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Entrapment of Milk Nutrients during Cholesterol Removal from Milk by Crosslinked β-Cyclodextrin

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Abstract

This study was designed to elucidate the quantities of milk nutrients entrapped during cholesterol removal from milk by crosslinked β-cyclodextrin (β-CD, 0.4~1.2%, w/v) and to evaluate the amounts of the residual β-CD in cholesterol-reduced milk treated by crosslinked β-CD. The content of lactose in the control milk (without treatment by crosslinked β-CD) was 4.86%, and the amounts of lactose entrapped by crosslinked β-CD ranged from 0.00 to 0.03%. The total amounts of the entrapped short-chain free fatty acid (FFA) and free amino acid (FAA) ranged from 0.03 to 0.09 ppm and from 0.28 to 0.71 μmol/mL, respectively. The amounts of the entrapped water-soluble vitamins (L-ascorbic acid, niacin, thiamine and riboflavin) ranged from 0.02 to 0.05 ppm, 0.01 to 0.06 ppm, 0.00 to 0.06 ppm and 0.01 to 0.06 ppm, respectively. The entrapped amounts of lactose, short-chain FFAs, FAAs and water-soluble vitamins were not remarkably affected by the concentrations of crosslinked β-CD (0.4~1.2%, w/v). Only very small amounts of residual β-CD in the cholesterol-removed milk were measured (1.22~3.00 ppm). Based on the data obtained from the present study, it was concluded that the amounts of entrapped nutrients were negligible during cholesterol removal from milk by crosslinked β-CD, and only trace amounts of residual β-CD were present in cholesterol-removed milk.

Key words: Milk nutrients, cholesterol removal, crosslinked β-cyclodextrin, residual β-cyclodextrin

Introduction

In recent years, a number of studies have indicated that cholesterol removal from dairy products such as milk, cream, butter, lard and cheese was most effectively achieved by powder β-cyclodextrin (β-CD) treatment (Alonso *et al*., 2009; Han *et al*., 2007; Kim *et al*., 2005, 2006, 2008; Lee *et al*., 2007). β-CD is a cyclic oligosaccharide composed of α (1→4) linkages of seven glucose units. It has a hydrophobic cavity at the center of its molecular arrangement, which forms an inclusion complex with various non-polar molecules including cholesterol. β-CD is also nontoxic, edible, nonhygroscopic and chemically stable and is easy to separate from the complex (Nagamoto, 1985).

Even though the β-CD treatment allows an effective removal of cholesterol (more than 90%) from milk, lots of β-CD was consumed for this process due to the ineffective recovery (Han *et al*., 2005). An example to overcome the problem is the crosslinking of β-CD. Crosslinking is a commonly used derivatization technique for manipulating starch functionality, and epichloroydrin and adipic anhydride have been extensively used to produce crosslinked starches, in which inter- or intramolecular mono- and diethers are formed with hydroxyl groups of starch (Cardwell, 1952). In our previous study, crosslinked β-CD made with adipic acid exhibited over 90% cholesterol removal and highly efficient recycling rate in milk (Han *et al*., 2005).

On the other hand, it is still questionable whether other milk nutrients (such as lactose, fatty acids, amino acids and vitamins) could be also removed during cholesterol removal from milk by crosslinked β-CD. Moreover, the literature on the amounts of residual β-CD in cholesterol-reduced milk treated by crosslinked β-CD is very limited. Therefore, the objectives of the present study were to examine the amounts of milk nutrients [lactose, short-chain free fatty acid (FFA), free amino acid (FAA) and water-soluble vitamin] entrapped during cholesterol removal from milk by crosslinked β-CD and to investigate the amounts of residual β-CD in cholesterol-removed milk.

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Materials and Methods

Materials

Commercial pasteurized milk (3.4% milk fat) was purchased from Pasteur Milk Co. (Hoeungseong, Korea). Commercial β-CD (purity 99.1%) was purchased from Nihon Shokuhin Cako Co. Ltd. (Japan). All chemicals and solvents were obtained from Sigma Chemical Co. (USA).

