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THE EFFECT OF TREATMENT CONDITIONS ON COLOR CHARACTERISTICS AND MEASURE OF CHOLESTEROL REMOVAL FROM MILK BY BETA-CYCLODEXTRIN APPLICATION

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ABSTRACT
Long-term high cholesterol intake is one of the most critical risk factors of cardiovascular diseases (CVD). As milk and dairy products are rich in cholesterol and are consumed on a large scale, the production of low-cholesterol content products could decrease effectively high cholesterol intake what would be one of the crucial steps in CVD prevention. Thus, this study is aimed at optimization of treatment conditions (mixing speed, time, and temperature) and β-cyclodextrin addition affecting the measure of cholesterol removal in milk. As found, the optimal conditions were identified such as mixing speed 840 rpm, mixing time 10 min, and the temperature of mixing 25 °C while the most effectivity in cholesterol decrease content (98.1%) was observed after 2.0% β-cyclodextrin addition. The cholesterol removal process did not affect considerably the lightness values L^* of treated milk, slight differences were noticed in terms of a^* and b^* color values but ΔE values were statistically insignificant, i.e., the process of cholesterol removal did not affect visual characteristics of treated milk. So, these conditions can be applied for the production of milk base functional foods with the decreased cholesterol content.

Keywords: milk; β-cyclodextrin; cholesterol; color; functional foods

INTRODUCTION
Milk fat is probably the most complex of all common fats consisting of numerous fatty acids, especially saturated. Because of high content of saturated fatty acids, milk has been associated with a variety of human diseases **(Seçkin et al., 2005)** thus several international dietary recommendations advise lowering of saturated fat and cholesterol intake mainly for the reduction of cardiovascular diseases (CVD) **(Larsson, Virtamo and Wolk, 2012)**. The most frequently proposed mechanism through which saturated fats negatively influence the risk for CVD is the increase of blood lipids content, especially total cholesterol, and low-density lipoproteins **(Pereira, 2014)**. As milk and dairy products are consumed on a large scale, the importance of the reduction of dietary cholesterol content in these products has risen in the past years. Several methods were developed for the reduction of cholesterol content in dairy products such as the enzymatic conversion of cholesterol, steam distillation, adsorption on several sorbents, supercritical extraction, or the removal of lipids and cholesterol using liquid solvents **(Mohamed et al., 2000)**. However, these methods lack specificity and affect negatively the nutritional and textural properties of final products. Therefore, the most promising method is based on the selective adsorption of cholesterol molecules onto a cyclodextrin cavity. Cyclodextrins (CDs) are cyclic oligomers of α-D-glucopyranose formed by the transformation of starch, and because of their structure, they can be used as encapsulating agents for protecting flavors, vitamins or natural colors, food preservatives, or cholesterol sequestrant, respectively **(Astray et al., 2009)**.

According to **Kukula, Kolarič and Šimko (2020)**, the application of CDs in the dairy industry could decrease the total per capita daily cholesterol intake in the Slovakian population from 369.8 mg to 296.3 mg, which is below the value (300 mg per day for adults) recommended by the World Health Organization **(Bertolín et al., 2018)**. The CD ring is a conical cylinder of an amphiphilic nature, with a hydrophilic outer part and lipophilic cavity **(Matencio et al., 2020)**, and because of that, they can form inclusion complexes with molecules of low hydrophilicity and proper geometrical size **(Fenyvesi, Vikmon and Szente, 2016)**. The most common CDs are the natural α, β, and γ-forms with six, seven, and eight glucose units, respectively **(Matencio et al., 2020)**. In the β-CD molecule, a complete secondary belt is formed by the hydrogen bonds, therefore, the β-CD has the lowest water solubility of all CDs **(Szejtli, 2004; Astray et al., 2009)**. The H-bond belt is incomplete in the α-CD molecule because one glucopyranose unit is in a distorted position, and the γ-CD is a non-coplanar with a more flexible structure thus it is the most soluble of the CDs **(Astray et al., 2009)**. According to the solubility in water, the β-CD is more preferred in cholesterol removal in milk.

The traditional procedure of cholesterol removal in milk by CDs consists of steps such as mixing the milk with the proper concentration of CD, settling the CD-cholesterol complex, centrifugation, and the removal of the CD-cholesterol complex. The measure of cholesterol removal will be thus influenced by the conditions of these processes. According to **Ahn and Kwak (1999)**, the effectiveness of cholesterol adsorption is dependent mainly on β-CD concentration, mixing time, and mixing speed. **Kwak et al. (2003)** reached a 79.3% reduction of cholesterol in raw milk by the application of 1% (w/v) β-CD, mixing time 10 min at 4 °C, and centrifugation speed 166 g. On the other side, **Alonso, Calvo and Fontecha (2019)** reached higher cholesterol removal in raw milk (up to 91.6%) by the application of a lower concentration of β-CD (0.8%, w/v) and longer mixing time (30 min). **Lee, Ahn and Kwak (1999)** suggested that the optimum conditions consisted of 1.5% β-CD, mixing time 10 min at 10 °C, and centrifugation at 166 g for 10 min. Based on their results, the removal rate was influenced neither by mixing time nor by the time of centrifugation.

