Production of Low-Fat Coconut Milk Using β-Cyclodextrin Column for Selective Removal of Saturated Fatty Acids

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Abstract

The objective of this study was to develop a low-fat coconut milk product by selectively removing saturated fatty acids from coconut milk using β-cyclodextrin (β-CD). The optimal β-CD loading was 50% weight per milk volume at a temperature of 4°C and contact time of 30 min. Approximately 48.9±0.8% of the total fatty acids, especially lauric acid, were removed. A continuous process for fat removal was developed using consecutive β-CD columns. The fat contents in the coconut milk after passing through the first β-CD column were reduced from 18.9±0.4% to 10.5±0.3% and passing through the second β-CD column reduced the fat contents by a further 6.7±0.1%. Interestingly, these fatty acids can be recovered, and β-CD can be reused after elution by ethanol. The sensory evaluation showed that the low-fat coconut milk from the 1st column was acceptable and comparable to the raw material while having a lower fat content. This study has shown the practical and effective application of β-CD for removing medium-chain saturated fatty acids and may apply to other types of high-fat raw materials, creating healthy choices for consumers.

Keywords: Coconut milk, saturated fatty acids, β-cyclodextrin, fatty acid recovery

Introduction

Coconut milk is an opaque white liquid obtained by pressing the grated or oil-in-water emulsion extracted ground coconut endosperm, with or without the addition of water. Coconut milk is an important ingredient in recipes in various parts of the world, especially in many traditional foods of the Asian and Pacific regions, such as curries and desserts, due to its pleasant taste and other desirable sensory characteristics (Karunasiri, Gunawardane, Senanayake, Jayathilaka, & Seneviratne, 2020). Coconut milk contains about 54% moisture, 35% fat, and 11% solid non-fat. Regular coconut milk is higher in fat and calories than cow's milk. It is rich in proteins such as albumin, globulin, prolamin and glutein. Emulsifying agents, such as phospholipids, cephalin, and lecithin, help to increase the stability of food emulsions and these agents are also found in coconut milk. However, the product is highly susceptible to chemical and biochemical spoilage such as fat oxidation (Alyaqoubi et al., 2015). As well, the saturated fatty acids in coconut milk exceed 90% of the total fatty acids which include 40-50% lauric acid, 13-19% myristic acid, and 4-18% palmitic acid. Consumption of these saturated fatty acids may increase the risk of coronary heart disease and ischemic stroke. Therefore, a low-fat coconut milk product would be beneficial to consumers. The separation of fatty acids can be performed using protein or membrane filtration (Priyananda & Chen, 2006). However, these methods cannot selectively remove saturated fatty acids and need high investment and operational costs.

Cyclodextrins (CDs) cyclic oligosaccharides consisting of α-1,4-glycosidic-linked glucosyl residues are produced by the cyclization activity of enzyme cyclodextrin glycosyltransferase (CGTase). There are three major types of CDs (alpha (α)-, beta (β)- and gamma (γ)-CDs), dependingon the number of glucosyl residues in the molecule. The α-CD contains 8 glucose units, β-CD contains 7, and γ-CD contains 8 glucose units, which also means that each type of CD has a different pore size and properties (Matencio, Navarro-Orcajada, García-Garmona, & L´opez-Nicol´as, 2020). Their torus-shaped molecules have a hydrophilic outside, which can dissolve in water, and a hydrophobic cavity, which can form inclusion complexes with a wide variety of poorly water-soluble compounds using hydrogen bonding, hydrophobic, and van der Waals interactions, called guest molecules (Astray, Mejuto, & Simal-Gandara, 2020). The characteristics of the CDs provide important parameters for complex formation with hydrophobic compounds. The formation of inclusion complexes leads to changes in the chemical and physical properties of the encapsulated compounds, mostly in terms of water solubility. Therefore, the guest molecules can be more stable from evaporation, heat, light, or chemical reactions when they are entrapped in CDs. Under suitable conditions, the guest molecules can be separated from the CDs, and their conventional properties are reinstated. For example, the separation of these complexes is controlled by the change of pH in a water solution, leading to the cleavage of the interactions between the CDs and the guest molecules. This has led to the various applications of CDs in chemistry, agriculture, pharmaceuticals, food, cosmetics, and biotechnology.

