin in butter before GLA addition. The thiobarbituric acid value of cholesterol-reduced and GLA-added butter increased slowly up to 4 week and plauteaued thereafter. TBA value was significantly increased with 2% GLA addition, compared with no GLA addition. The production of short-chain free fatty acids (FFA) increased with storage in all treatments. From 4 weeks storage, the amount of short-chain FFA in 2% GLA-added group was significantly higher than those in other groups. Among sensory characteristics, color, greasiness and overall acceptability were mostly affected by GLA addition, however, the rancidity value of 2% GLA addition was significantly different from those of control and GLA-unadded and cholesterol-reduced butter at 0, 6 and 8 week storage. Among groups, no difference was found in texture in all storage periods. The smallest increase of total blood cholesterol in rats was found in the group fed 2% GLA-added and cholesterol-reduced butter for 8 week, compared with that in controls. The present results showed the possibility of cholesterol-reduced and GLA-added butter development without much difference in chemical, rheological and sensory properties, and indicated a slow increase effect on blood total cholesterol in rats. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 11 : 1646-1654)***

Key Words : Cholesterol-reduced Butter, Gamma Linolenic Acid, Blood Total Cholesterol, β-Cyclodextrin

INTRODUCTION

In the past decades, 44% of Americans have changed habits to lower their cholesterol level (Authur, 1990). A 1990 Gallup poll reported that 60% of those people surveyed said they eliminated butter from their diet. Per capita sales of butter dropped to 3.5 pounds in 1989 (MIF, 1990) and in-home consumption of butter was down 13% in 1989 according to the National Milk Marketing Board (Kuhn, 1990).

This shift in eating habits has caused a dramatic increase in no-, low and reduced-cholesterol, and fat products available in the marketplace (Best, 1989). Futhermore, research into development of products of this type is the highest priority for food companies (Best, 1989). “Cholesterol-free” has become a labeling designation sought by many food manufacturers in the US (Kosikowski, 1990). Sales of low-cholesterol and low-fat foods are estimated to exceed $33 billion by 1991 (Schroder and Baer, 1991). In addition, a manufacturer in New Zealand reported trial production of reduced-cholesterol butter (Wilson, 1990) and consumer and industry demand has created the interest to manufacture reduced-cholesterol butter.

Ingestion of diets containing saturated fatty acids like butter elevates cholesterol concentrations on plasma of humans and experimental animals (McNamara, 1993). The hypercholesterolemic effect of dietary saturated fatty acids generally is thought to be due to lauric, myristic and palmitic acid (Hayes et al., 1991; McNamara, 1993). Butter is one of the high saturated fat products because it contains 13 major fatty acids (Lai et al., 1995) and is unique because it contains approximately 25% (moles per 100 mol) short- and medium-chain fatty acids. When butter is the major source of dietary fat (20 to 40% of energy), it is clearly hypercholesterolemic (Berner, 1993). However, questions remain about the effects of butter relative to other dietary saturated fats and in the context of typical, mixed fat human diet (Berner, 1993).

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 (GLA, all cis-6:9:12-octadecatrienoic acid) (Hudson,

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1984). Particular interest attaches to the recent observation that GLA is present in human milk fat (Hudson, 1984). At present, GLA 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).

Despite numerous demonstrations of the cholesterol-lowering property of PUFA like GLA, the precise mechanism of their abilities is still not fully understood (Sugano et al., 1986; Ihara-Watanabe et al., 1999). Fairly large amounts of PUFA are required to produce a substantial and meaningful reduction of plasma cholesterol.

Also, like many epidemiological surveys of dietary habits in diverse human populations, studies in the laboratory rat have shown that diets rich in PUFA markedly reduce the incidence and severity of cardiac dysfunction (Chanock et al., 1991). The addition of adequate amounts of PUFA to the diet of humans consistently lowers plasma cholesterol levels (Haung et al., 1984). The mechanism remains uncertain but the evidence is compelling enough to allow bodies such as the American Heart Association to recommend a general increase in dietary polyunsaturates.

