Functional Properties of Cholesterol-Removed Whipping Cream Treated by β -Cyclodextrin

S. Y. Shim, J. Ahn, and H. S. Kwak

Department of Food Science and Technology,
Sejong University, Seoul, Korea 143-747

ABSTRACT

The present study was carried out to examine the changes in functional properties of cholesterol-removed whipping cream by β -cyclodextrin (β -CD) treatment. The cholesterol removal rate reached over 90% in cream before whipping in all conditions (different stirring time and speed) applied. The apparent viscosity of β -CD treated cream after whipping increased with increased stirring time and speed. Comparatively, the overrun percentage reached to 150%, and foam instability was measured as 2.5 ml deformed cream with lower stirring time (10 min) and speed (400 rpm). The thiobarbituric acid value of cholesterol-removed whipping cream increased from 0.08 to 0.14 stored at 4°C during 4 wk; however, no difference was found compared with that of control. Above results indicated that β -CD treatment process for cholesterol removal did not show a profound adverse effect on functional properties of cream after whipping.

(Key words: whipping cream, cholesterol removal, β -CD treatment, functional properties)

Abbreviation key: β -CD = β -cyclodextrin; TBA = thiobarbituric acid.

INTRODUCTION

Public concern about cholesterol has increased due to the positive correlation of serum cholesterol concentration to the risk of developing coronary heart disease in addition to high dietary fat and low fiber (Grundy et al., 1982; Gurr, 1992; Law et al., 1994). Although the role of dietary cholesterol in human health has not been yet fully understood, factors raising serum cholesterol, such as dietary cholesterol, are generally considered to be unfavorable. Based on above information, physical, chemical, and biological methods to reduce cholesterol in foods including dairy products have been studied

(Szejtli, 1988; Ahn and Kwak, 1999; Lee et al., 1999; Kwak et al., 2002).

Recent studies have indicated that the cholesterol removal in milk, cream, and cheese was effectively conducted by β -cyclodextrin (β -CD) (Oakenfull and Sihdu, 1991; Makoto et al., 1992; Ahn and Kwak, 1999; Lee et al., 1999; Kwak et al., 2001). Because β -CD is nontoxic, edible, nonhygroscopic, chemically stable, and easy to separate (Nagamoto, 1985), it has positive attributes when used to remove cholesterol from foods. To apply this process to cream, it must be stirred with β -CD prior to the whipping process for a sufficient rate of cholesterol removal.

Whipped cream is a dispersion of gas bubbles that are surrounded by partially coalesced fat at the air/serum interface and supported by high viscosity in the serum phase (Smith et al., 2000a). Several factors affect the structural properties of whipped cream, including fat content, processing conditions, and the addition of stabilizers and emulsifiers (Bruhn and Bruhn, 1988).

Emulsion instability is sought in developing structure in whipped cream (Goff, 1997). The process of controlled partial coalescence of such emulsions during whipping and air incorporation leads to the formation of complex structures described both as protein-stabilized emulsions and fat-stabilized foams. Two distinct types of instability are found: 1) coalescence: a decrease in the number and an increase in the size of individual globules and 2) flocculation: a clustering of individual globules into a coherent unit in which the size and identity of individual globules are retained.

The β -CD treatment for effective cholesterol removal may extensively reduce cholesterol in cream but may impair foam stability, probably due to a size reduction of fat globules over the point at which they resist partial coalescence and inhibit stiff foam formation. The whipping of cream into stable foam relies on a combination of destabilization and structure-building mechanisms (Smith, 2000b). Destabilization occurs as the milk fat globule membranes are disrupted in the presence of shear (Stanley et al., 1996) and partial coalescence ensues.

Researchers agree that fat content in cream, homogenization and pasteurization conditions, and presence of

Received February 4, 2003.

Accepted April 14, 2003.

Corresponding Author: H. S. Kwak; e-mail: kwakhs@sejong.ac.kr.

stabilizers and emulsifiers influence functional properties of whipping cream. Higher milk fat increases foam firmness and stability but decreases overrun. Surface-active agents and stabilizers, often added to creams that undergo heat treatment, produce finer foam, and affect overrun and foam stability. However, there has been little literature regarding the whipping characteristics of the β -CD treatment processing in cholesterol-removed cream. Therefore, our objective in this study was to examine the effect of cholesterol-removed process by β -CD treatment on functional properties of whipping cream.