β-CD has been applied to remove cholesterol and some fatty acids from whole egg, milk and dairy products (Jeong, Sun, Chogsom, & Kwak, 2014; Mudassir, Nadeem, & Baig, 2017; Christoforides, Papaioannou, & Bethanis, 2018; Kolarič, Kántorová, & Šimko, 2022; Kolarič & Šimko, 2022). While other cyclodextrins are not used possibly due to β-CD being less soluble in water, and as milk matrices consist mainly of water, the complex of β-CD-cholesterol is more efficiently separated than other types of CD (di Cagno, 2017).

However, no research has been conducted on the use of β-CD to reduce fat content in coconut milk. Therefore, the objective of this study was to evaluate the application of β -CD for the selective removal of saturated fatty acids in coconut milk. The optimal conditions for batch removal of fat under room temperature and refrigerated temperature were determined. The continuous process for fat removal from coconut milk using the β-CD column was developed. The reusability of the β-CD column was also evaluated.

Methods and Materials

Materials

Coconut milk (16-19 % fat) was purchased from a wet market in Songkhla, Thailand, and used for the experiment within one hour. Commercial β-CD (purity 99.1%) was purchased from Nihon Shokuhin Kaku Co. Ltd. (Osaka, Japan). Lauric acid was purchased from Sigma-Aldrich Chemie Gmbh (Germany). The solvents used were sulfuric acid, amyl alcohol, and ethanol.

Experiment procedure

Fat removal from the coconut milk using β-CD

Different loadings of β-CD (0, 5, 15, 35, and 50%) were added to 20 mL of the coconut milk and mixed with a magnetic stirrer at 100 rpm. The temperature was set at 4°C and room temperature was 30°C. The samples were mixed for 30 min. The mixtures were then filtered with two layers of filter cloth. The filtered coconut milk fraction was determined for fat content, and the fat removal was calculated and compared against the initial fat content in the coconut milk.

Continuous process for fat removal from the coconut milk using β-CD column

Three β-CD columns, each with the same diameter of 1.0 cm, length of 15 cm, and volume of 15 mL, and each containing 5 gm of β-CD, were connected in series. The coconut milk was pumped through a column at a flow rate of 5 mL/min. The outlet coconut milk of each column was pumped to the following column. The fat contents in the coconut milk from each column were compared.

Sensory analysis and evaluation

The taste, flavor, viscosity, color, appearance and overall acceptability of the coconut milk samples were scored by a trained team of panelists using a nine-point hedonic scale, where 1 = extremely unfavorable, 2 = greatly unfavorable, 3 = moderately unfavorable, 4 = slightly unfavorable, 5 = not favorable or unfavorable, 6 = slightly favorable, 7 = moderately favorable, 8 = greatly favorable, and 9 = extremely favorable. The mean scores of each sample attribute were calculated and subjected to statistical analysis.

Reusability of β-CD

β-CD after fat removal was eluted using ethanol at a ratio of 3:1 (v/v) for 1 hr at room temperature. The β-CD after elution with ethanol was dried at temperature of 60°C for 8 hr and reused for the next batch of fat removal.

Analytical methods

In this study, the fat content in the coconut milk was determined using the modified Gerber method (Gurd, Jefferson, Villa, & Rodriguez, 2018). Briefly, the samples were digested in sulphuric acid to break down the proteins and release the lipids, and isoamyl alcohol was added to facilitate phase separation. The contents were mixed in a specially designed butyrometer and centrifuged to isolate the fat into the tube of the butyrometer where the percentage fat content was read from the graduated scale at a defined reading temperature of 65°C. The scale is based on a specific gravity of butterfat of 0.9 at the measuring temperature and a predefined volume of milk (10.75-11 mL).

Fatty acid compositions in the coconut milk were determined by conversion of fat into fatty acid methyl ester (FAME) before analysis by Gas Chromatography (GC) equipped with a cross-linked capillary FFAP column (0.32 mm I.D, 0.25 μm film thickness, length 30 m). The inlet temperature was set at 290°C and the oven temperature was set at 210°C. The oven temperature was held constant for 12 min before being increased to 250°C at a rate of 20°C/min and held for 8 min. The detector temperature was set at 300°C. The fatty acid profiles were quantified by comparing the retention time and peak area with those of the standard FAME (Srinuanpan, Cheirsilp, & Prasertsan, 2018).

Each determination was performed at least in duplicate. Data from each experiment was analyzed by analysis of variance (ANOVA) using a SPSS program, and differences among treatments were determined by Duncan's multiple test at $p<0.05$, unless otherwise stated.