Preparation of crosslinked β-CD

A 100 g sample of β-CD was dissolved in 80 mL of distilled water and placed in a stirrer at room temperature with constant agitation for 2 h. Adipic acid (2 g) was then incorporated into the β-CD solution and the pH was adjusted to pH 10.0 with 1N NaOH. The β-CD solution was stirred at room temperature for 90 min and then readjusted to pH 5.0 with 0.5% acetic acid. The β-CD was recovered by filtering through Whatman No. 2 filter paper and washing 3 times with 150 mL of distilled water. The product was dried at 60°C in a Lab-Line mechanical convection oven (O-Sung Scientific Co. Korea) for 20 h and passed through a 100-mesh sieve (Han *et al*., 2005).

Preparation of cholesterol-reduced milk

To remove cholesterol from milk, 500 mL of milk was placed in a 1,000 mL beaker, and 0.4, 0.6, 0.8, 1.0, and 1.2% (w/v) β-CD were added. The mixture was stirred at 800 rpm with a blender (Tops: Misung Co., Korea) in a temperature-controlled water bath at 10°C for 10 min. The mixture was centrifuged (HMR-220IV, Hanil Industrial Co., Korea) at 166 g for 10 min, and the supernatant, the cholesterol-reduced milk, was collected for the future study. All treatments were triplicated.

Extraction and determination of cholesterol

For the extraction of cholesterol from milk, 1 g of a milk 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 2M ethanolic potassium hydroxide solution (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 re-dissolved in 1 mL of hexane and was stored at -20°C until analysis.

The 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 chromatography (GC) equipped with a flame ionization detector. The injector and detector temperature 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/min with a split ratio of 1:50. Quantification of cholesterol was done by comparing the peak areas with the response of an internal standard.

The percentage of cholesterol reduction was calculated as follows:

$$\text{Cholesterol reduction (\%)} = 100 - [\text{the amount of cholesterol in β-CD treated milk} \times 100/\text{the amount of cholesterol in the untreated milk (control)}] \quad (1)$$

Cholesterol determination for milk samples was averaged with each batch of treatments.

Lactose

Determination of lactose in each sample using high performance liquid chromatography (HPLC) was done by the procedure described by Kwak and Jeon (1988). All samples were analyzed in triplicate.

Short-chain free fatty acids

Milk samples (1 g) were mixed with diethyl ether and hexane for 2 h and eluted through a 10 mm I. D. glass column containing neutral alumina, as described by Ikins *et al*. (1988). A Hewlett-Packard GC (Model 5880A, USA) 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 (Supelco Inc., USA). The GC was operated with helium carrier gas at 2 mL/min, hydrogen gas at 37 mL/min, and air at 300 mL/min. The column oven was programmed for an initial holding for 1 min at 110°C, heating to 180°C at 5 °C/min for 10 min and holding for 20 min. The temperature for both the injector and detector was 250°C. All quantitative analyses were carried out 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 the standard. All samples were analyzed in triplicate.

Free amino acids (FAA)

To determine FAA, 5 g of milk were mixed with 5 mL distilled water. Then 500 mg of sulfosalicyclic acid were added to the mixture, after which the mixture was stored at 4°C for 1 h and centrifuged at 1,300 g for 15 min. The

supernatant was filtered through a 0.45 μm filter paper (MILLEX-HV13, Millipore, Ireland) and pre-treated by the method described by Lindroth and Mopper (1979). Determination of FAA by using HPLC was done by the modified method of Hodgin *et al*. (1983). Flow rate was 2 mL/min and two mobile phases were used: solvent A was 0.05 M sodium acetate (pH 6.3), and solvent B, methanol:tetrahydrofuran (90:10, v/v). The linear gradient of solvent B was programmed at 5 levels as follows: initial starting at 20%, then increasing of 40% for 6 min, to 42% for 9 min, to 50% for 3 min and finally to 70% for 12 min. FAA was analyzed on an ODS-μ-Bondpak C column (3.9 mm×300 mm, Waters, USA), and HPLC (Waters, USA) equipped with a refractive index detector was used. All quantitative analyses were performed by relating peak areas of individual FAA to those of external standard amino acids. All samples were analyzed in triplicate.

Water-soluble vitamins

For the analysis of water-soluble vitamins (L-ascorbic acid, niacin, thiamine and riboflavin), 0.5 mL of milk was placed in a 50 mL volumetric flask, mixed with mobile phase and sonicated for 20 min. The mixed solution was centrifuged (HMR-220IV, Hanil Industrial Co., Korea) at 452 g for 20 min, filtered through MILLEX-HV13 (0.45 μm, Millipore, Ireland), and analyzed by HPLC equipped with a refractive index detector using a Shodex RSpak DE-413L column (4.6 mm×250 mm, Japan). The flow rate was 0.5 mL/min and two mobile phases were used: solvent A was 0.005 M PIC B_6 and solvent B was a mixture of 0.4 mL triethylamine in 15 mL methanol:acetic acid (1:1, v/v). The liner gradient of solvent B was programmed at 5 levels as follows: the initial level was 20%, followed by an increase to 40% for 6 min, 42% for 9 min, 50% for 3 min, and finally to 70% for 12 min. All quantitative analyses were performed by relating peak areas of individual vitamins to those of external standards. All samples were analyzed in triplicate.