Based on above information, we proposed that the effect of GLA on lowering plasma cholesterol level could be more effective when added in cholesterol-reduced food products. Previously, several studies including our laboratory have been indicated that the cholesterol in food, including milk, yogurt, cream and cheese, was effectively reduced by β-cyclodextrin (β-CD) (Oakenfull and Sidhu, 1991; Kwak et al., 2002; Shim et al., 2003; Hwang et al., 2005; Kim et al., 2005; Kwak et al., 2005; Lee et al., 2005). 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 develop the cholesterol-reduced and GLA-added butter and to examine the effects of GLA addition on chemical and sensory properties of butter, and blood cholesterol level in rat.

MATERIALS AND METHODS

Materials

Separated cream (36% milk fat) was obtained from Binggare Dairy Plant (Kyonggi-do, Korea). Commercial β-CD (purity 99.1%) was purchased from Nihon Shokuhin Cako Co. Ltd. (Osaka, Japan). Gamma linolenic acid (GLA, 80%) 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 cream

Bulk raw cream (15 kg) was stirred with 10% β-CD at 400 rpm with a blender (Tops, Misung Co., Seoul, Korea) in a temperature-controlled water bath at 5°C for 10 min (Ahn and Kwak, 1999). Then, it was centrifuged at 166×g for β-CD removal. All treatments were run in triplicate. The whole cream was not treated with β-CD as the control. The cream was pasteurized at 75°C for 15 sec.

Manufacture of butter

Three different experimental butters were manufactured as followed; 1) Control: made by cream with no β-CD treated and no GLA added, 2) Trt A: cream with 10% β-CD treated and no GLA added, and 3) Trt B: cream with 10% β-CD treated and 2% GLA added.

The treated cream was stored overnight in refrigerator as 0 day sample. Raw 36% milk fat cream was tempered to 13-14°C and churned using a mechanical butter churn (MANVELB A20, Elecrem, Navnes, France) until butter granule were visible. Buttermilk was drained and the butter was washed in 10°C water. Then, 1% salt was added and the butter was pressed. After manufacturing, pressed butter was weighed, placed in suitable container and stored at 5°C for 8 weeks to study the changes in chemical, rheological and sensory aspects, and freezed for animal study. The butter making was carried out in triplicate on different days using different batches of treatments. Each batch of butter making was done in triplicate.

Extraction and determination of cholesterol

For the extraction of cholesterol, 1 g of butter sample 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 chromatography (Palo Alto, CA, USA) equipped with a flame ionization detector. The temperatures of injector and detector were 270 and 300°C, respectively. The oven temperatures were programmed from 200 to 300°C at 10°C/min and 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 butter×100/amount of

Table 1. Composition of modified AIN-76A purified rodent diet (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.

cholesterol in Control). Cholesterol determination for control was averaged with each batch of treatments.

Analysis of chemical composition and yield of cheese

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

Thiobarbituric acid (TBA) test

Oxidation products 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 1 N NaOH solution and 2 M H_3PO_4/2 M citric acid. Reactions of the TBA test were started with 1 g of butter sample containing GLA 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 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 acid (FFA)

The treated butter sample (1 g) was taken periodically, which was stored for 0, 2, 4, 6 and 8 weeks for experimental groups 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. (2002). 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 x elasticity, respectively.

Sensory analysis

For the sensory test, cholesterol-reduced and GLA-added butter was stored at 4°C for 0, 2, 4, 6 and 8 weeks. A ten-trained panel evaluated randomly coded butter. The texture, color, rancidity, acidity and greasiness were evaluated on a 7-point scale (1 = very weak, 4 = moderate, 7 = very strong). Overall preference was scored on a 3-point scale (1 = like extremely, 2 = neither like or dislike and 3 = dislike extremely). A randomized, balanced, complete block design was used (Cochran and Cox, 1957) that resulted in two replications for all samples.

