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Cholesterol Removal from Lard with Crosslinked β-Cyclodextrin

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ABSTRACT : The present study was carried out to determine the optimum conditions of different factors (ratio of lard to water, β-CD concentrations, mixing temperature, mixing time and mixing speed) on cholesterol reduction from lard by using crosslinked β-CD. Crosslinked β-CD was prepared with adipic acid. When the lard was treated under different conditions, the range of cholesterol removal was 91.2 to 93.0% with 5% crosslinked β-CD, which was not significantly different among treatments. In a recycling study, cholesterol removal with crosslinked β-CD in the first trial was 92.1%, which was similar to that with new crosslinked β-CD. In up to eight time trials, over 90% of cholesterol removal was found. The present study indicated that the optimum conditions for cholesterol removal using crosslinked β-CD were a 1:3 ratio of lard to water, 5% crosslinked β-CD concentration, 40°C mixing temperature, 1 h mixing time and 150 rpm mixing speed. In addition, crosslinked β-CD made by adipic acid resulted in an effective recycling efficiency. (**Key Words :** Crosslinked β-CD, Cholesterol Removal, Lard, Recycling)

INTRODUCTION

Since a strong positive correlation exists between increased serum cholesterol concentrations and risk of coronary heart disease, most consumers are concerned about excessive intake of cholesterol (Grundy et al., 1982; Gurr, 1992). Experiments on animals and human have shown that plasma cholesterol can be raised by increased intake of cholesterol and saturated fat (Gurr, 1982; Pyorala, 1987; Sieber, 1990; Carleton et al., 1991). Most consumers are concerned about excessive intakes of cholesterol and fat in their daily diets because of the risk of coronary heart disease (Grundy et al., 1982; Gurr, 1992). There have been dramatic increases in no-, low and reduced-cholesterol products in the market place (Schroder and Baer, 1990).

A number of studies have indicated that cholesterol removal from milk, butter and cheese was most effectively achieved by β-CD treatment (Oakenfull and Sihdu, 1991; Makoto et al., 1992; Hwang et al., 2005; Jung et al., 2005; Kwak et al., 2005; Kim et al., 2006). Thus, β-CD provides advantages when used for removal of cholesterol from various foods. While this method allows cholesterol removal in milk (about 90%), using β-CD powder is an ineffective way for β-CD recovery.

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Crosslinking is a commonly used derivatization technique for manipulating starch functionality, and epichlorohydrin acid have been extensively used to produce crosslinked starches, which inter- or intramolecular mono- and diethers are formed with hydroxyl groups of starch (Hamerstrand et al., 1959), This modification produces important changes in the starch functional properties, such as an increase or decrease in viscosity (Wister and Daniel, 1990). Crosslinked starches are employed mainly as thickening agents and stabilizers in most food systems, such as sauces and dressings for pizzas, spaghetti, jams and pie fillings (Fleche, 1985; Luallen, 1985; Whistler and Daniel, 1990). Modification of the starch by crosslinkage formation has been proposed to provide the pastes with more stability when overheated, more mechanical power and pH changes, which are indispensable for food manufacture (Hosney, 1986).

Edible commercial lard, used for hardening agents, shortening cocoa butter substitutes and cooking oils, contains a high levels of cholesterol (ca, 50-120 mg/100 g) (Corregelongue and Maffrand, 1989). Several studies have been reported on removal of cholesterol from animal fats with β-CD (Corregelongue and Maffrand, 1989; Davidson, 1990; Oakenfull and Sidhu, 1990; Makoto et al., 1992), but little information is available on cholesterol removal from lard by crosslinked β-CD. Our primary objective was involved investigating the conditions for removal of

Table 1. Effect of the ratio of lard to water on cholesterol removal in lard[1]

Ratio of lard to distilled water	Cholesterol removal (%)
3:1	73.05 [c]
2:1	80.11 [b]
1:1	91.08 [a]
1:2	90.45 [a]
1:3	91.21 [a]

[1] Means within a column with different superscript letters differ ($p<0.05$). Means of triplicate. Factors of cholesterol removal: Crosslinked β-cyclodextrin; 5%, mixing temp.; 40°C, mixing time; 1 h, mixing speed; 150 rpm, average cholesterol in lard; 27%.

cholesterol from lard with crosslinked β-CD.