Residual β-cyclodextrin

The amounts of residual β-cyclodextrin in the cholesterol-reduced milk samples were investigated using the procedure described by Lüpez *et al*. (2009) with some modifications. Ten g of milk were mixed with 5 mg of α-CD dissolved in 1 mL of water (an internal standard for the quantitative analysis) for 2 min at 40°C. The mixture was centrifuged (HMR-220IV, Hanil Industrial Co., Korea) at 26,000 g for 30 min. The supernatant was collected and filtered through a 0.45 μm membrane filter (MILLEX-HV13, Millipore, Ireland).

HPLC was carried out on an Agilent 1100 series GPC-SEC system, consisting of an Agilent 1100 series refractive index detector (Agilent Technologies, USA). Separation was performed on a Shodex Asahipak NH_2P-50 4E column (4.6×250 mm, Japan). The mobile phase composition was an isocratic mixture of methanol and water (7:93, v/v) at a flow rate of 1 mL/min. The standard solutions were prepared in water to establish elution time, and the quantification of β-CD was conducted by comparing sample peak area of β-CD with α-CD as an internal standard. All samples were analyzed in triplicate.

Statistical analysis

All statistical analyses were performed using SAS version 9.0 (SAS Institute Inc., USA). Analysis of variance (ANOVA) was performed using the general linear models (GLM) procedure to determine significant differences among the samples. Means were compared by using Fisher's least significant difference (LSD) procedure. Significance was defined at the 5% level.

Results and Discussion

Cholesterol removal

The effects of different concentrations of crosslinked or powdered β-CD on cholesterol removal from milk are presented in Table 1. The cholesterol content of the control milk was 13.2 mg/100 g (data not shown). The crosslinked and powdered β-CD (0.4-1.2%, w/v) removed the cholesterol from 85.3 to 92.7% and from 84.6 to 92.3%, respectively, when mixed with milk at 10°C for 10 min. Addition of crosslinked and powdered β-CD at 0.4% (w/v) into milk showed the least efficiency of cholesterol removal (85.3 and 84.6%, respectively), while 1.0% addition resulted in the highest removal rate as 92.7 and 92.3%, respectively. The finding was in good agreement with our previous work conducted by Han *et al*. (2005), who reported the optimum concentration (1.0%, w/v) of crosslinked β-CD for cholesterol removal from commercial homogenized milk (3.6% milk fat).

Milk nutrients entrapped during cholesterol removal from milk by crosslinked β-CD

The quantities of lactose entrapped during cholesterol removal from milk by crosslinked or powdered β-CD are shown in Table 2. The average lactose content in the control milk was 4.86%. Kwak and Jeon (1988) also observ-

Table 1. Effects of different concentrations of crosslinked or powdered β-cyclodextrin (β-CD) on cholesterol removal from milk[1]

Treatment	Concentrations (%, w/v) of β-CD	Cholesterol removal (%)
Crosslinked[2]	0.4	85.3[f]
Powdered[3]		84.6[g]
Crosslinked	0.6	90.8[d]
Powdered		89.6[e]
Crosslinked	0.8	91.9[bc]
Powdered		91.5[c]
Crosslinked	1.0	92.7[a]
Powdered		92.3[ab]
Crosslinked	1.2	90.8[d]
Powdered		89.4[e]

[1]Values with different letters within the same column differ significantly ($p<0.05$).
[2]β-CD was crosslinked with adipic acid.
[3]β-CD was not crosslinked.