MATERIALS AND METHODS

Materials

Raw milk was obtained from Binggare Dairy Plant (Kyonggi-do, Korea), pasteurized at 72°C for 16 s and cooled to 55°C, and cream was separated using a cream separator (Elecram, Vanves, France), and standardized to 36% milk fat content with skim milk. The cream was refrigerated overnight at 5°C.

Emulsifiers and stabilizers were purchased from Il-Shin Company (Seoul, Korea). Commercial β -CD (purity 99.1%) was purchased from Nihon Shokuhin Kaku Co. LTD. (Osaka, Japan). Cholesterol and 5- α cholestane were purchased from Sigma Chemical Co. (St. Louis, MO), and all solvents were gas chromatographic grade.

Cholesterol Removal

Cream was treated with 10% (wt/vol) β -CD to remove cholesterol as described earlier (Ahn and Kwak, 1999). The mixture was stirred at 400, 800, or 1200 rpm for 10, 20, or 30 min with a blender (Tops, Misung Co., Seoul, Korea) in a temperature-controlled water bath at 40°C. The mixture was centrifuged (HMR-220IV; Hanil Industrial Co., Seoul, Korea) with 166 $\times$ g for 10 min to remove β -CD-cholesterol complex (Lee et al., 1999). All treatments were run in triplicate.

Manufacture of Whipping Cream

To study the effect of three different kinds of stabilizer, the cholesterol was removed, and refrigerated cream was stabilized as follows: 1) group I: 0.1% monoglyceride, 0.2% sugar ester, 0.5% lecithin (liquid type), and 0.1% phosphate, 2) group II: 0.2% avicell, 0.1% sugar ester, 0.2% α -cellulose, 0.3% sodium alginate, and 0.3% sucrose, and 3) group III: 0.2% trisodium citrate, 0.1% sugar ester, 0.2% sodium alginate, and 0.3% sucrose. Stabilizer unadded (control) and added creams were processed with 100 psi homogenization pressure

using HC 5000 (Microfluidics Corp. Newton, MA) at 60°C. After cooling to 4°C, the samples were aged for 24 h and then the cholesterol-removed whipping cream was whipped by EGS type 06 (E3290 Model 296, Germany) with the third step speed for 2 and 1/2 min. Three replicates were tested on each of three treatments.

Extraction and Determination of Cholesterol

For the extraction of cholesterol from whipped cream, 1 g of sample was placed in a screw-capped glass tube (15 $\times$ 180 mm), and 1 ml of 5- α -cholestane (1 mg/ml) was added as an internal standard (Adams et al., 1986). The sample was saponified at 60°C for 30 min with 5 ml of 2 M ethanolic potassium hydroxide solution. 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 $\times$ 0.32-mm i.d. $\times$ 0.25- μ m thickness) using a gas chromatograph (5880A; Hewlett-Packard, Palo Alto, CA) equipped with a flame-ionization detector. Temperatures of the injector and detector were 270 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 μ l 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 β -CD-treated cream $\times$ 100/amount of cholesterol in untreated cream (control). Cholesterol determination for a control was done with each treatment batch.

Apparent Viscosity

All measurements were made with a Brookfield viscometer model DVII+, Version 3.0 (Brookfield Engineering Labs, Inc., Staughton, MA), with spindle number 3 at 0.3 rpm. Apparent viscosities of the samples were measured at 22°C. (Kailasapathy and Sellean, 1998).

Overrun

Samples (200 ml) were whipped for 2 and 1/2 min to maximum overrun, according to the following equation (Smith et al., 2000a).

Overrun % = (volume of whipped cream – volume of unwhipped cream) × 100/volume of unwhipped cream.

Foam Instability

Foam instability for the cholesterol-removed whipping cream was measured as the rate of foam drainage (Mangino et al., 1987). A 100-g foam was let stand for 2 h at 24°C, and the defoamed cream as liquid form was collected in mass cylinder and measured as milliliters per unit.

Deemulsification

The whipping cream (1 g) was diluted with 50 ml of distilled water, and 5 ml was transferred to a screw-capped glass tube (15 × 180 mm). Four and half milliliters of distilled water was additionally added, centrifuged at 1000 rpm for 5 min, and let stand for 10 min. Deemulsification was measured spectrophotometrically by absorbance at 540 nm as follows:

% Deemulsification = (absorbance of unwhipped cream – absorbance of whipped cream) × 100/absorbance of unwhipped cream.

