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Passer Journal of Basic and Applied Sciences

Passer

Passer 7 (Issue 1) (2025) 495-498

Original Article

Cholesterol Reduction in Milk/Queso Blanco Cheese via β-Cyclodextrin

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Received 21 December 2024; revised 14 February 2025;
accepted 13 April 2025; available online 24 June 2025

DOI: 10.24271/PSR.2025.495169.1866

ABSTRACT

Since milk and its products raise the risk of cardiovascular disease, there has been an annual growth in the demand for low-cholesterol dairy products. Therefore, this study focuses on developing Queso Blanco cheese by treating prepared bovine milk with beta-cyclodextrin (β-CD). An HPLC analysis of treated bovine milk (TBM) and untreated bovine milk (UBM) showed a considerable reduction in cholesterol from 2.37 mg/100g to 1.13 mg/100g, with a reduction ratio of 52.32%. Experimental bovine milk (EBM) and control bovine milk (CBM) did not differ substantially ($p<0.05$) in physical characteristics, including pH, acidity, and viscosity, suggesting that β-CD treatment did not negatively impact these attributes. Experimental Queso Blanco cheese (QEC) was made with CBM, and control cheese (QCC) was made with EBM. A proximate study revealed no discernible differences in the amounts of protein, fat, and moisture between QCC and QEC. Likewise, texture analysis verified that the cheese's hardness remained unchanged after the β-CD treatment. These results demonstrate that β-CD can successfully reduce the cholesterol in cow's milk without changing the final texture, color, or flavor of Queso Blanco cheese. Further research into this technology for large-scale production and an evaluation of its suitability for other dairy products is advised.

Keywords: Low-cholesterol dairy products, β-cyclodextrin, Queso Blanco cheese, HPLC analysis.

1 Introduction

Although primary prevention and medical care have greatly improved, cardiovascular diseases (CVD) continue to be the world's leading cause of death, accounting for about 1.8 million deaths annually in the European Union[1]. Healthy eating patterns like the Mediterranean style dish (Dietary Approaches to Stop Hypertension), which stresses items that are relatively low in cholesterol content, such fruits, vegetables, whole grains, low-fat or fat-free dairy products, etc., should be emphasized in current dietary guidelines because nutritional habits hurt CVD[2]. Cardiovascular disorders rank first among the top 10 causes of death, according to a World Health Organization assessment. According to WHO estimates, the various forms of cardiovascular disease (CVD) account for 29.2% of all deaths worldwide, or 16.7 million people [3]. The direct correlation between high blood cholesterol and CVD has been proven. For instance, 95% to 98% of milk fat contains cholesterol, a common animal sterol [4]. Milk and dairy products are rich providers of nutrients, but in recent years, their saturated fatty acid content has been linked to several health issues, such as elevated LDL-C levels and a higher risk of cardiovascular disease (CVD)[1]. A population-based cohort study and meta-analysis found a favorable correlation between milk intake and coronary heart disease, but no significant relationship between milk consumption and overall mortality. The significance of lowering the dietary cholesterol content of these products may contribute to the incidence of CVD because of their high consumption [5].

Due to health-conscious attitudes, there has been a sharp rise in demand for dairy products with reduced cholesterol levels [6]. The cholesterol content of dairy products can be reduced using several techniques, including steam distillation, supercritical extraction, enzymatic conversion, and the adsorption of different sorbents. However, these methods are mainly non-selective and negatively affect the mechanical and nutritional properties of the finished products [7]. β-cyclodextrin (β-CD) can also be used to eliminate cholesterol because it is an effective method that does not significantly alter the quantity of other flavoring and nutritional components [8].

Regarding safety and structure, β-CD is a doughnut-shaped cyclic oligosaccharide composed of seven glucose units that, due to its proper width, may nearly perfectly entrap a cholesterol molecule [9]. The World Health Organization and the Joint Expert

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Peer-reviewed under the responsibility of the University of Garmian.

Committee on Food Additives (JECFA) have deemed β-CD to be "safe". In many tests, this component has been successfully employed to reduce the cholesterol level of different types of cheese. Consider Manchego cheese as an example [10]. The production of cheese also increases every year. Overall, cheese consumption is highest in Europe and North America. However, it is also expected that cheese consumption would rise in regions like Southeast Asian nations where it was not historically a staple food [11]. As a result, eating cheese also dramatically increases the amount of cholesterol in the diet.

The primary objective of this study is to create Queso Blanco cheese with a lower cholesterol content because, as far as we are aware, no research has been done on the production of cholesterol-reduced Queso Blanco cheese.

2 Materials and methods

2.1 Materials and reagents

Fresh bovine milk was supplied by the Agricultural College of Suleimani/Iraq. A local market was the source of the citric acid. B-cyclodextrin (B-CD) was purchased from the German company Wacker Chemic. Analytical-grade potassium hydroxide (KOH), chloroform, n-hexane, ethanol, and anhydrous sodium sulfate were acquired from Chem-Lab (Belgium), at the same time, Fisher Chemical in Loughborough, UK, provided methanol and acetonitrile for HPLC analysis.

2.2 Low-cholesterol bovine milk preparation

After cooling at 10°C, 5 kg of bulk bovine milk was agitated for 10 minutes at 800 rpm using 1% β-CD in a blender (NJ-Rong Hua-Scientific, China). B-CD was subsequently extracted from the milk by centrifuging it at 166 x g. Every treatment was made in duplicate [8].

2.3 Queso Blanco cheese production

For making Queso Blanco cheese, a recommended method by [12] was adopted with some modifications. The citric acid solution (10%) was added to the heated bovine milk (83 °C), in three batches with continuous stirring for half a minute after each batch. Until all of the milk has formed curds, keep stirring. After adding 3% salt, the curds were placed in cheesecloth and special aluminum hoops and pressed for at least five hrs. Stored in the refrigerator at 5 °C for 7 days. For both treated milk (TM) and non-treated milk (NTM), each batch of cheese was made in triplicate.