MATERIALS AND METHODS

Materials

Lard was obtained from a retail store, and β-CD (purity 99.1%) was obtained from Nihon Shokunin Cako Co. LTD. (Osaka, Japan). Cholesterol and 5α-cholestane were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and all solvents were gas-chromatographic grade.

Preparation of crosslinked β-CD

A sample of 100 g β-CD was prepared in a 80 ml distilled water and placed in a stirrer at room temperature with constant agitation for 2 h. Then 5 g of adipic acid was incorporated with 100 g β-CD, and pH was adjusted to 10 with 1 N NaOH. The β-CD solution was stirred at room temperature for 90 min and then readjusted to pH 5 with acetic acid. β-CD was recovered by filtering with Whatman paper No. 2 and washed three times with 150 ml of distilled water. The product was dried at 60°C in a Lab-line mechanical convection oven for 20 h and passed through a 100 mesh (Han et al., 2005).

Cholesterol removal

To study the effects of five different factors, 100 ml mixture of different ratios of lard to distilled water (3:1, 2:1, 1:1, 1:2 or 1:3) was placed in a 250 ml beaker and different concentrations of β-CD (1, 3, 5, 7 or 9%) were added. The mixture was stirred at different mixing speeds (50, 100, 150, 200 or 250 rpm) with a blender (Tops; Misung Co., Seoul, Korea) in a temperature-controlled water bath with different mixing temperatures (20, 30, 40, 50 or 60°C) and mixing times (0.5, 1.0, 1.5 or 2.0 h). Then, the mixture was centrifuged (HMR-220IV; Hanil Industrial Co., Seoul, Korea) with 166×g for 10 min.

For each treatment after centrifugation, the supernatant fraction containing cholesterol-reduced lard was decanted and was used for cholesterol determination. All treatments were duplicated.

Extraction and determination of cholesterol

For the extraction of cholesterol, 1 ml of lard sample was placed in a screw-capped glass tube (15 mm×180 mm), and 1 ml of the 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. 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 a gas chromatograph (5890A: Hewlett-Packard, Palo Alto, CA, USA) equipped with a flame-ionization detector. Temperatures of the injector and detector were 170 and 300°C, respectively. Oven temperature was programmed to increase from 200 to 300°C, at 10°C/min, and then was constant for 20 min. Nitrogen was used as carrier gas at a flow rate of 2 ml/min. The sample injection volume was 2 μl with a split ratio of 1/50. Quantitation of cholesterol was done by comparing sample peak areas with the response of an internal standard.

The percentage of cholesterol reduction was calculated as follows:

Cholesterol reduction (%) = amount of cholesterol in lard mixture sample×100/amount of cholesterol in untreated lard sample (control). Cholesterol determination for a control was done with each treatment batch.

Recycling of β-CD

The study how effective the recycled β-CD was for cholesterol reduction in lard was carried out. The used crosslinked β-CD was washed in acetic acid:butanol = 3:1 (v/v) (Kwak et al., 2001) for 24 h at room temperature. The recycled β-CD was dried at room temperature and reused for recycling study.

Statistical analysis

Data from each experiment were analyzed by analysis of variance (ANOVA) using a SAS program (Cary, NC, 1985) and differences among treatments were determined by Duncan's multiple test at $p<0.05$, unless otherwise stated.

RESULTS AND DISCUSSION

In the present study, the optimum conditions of five different factors (the ratio of lard to distilled water, β-CD concentration, mixing time, mixing temperature and mixing speed) were examined in cholesterol removal in lard using crosslinked β-CD.