Thiobarbituric Acid Test

The cholesterol-removed whipping cream was measured using thiobarbituric acid (TBA) test for fat oxidation during storage at 4°C for 4 wk (Hegenauer et al., 1979). The reagent for TBA test was prepared immediately before use by mixing equal volumes of freshly prepared 0.025 M TBA, which was neutralized with NaOH and 2 M H₃PO₄/2 M citric acid. Reactions of TBA test were started by pipetting 1 g of whipped cream 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 cyclohexane and 1 ml of 4 M ammonium sulfate were added and centrifuged at 2490 × g for 5 min at room temperature. The orange-red cyclohexane supernatant was decanted and its absorbance at 532 nm was measured spectrophotometrically in a 1-cm length path. All measurements run in triplicate.

Statistical Analysis

Data for the determination of optimum conditions of cholesterol-removed whipping cream, one-way ANOVA (SAS Institute Inc., 1985) was used. The significance of the results was analyzed by the least significant difference (LSD) test. A difference of *P* < 0.05 were considered to be significant.

Table 1. Effect of various groups of emulsifier and stabilizer on characteristics of whipping cream.

Groups of emulsifier and stabilizer	Overrun (%)	Foam instability (ml)
I ¹	130 ^a	5 ^a
II ²	120 ^a	1 ^b
III ³	130 ^a	5 ^a

^{a,b}Means of triplicate. Means in a column with different letters differ significantly (*P* < 0.05).

¹0.1% monoglyceride, 0.2% sugar ester, 0.5% lecithin, and 0.1% phosphate.

²0.2% avicell, 0.1% sugar ester, 0.2% α-cellulose, and 0.3% sodium alginate.

³0.2% trisodium citrate, 0.1% sugar ester, 0.2% sodium alginate, and 0.3% sucrose.

RESULTS AND DISCUSSION

Emulsifiers and Stabilizers

To choose appropriate emulsifiers and stabilizers on whipping properties, three different powder-type mixtures were tested (Table 1). When cholesterol-removed whipping cream was manufactured, overrun and foam instability were 130% and 5 ml in groups I and III, respectively. Those values in group II showed a slightly lower overrun value (120%), but not significant (*P* > 0.05), however, higher foam instability (1 ml) than those in others.

Based on results, groups II containing α-cellulose 0.2%, avicell 0.2%, sodium alginate 0.2%, sugar ester 0.1%, and sucrose 0.3% (vol/wt) was an optimum emulsifier and stabilizer, which showed a greater stability. In a subsequent experiment, selected mixture of emulsifier and stabilizer was used.

Among several factors influencing on functional properties of whipping cream, surface-active agents and stabilizers may play an important role, producing a finer foam and stable overrun value and foam stability. Also, microstructure and rheological properties such as an increased viscosity of whipped cream are affected by the addition of stabilizer (Smith, 2000a).

It is well explained that emulsifiers compete for fat interface and hence displace proteins from the interface, which renders it less sterically stable and more susceptible to partial coalescence. These properties allow emulsifiers to enhance desirable qualities by enhancing whipping ability, increasing overrun capacity, reducing whipping time, and enhancing product uniformity. In addition, stabilizer increased foam stability, viscosity, and resistance to shear, resulting in decreased drainage and increased separation of air bubbles (Stanley et al., 1996).

Figure 1. Change of apparent viscosity with different stirring speeds and stirring times in β -cyclodextrin (β -CD) treatment for making cholesterol-removed whipping cream. Other factors: 10% β -CD added, $166 \times g$ of centrifugation speed, and 10-min centrifugation time.

Figure 2. Change of overrun with different stirring speeds and stirring times in β -cyclodextrin (β -CD) treatment for making cholesterol-removed whipping cream. Other factors: 10% β -CD added, $166 \times g$ of centrifugation speed, and 10-min centrifugation time.

Cholesterol Removal Rate

The cholesterol removal process was performed according to our previous study (Ahn and Kwak, 1999) as follows: 10% β -CD, 40°C stirring temperature, 10-, 20-, or 30-min stirring time, 400, 800, or 1,200 rpm stirring speed, $166 \times g$ centrifugal speed, and 10 min centrifugal time. The cholesterol removal rate of the cream under the above conditions reached 90% or higher (data not shown).

