Optimization of β-Cyclodextrin Recycling Process for Cholesterol Removal in Cream

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ABSTRACT : This study was designed to find optimum conditions of four different factors (ratio of solvent to cholesterol-β-cyclodextrin complex, mixing speed, mixing temperature, and mixing time) for cholesterol dissociation in cream. Using the ratio of 6 to 1 (solvent to the complex) showed the highest cholesterol dissociation rate (82.50%) when mixed at 100 rpm at 50℃ for 1 h. Mixing speed did not significantly affect the cholesterol dissociation. Also, mixing time appeared to be insignificant. The optimum mixing temperature was 50℃, and mixing at 40℃ resulted in a significantly lower rate, compared with that at 50℃. In a subsequent experiment, using recycled β-cyclodextrin only showed 75.07% of cholesterol removal in cream, while the mixture of recycled to unused β-CD with the ratio of 6 to 4 increased cholesterol removal to 95.59%, which is highly close to that of 100% unused β-CD. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 4 : 548-552*)

Key Words : β-Cyclodextrin, Recycling, Cholesterol Removal, Cream

INTRODUCTION

Cholesterol has been removed from dairy, meat and egg products because most consumers are concerned about the excessive intake of cholesterol causing coronary heart disease (Grundy and Brheimer, 1982 and Gurr et al., 1992). Adsorption of cholesterol with β-cyclodextrin (β-CD) was found to be the most effective method to reduce the amount of cholesterol from these animal food products (Micich et al., 1990; Oakenfull and Sidhu, 1991; Makoto et al., 1992; Smith et al., 1995; Yen and Tsui, 1995; Lee et al., 1999 and Ahn and Kwak, 1999). However, commercial β-CD is expensive and waste in the process results in an environmental problem. Therefore, β-CD recycling needs to be emphasized.

Several methods have been developed for the dissociation of cholesterol from cholesterol-β-CD complex. Using a hydrogen bond inhibitor (Wen Shieh, 1994), resin (Nobnyuki et al., 1995), heating (Mentink et al., 1990), sodium chloride (Wen Shieh et al., 1995) and organic solvent (Comini and Mentink, 1991; Oakenfull and Sihdu, 1991; Oakenfull et al., 1991 and Yoo et al., 2000) are examples. Among those, the method using organic solvent is recognized as the simplest, most economical, and easiest for industrial application.

Therefore, we designed this study to determine optimum conditions (kind of solvent, mixing time, mixing temperature and speed) for β-CD recycling from cholesterol-β-CD complex, and to enhance the possibility of using the recycled β-CD in cholesterol removal in cream.

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MATERIALS AND METHODS

Materials

Cream (36% milk fat) was obtained from Samik Dairy Company (Seoul, Korea) and stored at -20℃ until use. Commercial β-CD (purity 99.1%) was purchased from Nihon Shokuhin Kaku Co. Ltd. (Osaka, Japan). Cholesterol and 5α-cholestane were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Solvents were acetic acid and butanol (purity 99.00%).

Preparation of cholesterol-β-CD complex

To obtain cholesterol-β-CD complex, 10% β-CD was added into cream and blended at 50℃ for 30 min. it was then centrifuged (444×g) for 10 min to precipitate the cholesterol-β-CD complex (Ahn and Kwak, 1999). The supernatant was separated to quantify the cholesterol content and the precipitated complex was lyophilized for 6 h, and stored at a refrigerated temperature.

Selection of solvent

To find an efficient solvent for dissociation of cholesterol from the complex, 5 different kinds of solvent mixtures were tested: 1) acetic acid:butanol=3:1, 2) chloroform:butanol=2:1, 3) hexane:butanol=2:1, 4) chloroform:ethanol=2:1, and 5) hexane:isopropanol=3:2. Five g of cholesterol-β-CD complex, containing a known cholesterol amount was stirred with 30 ml of the above solvent mixtures at 100 rpm at 50℃ for 2 h, then cooled at room temperature, and centrifuged at 444×g for 10 min. The supernatant containing the cholesterol was decanted and used for cholesterol

determination.