Table 2. Effect of various crosslinked β-cyclodextrin concentrations on cholesterol removal in lard[1]

β-Cyclodextrin (%)	Cholesterol removal (%)
1	64.05 [c]
3	85.13 [b]
5	93.02 [a]
7	92.92 [a]
9	92.58 [a]

[1] Means within a column with different superscript letters differ ($p<0.05$). Means of triplicate. Factors of cholesterol removal: Ratio of lard to water; 1:1, mixing temp.; 40°C, mixing time; 1 h, mixing speed; 150 rpm, average cholesterol in lard; 27%.

Table 3. Effect of various mixing temperatures of crosslinked β-cyclodextrin on cholesterol removal in lard[1]

Mixing temp. (°C)	Cholesterol removal (%)
20	90.11 [b]
30	90.32 [b]
40	92.38 [a]
50	92.31 [a]
60	92.08 [a]

[1] Means within a column with different superscript letters differ ($p<0.05$). Means of triplicate. Factors of cholesterol removal: Crosslinked β-cyclodextrin; 5%, ratio of lard to distilled water; 1:1, mixing time; 1 h, mixing speed; 150 rpm, average cholesterol in lard; 27%.

Effect of the ratio of lard to distilled water

Cholesterol removal from lard by crosslinked β-CD was markedly related to the lard to water ratio (Table 1). The highest removal of cholesterol occurred at 1:1 ratio of lard to water and 91.1% cholesterol was removed by mixing with 5% crosslinked β-CD for 1 h. The lower cholesterol removal appeared in the higher ratio of lard to water. This may be due to the fact that higher water content would reduce the inclusion between crosslinked β-CD and cholesterol. A small amount of cholesterol was removed by crosslinked β-CD with less addition of water (3:1 and 2:1). Since the outer shell of crosslinked β-CD has hydrophilic properties, the inner hydrophobic layer of crosslinked β-CD would become exposed when directly interacting with oil, as well as reducing its inclusion capability. Therefore, the proper addition of water during operation is necessary. According to the result of Courregelongue et al. (1989), only 26 and 33% of cholesterol was removed in butter by 5 and 10% β-CD, respectively, without addition of water.

In similar study (Jung et al., 2005), cholesterol removal in egg yolk was significantly related to the ratio of egg yolk-to-water. The 94.6% cholesterol removal was found at 1:1 ratio of egg yolk-to-water in the sample treated with 20% crosslinked β-CD at 800 rpm for 30 min, and more addition of water showed no significant effect. Based on the present results, the ratio of 1:1 ratio of lard to water may be optimum for the effective cholesterol removal.

Effect of crosslinked β-CD concentration

Table 2 showed the cholesterol removal of lard was

Table 4. Effect of various mixing times of crosslinked β-cyclodextrin on cholesterol removal in lard[1]

Mixing time (h)	Cholesterol removal (%)
0.5	87.14 [b]
1.0	92.01 [a]
1.5	91.47 [a]
2.0	91.48 [a]

[1] Means within a column with different superscript letters differ ($p<0.05$). Means of triplicate. Factors of cholesterol removal: Crosslinked β-cyclodextrin; 5%, ratio of lard to distilled water; 1:1, mixing temp.; 40°C, mixing speed; 150 rpm, average cholesterol in lard; 27%.

significantly affected by crosslinked β-CD concentration (1, 3, 5, 7 or 9%). The range of 64-93% cholesterol was removed by stirring with 10% crosslinked β-CD for 1 h. According to Oakenfull et al. (1991), 9.8 and 23.5% cholesterol were removed from yolk plasma by 1 and 2% β-CD, respectively. Recently, Jung et al. (2005) indicated that crosslinked β-CD showed over 90% of cholesterol removal in egg yolk. Therefore, crosslniked β-CD treatment could be an effective way to reduce cholesterol, and 5% of crosslinked β-CD may be a sufficient amount for cholesterol removal in lard.