Results and Discussion

Efficacy of β-CD for entrapment of lauric acid

Firstly, the efficacy of β-CD to entrap lauric acid, which is the main saturated fatty acid in coconut milk, was studied by Phenolphthalein Displacement (Skoulika, Georgiou & Polissiou, 1999), using the principle that phenolphthalein entrapped in β-CD shows a colorless solution. When lauric acid is added to the solution, the lauric acid replaces phenolphthalein in the β-CD cavity and phenolphthalein released in the solution can be detected at 550 nm. In our study, the linear correlation between added lauric acid concentration and increased absorbance, which occurred by phenolphthalein replacement in the β-CD cavity, was found, indicating the ability of β-CD to entrap lauric acid. These results are consistent with those reported by Skoulika et al. (1999), who found that β-CD could entrap fatty acids and proportionally release phenolphthalein. Therefore, it could be concluded that the β-CD has the potential to be used to remove lauric acid from coconut milk.

Optimal conditions for fat removal from the coconut milk using β-CD

The effect of β-CD loading (5, 15, 35, and 50%) on fat removal from the coconut milk was investigated. The results at refrigerated temperature (4°C) and room temperature (30°) were compared (Figure 1 and Table 1). The fat content in the coconut milk significantly decreased with increasing β-CD loading (Figure 1). At 4°C using the maximum β-CD loading of 50% and mixing time for 30 min, the fat content decreased from 18.4% to 9.4%, which corresponded to the maximum fat removal of 48.9±0.8%. The higher β-CD loading could not be tested due to the mass transfer limitation. The results were consistent with those reported by Maskooki, Beheshti, Valibeigi, and Feizi (2013) who found that the β-CD loading affected the absorption capacity of the substance. However, increasing the mixing time from 15 min to 30 min only slightly increased the percentage of fat removal (Table 1). Therefore, the β-CD loading significantly influenced fat removal more than did the mixing time. The requirement for β-CD loading also depends on the amount of substance to be entrapped and the modification of β-CD (Kukula, Kolarič, & Šimko, 2020). In the study using crosslinked β-CD by adipic acid for cholesterol removal from milk, only 1% β-CD was required for the removal of 13.4 mg of cholesterol and the mixing time was only 10 min (Han, Kim, Ahn, & Kwak, 2005). Jeong et al. (2014) used crosslinked β-CD to remove cholesterol from whole eggs and found that the addition of β-CD at 25% by weight gave the highest cholesterol removal efficiency of 91.9%.

The effect of temperature was studied by mixing β-CD and the coconut milk for 30 min at different temperatures of 4°C and 30°C. The fat removal from the coconut milk was more effective at 4°C. This might be because the lower temperature of 4°C led to the more stable fatty acids entrapped in the β-CD cavity. Similarly, other research using crosslinked β-CD also found that the removal of cholesterol at the low temperature was higher than that at the high temperature. In a previous study, cholesterol was reduced by 77% at 4°C, 63% at 8°C and 62% at 40°C (Jeong et al., 2014). However, another study using crosslinked β-CD by adipic acid found that temperatures of 0°C and 5°C were less effective in removing cholesterol than higher temperatures of 10°C to 20°C (Han et al., 2005).

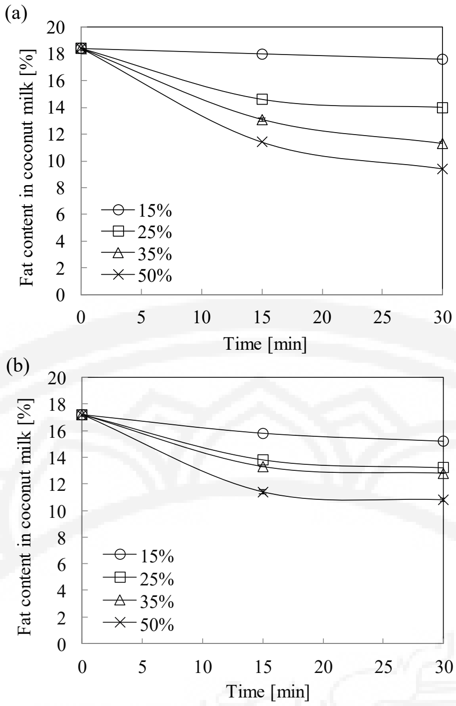

Figure 1 Effect of β-cyclodextrin loadings (% β-CD weight per milk volume) on fat content in the coconut milk at 4°C (a) and 30°C (b)