Table 2. Quantities of lactose entrapped during cholesterol removal from milk by crosslinked or powdered β-cyclodextrin (β-CD)

Treatment	Concentrations (%, w/v) of β-CD	Lactose (%)	Entrapped lactose (%)
Control[1]	0.0	4.86	0.00
Crosslinked[2]	0.4	4.84	0.02
Powdered[3]		4.85	0.01
Crosslinked	0.6	4.83	0.03
Powdered		4.85	0.01
Crosslinked	0.8	4.85	0.01
Powdered		4.86	0.00
Crosslinked	1.0	4.86	0.00
Powdered		4.85	0.01
Crosslinked	1.2	4.85	0.01
Powdered		4.86	0.00

[1]All samples were treated by low temperature long time (LTLT).
[2]β-CD was crosslinked with adipic acid.
[3]β-CD was not crosslinked.

ed the similar results that the lactose contents in whole milk samples determined by the HPLC method ranged from 4.87 to 4.96%.

In the present study, the content of lactose entrapped during cholesterol removal from milk by crosslinked or powdered β-CD was calculated as follows:

The entrapped lactose content (%) = lactose content (4.86%) in the control milk – lactose content (%) in the cholesterol-removed milk treated by crosslinked or powdered β-CD (2)

The calculated amounts of lactose entrapped by crosslinked β-CD (0.4-1.2%, w/v) ranged from 0.00 to 0.03%, indicating that the amounts of lactose entrapped during cholesterol removal from milk by crosslinked β-CD were negligible. The calculated amounts of the entrapped lactose were not remarkably affected by the concentrations (0.4-1.2%, w/v) of crosslinked β-CD studied. The calculated quantities of lactose entrapped by crosslinked β-CD at each concentration (0.4-1.2%, w/v) were not considerably different from those entrapped by powdered β-CD.

Table 3 shows the quantities of the short-chain FFA entrapped during cholesterol removal from milk by crosslinked or powdered β-CD. The amounts of the butyric, caproic, caprylic and capric acid in the control milk were 4.45, 2.26, 1.78 and 3.21 ppm, respectively. The quantities of each short-chain FFA entrapped during cholesterol removal from milk by crosslinked or powdered β-CD were calculated in the same way as the Eq. (2). The calculated amounts of the total short-chain FFA entrapped during cholesterol removal from milk by crosslinked or powdered β-CD ranged from 0.03 to 0.09 ppm and from 0.04 to 0.07 ppm, respectively, thereby explaining that the calculated amounts of the total FFA entrapped by crosslinked β-CD were not markedly different from those entrapped by powdered β-CD. There were not remarkable differences in the calculated amounts of the entrapped butyric, caproic, caprylic and capric acid with increasing the concentrations (0.4-1.2%, w/v) of crosslinked β-CD.

The total amount of FAA in the control milk was 44.40 μmol/mL (Table 4). The quantities of the individual FAA entrapped during cholesterol removal from milk by crosslinked or powdered β-CD were calculated in the same way as the Eq. (2). The calculated amounts of the total FAA entrapped by crosslinked and powdered β-CD (0.4-1.2%, w/v) ranged from 0.28 to 0.71 μmol/mL and from 0.31 to 0.49 μmol/mL, respectively. The concentrations of crosslinked β-CD (0.4-1.2%, w/v) used during cholesterol removal from milk did not remarkably influence the calculated amounts of the total FAA entrapped.

The quantities of water-soluble vitamins entrapped during cholesterol removal from milk by crosslinked or powdered β-CD are listed in Table 5. The amounts of L-ascorbic acid, niacin, thiamine and riboflavin in the control milk were 3.24, 1.33, 0.51 and 1.31 ppm, respectively. The quantities of L-ascorbic acid, niacin, thiamine and riboflavin entrapped during cholesterol removal from milk by crosslinked or powdered β-CD were calculated in

Table 3. Quantities of short-chain free fatty acid (FFA) entrapped during cholesterol removal from milk by crosslinked or powdered β-cyclodextrin (β-CD)