Animals and diets

Eighteen 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

Table 2. Mean chemical composition of cholesterol-reduced and gamma linolenic acid (GLA)-added butter

Component	Control[1] (%)	Trt A[2] (%)	Trt B[3] (%)
Fat	77.3	80.6	78.2
Moisture	18.8	16.9	19.6
Protein	1.4	1.3	1.0

[1] Control: no β-CD treatment and no GLA addition.
[2] Trt A: cream was treated by 10% β-CD and no GLA addition.
[3] Trt B: cream was treated by 10% β-CD and 2% GLA addition.

tallow modified rodent diet with 5% cholesterol and 0.5% cholic acid for 6 weeks, and normal rodent diet containing 2 different butter for 6 week *ad libitum*. Animals were given free access to tap water via a stainless steel delivery system.

Two different groups were as followed: 1) Control: fed butter containing no GLA, and 2) GLA: fed a cholesterol-reduced butters containing 2% GLA. 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 butter, one-way ANOVA (SAS, 1985) was used. The 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

Rate of cholesterol removal and composition

The cholesterol content of control butter was 210 mg/100 g and the cholesterol reduction reached 92.7-93.2% 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 (Kwak et al., 2003; Shim et al., 2003; Hwang et al., 2005; Kim et al., 2005; Kwak et al., 2005; Lee et al., 2005).

The moisture content was the highest in Trt B, which was treated with 10% β-CD and 2% GLA-added group as 19.6% (Table 2). Trt A group showed slightly lower moisture content, while higher fat content, compared with those in Control and Trt B. These results indicated that fat was substituted for moisture in Trt A butter in 10% β-CD treated and 0% GLA added group. Also, yield after manufacture was significantly lower in Trt A, which may be due to moisture or fat substitution (data not shown).

TBA test during storage

Polyunsaturated fatty acids like GLA 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 GLA-added butter. Therefore, we determined the effect of GLA oxidation (as measured by the TBA test) in GLA-added butter and Control during 8 week storage, as shown in Figure 1. During storage, TBA absorbance increased slightly in all groups regardless of GLA addition. However, TBA absorbance was significantly different between the GLA-added (Trt B) and GLA-unadded groups (Control and Trt A) in every storage periods. In Trt B (β-CD-treated and 2% GLA-added), TBA absorbance increased slowly from 0.14 to 0.18 for initial 4 weeks, and maintained steadily up to 8 weeks. Even though 2% GLA was added, the TBA value reached at most 0.18 during 8 week storage when the TBA was 0.09 in Control. Therefore, this result indicated that lipid oxidation proceeded more highly in GLA-added butter than in GLA-unadded butter regardless of β-CD treatment, however, that value may not cause a significantly adverse effect on quality of butter.

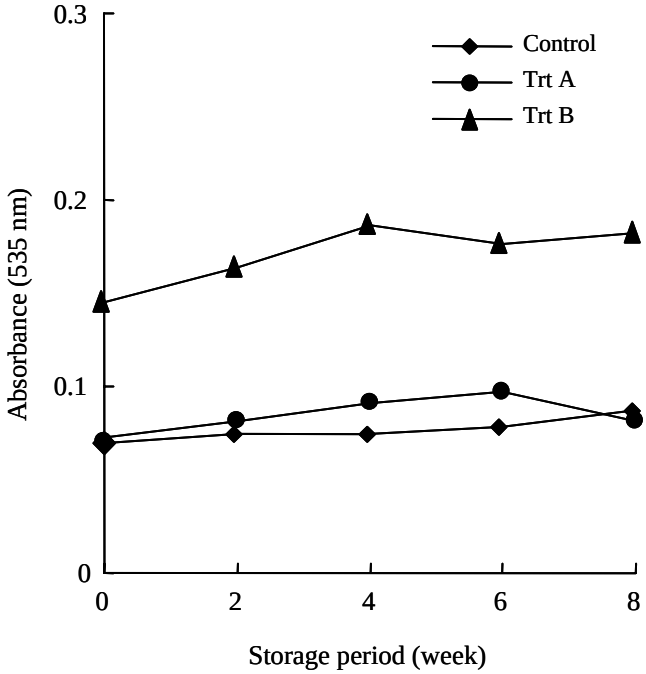

Figure 1. Changes of thiobarbituric acid values for cholesterol-reduced and gamma linolenic acid (GLA)-added butter stored at 4°C for 8 weeks[1]. Control, not β-CD treated and GLA added; Trt A, cream was treated with 10% β-CD and GLA-unadded; Trt B, 2% GLA was added in cholesterol-reduced butter.