Functional Properties

Apparent viscosity. To find out the effects of stirring time and speed on apparent viscosity of cholesterol-removed cream after whipping, different stirring times (10, 20, or 30 min) and stirring speeds (400, 800, or 1200 rpm) to remove cholesterol from cream were tested (Figure 1). With 400 rpm stirring for 30 min, no change was found in viscosity. However, with 800 or 1200 rpm stirring, significant increases were found at both 20 and 30 min. At 30 min, apparent viscosity in whipping cream stirred at 1200 rpm was the highest among other groups, which may be desirable for whipping process. The apparent viscosity of cholesterol-removed whipping cream increased with an increase of stirring time and speed during β -CD treatment. These data suggested

that the fat somewhat flocculated in the cream before whipping by β -CD treatment.

Overrun. When cream was stirred at 400 rpm to remove cholesterol, the overrun value of cholesterol-removed whipping cream was 150% and decreased at both 20 and 30 min as 140% (Figure 2). When stirred at 800 rpm, only 10 min showed 140%, and decreased dramatically to 120% at 20 and 30 min. The overrun decreased with both an increased stirring speed and stirring time for β -CD treatment.

Bruhn and Bruhn (1988) indicated that whipping times required to reach maximum overrun varied significantly according to processing method and stabilizer addition. The addition of stabilizer resulted in a lower overrun as seen in our whipping data and also previously reported (Bruhn and Bruhn, 1988). This effect is supported by an expected decrease in bubble size.

Foam instability. The effects of stirring speed and time to remove cholesterol from cream on foam instability of cholesterol-removed whipping cream were shown in Figure 3. When stirred for 10 min, no difference was found with stirring speed. However, with 30 min of stirring, the instability was decreased as 3, 4, and 6 ml at 400, 800, and 1200 rpm, respectively. These data indicated that lower stirring speed makes better foam instability in cream.

Figure 3. Change of foam instability with different stirring speeds and stirring times in β -cyclodextrin (β -CD) treatment for making cholesterol-removed whipping cream. Other factors: 10% β -CD added, $166 \times g$ of centrifugation speed, and 10-min centrifugation time.

Foam instability is greatly affected by rheological properties of the continuous phase of air bubble as well as by the viscoelastic properties of the interfacial film. In whipped dairy creams, fat globules are partially aggregated in the aqueous phase and evenly distributed around the air/serum interface, thus giving stability and firmness to the foam (Noda and Shiinoki, 1986).

Graf and Muller (1965) defined ideal foam as one that has a rigid structure and a high overrun (100 to 120%) and is stable against deformation. Emulsifiers/stabilizers are usually added to cream to improve the whipping behavior and enhance foam instability. The function of emulsifiers on foam instability attributes to promote adsorption of partially coalesced fat at the air/serum interface through a lowering an interfacial tension (Anderson et al., 1988).

Deemulsification. In all β -CD treatments except for 1200 rpm, similar trend as a decrease with stirring time was found (Figure 4). With 400 rpm stirring, the deemulsification decreased with stirring time as 29.46, 20.04, and 6.72% at 10, 20, and 30 min, respectively. With 1200 rpm stirring, only 10 min of stirring showed a dramatically lower deemulsification. The present data indicated that cream was flocculated before whipping by β -CD treatment, therefore, lower stirring time and

Figure 4. Change of deemulsification with different stirring speeds and stirring times in β -cyclodextrin (β -CD) treatment for making cholesterol-removed whipping cream. Other factors: 10% β -CD added, $166 \times g$ of centrifugation speed, and 10-min centrifugation time.

speed were needed to result in a similar deemulsification in cholesterol-removed whipping cream, compared with control (no stirring and 2 and $\frac{1}{2}$ min whipping).

TBA test during storage. The effect of whipping in cream on chemical oxidation (as measured by the TBA test) during 4 wk of storage is shown in Figure 5. Thiobarbituric acid absorbance was not significantly different between control and β -CD-treated cream until 2 wk. However, a significantly higher TBA value was observed in cholesterol-removed cream at 3 and 4 wk of storage.

O'Sullivan and Keogh (1967) reported that the chemical problem limiting the storage life of whipping cream was the development of oxidative rancidity which resulted in an objectionable oxidized or stale flavor. In addition, they detected a pronounced cooked and oxidized flavor during storage.

CONCLUSIONS

The present study indicated that the cholesterol-removed whipping cream showed the stable functional properties, even with β -CD treatment. Among the functional properties, the apparent viscosity of cholesterol-removed whipping cream increased with a higher stirring time and speed in β -CD treatment. In comparison, the overrun and foam instability characteristics with lower stirring time and speed were close to those of control. Therefore, the present study showed that β -CD treatment process itself caused a somewhat flocculation

Figure 5. Change of thiobarbituric acid value in cholesterol-removed whipping cream stored at 4°C for 4 wk.

in fat of cream, resulting in less whipping time needed for cream whipping. In addition, it may be considered as first evidence, which provides the possibility of cholesterol-removed whipping cream manufacture effectively in industry.