Treatment	Concentrations (%, w/v) of β-CD	Short-chain free fatty acids (ppm)				
		C_4	C_6	C_8	C_{10}	Total
Control[1]	0.0	4.45 (0.00)[4]	2.26 (0.00)	1.78 (0.00)	3.21 (0.00)	11.70 (0.00)
Crosslinked[2]	0.4	4.45 (0.00)	2.25 (0.01)	1.76(0.02)	3.19 (0.02)	11.65 (0.05)
Powdered[3]		4.44 (0.01)	2.24 (0.02)	1.77 (0.01)	3.21 (0.00)	11.66 (0.04)
Crosslinked	0.6	4.42 (0.03)	2.22 (0.04)	1.77 (0.01)	3.20 (0.01)	11.61 (0.09)
Powdered		4.44 (0.01)	2.25 (0.01)	1.78 (0.00)	3.19 (0.02)	11.66 (0.04)
Crosslinked	0.8	4.45 (0.00)	2.24 (0.02)	1.78 (0.00)	3.20(0.01)	11.67 (0.03)
Powdered		4.42 (0.03)	2.25 (0.01)	1.77 (0.01)	3.20 (0.01)	11.64 (0.06)
Crosslinked	1.0	4.44 (0.01)	2.25 (0.03)	1.77 (0.01)	3.17 (0.04)	11.61 (0.09)
Powdered		4.44 (0.01)	2.24 (0.00)	1.76 (0.02)	3.19 (0.02)	11.65 (0.05)
Crosslinked	1.2	4.43 (0.02)	2.25 (0.01)	1.77 (0.01)	3.20 (0.01)	11.65 (0.05)
Powdered		4.45 (0.00)	2.20 (0.02)	1.74 (0.04)	3.20 (0.01)	11.63 (0.07)

[1]All samples were treated by low temperature long time (LTLT).
[2]β-CD was crosslinked with adipic acid.
[3]β-CD was not crosslinked.
[4]The number in parenthesis means the amounts of short-chain FFA entrapped during cholesterol removal from milk by crosslinked or powder β-CD.

Table 4. Quantities of free amino acid (FAA) entrapped during cholesterol removal from milk by crosslinked or powdered β-cyclodextrin (β-CD)

Treatment	Concentrations (%, w/v) of β-CD	Free amino acids (μmol/mL)															
		Asp	Glu	Ser	Asn	Thr	Ala	Arg	Tyr	Val	Me	Ile	Leu	Phe	Trp	Lys	Total
Control[1]	0.0	3.2 (0.00)[4]	3.25 (0.00)	4.35 (0.00)	3.55 (0.00)	3.40 (0.0)	5.00 (0.00)	1.85 (0.00)	1.95 (0.00)	3.55 (0.00)	2.35 (0.00)	2.95 (0.00)	2.80 (0.00)	2.15 (0.00)	1.00 (0.00)	3.04 (0.00)	44.40 (0.00)
Crosslinked[2]	0.4	3.19 (0.01)	3.23 (0.02)	4.35 (0.00)	3.54 (0.01)	3.38 (0.02)	5.00 (0.00)	1.81 (0.04)	1.94 (0.01)	3.53 (0.02)	2.35 (0.00)	2.91 (0.04)	2.76 (0.04)	2.11 (0.04)	0.99 (0.01)	3.02 (0.02)	44.12 (0.28)
Powdered[3]		3.19 (0.01)	3.22 (0.03)	4.34 (0.01)	(3.51 0.04)	3.36 (0.04)	4.90 (0.10)	1.85 (0.00)	1.93 (0.02)	3.46 (0.09)	2.35 (0.00)	2.89 (0.06)	2.75 (0.05)	2.13 (0.02)	0.99 (0.01)	3.03 (0.01)	43.91 (0.49)
Crosslinked	0.6	3.15 (0.05)	3.18 (0.07)	4.34 (0.01)	3.49 (0.06)	3.35 (0.05)	4.94 (0.06)	1.81 (0.04)	1.95 (0.00)	3.53 (0.02)	2.34 (0.01)	2.90 (0.05)	2.78 (0.02)	2.15 (0.00)	0.98 (0.02)	3.04 (0.00)	43.99 (0.41)
Powdered		3.20 (0.00)	3.23 (0.02)	4.32 (0.03)	3.50 (0.05)	3.37 (0.03)	4.94 (0.06)	1.81 (0.01)	1.93 (0.02)	3.53 (0.02)	2.31 (0.04)	2.91 (0.04)	2.79 (0.01)	2.14 (0.01)	0.99 (0.01)	3.03 (0.01)	44.09 (0.31)
Crosslinked	0.8	3.16 (0.04)	3.23 (0.02)	4.34 (0.01)	3.54 (0.01)	3.39 (0.01)	4.96 (0.04)	1.80 (0.05)	1.94 (0.01)	3.55 (0.00)	2.32 (0.03)	2.93 (0.02)	2.80 (0.00)	2.10 (0.05)	0.98 (0.02)	3.02 (0.02)	44.07 (0.33)
Powdered		3.19 (0.01)	3.17 (0.08)	4.31 (0.04)	3.54 (0.01)	3.34 (0.06)	4.99 (0.01)	1.80 (0.05)	1.94 (0.01)	3.51 (0.04)	2.34 (0.01)	2.92 (0.03)	2.79 (0.01)	2.13 (0.02)	0.96 (0.04)	3.00 (0.04)	43.94 (0.46)
Crosslinked	1.0	3.19 (0.01)	3.16 (0.09)	4.28 (0.07)	3.50 (0.05)	3.36 (0.04)	4.98 (0.02)	1.83 (0.02)	1.95 (0.00)	3.49 (0.06)	2.33 (0.02)	2.89 (0.06)	2.80 (0.00)	2.11 (0.04)	0.99 (0.01)	3.04 (0.00)	43.91 (0.49)
Powdered		3.20 (0.00)	3.21 (0.04)	4.31 (0.04)	3.49 (0.06)	3.37 (0.03)	4.94 (0.06)	1.84 (0.01)	1.95 (0.00)	3.54 (0.01)	2.29 (0.06)	2.91 (0.04)	2.80 (0.00)	2.15 (0.00)	0.98 (0.02)	2.99 (0.05)	44.04 (0.36)
Crosslinked	1.2	3.19 (0.01)	3.18 (0.07)	4.27 (0.08)	3.51 (0.04)	3.35 (0.05)	4.93 (0.07)	1.84 (0.01)	1.94 (0.01)	3.46 (0.09)	2.33 (0.02)	2.90 (0.05)	2.75 (0.05)	2.12 (0.03)	0.94 (0.06)	2.97 (0.07)	43.69 (0.71)
Powdered		3.20 (0.00)	3.22 (0.03)	4.23 (0.02)	3.48 (0.07)	3.39 (0.01)	4.94 (0.06)	1.79 (0.06)	1.94 (0.01)	3.53 (0.02)	2.22 (0.03)	2.95 (0.00)	2.80 (0.00)	2.15 (0.00)	0.94 (0.06)	2.96 (0.08)	43.95 (0.45)