Solvent for cholesterol dissociation

To find the effective ratio of acetic acid and butanol on cholesterol-β-CD complex dissociation in cream, five different ratios (4:1, 3:1, 2:1, 1:1, and 1:2) were applied. The ratio of cholesterol-β-CD complex to solvent was 1 to 6 and the mix was stirred in a temperature-controlled waterbath at 100 rpm at 50℃ for 2 h, cooled at room temperature, and centrifuged at 444×g for 10 min. The supernatant containing the cholesterol was decanted and used for cholesterol determination.

Cholesterol dissociation and determination

To examine the effect of each combination of four factors on cholesterol dissociation, the complex was placed in a screw-capped glass tube (180 mm×15 mm). The four factors were applied in this process, were ratio of complex to solvent (w/v), mixing speed, mixing temperature and mixing time. After processing, each mixture was centrifuged at 444×g for 5 min at 5℃ in HMR-220IV Centrifuge (Hanil Industrial Co., Seoul, Korea). β-CD was then precipitated and supernatant was decanted.

Five mL of supernatant was placed in a screw-capped glass tube (180 mm×15 mm) and 250 μL of 5α-cholestane (1 mg/mL) was added as an internal standard. The sample was saponified with 5 mL of 2 M ethanolic KOH solution at 60℃ for 30 min (Adams et al., 1986 and Lee et al., 1997). After cooling to room temperature, cholesterol was extracted with 5 mL hexane and 5 mL distilled water. The process was repeated 3 times. The hexane layer was transferred to a round-bottom flask and dried under vacuum. The extract was re-dissolved in 1 mL hexane and stored at -20℃ 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 a Hewlett-Packard 5890A gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionization detector. The temperature of injector and detector were 270 and 300℃ at 10℃/min and then held for 20 min. Nitrogen was used as the carrier at 2 mL/min. The sample injection volume was 2 μL with a split ratio of 1/50. Quantitation of cholesterol was conducted by comparing sample peak areas with responses of an internal standard. The percentage of cholesterol reduction was calculated as follows: cholesterol reduction (%) = 100 - amount of cholesterol in β-CD-treated cream×100/amount of cholesterol in untreated-cream (control). Determination of cholesterol in the control was done with each batch of cream used for all treatments and control. All experiments were conducted in triplicate.

Recycling of β-CD

To study how effective the recycled β-CD was for cholesterol reduction, different ratios (w/w) of recycled and unused β-CD mixture (10:0, 8:2, 7:3, 6:4, and 0:10) were tested as described above in preparation of cholesterol-β-CD complex and cholesterol determination.

Statistical analysis

The analysis of variance (ANOVA) and the least significant difference (LSD) test from a statistical package (SAS Institute Inc., Cary, NC, USA) were used for comparison among multiple means.

RESULTS AND DISCUSSION

Selection of solvent

To find an efficient solvent for dissociation of cholesterol from the complex, different kinds of solvent mixture were tested as shown in table 1. The relative rates were 1.00 and 1.03 in the mixture of acetic acid and butanol (3:1), and hexane and isopropanol (3:2), respectively. The rates of other mixtures were lower than those two. Among them, acetic acid:butanol=3:1 was the most effective on the dissociation of cholesterol considering industrial usage.

Comini and Mentink (1991) reported that β-CD-cholesterol complex was subjected to an apolar solvent suitable for extracting lipophilic compounds which are most weakly attached to cyclodextrin before the treatment with one polar solvent is carried out. The preliminary treatment may result in the formation of apolar phase containing the lipophilic compounds, and of a residual mixture to be treated with a polar solvent. The lipophilic compounds were thus selectively separated (Comini and Mnetink, 1991).