Therefore, the objective of this study was to find suitable treatment conditions and β-CD concentration on the measure of cholesterol removal from homogenized milk as well as the effect of the cholesterol removal on color characteristics of treated milk.

Scientific hypothesis

The measure of cholesterol removal from homogenized milk is affected by the conditions of mixing and the concentration of β-CD. The application of β-CD does not affect the milk color characteristics.

MATERIAL AND METHODOLOGY

Samples

Commercial milk (3.5% declared fat content) bought in a local market.

Chemicals

β-cyclodextrin (Wacker Chemie AG, Burghausen, Germany, ≥95.0%, HPLC).
Cholesterol (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany, Sigma Grade, ≥99%).
Chloroform (Centralchem s.r.o., Bratislava, Slovakia, p.a.).
n-hexane (Centralchem s.r.o., Bratislava, Slovakia, p.a.).
Ethanol (Centralchem s.r.o., Bratislava, Slovakia, p.a.).
Sodium sulfate anhydrous (Centralchem s.r.o., Bratislava, Slovakia, p.a.).
Potassium hydroxide (Mikrochem, Pezinok, Slovakia, p.a.).
Methanol (Fisher Chemical, Loughborough, UK, HPLC grade).
Acetonitrile (Fisher Chemical, Loughborough, UK, HPLC grade).

Instruments

HPLC system (Agilent Technologies 1260 Infinity, Santa Clara, CA, USA).
Vacuum degasser (Agilent Technologies, Santa Clara, CA, USA).
Quarterly pump (Agilent Technologies, Santa Clara, CA, USA).
Autosampler (Agilent Technologies, Santa Clara, CA, USA).
UV-DAD detector (Agilent Technologies, Santa Clara, CA, USA).
Chromatography column (Zorbax Eclipse Plus C_{18}, 2.1x50 mm, 5 µm particle size, Agilent, Santa Clara, CA, USA).
Guard column (Zorbax SB-C_{18}, 4.6x12.5 mm, 5 µm particle size, Agilent, Santa Clara, CA, USA).
Rotary vacuum evaporator (Heidolph, Germany).
Centrifuge (Hettich Zentrifugen, Germany)
Magnetic stirrer (Arex-6 Connect Pro, Velp Scientifica, Italy).
Analytical balance (Sartorius, Goettingen, Germany)
UV-VIS spectrophotometer (Cary 300, Agilent Technologies, Santa Clara, CA, USA).

Laboratory Methods

HPLC conditions for the determination of cholesterol content

The determination of cholesterol content in milk samples was performed by HPLC analysis using an Agilent Technologies 1260 infinity system (USA) equipped with a vacuum degasser, a quarterly pump, an autosampler, and the UV-DAD detector, set at 205 nm. Isocratic elution was performed at a flow rate of 0.5 $mL.min^{-1}$ using the mobile phase consisted of acetonitrile/methanol 60:40 (v/v). The injection volume was 10 µL and the temperature was set at 30 °C. As a stationary phase, Zorbax Eclipse Plus C_{18} column (2.1×50 mm, 5 µm particle size, Agilent, USA) was used with the guard column Zorbax SB-C_{18} (4.6×12.5 mm, 5 µm particle size, Agilent, USA). The total run time of analysis was 5 min with the retention time of cholesterol in 2.2 min. The calibration curve was recorded from 10 working standard solutions with the concentrations of 2, 6, 10, 25, 40, 50, 75, 100, 300, and 350 $mg.L^{-1}$.

The results were recorded using the OpenLab CDS software, ChemStationEdition for LC, and LC/MS systems (product version A.01.08.108).

Analysis of color

The color of the treated milk was measured using a UV-VIS spectrophotometer Cary 300 (Agilent Technologies, USA) with DRA-CA-30I (internal) sphere accessory. The color itself was evaluated using the CIELAB coordinate system regarding illuminant D65 and a visual angle of 10°. The parameters measured were: L* (lightness factor), a* (–a* = green, +a* = red) and b* (–b* = blue, +b* = yellow). The spectrophotometer was standardized with a white plate, supplied with the equipment. The results were treated with Cary WinUV software (version 4.20(468)) **(Kolarič et al., 2020)**. All procedures were triplicated. ΔE was calculated to estimate the visual differences between the control milk and treated milk using Equation (1):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (1)$$

Description of the Experiment

Sample preparation:

Cholesterol removal:

Cholesterol removal in milk samples was performed according to the modified method by **Kukula, Kolarič and Šimko (2020)**. To study the effects of mixing conditions, 25 g of milk was placed in a beaker, and 1.0% of β-CD was added. The mixtures were stirred at 5 different mixing speeds (480, 600, 720, 840, and 960 rpm) using a magnetic stirrer (Arex-6 Connect Pro, Velp Scientifica, Italy) for 4 different times (5, 10, 15, and 20 min), and at 3 different

temperatures (25, 40, and 50 °C). The mixtures were then centrifuged (Hettich Zentrifugen, Germany) at 200 g for 20 min. After centrifugation, the milk supernatant was analyzed for cholesterol content and color. After setting optimal mixing conditions, the milk samples were stirred with 5 different β-CD concentrations (0.5, 1.0, 1.5, 2.0, and 2.5%). All procedures were triplicated.