[1]All samples were treated by low temperature long time (LTLT).
[2]β-CD was crosslinked with adipic acid.
[3]β-CD was not crosslinked.
[4]The number in parenthesis means the amounts of FAA entrapped during cholesterol removal from milk by crosslinked or powder β-CD.

the same way as the Eq. (2). The calculated amounts of the entrapped L-ascorbic acid, niacin, thiamine and riboflavin by crosslinked β-CD (0.4-1.2%, w/v) ranged from 0.02 to 0.05 ppm, from 0.01 to 0.06 ppm, from 0.00 to 0.06 ppm, and from 0.01 to 0.06 ppm, respectively. The calculated amounts of all the water-soluble vitamins studied in the present study were not markedly dependent upon the concentrations of crosslinked β-CD (0.4-1.2%, w/v).

On the basis of all of the results obtained from the present study (on the entrapment of milk nutrients during cholesterol removal from milk by crosslinked β-CD), it was suggested that the amounts of milk nutrients, such as lactose, short-chain FFA, FAA and water-soluble vitamins, entrapped during cholesterol removal from milk by crosslinked β-CD were minimal. Alonso *et al*. (2009) studied the effect of β-CD on trans C18:1 fatty acid isomers, conjugated linoleic acid, polyunsaturated fatty acids and phospholipids in pasteurized milk and noted that β-CD is an effective oligosaccharide for cholesterol removal from milk and did not significantly influence the lipid components of the milk. According to them, the size of β-CD cavity is the almost exactly same as that of a cholesterol molecule; therefore, β-CD specifically forms an inclusion complex with cholesterol during cholesterol removal from milk. In the present study, it is suggested that β-CD could more preferably form an inclusion complex with milk cholesterol than other milk nutrients when blended at 10°C for 10 min.