Ratio of acetic acid and butanol

The effect of different ratios of acetic acid and butanol for the cholesterol dissociation rate from cholesterol-β-CD complex in cream is shown in table 2. When the ratio of acetic acid to butanol was 3 to 1, the dissociation of cholesterol reached the highest rate of 78.31%. When the ratio of butanol decreased, the dissociation tended to be decreased (69.80%), but

Table 1. Relative rate of cholesterol dissociation from cholesterol-β-cyclodextrin complex with various solvents

Solvent	Mixing ratio	Relative rate of dissociation
Acetic acid : Butanol	3:1	1.00
Chloroform : Butanol	2:1	0.10
Hexane : Butanol	2:1	0.15
Chloroform : Ethanol	2:1	0.40
Hexane : Isopropanol	3:2	1.03

Table 2. Effects of different ratios of solvent mixture on dissociation of cholesterol-β-cyclodextrin complex in cream

Solvent ratio (v/v)	Cholesterol dissociation (%)		
Acetic acid : butanol	1st	2nd	Sum
4:1	51.63	18.16	69.80
3:1	70.23	8.08	78.31
2:1	43.18	15.06	58.24
1:1	44.56	12.08	56.64
1:2	36.10	7.09	43.19
SEM		4.06	

Means of triplicate. Means in a column with different letter are significant ($p<0.05$).
Other experiment factors included solvent: β-CD, 6:1; mixing speed, 100 rpm; mixing temperature, 50℃; and mixing time, 2 h.

was not significantly different ($p>0.05$). With the increasing ratio of butanol, the dissociation of cholesterol from the complex was also decreased, compared with that of 3 to 1 (acetic acid : butanol). Based on this result, the optimum ratio of acetic acid to butanol on the cholesterol dissociation from cholesterol-β-CD complex is 3 to 1.

Ratio of solvent and complex

The effect of ratio of the solvent to cholesterol-β-CD complex mixture on the cholesterol dissociation is shown in table 3. The maximal dissociation of cholesterol reached 82.50% with the mixture ratio of 6 to 1 (solvent to the complex). However, values from other ratios such as 9 to 1 (73.59%), 8 to 1 (76.48%) and 7 to 1 (76.73%) were not significantly different ($p>0.05$). The dissociation of cholesterol was not improved with an increasing amount of solvent up to 20 to 1 (70.94%), nor with a decrease in the mixture ratios of 5 to 1 and 4 to 1. Therefore, we decided to use 6 to 1 as an optimum ratio for the dissociation of cholesterol.

Mixing speed

Mixing speed, except 50 rpm, did not affect significantly the cholesterol dissociation, Among various mixing speeds tested, 100 rpm showed the highest dissociation of cholesterol with 76.59% (table 4). Others (75, 125, and 150 rpm) showed a little decrease, but were not significant, compared with that of 100 rpm. However, 50 rpm of mixing showed a significant decrease ($p<0.05$). Therefore, the optimum mixing speed was chosen as 100 rpm for the following studies.

Mixing time

The effect of mixing time for cholesterol dissociation from cholesterol-β-CD complex is shown in table 5. The optimum mixing time was 2 h, which resulted in 81.84% cholesterol dissociation. The rate of cholesterol dissociation was not significantly different between 1 and 3 h mixing ($p>0.05$). However, 30 min mixing. showed little decrease in the dissociation rate

Table 3. Effect of ratio of solvents to β-cyclodextrin on dissociation of cholesterol-β-cyclodextrin complex in cream

Ratio (v/w)	Cholesterol dissociation (%)		
Solvent : β-cyclodextrin	1st	2nd	Sum
9:1	43.79	29.80	73.59
8:1	42.77	33.71	76.48
7:1	43.91	32.83	76.73
6:1	53.16	29.34	82.50
5:1	40.76	28.90	69.86
4:1	39.40	29.93	69.33
SEM		3.22	

Means of triplicate. Means in a column with different letter are significant ($p<0.05$).
Other experimental factors included solvent ratio of acetic acid:butanol, 3:1; mixing speed, 100 rpm; mixing temperature, 50℃; and mixing time, 1 h.