Production of short-chain free fatty acids (FFA)

The production of short-chain FFA in Control and experimental butters stored for 8 weeks at 4°C is shown in Table 3. Among individual short-chain FFA, C_4 and C_8 were slightly higher in Trt A and B, compared with those in

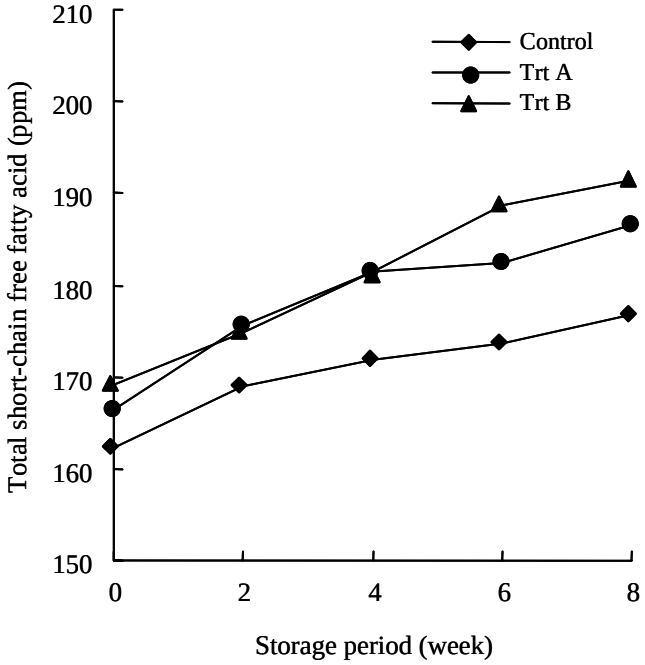

Figure 2. Production of total short-chain free fatty acid in cholesterol-reduced and gamma linolenic acid (GLA)-added butter stored 4°C for 8 weeks[1]. Control, not β-CD treated and GLA added; Trt A, cream was treated with 10% β-CD and GLA-unadded; Trt B, 2% GLA was added in cholesterol-reduced butter.

Control ($p<0.05$) throughout storage period. Within GLA-added groups in all periods, there was no difference in productions of short-chain FFA. The releases of butyric acid (C_4) and caproic acid (C_{10}) contributed to the increase in total amount of FFA.

The production of short-chain FFA increased with storage period. During 8 weeks, the production of short-chain FFA was slightly higher in β-CD-treated butter (Trt A and B) compared with that in Control at 1 and 8 week storage, however, there was no difference in short-chain FFA release at other periods (Figure 2). In Control, the amount of total short-chain FFA increased from 162.3 to 176.7 ppm during 8 weeks. In 0 and 2% GLA-added butter (Trt A and B, respectively), 175.5 and 169.2 ppm of total short-chain FFA were found at 0 week, and 186.6 to 191.5 ppm were produced at 8 weeks, respectively. The present results indicated that the GLA-added butter produced more amount of short-chain FFA than the GLA-unadded groups, regardless of β-CD treatment. Another aspects was that even in Control and Trt A, total FFA increased with storage, which indicated that the production of FFA may be enhanced during storage regardless of amount of GLA addition.

Rheological characteristics

Effects of GLA addition and β-CD treatment on textural properties of cholesterol-reduced butter are shown in Table 4. Hardness increased dramatically in Control, while increased slowly in experimental groups throughout 8 week storage. In Control, hardness was significantly lower than those in Trt A and B. Elasticity and cohesiveness were significantly higher in Trt A, compared with other groups up to 6 weeks and 2 weeks, respectively, Gumminess and chewiness were not profoundly different between Control and treated groups regardless of storage periods.