Table 1 Fat removal in the coconut milk using various β-cyclodextrin loadings

Temperature	β-CD loading [% weight per milk volume]	Fat removal [%] 15 min	Fat removal [%] 30 min
Refrigerated temperature at 4°C	15	2.2 ± 0.0^{l}	4.4 ± 0.0^{k}
	25	20.7 ± 0.0^{h}	23.9 ± 0.0^{fg}
	35	28.8 ± 0.8^{e}	38.6 ± 0.0^{b}
	50	38.0 ± 0.8^{b}	48.9 ± 0.8^{a}
Room temperature at 30°C	15	8.1 ± 0.0^{j}	11.6 ± 0.0^{i}
	25	19.7 ± 0.0^{h}	23.3 ± 0.0^{g}
	35	22.7 ± 0.8^{g}	25.6 ± 0.8^{f}
	50	34.3 ± 0.0^{d}	36.6 ± 0.0^{c}

Superscript letters indicate significant differences between treatments.

Two-consecutive steps for selective removal of fatty acids in the coconut milk by β-CD

As there was a limitation to adding more β-CD into the coconut milk, the two-consecutive step process for fat removal was conducted. In the first step, 10 g of β-CD was added to 20 mL of coconut milk (50% β-CD) and mixed at 4°C for 15 min, and in the second step, after separating the used β-CD, the new batch of β-CD was added and mixed at 4°C for another 15 min (Table 2). After the first batch, the fat content in coconut milk was reduced by 42.3% of the initial fat content, and after two consecutive steps, the fat content was further reduced. The overall fat removal was as high as 71.7% of the initial fat content. As a result, the fat content in coconut milk was reduced to less than 10% and can be claimed as low-fat coconut milk.

Table 3 shows the fatty acid compositions in the coconut milk before and after the first and second batches of fat removal by β-CD. The coconut milk was composed of C8-C22 fatty acids. The main fatty acids were lauric acid (C12:0) 48.4±0.3% of total fatty, acids followed by myristic acid (C14:0) 20.1±0.3% and palmitic acid (C16:0) 10.3±0.3%. In addition, the content of saturated fatty acids was as high as 92.6% of total fatty acids, while the content of unsaturated fatty acids was only 7.5%. After the first batch of fat removal by β-CD, C8-C14 fatty acids were significantly removed due to the adsorption in the cavity of β-CD. The adsorption in the β-CD cavity depends mainly on the hydrophobicity and the size of the substances. As the content of C8-C14 reduced, the content of C16-C18 increased drastically. These results show that β-CD could be used to selectively reduce medium-chain saturated fatty acids in coconut milk.

Table 2 Fat removal in coconut milk using β-cyclodextrin at 50% β-CD weight per milk volume

Sample	Fat in coconut milk [%]	Fat removal [%]
Coconut milk	17.2±0.0[a]	-
Coconut milk after 1st batch	9.9±0.1[b]	42.3±0.7[b]
Coconut milk after 2nd batch	4.9±0.1[c]	71.7±0.7[a]

Superscript letters indicate significant different between treatments

Table 3 Fatty acid composition in coconut milk after fat removal using β-cyclodextrin column

Fatty acid composition	Control [%]	After 1st batch [%]	After 2nd batch [%]
Caprylic acid (C8:0)	4.7±0.8	-	-
Capric acid (C10:0)	5.6±0.2	4.0±0.3	-
Lauric acid (C12:0)	48.4±0.3	16.1±6.2	6.6±0.0
Myristic acid (C14:0)	20.1±0.3	10.7±5.2	5.8±0.0
Palmitic acid (C16:0)	10.2±0.2	28.2±6.2	34.5±10.1
Stearic acid (C18:0)	3.5±0.1	9.3±2.6	15.7±5.8
Oleic acid (C18:1)	6.2±0.2	22.6±1.5	12.7±0.0
Linoleic acid (C18:2)	1.3±0.0	9.1±3.4	30.2±8.3
Arachidic acid (C20:0)	0.1±0.0	-	-
Total saturated fatty acids	92.6	68.3	62.6

Note: Analysis type of fatty acids by gas chromatography
" - ": Not detected.