Table 4. Effect of various speeds on dissociation of cholesterol-β-cyclodextrin complex in cream

Mixing speed (rpm)	Cholesterol dissociation (%)		
	1st	2nd	Sum
50	33.81	33.98	67.79
75	35.17	35.40	70.58
100	51.63	24.96	76.59
125	47.17	26.71	73.88
150	49.92	23.13	73.05
SEM		1.95	

Means of triplicate. Means in a column with different letter are significant ($p<0.05$).
Other experimental factors included solvent ratio of acetic acid:butanol, 3:1; solvent: β-CD, 6:1; mixing temperature, 50℃; and mixing time, 1 h.

Table 5. Effect of various mixing times on dissociation of cholesterol-β-cyclodextrin complex in cream

Mixing time (h)	Cholesterol dissociation (%)		
	1st	2nd	Sum
0.5	42.49	19.77	62.26
1.0	46.84	29.21	76.05
2.0	52.77	29.07	81.84
3.0	50.48	29.34	79.82
4.0	45.41	27.33	72.74
5.0	44.28	26.18	70.46
SEM		2.22	

Means of triplicate. Means in a column with different letter are significant ($p<0.05$).
Other experimental factors included solvent: β-CD, 6:1; mixing speed, 100 rpm; and mixing temperature, 50℃.

compared with those in 1, 2, and 3 h mixing (62.26%). This result agreed with the study by Umino and Kudo (1995), who reported that at least 2-4 h was required for dissociation of cholesterol from a cholesterol-β-CD complex of food.

Mixing temperature

Mixing temperature for effective dissociation from cholesterol-β-CD complex is shown in table 6. The optimum temperature appeared to be 50℃ with 80% of cholesterol dissociation. Temperatures higher than 50℃ resulted in low yield on the cholesterol dissociation. Although the dissociation was not significantly different at 60℃, it was different at 79 and 80℃ ($p<0.05$). Also mixing at 40℃ was not sufficient to dissociate cholesterol.

Poech (1990) reported that cholesterol-β-CD complex in egg yolk became unstable above 40℃, when 80% cholesterol was dissociated from β-CD. This agreed with our study showing an 80% cholesterol dissociation in cream at 50℃ mixing temperature. Therefore, we decided to apply 50℃ as an optimum temperature for cholesterol dissociation process.

Table 6. Effect of various mixing temperatures on dissociation of cholesterol-β-cyclodextrin complex in cream

Mixing temperature (℃)	Cholesterol dissociation (%)		
	1st	2nd	Sum
40	27.51	24.96	52.47
50	57.93	22.07	80.00
60	57.62	16.50	74.12
70	51.72	20.15	71.87
80	49.33	20.22	69.56
SEM		1.43	

Means of triplicate. Means in a column with different letter are significant ($p<0.05$).
Other experiment factors included solvent: β-CD, 9:1; mixing speed, 100 rpm; and mixing time, 2 h.

Table 7. Effect of ratio recycled to unused β-cyclodextrin on cholesterol reduction in cream

β-CD ratio (w/w)		Cholesterol Reduction (%)
Recycled	Unused	
10	0	75.07
8	2	81.83
7	3	84.71
6	4	95.59
0	10	96.17
SEM		2.33

Means of triplicate. Means in a column with different letter are significant ($p<0.05$).
Other experimental factors included β-cyclodextrin added, 10%; mixing speed, 800 rpm; mixing temperature, 10℃; and mixing time, 10 min, centrifugal speed, 111 × g, and centrifugal time, 10 min..

Recycling of β-CD

Since the optimum conditions were chosen for collecting recycled β-CD, we decided to examine how effective the recycled β-CD would be in cholesterol removal. For this study, various mixing ratios of recycled β-CD to unused β-CD (10:0, 8:2, 7:3, 6:4, and 0:10) were applied to cream containing 36% milk fat as shown in table 7. In a previous study (Ahn and Kwak, 1999), cholesterol removal of β-CD was 96.17% with a 10% unused β-CD addition to cream. The content of cholesterol in the cream was 123.5 mg/100 g.

In this study, the addition of 10% recycled β-CD alone showed only 75.07% (table 7), which was significantly lower than with 10% unused β-CD. With the ratio of 6 to 4 (recycled to unused β-CD), cholesterol reduction was 95.59%, which almost identical with that when applying unused β-CD alone ($p<0.05$).

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