Analysis of cholesterol content:

The analysis of cholesterol content in treated milk was performed according to **Kolarič and Šimko (2020)**. 5.0 g of samples were refluxed with 0.015 L of 1 mol.L^{-1} KOH methanolic solution for 15 min. The extraction process was performed with the 0.015 L of the extraction solvent composed of n-hexane and chloroform (1:1, v/v) in duplicate. The extracts were filtrated through anhydrous Na_2SO_4 and evaporated using a rotary vacuum evaporator (Heidolph, Germany). The residue was then dissolved in 5 mL of methanol and filtered using a syringe PTFE filter with 0.2 μm membrane (Agilent Technologies, USA), and analyzed by HPLC. All procedures were triplicated.

Number of samples analyzed: 17
Number of repeated analyses: 3
Number of experiment replication: 1

Statistical Analysis

Results are expressed as mean ±standard deviation or as a percentage. Statistical analysis was performed using the XLSTAT tool of Microsoft Excel 365 (version 2012, Microsoft, USA). The obtained data from the cholesterol content analysis and the analysis of color were subjected to one-way analysis of variance (ANOVA) and the Student´s test, and the values were considered significantly different when p <0.05. From the ANOVA and Student´s test, it was able to determine if there are some significant differences between the results obtained by the analysis of the effect of mixing conditions (speed, time, and temperature), CD concentration, and color changes.

RESULTS AND DISCUSSION

Effect of mixing speed

The cholesterol removal was significantly affected by mixing speed. It was noticed significant (p <0.05) differences between the results obtained at 480 to 600 rpm, and 720, 840, and 960 rpm. The results were not significantly different at 720, 840, and 960 rpm, suggested that the optimum mixing speed begins at 720 rpm. However, the highest removal efficiency (73.3%) was observed at 840 rpm thus this speed was set as the most suitable one, as shown in Figure 1A. The results are similar to other studies. **Kwak, Nam and Ahn (2001)** stirred homogenized milk with β-CD at 800 rpm and reached cholesterol removal up to 78.2% (1.0% β-CD). **Lee, Ahn and Kwak (1999)** reported that mixing speed at 800 rpm is efficient to remove 94.0% of the cholesterol in commercial milk by β-CD (1.0%), also supported by **Lee et al. (2007)**. According to **Maskooki et al. (2013)**, by the application of mixing speed at 700 rpm, it is possible to remove 67.7% of the cholesterol in homogenized milk by β-CD in the same concentration. Lower mixing speed (530 rpm) is reported by **Gianni et al. (2020)** with the cholesterol removal rate varied from 33 to 89%. According to **Kim, Ahn and Kwak (2004)**, the mixing speed is not an important factor in cholesterol removal in milk by crosslinked β-CD as it was not noticed a significant difference with various mixing speeds. The mixing speed is also influenced by the type of sample. E.g., higher speed is required in cream treatment as **Jung, Ko and Kwak (2013)** used 1400 rpm, **Ahn and Kwak (1999)** 800 – 1600 rpm, or **Shim, Ahn and Kwak (2003)** 400 – 1200 rpm. **Tahir, Bokhari and Adnan (2015)** extracted the cholesterol from ghee by mixing with β-CD immobilized on glass beads at 170 rpm.

Effect of mixing time

A significant difference (p <0.05) was noticed between the mixing time of 5 min and 10, 15, and 20 min. The highest measure of cholesterol removal was observed after 20 min of mixing (79.8%) but similar values were monitored in 10 and 15 min (79.3 and 79.2%, respectively). The lowest value of 73.0% was noticed at a mixing time of 5 min (Figure 1B). As found, a mixing time over 10 min is enough for sufficient removal. In comparison to this, slightly different results were reported by **Lee, Ahn and Kwak (1999)**. Based on their results, the cholesterol removal was not affected by a mixing time of 5 to 20 min, but it was noticed a reduction at 25 min of mixing. A similar trend was also mentioned by **Alonso et al. (2009)**. A slight reduction was observed when the mixing time increased to >20 min for 0.8 and 1.0% β-CD. The above results suggest that at longer mixing times, the inclusive complex between β-CD and cholesterol could be unstable. Therefore, from the other studies, it can be concluded that the mixing time between 10 and 20 min is the most suitable for the sufficient removal of cholesterol from milk. **Kwak et al. (2003)** reported that by the mixing time of 10 min it is possible to remove 79.3% of cholesterol content in milk by β-CD addition (1.0%), which is similar to our study. **Lee, Ganesan and Kwak (2012)** used 10 min mixing of milk with crosslinked β-CD and reached the removal of cholesterol up to 93%. Higher mixing times are reported in the case of cream. **Ahn and Kwak (1999)** achieved the highest removal of cholesterol in cream by the mixing time of 30 min. This time was also used in other studies **(Shim, Ahn and Kwak, 2003**; **Jeon et al., 2012**; **Galante et al., 2017)**.