Table 3. Concentrations of short-chain free fatty acid in cholesterol-reduced and gamma linolenic acid (GLA)-added butter stored at 4°C for 8 weeks[1]

Storage period (week)	SFFA concentration (ppm)	Treatment		
		Control[2]	Trt A[3]	Trt B[4]
0	C_4	50.7[a]	52.7[a]	53.2[a]
	C_6	13.8[a]	12.9[a]	13.8[a]
	C_8	10.5[a]	11.4[a]	12.3[a]
	C_{10}	87.3[b]	89.5[a]	89.9[a]
	Total	162.3[b]	166.5[a]	169.2[a]
2	C_4	52.5[b]	55.4[a]	55.3[a]
	C_6	13.9[a]	13.8[a]	14.5[a]
	C_8	11.1[a]	12.7[a]	12.5[a]
	C_{10}	91.5[a]	93.7[a]	92.5[a]
	Total	169.0[b]	175.6[a]	174.8[a]
4	C_4	53.1[b]	57.1[a]	56.9[a]
	C_6	13.7[a]	14.2[a]	15.3[a]
	C_8	11.9[b]	13.4[a]	14.1[a]
	C_{10}	93.1[b]	96.8[a]	95.2[a]
	Total	171.8[b]	181.5[a]	181.5[a]
6	C_4	53.9[b]	57.2[a]	58.8[a]
	C_6	14.1[b]	14.6[b]	16.8[a]
	C_8	12.2[b]	13.3[b]	15.6[a]
	C_{10}	93.5[b]	97.3[a]	97.4[a]
	Total	173.7[b]	182.4[a]	188.6[a]
8	C_4	54.7[b]	58.5[a]	59.7[a]
	C_6	14.5[a]	15.3[a]	16.5[a]
	C_8	13.3[a]	13.9[a]	15.8[a]
	C_{10}	94.2[b]	98.9[a]	99.5[a]
	Total	176.7[b]	186.6[a]	191.5[a]

[1] Means within row by the same letter are not significantly different ($p<0.05$).
[2] Control: no β-CD treatment and no GLA addition.
[3] Trt A: cream was treated by 10% β-CD and no GLA addition.
[4] Trt B: cream was treated by 10% β-CD and 2% GLA addition.

Sensory evaluation

The sensory attributes of the GLA-added butter were shown in Table 5. Texture score was not significantly different among groups in all storage periods. However, color among three different treatments was significantly different throughout storage and was the highest in Trt B and lowest in Control. The rancidity was slightly higher in Trt B, compared with those in Control and Trt A. There was no difference in acidity among groups, except for 0 week. Greasiness score was significantly higher in Trt B than those in Control and Trt A, except for 4 and 6 weeks.

Table 4. Textural properties in cholesterol-reduced and gamma linolenic acid (GLA)-added butter stored at 4°C for 8 weeks[1]

Storage period (week)	Treatment	Hardness	Elasticity	Cohesiveness	Gumminess	Chewiness
0	Control[2]	457.1[c]	47.4[b]	27.2[b]	17.6[a]	13.6[a]
	Trt A[3]	666.2[a]	86.6[a]	46.8[a]	21.1[a]	18.3[a]
	Trt B[4]	596.0[b]	43.3[b]	24.1[b]	18.0[a]	13.5[a]
2	Control	515.7[c]	57.0[b]	36.7[b]	21.7[a]	16.7[a]
	Trt A	683.2[a]	93.3[a]	46.3[a]	20.4[a]	19.0[a]
	Trt B	564.1[b]	39.2[b]	32.1[b]	24.2[a]	21.7[a]
4	Control	683.1[a]	63.8[b]	41.8[a]	26.2[a]	19.6[a]
	Trt A	686.5[a]	96.0[a]	50.6[a]	30.2[a]	20.0[a]
	Trt B	628.1[b]	45.4[c]	43.6[a]	25.3[a]	21.5[a]
6	Control	693.2[b]	76.5[b]	48.3[a]	25.1[a]	20.5[a]
	Trt A	730.5[a]	97.2[a]	51.2[a]	27.4[a]	23.7[a]
	Trt B	665.8[c]	61.2[b]	44.7[a]	25.2[a]	21.4[a]
8	Control	728.1[b]	92.2[a]	52.9[a]	25.7[a]	23.9[a]
	Trt A	761.0[a]	95.0[a]	54.9[a]	28.5[a]	27.3[a]
	Trt B	749.4[a]	88.0[a]	48.1[a]	23.9[a]	21.0[a]