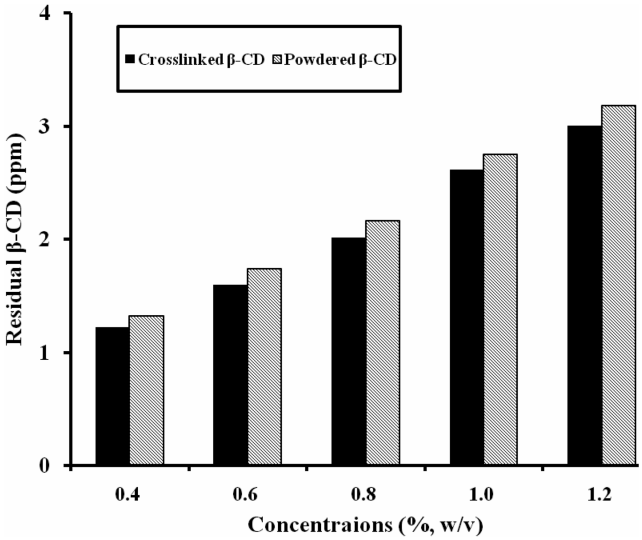

Fig. 1. Residual β-cyclodextrin (β-CD) after cholesterol removal from milk by crosslinked or powdered β-CD.

Residual β-cyclodextrin

The amounts of residual β-CD after cholesterol removal from milk by crosslinked or powdered β-CD are shown in Fig. 1. The amounts of residual β-CD in the cholesterol-removed milk were proportionally increased from 1.22 to 3.00 ppm and from 1.30 to 3.08 ppm, respectively, with increasing the concentrations from 0.4 to 1.2% (w/v) of crosslinked and powdered β-CD.

In the present study, the recovery (%) of β-CD was calculated as follows:

Table 5. Quantities of water-soluble vitamins entrapped during cholesterol removal from milk by crosslinked or powdered β-cyclodextrin (β-CD)

Treatment	Concentrations (%, w/v) of β-CD	Water-soluble vitamins (ppm)			
		L-Ascorbic acid	Niacin	Thiamine	Riboflavin
Control[1]	0.0	3.24 (0.00)	1.33 (0.00)	0.51 (0.00)	1.31 (0.00)
Crosslinked[2]	0.4	3.22 (0.02)	1.27 (0.06)	0.51 (0.00)	1.27 (0.04)
Powdered[3]		3.20 (0.04)	1.29 (0.04)	0.50 (0.01)	1.29 (0.02)
Crosslinked	0.6	3.21 (0.03)	1.29 (0.04)	0.47 (0.04)	1.29 (0.02)
Powdered		3.19 (0.05)	1.30 (0.03)	0.49 (0.02)	1.30 (0.01)
Crosslinked	0.8	3.20 (0.04)	1.32 (0.01)	0.48 (0.03)	1.28 (0.03)
Powdered		3.21 (0.03)	1.31 (0.02)	0.47 (0.04)	1.25 (0.06)
Crosslinked	1.0	3.20 (0.04)	1.31 (0.02)	0.45 (0.06)	1.29 (0.02)
Powdered		3.22 (0.02)	1.29 (0.04)	0.49 (0.02)	1.28 (0.03)
Crosslinked	1.2	3.21 (0.03)	1.30 (0.03)	0.47 (0.04)	1.27 (0.04)
Powdered		3.22 (0.02)	1.29 (0.04)	0.48 (0.03)	1.29 (0.02)

[1]All samples were treated by low temperature long time (LTLT).
[2]β-CD was crosslinked with adipic acid.
[3]β-CD was not crosslinked.
[4]The number in parenthesis means the amounts of water-soluble vitamins entrapped during cholesterol removal from milk by crosslinked or powder β-CD.

Recovery (%) of β-CD = [the amount of β-CD added (g) – the amount of β-CD recovered (g)] × 100 (3)

The calculated recoveries of β-CD for all the samples ranged from 99.9 to 100%. The finding was consistent with López *et al*. (2009) investigating the recovery (%) of β-CD after cholesterol removal from milk by powdered β-CD. They reported that the recovery of β-CD ranged from 98.61-101.63%. Therefore, in the present study, it is plausible that crosslinkd β-CD used for the cholesterol removal from milk could be almost recovered by the centrifugation method.

Conclusions

The present study investigated the quantities of milk nutrients entrapped during cholesterol removal from milk by crosslinked β-CD and measured the quantities of residual β-CD in cholesterol-reduced milk treated by crosslinked β-CD. The data on the lactose, short-chain FFA, FAA, water-soluble vitamins and residual β-CD obtained from the present study indicated that the very small amounts of lactose, short-chain FFA, FAA and water-soluble vitamins were entrapped during cholesterol removal from milk by crosslinked β-CD, and the residual β-CD in the cholesterol-removed milk was trace amounts. It is suggested in the present study that the cholesterol-removal by crosslinked β-CD treatment can be applicable to produce a cholesterol-removed milk without altering any nutritional properties of the final products.

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