Effect of mixing temperature

Besides the importance of mixing speed and duration, temperature is reported as another factor affecting the measure of cholesterol removal. According to **Matencio et al. (2020)**, the most important factors to consider in the mechanism of CD/guest reaction are the structure of the guest molecule, solvent, and temperature. The various results are noticed among published reports. According to this study (Figure 1C), no significant differences (p <0.05) between the mixing temperatures of 25, 40, and 50 °C, were observed, when the measure of cholesterol removal varied at 79.3, 78.2, and 77.9%, respectively. Similar results were mentioned by **Lee, Ahn and Kwak (1999)** as they also found no differences in cholesterol removal at different temperatures but suggested that mixing at 10 and 25 °C tends to increase cholesterol removal compared to lower values of temperature. A different mixing temperature was used in studies by **Alonso et al. (2009)**, **Alonso et al. (2018)**, and **Alonso, Calvo and Fontecha (2019)**. According to them, the mixing in at 4 °C is sufficient for

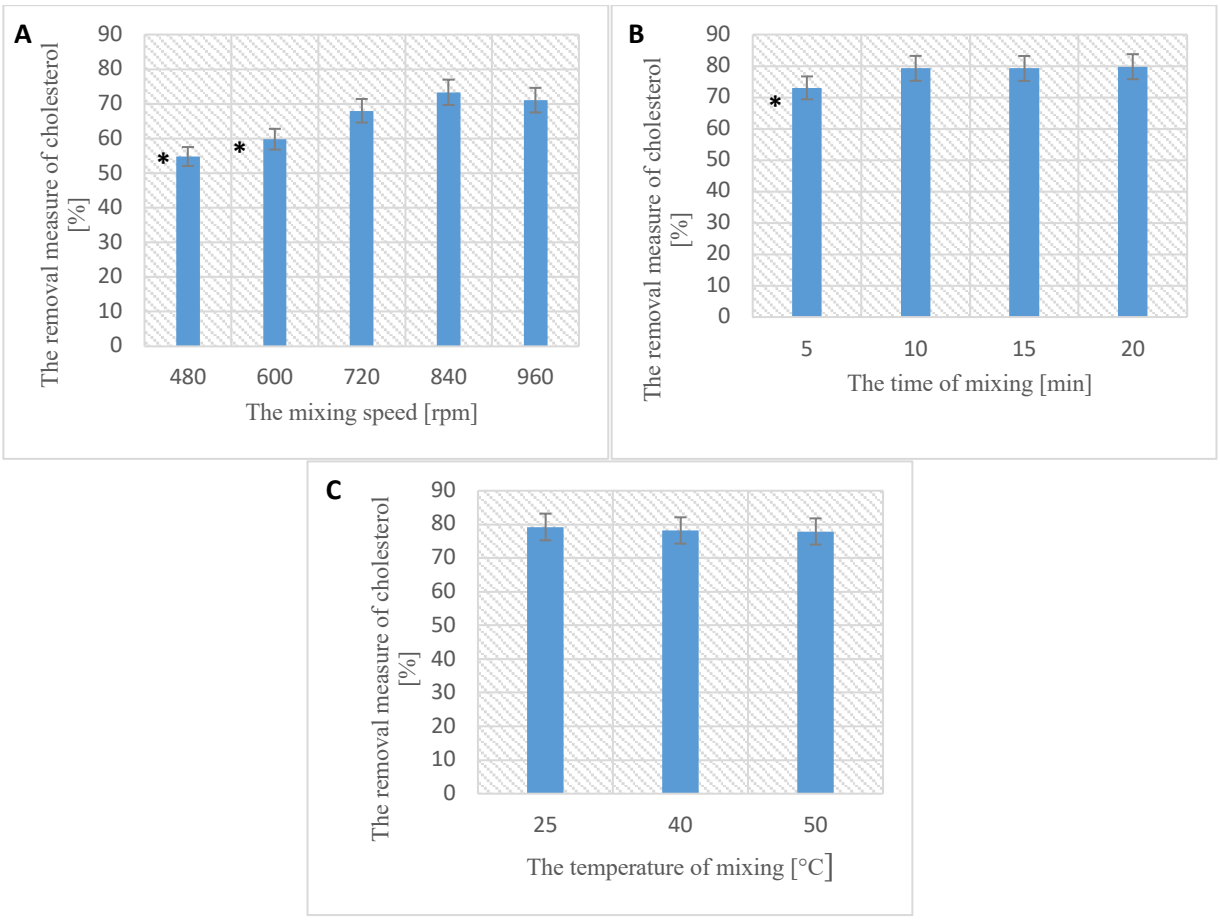

Figure 1 The influence of the mixing conditions (speed (A), time (B), and temperature (C)) on the measure of cholesterol removal. Note: *indicates a statistically significant differences ($p < 0.05$). The other conditions were as follow: A - the concentration of β-CD 1%, mixing for 5 min at 25 °C, centrifuging at 200 g for 10 min. B – the concentration of β-CD 1%, mixing at 840 rpm at 25 °C, centrifuging at 200 g for 10 min, C – the concentration of β-CD 1%, mixing at 840 rpm for 10 min, centrifuging at 200 g for 10 min.