[1] Means within column by the same letter are not significantly different ($p<0.05$).
[2] Control: no β-CD treatment and no GLA addition.
[3] Trt A: cream was treated by 10% β-CD and no GLA addition.
[4] Trt B: cream was treated by 10% β-CD and 2% GLA addition.

Table 5. Sensory characteristics for cholesterol-reduced and gamma linolenic acid (GLA) added butter stored at 4°C for 8 weeks[1]

Storage period (week)	Treatment	Sensory description					
		Texture	Color	Rancidity	Acidity	Greasiness	Overall acceptability[2]
0	Control[3]	4.0[a]	4.0[c]	4.0[b]	4.0[b]	4.0[b]	1.4[b]
	Trt A[4]	4.2[a]	4.7[b]	3.4[b]	4.1[b]	4.1[b]	1.5[b]
	Trt B[5]	4.0[a]	5.5[a]	4.8[a]	4.6[a]	5.5[a]	3.0[a]
2	Control	4.0[a]	4.1[c]	4.8[a]	4.1[a]	4.5[b]	1.7[b]
	Trt A	3.7[a]	4.8[b]	4.5[a]	4.2[a]	4.6[b]	1.4[b]
	Trt B	3.7[a]	5.5[a]	4.9[a]	4.3[a]	5.7[a]	2.9[a]
4	Control	4.4[a]	4.2[c]	4.2[a]	4.4[a]	4.9[a]	1.2[c]
	Trt A	4.4[a]	5.0[b]	4.5[a]	4.4[a]	5.0[a]	1.8[b]
	Trt B	4.2[a]	6.0[a]	4.9[a]	4.7[a]	6.0[a]	3.0[a]
6	Control	4.4[a]	4.2[c]	4.4[b]	4.4[a]	5.0[b]	1.4[b]
	Trt A	4.2[a]	4.8[b]	4.6[b]	4.4[a]	5.2[b]	1.6[b]
	Trt B	4.2[a]	6.3[a]	5.3[a]	4.8[a]	6.5[a]	3.5[a]
8	Control	4.0[a]	4.2[c]	4.6[b]	4.5[a]	5.0[b]	1.5[b]
	Trt A	4.4[a]	5.0[b]	4.6[b]	4.6[a]	5.3[b]	1.5[b]
	Trt B	4.2[a]	6.5[a]	5.5[a]	4.8[a]	6.8[a]	4.0[a]

[1] Means within column by the same letter are not significantly different ($p<0.05$).
The scale of sensory score: 1 = very slight, 2 = slight, 3 = slight-moderate, 4 = moderate, 5 = moderate-strong, 6 = strong and 7 = very strong.
[2] The scale of overall score; 1 = like extremely, 4 = neither like nor dislike and 7 = dislike extremely.
[3] Control: no β-CD treatment and no GLA addition
[4] Trt A: cream was treated by 10% β-CD and no GLA addition.
[5] Trt B: cream was treated by 10% β-CD and 2% GLA addition.

Similarly, overall acceptability score was significantly higher in Trt B, compared with those in Control and Trt A. The overall acceptability was highly affected by 2% GLA addition. However, no difference was found between Control and Trt A, which was only β-CD-treated group in every periods of storage.

Similar study (Kim et al., 2005) indicated that the higher amount of 7% containing EPO addition (3 and 5%) resulted in a profound adverse effect on overall quality of Cheddar cheese throughout the 8 week ripening. However, small amount of EPO (1%) did not result in any adverse effect on sensory characteristics.