Effect of mixing temperature

To examine conditions that might affect cholesterol removal by temperature, five different mixing temperatures were tested. No difference was found in cholesterol removal at 40, 50 or 60°C (Table 3). The range of cholesterol removal was from 92.08 to 92.38% when the lard was mixed with 5% crosslinked β-CD at 150 rpm for 1 h.

Yen and Tsai (1995) reported that tempeature was also important for removing cholesterol from lard with β-CD. Removal of cholesterol from lard with β-CD stirred at 50°C was greater than when stirred at other temperatures. However, the efficiency was increased with increasing stirring time when treated at 40°C, but it decreased with increased stirring time when treated at 60°C. Oakenfull et al. (1991) indicated that removal of cholesterol from milk with β-CD was markedly influenced by temperature. The higher removal was found at a lower temperature, i.e., 77, 63 and 62% cholesterol in milk were removed when treated with β-CD at 4, 8 and 40°C, respectively.

Effect of mixing time

Cholesterol removal was not significantly affected by mixing time after 1 h mixing treatment (Table 4). The crosslinked β-CD removed 92.01% of the cholesterol for 1 h when mixed with 150 rpm at 40°C with 1:1 ratio of lard to water. More time of mixing did not enhance the cholesterol removal rate. Therefore, it appears that 1hr of mixing may be sufficiently effective to remove cholesterol from lard.

In lard, cholesterol reduction dramatically increased up to 30 min at all temperatures and plateaued thereafter up to

Table 5. Effect of various mixing speeds of crosslinked β-cyclodextrin on cholesterol removal in lard[1]

Mixing speed (rpm)	Cholesterol removal (%)
50	89.05 [b]
100	90.48 [b]
150	93.11 [a]
200	93.00 [a]
250	92.85 [a]

[1] Means within a column with different superscript letters differ ($p<0.05$). Means of triplicate. Factors of cholesterol removal: Crosslinked β-cyclodextrin; 5%, ratio of lard to distilled water; 1:1, mixing time; 1 h, mixing temp.; 40°C; average cholesterol in lard; 27%.

2 h (Yen and Tsui, 1995). About 90 to 95% cholesterol was removed from lard with 10% β-CD after 30 min of mixing. However, cholesterol removal slightly decreased when samples were mixed for 2 h. This finding may be due to the instability of an inclusive complex between β-CD and cholesterol during longer mixing time (Yen and Tsui, 1995).

Effect of mixing speed

The removal was increased with an increasing mixing speed (Table 5). In addition, 93% cholesterol in lard was removed with 5% crosslinked β-CD when mixed at 40°C and 150 rpm for 1 h. Although mixing speed might not be an important factor on cholesterol removal using crosslinked β-CD at over 150 rpm mixing speed, cholesterol removal was significantly lower below 100 rpm mixing speed in lard.

Recycling of β-CD

Since the optimum conditions were chosen for cholesterol removal of lard, we tried to examine whether the recycled crosslinked β-CD could remove cholesterol effectively or not. The β-CD for recycling were applied to lard 10 times repeatedly, and results are shown in Table 6. The cholesterol removal was found between 92.11 and 90.72% from 1 and 8 time uses and it was not significantly different compared with that of unused crosslinked β-CD. A slightly lower removal was shown in 9 and 10 time uses as 88.71 and 87.34%, respectively.

In similar recycling study (Jung et al., 2005), one time recycled crosslinked β-CD showed 84.9% of cholesterol removal in egg yolk, while unused crosslinked β-CD resulted in 92.7% cholesterol removal. Also, over 80% cholesterol removal was maintained with up to 8 times used crosslinked β-CD, and no significant differences were observed depending on how many times the crosslinked β-CD were recycled. When 9 and 10 time used crosslinked β-CD were applied, cholesterol removal significantly decreased to 78.7 and 75.7%, respectively.