Table 1 The effect of β-CD concentration on the cholesterol removal and color of milk.

β-CD concentration [%]	Cholesterol content [mg.kg^{-1}]	$^{++}$Cholesterol removal [%]	L^* coordinate	a^* coordinate	b^* coordinate	ΔE
0.0	127.3 ±2.1	-	67.01 ±0.18	-0.70 ±0.03	1.57 ±0.11	-
0.5	119.4 ±0.5	$^{+}$6.2 ±0.4	67.53 ±0.37	-0.82 ±0.05	$^{+}$1.18 ±0.05	0.77 ±0.47
1.0	27.2 ±0.7	$^{+}$78.6 ±4.3	67.26 ±0.48	$^{+}$-1.00 ±0.06	$^{+}$1.13 ±0.02	0.59 ±0.29
1.5	2.4 ±0.9	98.1 ±0.7	67.32 ±0.48	-0.76 ±0.02	$^{+}$2.20 ±0.04	0.52 ±0.01
2.0	2.4 ±0.4	98.1 ±0.3	66.86 ±0.27	-0.75 ±0.02	$^{+}$2.33 ±0.13	0.79 ±0.03
2.5	2.6 ±0.5	97.9 ±0.4	67.25 ±0.04	-0.79 ±0.03	$^{+}$2.32 ±0.09	0.69 ±0.04

Note: mean ±standard deviation (n = 3). Color coordinates: L (lightness factor), a (–a = green, +a = red), b (–b = blue, +b = yellow); $^{+}$indicates a statistically significant differences ($p < 0.05$); $^{++}$ The conditions of removal were as follow: mixing at 840 rpm for 10 min at 25 °C, centrifuging at 200 g for 10 min.

cholesterol removal up to 95%, which is effective in maintaining the quality of milk. **Maskooki et al. (2013)** reported that the mixing temperature of 20 °C is more suitable than 8 °C because the aggregations of milk lipoproteins at higher temperatures are lower, and consequently there is a better chance for the entrapment of cholesterol molecules in β-CD cavities.

The effect of milk lipoproteins aggregation may play a greater role by the processing of cream as the cream is mixing with β-CD usually at temperatures between 40 to 50 °C. E.g., **Shim, Ahn and Kwak, 2003**, **Ha et al. (2010)**, or **Lee, Ganesan and Kwak (2012)** used the temperature of mixing at 40 °C and achieved effective removal of cholesterol content in cream.

Effect of β-CD concentration

After the optimization of mixing conditions, the influence of β-CD concentration was also studied, as shown in Table 1. The chromatographic records of the analysis of cholesterol in control milk without the addition of β-CD and with 2.0% β-CD addition are shown in Figure 2. As follows from Table 1, the lowest value was observed at 0.5% of β-