ACKNOWLEDGMENTS

This research was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (02-PJ1-PG10-220055-0002).

REFERENCES

- Adams, M. L., P. M. Sullivan, and E. F. Richter. 1986. Evaluation of direct saponification method for determination of cholesterol in meats. *J. AOAC* 69(5):842–846.
- Ahn, J., and H. S. Kwak. 1999. Optimizing cholesterol removal in cream using β -cyclodextrin and response surface methodology. *J. Food Sci.* 64:629–632.
- Anderson, M., B. E. Brooker, and E. C. Needs. 1987. The role of proteins in the stabilization/destabilization of dairy foams. Pages 100–109 in *Food Emulsions and Foams*. E. Dickinson, ed. Royal Society of Chemistry, London.
- Bruhn, C. M., and J. C. Bruhn. 1988. Observations on the whipping characteristics of cream. *J. Dairy Sci.* 71:857–862.
- Goff, H. D. 1997. Instability and partial coalescence in whippable dairy emulsions. *J. Dairy Sci.* 80:2620–2630.
- Graf, E., and H. R. Muller. 1965. Fine structure and whippability of sterilized cream. *Milchwissenschaft* 20:302–308.
- 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.
- Hegenauer, J., P., Saltman, D. Ludwig, L. Ripley, and P. Bajo. 1979. Effects of supplemental iron and copper on lipid oxidation in milk. 1. Composition of metal complexes in emulsified and homogenized milk. *J. Agric. Food Chem.* 27:860–867.
- Kailasapathy, K., and C. D. Sellpan. 1988. Effect of single and integrated emulsifier-stabilizer on soy-ice confection. *Food Chem.* 63:181–186.
- 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.
- Law, M. R., N. J. Wald, T. Wu, A. Hackshaw, and A. Bailey. 1994. Systematic underestimation of association between serum cholesterol concentration and ischaemic heart disease in observational studies: data from the BUPA study. *Br. Med. J.* 308:363–366.
- Lee, D. K., J. Ahn, and H. S. Kwak. 1999. Cholesterol removal from homogenized milk with β -cyclodextrin. *J. Dairy Sci.* 82:2327–2330.
- Makoto, K., O. Akio, and S. Reijiro. 1992. Cholesterol removal from animal with cyclodextrin by inclusion. Honnen LTD., assigned Japan Pat. No. 4,168,198.
- Mangino, M. E., Y. Y. Liao, N. J. Harper, C. V. Morr, and J. G. Zadow. 1987. Effects of heat processing on the functionality of whey protein concentrates. *J. Food Sci.* 52:1522–1524.
- Nagamoto, S. 1985. Cyclodextrin-expanding the development of their functions and applications. *Chem. Econ. Eng. Rev.* 17:28–34.
- Noda, M., and Y. Shiinoki. 1986. Microstructure and rheological behavior of whipping cream. *J. Texture Stud.* 17:189–196.
- Oakenfull, D. G., and G. S. Sihdu. 1991. Cholesterol reduction. Commonwealth scientific and industrial research organization. Int. Pat. No. 91/11114.
- O'Sullivan A. C., and M. K. Keogh. 1967. Ultra high temperature whipping cream. *J. Dairy Sci.* 51:1324–1329.
- SAS User's Guide: Statistics. 1985. Version 5 Edition. SAS Inst., Inc., Cary, NC.
- Smith, A. K., H. D. Goff, and Y. Kakuda. 2000a. Microstructure and rheological properties of whipped cream as affected by heat treatment and addition of stabilizer. *Int. Dairy J.* 10:295–301.
- Smith, A. K., Y. Kakuda, and H. D. Goff. 2000b. Changes in protein and fat structure in whipped cream caused by heat treatment and addition of stabilizer to the cream. *Food Res. Int.* 33:697–706.
- Stanley, D. W., H. D. Goff, and A. S. Smith. 1996. Texture-structure relationships in foamed dairy emulsions. *Food Res. Int.* 29:1–10.
- Szejtli, J. 1988. Chapter 2: Cyclodextrin inclusion complexes. Pages 79–170 in *Cyclodextrin Technology*. J. Szejtli, ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.