Another study applied to milk using crosslinked β-CD (Han et al., 2005) showed that the cholesterol removal rate was in the range of 92.1 to 93.1% with 1% crosslinked

Table 6. The change of cholesterol removal rate using crosslinked β-cyclodextrin with repeated times of recycling in lard[1]

Number of repeated recycling	Cholesterol removal (%)
Initial	92.32
1st	92.11 [a]
2nd	91.83 [a]
3rd	91.27 [a]
4th	91.10 [a]
5th	90.82 [a]
6th	90.71 [a]
7th	90.62 [a]
8th	90.72 [a]
9th	88.71 [b]
10th	87.34 [b]
Average	90.52

[1] Means within a column with different superscript letters differ ($p<0.05$). Means of triplicated cholesterol extraction.

Recycled crosslinked β-CD was washed as following factors: Acetic acid:isopropanol = 3:1 as solvent, solvent:crosslinked β-cyclodextrin = 6:1, centrifugal speed; 1,500 rpm, centrifugal time; 5min, drying time; 6 h.

β-CD addition. Therefore, the present study indicated the crosslinked β-CD could be applied into lard on cholesterol removal process with an effective reproductivity.

CONCLUSION

Crosslinked β-CD was prepared with adipic acid. When lard was treated with different conditions (ratio of lard to water, β-CD concentration, mixing temperature, mixing time and mixing speed). The cholesterol removal rate was in the range of 91.2 to 93.0% with 5% crosslinked β-CD addition. Not much difference was found among other conditions. Interestingly, in recycling study, the cholesterol removal in the first trial was 92.1%, which was mostly same as that using new crosslinked β-CD (92.3%). Up to 8 time use, the cholesterol removal rate was not significantly different. The present study indicated that the optimum conditions of cholesterol removal crosslinked β-CD by adipic acid were 1:3 ratio of lard to water, 5% crosslinked β-CD concentration, 40°C mixing temperature, 1 hr mixing time and 150 rpm mixing speed with over 92% cholesterol removal. In addition, this study showed the first evidence of possibility for applying crosslinked β-CD by adipic acid in lard, and further study would be necessary in future.

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REFERENCES

Adams, M. L., D. M. Sullivan, R. L. Smith and E. F. Richter. 1986.

Evaluation of direct saponification method for determination of cholesterol in meats. J. AOAC. 69:844-846.

Carleton, R. A., L. Finberg, J. Flora, D. S. Goodman, S. M. Grundy, S. Havas, G. T. Hunter, D. Kritchevsky, R. M. Lauer, R. V. Lueoker, A. G. Ramirez, L. Van Horn, W. B. Stason and J. Stokes. 1991. Report of the expert panel on population strategies for blood cholesterol reduction. Circulation 83:2154-2232.

Courregelongue, J. and J. P. Maffrand. 1989. Process for eliminating cholesterol contained in a fatty substance of animal origin and the fatty substance with reduced cholesterol obtained. United States Patent USP 4880573.

Davidson, S. 1990. Low cholesterol milk and eggs. Rural Res. 148:27-28.

Fleche, G. 1985. Chemical modification and degradation of starch. In: (Ed. G. M. Van Beynum and J. A. Roel) Starch conversion technology. Deckker, New York, NY, USA, pp. 73-99.

Grundy, S. M., D. Brheimer, H. Blackburn, W. V. Brown, P. O. Kwiterovich, F. Mattson, G. Schonfeld and W. H. Weidman. 1982. Rationale of the diet-heart statement of the American Heart Association Report of the Nutrition Committee. Circulation 65:839A-854A.

Gurr, M. I. 1992. Dietary lipids and coronary disease: old evidence, new perspectives and progress. Lipid Res. 31:195-243.

Hamerstrand, G. E., B. T. Hofreiter and C. L. Mehltretter. 1959. Determination of the extent of reaction between epichlorohydrin and starch. 37:519-524.

Han, E. M., S. H. Kim, J. Ahn and H. S. Kwak. 2005. Cholesterol removal from homogenized milk with crosslinked β-cyclodextrin by adipic acid. Asian-Aust. J. Anim. Sci. 18: 1794-1799.