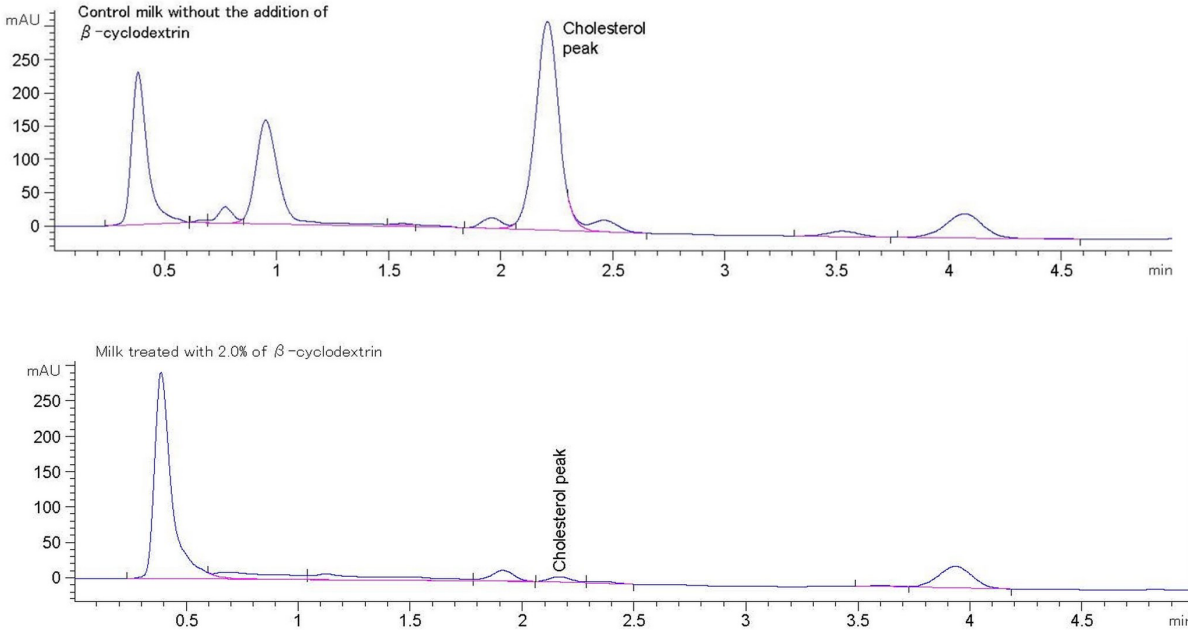

Figure 2 The chromatographic records of the analysis of cholesterol in control milk without the addition of β-cyclodextrin and with 2.0% addition of β-cyclodextrin.

CD (6.2%) and the highest at 2.0% (98.1%). A slight decrease was noticed at 2.5% of β-CD (97.4%). The results were significantly (p <0.05) different at 0.5 and 1.0% of β-CD addition and did not differ at the concentrations of 1.5, 2.0, and 2.5%. Therefore, the optimal β-CD concentration was thus determined at 1.5%. Similar results are reported by **Kwak, Nam and Ahn (2001)**. According to them, a high increase of cholesterol removal was also found between 0.5 and 1.0% β-CD addition. They suggested that 1.0% of β-CD addition may be effective enough for cholesterol removal. However, lower optimal concentrations are published by other authors. **Lee, Ahn and Kwak (1999)** noticed that the least efficiency of removal showed the addition of β-CD at 2.0%. According to **Alonso et al. (2009)**, no significant differences in cholesterol reduction were observed at concentrations of 0.6, 0.8, and 1.0%. According to **Maskooki et al. (2013)**, the measure of cholesterol adsorption was reduced at 1.5% β-CD due to excess of β-CD, which could compete with itself to bind to cholesterol molecules, and consequently reduced the cholesterol adsorption effectivity **(Lee, Ahn and Kwak, 1999; Maskooki et al., 2013)**. Besides that, from the published results, it can be stated that the variation of the optimal β-CD concentrations may be caused by different operational conditions, e.g., mixing temperature or mixing and centrifugal speeds. The β-CD concentration depends also on the fat/cholesterol content of food items. E.g., higher additions are required by the processing of cream. **Shim, Ahn and Kwak (2003)** used 10% of β-CD in the extraction of cholesterol in cream while **Dias et al. (2010)** reported that 20% of β-CD addition showed the best removal rate of cholesterol in commercial butter.

Effect of treatment on color characteristics

Since the color characteristics of treated milk are scarce, these were studied by analyzing measured values in *CIEL*a*b** color space. As follows from Table 1, treatment with β-CD did not affect the lightness values (L^*), which is in accordance with results reported by **Gianni et al. (2020)**. Slight differences were noticed in a^* coordinate values between the control sample and the sample treated with 1% of β-CD. The significant differences (p <0.05) were shown between the control and 1.5, 2, and 2.5% of β-CD in b^* coordinate values. In previous studies, there is no evidence about the influence of β-CD on the yellowness factor of milk. Despite higher values of this coordinate, it was not noticed any significant changes in the visual appearance of treated milk, which was also proven by the calculation of ΔE values. According to **Bhatia et al. (2019)**, the process of cholesterol removal by β-CD does not affect the typical color of cow ghee so it has no impact on the visual consumer perception of the product.

CONCLUSION

This study proved that the removal of cholesterol from milk is affected by treatment parameters such as mixing duration, mixing speed, and the temperature of mixing as well as β-CD concentration. On the basis of experimental data, it was found that optimal mixing conditions were the speed at 840 rpm, the time 10 min, and the temperature at 25 °C when the mixing speed was noticed as the most significant (p <0.05). After the optimization of mixing conditions, the effect of β-CD addition was monitored. The highest decrease of cholesterol content was found at 2.0% β-CD addition (98.1%), but the results between 1.5, 2.0, and 2.5% were insignificant (p <0.05). The treatment did not affect the lightness values L^* of treated milk, the slight differences were observed in both a^* and b^* color values but ΔE values were statistically insignificant (p <0.05). Thus, this study confirmed the excellent possibility of the production of cholesterol-free milk by application of β-CD without any effect of this treatment on milk color characteristics.