Continuous fat removal from coconut milk using β-CD column and sensory evaluation

Figure 2 shows the schematic diagram of three β-CD columns, each with a diameter of 1.0 cm, a length of 15 cm, and a volume of 15 mL. Each contained 5 gm of β-CD. The coconut milk at 4°C was pumped through a column at a flow rate of 5 $mL{\cdot}min^{-1}$. Table 4 shows the results of fat removal from coconut milk using the β-CD column. The fat content of the inlet coconut milk was 18.9±0.4%. After passing through the first, second and third β-CD columns, the fat content was reduced to 14.9±0.4%, 10.5±0.3% and 6.7±0.1%, respectively, corresponding to the fat removal of 21.2±1.0%, 44.4±1.0% and 63.6±0.8%, respectively. In addition, to evaluate the reusability of β-CD, the fatty acids were eluted using ethanol for 30 min. It was found that the fat removal using fresh β-CD (55.8±0.7%) and reused β-CD (58.8±0.0%) was comparable indicating the possibility to reuse β-CD. It should also be noted that the recovered fatty acids could be applied as an intermediate and surface active agent in industry and in the manufacture of personal care products in the consumer market (Mahbub, Octaviani, Astuti, Sisunandar, & Dhiani, 2022).

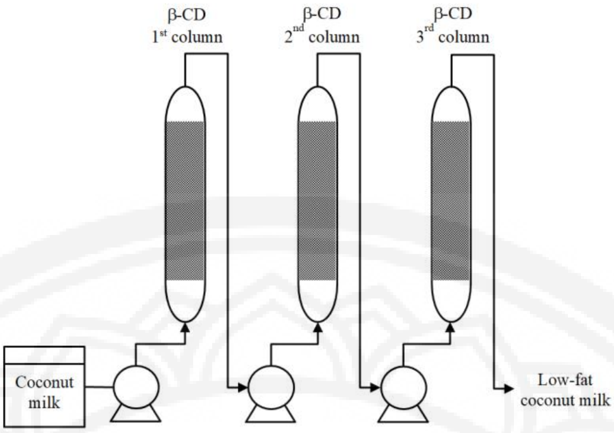

Figure 2 Flow diagram of fat removal in coconut milk by β-cyclodextrin column

Table 4 Continuous process for fat removal in coconut milk using β-cyclodextrin column

Coconut milk	Fat in coconut milk [%] Lipid removal (%)	Fat removal [%]
Inlet coconut milk	18.9±0.4[a]	-
Outlet from 1st column	14.9±0.4[b]	21.2±1.0[c]
Outlet from 2rd column	10.5±0.3[c]	44.4±1.0[b]
Outlet from 3rd column	6.7±0.1[d]	63.6±0.8[a]

Superscript letters indicate significant differences between treatments

Figure 3 shows the sensory evaluation of coconut milk before (control) and after fat removal by β-CD columns. The control coconut milk recorded the highest scores for all evaluation items. There was no significant difference in color score for the control and samples after the 1st and 2nd β-CD columns. The coconut milk after the 1st column received a slightly lower score than the control. The appearance and viscosity of the sample after the 1st and 2nd β-CD columns were similar. However, the flavor, taste, and overall liking scores of the sample after the 2nd β-CD columns reduced significantly, probably due to aroma and texture changes after 44.4% of the fat was removed. This demonstrated that the low-fat coconut milk after the 1st β-CD columns was more acceptable and comparable to the control.

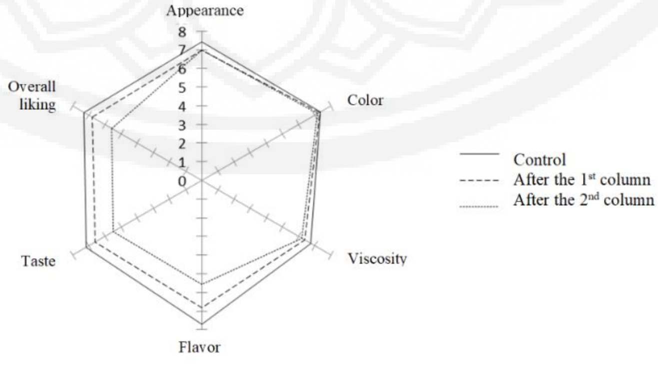

Figure 3 The sensory evaluation of coconut milk before (control) and after fat removal by β-cyclodextrin columns

Conclusion and Suggestions

We have shown that it is possible to use β-CD to reduce the fat content in coconut milk. β-CD significantly reduced the C8-C14 fatty acids particularly, and increased the unsaturated fatty acids in coconut milk. The optimal conditions for the removal of fat were determined and applied in a continuous process using the β-CD column. The optimal β-CD loading and the number of columns depend on the target content of the fat in the final products. The reusability test confirmed the effectiveness of the reused β-CD. Our results suggest that the practical and effective application of β-CD for removing medium-chain fatty acids may apply to other types of high-fat raw materials, which would then increase the choices for consumers who have health problems or want to limit consumption of high-fat foods.

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