Hwang, J. H., S. J. Lee and H. S. Kwak. 2005. Properties and cholesterol lowering effect of cholesterol-reduced milk supplemented with evening primrose oil. Asian-Aust. J. Anim. Sci. 18:1041-1047.

Jung, T. H., H. S. Park and H. S. Kwak. 2005. Optimization of cholesterol removal by crosslinked β-cyclodextrin in egg yolk. Food Sci. Biotechnol. 14:79-797.

Jung, T. H., J. J. Kim, S. H. Yu, J. Ahn and H. S. Kwak. 2005. Properties of chosleterol-reduced butter and effect of gamma-linolenic acid added butter on blood cholesterol. Asian-Aust. J. Anim. Sci. 18:1646-1654.

Jung, T. H., H. S. Park and H. S. Kwak. 2005. Optimization of cholesterol removal by crosslinked β-cyclodextrin in egg yolk. Food Sci. Biotechnol. 14:79-797.

Kim, J. J., S. H. Yu, W. M. Jeon and H. S. Kwak. 2006. The effect of evening primrose oil on chemical and blood cholesterol lowering properties of Cheddar cheese. Asian-Aust. J. Anim. Sci. 19:450-458.

Kwak, H. S., C. S. Chung and J. Ahn. 2002. Flavor compounds of cholesterol-reduced Cheddar cheese slurries. Asian-Aust. J. Anim. Sci. 15:117-123.

Kwak, H. S., S. H. Kim, E. M. Han and J. Ahn. 2005. Effect of crosslinked β-cyclodextrin on quality of cholesterol-reduced cream cheese. Asian-Aust. J. Anim. Sci. 18:584-589.

Kwak, H. S., C. S. Chung and J. Ahn. 2002. Flavor compounds of cholesterol-reduced Cheddar cheese slurries. Asian-Aust. J. Anim. Sci. 15:117-123.

Kwak, H. S., C. G. Nam and J. Ahn. 2001. Low cholesterol Mozzarella cheese obtained from homogenized and β-cyclodextrin-treated milk. Asian-Aust. J. Anim. Sci. 14:268-275.

Kwak, H. S., H. M. Suh, J. Ahn and H. J. Kwon. 2001. Optimization of β-cyclodextrin recycling process for cholesterol removal in cream. Asian-Aust. J. Anim. Sci. 14:548-552.

Luallen, E. T. 1985. Starch as a functional ingredient. Food Technol. 39:59-63.

Makoto, K., O. Akio and S. Reijiro. 1992. Cholesterol removal from animal with cyclodextrin by inclusion. Japan Patent 4,168,198.

Oakenfull, D. G. and G. S. Sihdu. 1991. Cholesteorl reduction. International Patent, 91/11114.

Pyorala, K. 1987. Dietary cholesterol in relation to plasma cholesterol and coronary heart disease. Am. J. Clin. Nutr. 45:1176-1184.

Schroder, B. G. and R. J. Baer. 1990. Utilization of cholesterol-reduced milk fat in fluid milks. Food Technol. Nov. 145-148.

Sieber, R.1990. Cholesterol removal from animal food-Can it be justified? Fed. Dairy Res. Inst., Libefeld, Switzerland, pp. 375-387.

SAS Users Guide. 1985. Statistics Version 5; SAS Institute, Inc., Cary, NC.

Szjetli, J. 1988. Cyclodextrin inclusion complexes. In (J. Szejtli) Cyclodextrin technology; Kluwer Akademic Publishers: Dordrecht, The Netherlands, pp. 79-170.

Whistler, R. and R. Daniel. 1990. Function of polysaccharide. In: Food additives, Dekker, New York, NY, USA. pp. 399-406.

Yen, G. C. and L. T. Tsui. 1995. Cholesterol removal from a lard-mixture with β-cyclodextrin. J. Food Sci. 60:561-564.