REFERENCES

Ahn, J., Kwak, H. S. 1999. Optimizing cholesterol removal in cream using β-cyclodextrin and response surface methodology. *Journal of Food Science*, vol. 64, no. 4, p. 629-632. https://doi.org/10.1111/j.1365-2621.1999.tb15098.x

Alonso, L., Cuesta, P., Fontecha, J., Juarez, M., Gilliland, S. E. 2009. Use of β-cyclodextrin to decrease the level of cholesterol in milk fat. *Journal of Dairy Science*, vol. 92, no. 3, p. 863-869. https://doi.org/10.3168/jds.2008-1452

Alonso, L., Fox, P. F., Calvo, M. V., Fontecha, J. 2018. Effect of beta cyclodextrin on the reduction of cholesterol in ewe´s milk manchego cheese. *Molecules*, vol. 23, no. 7, p. 1789. https://doi.org/10.3390/molecules23071789

Alonso, L., Calvo, M. V., Fontecha, J. 2019. A scale-up process for the manufacture of reduced-cholesterol butter using beta-cyclodextrin. *Journal of Food Process Engineering*, vol. 42, no. 3, p. e13009. https://doi.org/10.1111/jfpe.13009

Astray, G., Gonzalez-Barreiro, C., Mejuto, J. C., Rial-Otero, R., Simal-Gándara, J. 2009. A review on the use of cyclodextrins in foods. *Food Hydrocolloids*, vol. 23, no. 7, p. 1631-1640. https://doi.org/10.1016/j.foodhyd.2009.01.001

Bertolín, J. R., Joy, M., Rufino-Moya, P. J., Lobón, S., Blanco, M. 2018. Simultaneous determination of carotenoids, tocopherols, retinol and cholesterol in ovine lyophilised samples of milk, meat, and liver and in unprocessed/raw samples of fat. *Food Chemistry,* vol. 257, p. 182-188. https://doi.org/10.1016/j.foodchem.2018.02.139

Bhatia, P., Sharma, V., Arora, S., Rao, P. S. 2019. Effect of cholesterol removal on compositional and the physicochemical characteristics of anhydrous cow milk fat (cow ghee). *International Journal of Food Properties*, vol. 22, no. 1, p. 1-8. https://doi.org/10.1080/10942912.2018.1564762

Dias, H. M. A. M., Berbicz, F., Pedrochi, F., Baesso, M. L., Matioli, G. 2010. Butter cholesterol removal using different complexation methods with beta-cyclodextrin, and the contribution of photoacoustic spectroscopy to the evaluation of the complex. *Food Research International*, vol. 43, no. 4, p. 1104-1110. https://doi.org/10.1016/j.foodres.2010.02.002

Fenyvesi, É., Vikmon, M., Szente, L. 2016. Cyclodextrins in food technology and human nutrition: Benefits and limitations. *Critical Reviews in Food Science and Nutrition*, vol. 56, no. 12, p. 1981-2004. https://doi.org/10.1080/10408398.2013.809513

Galante, M., Pavón, Y., Lazzaroni, S., Soazo, M., Costa, S., Boeris, V., Risso, P., Rozycki, S. 2017. Effect of cholesterol-reduced and zinc fortification treatments on physicochemical, functional, textural, microstructural and sensory properties of soft cheese. *International Journal of Dairy Technology*, vol. 70, no. 4, p. 533-41. https://doi.org/10.1111/1471-0307.12361

Gianni, D. E., Jorcin, S., Lema, P., Olazabal, L., Medrano, A., Lopez-Pedemonte, T. 2020. Effect of ultra-high pressure homogenization combined with β-cyclodextrin in the development of a cholesterol-reduced whole milk. *Journal of Food Processing and Preservation*, vol. 44, no. 11, p. e14845. https://doi.org/10.1111/jfpp.14845

Ha, H. J., Lee, J. E., Chang, Y. H., Kwak, H-S. 2010. Entrapment of nutrients during cholesterol removal from cream by crosslinked β-cyclodextrin. *International Journal of Dairy Technology*, vol. 63, no. 1, p. 119-126. https://doi.org/10.1111/j.1471-0307.2009.00538.x

Jeon, S-S., Ganesan, P., Lee, Y-S., Yoo, S-H., Kwak, H-S. 2012. Texture and sensory properties of cream cheese and cholesterol-removed cream cheese made from whole milk powder. *Korean Journal for Food Science of Animal Resources*, vol. 32, no. 1, p. 49-53. https://doi.org/10.5851/kosfa.2012.32.1.49

Jung, H-J., Ko, E-J., Kwak, H-S. 2013. Comparison of physicochemical and sensory properties between cholesterol-removed gouda cheese and gouda cheese during ripening. *Asian-Australasian Journal of Animal Science*, vol. 26, no. 12, p. 1773-1780. https://doi.org/10.5713/ajas.2013.13255

Kim, S. H., Ahn, J., Kwak, H. S. 2004. Crosslinking of β-cyclodextrin on cholesterol removal from milk. *Archives of Pharmacal Research*, vol. 27, p. 1183-1187. https://doi.org/10.1007/BF02975126