The significant high scores of rancidity and overall acceptability in β-CD-treated and 2% GLA-added butter probably resulted from the EPO itself or derived from the fat oxidation. Interestingly, there was no difference in texture score between experimental butter and Control, and color was even more yellowish in EPO-added group, which may be more preferred by consumers in retail.

When another study examined the effect of EPO on

Table 6. Effects of experimental diets on the change of blood triacylglycerol, total cholesterol and high density lipoprotein in rats fed for 6 weeks[1] (mg/dL)

Treatment	Total cholesterol		Triglyceride		High-density lipoprotein	
	Initial	Final	Initial	Final	Initial	Final
Control[2]	151.2±37.9[a]	230.0±47.7[a]	35.5±14.0[a]	52.0±24.4[a]	37.5±7.0[a]	36.5±12.0[b]
Trt A[3]	153.8±34.4[a]	201.8±24.2[b]	30.2±8.2[a]	27.5±5.0[b]	37.8±3.1[a]	43.8±11.0[a]
Trt B[4]	144.4±37.7[b]	190.5±36.7[b]	22.2±7.8[b]	29.0±7.6[b]	34.7±8.5[a]	38.5±9.1[b]

[1] Means within column by the same letter are not significantly different ($p<0.05$).
[2] Control: no β-CD treatment and no gamma linolenic acid (GLA) addition (0.5 g/day).
[3] Trt A: cream was treated by 10% β-CD and no GLA addition (0.5 g/day).
[4] Trt B: cream was treated by 10% β-CD and 2% GLA addition (0.5 g/day).

sensory properties in yogurt (Lee et al., 2005), 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 periods ($p<0.05$). This may be mainly due 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 butter.

Animal study

After 6 week feeding of 40% beef tallow and 5% cholesterol containing diet, the average food intake was about 22 g/day in groups during next 6 weeks. There was no difference in body weight gain among groups, which were Control (125.65 g), Trt A (120.43 g) and Trt B group (115.17 g) for 6 week period.

In blood analysis, after 6 week of high cholesterol and high fat diet feeding, the average total serum cholesterol were 151.2, 153.8 and 144.5 mg/dL in Control, Trt A (β-CD-treated and 0% GLA added) and Trt B (β-CD-treated and 2% GLA-added) groups, respectively (Table 6). During next 6 week of experimental butter feeding, the blood cholesterol fed 2% GLA-added butter increased from 144.4 to 190.5 mg/dL. Comparatively, a dramatic increase was found in Control from 151.2 to 230.0 mg/dL. There was no difference in total cholesterol between Trt A and B. Based on these results, the increase in total cholesterol was significantly greater in Control group although those in both groups which were fed the cholesterol-reduced or GLA-added butter also increased during the feeding periods. These are not accordance to other similar studies and probably due to high fat and high cholesterol aspects of butter.

Similar study 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 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, higher increase was found in Control, compared with those of Trt A and B. Also, there was no difference in blood high density lipoprotein (HDL) between groups. The present data indicated that GLA addition showed a slower increase effect in blood total cholesterol in rat, even though several other studies have been proposed the lowering effect of EPO (Horrobin and Manku, 1983; Sugano et al., 1986; Lee et al., 2005).

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. Haung 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 develop the cholesterol-reduced and GLA-added butter and to examine the effect of GLA addition and β-CD treatment on total blood cholesterol, and the changes of chemical and sensory properties during storage. As expected, TBA value increased with GLA addition in cholesterol-reduced butter. During storage, the production of short-chain FFA increased significantly in GLA-added group, compared with those of Control and cholesterol-reduced and GLA-unadded butter. Color and overall acceptability among several sensory characteristics were significantly different in cholesterol-

reduced and GLA-added group (Trt B) from those in Control and cholesterol-reduced and GLA-unadded group (Trt A). Total blood cholesterol increased slowly and triglyceride level decreased significantly in rat fed cholesterol-reduced and GLA-added butter. The present study indicated that GLA addition in butter did not enhance the total blood cholesterol level and did not affect sensory properties profoundly. Also, it may be considered as first evidence that provides the possibility of GLA-added and cholesterol-reduced butter.

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