Kolarič, L., Minarovičová, L., Lauková, M., Karovičová, J., Kohajdová, Z. 2020. Pasta noodles enriched with sweet potato starch: Impact on quality parameters and resistant starch content. *Journal of Texture Studies*, vol. 51, no. 3, p. 464-474. https://doi.org/10.1111/jtxs.12489

Kolarič, L., Šimko, P. 2020. Determination of cholesterol content in butter by HPLC: Up-to-date optimization, and in-house validation using reference materials. *Foods*, vol. 9, no. 10, p. 1378. https://doi.org/10.3390/foods9101378

Kukula, M., Kolarič, L., Šimko, P. 2020. Decrease of cholesterol content in milk by sorption onto β-cyclodextrin crosslinked by tartaric acid, considerations and implications. *Acta Chimica Slovaca*, vol. 13, no. 2, p. 1-6. https://doi.org/10.2478/acs-2020-0024

Kwak, H. S., Nam, C. G., Ahn, J. 2001. Low cholesterol mozzarella cheese obtained from homogenized and β-cyclodextrin-treated milk. *Asian-Australasian Journal of Animal Sciences*, vol. 14, no. 2, p. 268-275. https://doi.org/10.5713/ajas.2001.268

Kwak, H. S., Jung, C. S., Seok, J. S., Ahn, J. 2003. Cholesterol removal and flavor development in cheddar cheese. *Asian-Australasian Journal of Animal Sciences,* vol. 16, no. 3, p. 409-416. https://doi.org/10.5713/ajas.2003.409

Larsson, S. C., Virtamo, J., Wolk, A. 2012. Dietary fats and dietary cholesterol and risk of stroke in women. *Atherosclerosis*, vol. 221, no. 1, p. 282-286. https://doi.org/10.1016/j.atherosclerosis.2011.12.043

Lee, D. K., Ahn, J., Kwak, H. S. 1999. Cholesterol removal from homogenized milk with β-cyclodextrin. *Journal of Dairy Science*, vol. 82, p. 2327-2330. https://doi.org/10.3168/jds.S0022-0302(99)75481-0

Lee, S-J., Hwang, J-H., Lee, S., Ahn, J., Kwak, H-S. 2007. Property changes and cholesterol-lowering effects in evening primrose oil-enriched and cholesterol-reduced yogurt. International Journal of Dairy Technology, vol. 60, no. 1, p. 22-30. https://doi.org/10.1111/j.1471-0307.2007.00294.x

Lee, Y-K., Ganesan, P., Kwak, H-S. 2012. Optimisation of cross-linking β-cyclodextrin and its recycling efficiency for cholesterol removal in milk and cream. *International Journal of Food Science and Technology*, vol. 47, no. 5, p. 933-939. https://doi.org/10.1111/j.1365-2621.2011.02924.x

Maskooki, A. M., Beheshti, S. H. R., Valibeigi, S., Feizi, J. 2013. Effect of cholesterol removal processing using β-cyclodextrin on main components of milk. *International Journal of Food Science*, vol. 2013, 6 p. https://doi.org/10.1155/2013/215305

Matencio, A., Navarro-Orcajada, S., García-Garmona, F., López-Nicolás, J. M. 2020. Applications of cyclodextrins in food science. A review. *Trends in Food Science & Technology*, vol. 104, p. 132-143. https://doi.org/10.1016/j.tifs.2020.08.009

Mohamed, R. S., Saldaña, M. D. A., Socantaype, F. H., Kieckbusch, T. G. 2000. Reduction in the cholesterol content of butter oil using supercritical ethane extraction and adsorption on alumina. *Journal of Supercritical Fluids*, vol. 16, no. 3, p. 225-233. https://doi.org/10.1016/S0896-8446(99)00034-0

Pereira, P.C. 2014. Milk nutritional composition and its role in human health. *Nutrition,* vol. 30, no. 6, p. 619-627. https://doi.org/10.1016/j.nut.2013.10.011

Seçkin, A. K., Gursoy, O., Kinik, O., Akbulut, N. 2005. Conjugated linoleic acid (CLA) concentration, fatty acid composition and cholesterol of some Turkish dairy products. *LWT-Food Science and Technology*, vol. 38, no. 8, p. 909-915. https://doi.org/10.1016/j.lwt.2004.09.011

Shim, S. Y., Ahn, J., Kwak, H. S. 2003. Functional properties of cholesterol-removed whipping cream treated by β-cyclodextrin. *Journal of Dairy Science*, vol. 86, no. 9, p. 2767-2772. https://doi.org/10.3168/jds.S0022-0302(03)73873-9

Szejtli, J. 2004. Past, present, and future of cyclodextrin research. *Pure and Applied Chemistry*, vol. 76, no. 10, p. 1825-1845. https://doi.org/10.1351/pac200476101825

Tahir, M. N., Bokhari, S. A., Adnan, A. 2015. Cholesterol extraction from ghee using glass beads functionalized with beta cyclodextrin. *Journal of Food Science and Technology*, vol. 52, p. 1040-1046. https://doi.org/10.1007/s13197-013-1039-2

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