2.4 Cholesterol Analysis

The HPLC method was used to determine cholesterol using a capillary column of 100x4 mm, nuclear C18 PAR measuring 3 μm, and a UV detector set to 205 nm (Shimadzu, Kyoto). A temperature of 35°C was established for the column. The HPLC procedure's approach for preparing solutions and reagents was explained by [13]. For the sample preparation, 1 g of the methanolic solution of 10 mol/l potassium hydroxide (9:1) was added and refluxed for 30 minutes. An internal standard of 0.1% stigmasterol solution was applied to the milk samples. The samples were dissolved in 10 ml of isopropanol before the saponification, and an aliquot of 1 ml was used. After cooling, 5 ml of deionized water and 10 ml of n-hexane were added and intensively shaken for 20 minutes. The organic layer was separated, washed with deionized water to the neutral reaction, and dried with sodium sulfate. For HPLC analysis, the hexane solution was evaporated, and the residue was then dissolved in one milliliter of methanol. The reduction % in cholesterol was calculated using the following formula:

$$\text{cholesterol reduction\%} = \frac{\text{amount of cholesterol in the untreated milk} - \text{amount of cholesterol in the treated milk}}{\text{cholesterol content in the untreated milk}} * 100$$

There were HPLC curves in the supplemental file.

2.5 Proximate Analysis

Moisture, crude protein (N*6.38), and fat proximate compositions were calculated using [14].

2.6 Physical properties

The texture, viscosity, and pH levels were evaluated using the method described by [15]. An Inolab WTW Seirs 720 pH meter was used to measure the pH readings. The Brookfield viscometer (type DV-E, Brookfield Engineering Laboratories) was used to measure the viscosity. Using spindle No. 7 at 100 rpm, and at room temperature. In the meantime, a texture analyzer (CT3, 4500, Brookfield Engineering Lab) was used to determine the texture.

2.7 Sensory evaluation

A team of ten skilled sensory experts assessed Queso Blanco cheeses. The intensity of texture, color, and flavor was evaluated on a ten-point scale, with 10 denoting good and 1 denoting bad.

2.8 Statistical evaluation

The results are shown as a percentage and as mean ± standard deviation. Origin Pro 9.0 was used for the statistical analysis (OriginLab Corp., USA). All the gathered data were subjected to a one-way analysis of variance (ANOVA), and a difference of $p < 0.05$ was considered significant.

3 Results and discussion

Table 1 summarizes the amount of cholesterol in untreated bovine milk (UBM) and treated bovine milk (TBM) with %1% β-CD. The former's cholesterol score was approximately 2.37 mg/100g, while the latter was approximately 1.13 mg/100g. Additionally, the table indicates that treating bovine milk with 1% beta-cyclodextrin significantly lowers the cholesterol content by roughly 52.33%. A study by [8] utilized the same concentration of %1 β-CD and obtained a larger reduction of about 97.2%; this discrepancy is probably because the milk used in our investigation is not homogenized. When milk was homogenized, the rate at which β-CD removed cholesterol was higher than when milk was not homogenized.

Table 1: Amount of cholesterol and cholesterol removal percent, in the control bovine milk (CBM), and experimental bovine milk (EBC).

Parameters	CBM	EBM
Cholesterol (mg/100g fat)	2.37 ±0.05a	1.13 ±0.02b
Cholesterol removal (%fat)	0.00	52.33

Values are expressed in mean ± standard deviation (n=3). Means within the same row followed by different superscript lowercase letters indicate significant differences at $p<0.05$ by Tukey's multiple comparisons tests.

An investigation into the Ewe's Milk Manchego cheese was conducted by [16]. They suggested that due to the structural characteristics of β-CD and the specific processing conditions used during cholesterol removal, it is likely that some milk components are also retained and eliminated along with the cholesterol. Therefore, it is crucial to look into the compositional changes that occur when the cholesterol in Manchego cheese is removed. In light of this, we examined the physical and chemical characteristics of milk and Queso Blanco cheese in our study. The physical characteristics of CBM and EBM are compared in Table 2 using the parameters of pH, acidity percentage, and viscosity. The pH of CBM and EBM is roughly 6.4, meaning there is no discernible ($p<0.05$) difference between the two samples. Additionally, the acidity percentage indicates how much acid is in the milk. The acidity of the CBM is 0.183%, whereas that of the EBM is 0.186%. There is no discernible ($p<0.05$) difference between these results, which are extremely nearby.

Table 2. Physical properties of control bovine milk (CBM), and experimental bovine milk (EBM).

Test	CBM	EBC
pH	6.4 ± 0.2a	6.4 ± 0.1a
Acidity%	0.183 ± 0.5a	0.186 ± 0.5a
Viscosity (poise)	13.95 ± 0.3a	14.62 ± 0.5a

Values are expressed in mean ± standard deviation (n=3). Means within the same row followed by same superscript lowercase letters indicate significant differences at $p<0.05$ by Tukey's multiple comparisons tests.

Viscosity was the third physical property parameter. The thickness of flow resistance is measured by viscosity [17]. The EBM had a slightly higher viscosity of 14.62 poise than the CBM, which had a viscosity of 13.95 poise. Despite the EBM's slight rise in viscosity, the difference is not statistically significant. The results of the physical properties of CBM and EBM indicate that using 1% β-CD did not affect the milk's physical properties. This result is in agreement with[18].

Following the preparation and analysis of CBM and EBC, Queso Blanco cheese was made from the two types of milk mentioned above to examine the effects of adding 1% of β-CD on the cholesterol ratio and its elimination. To examine the impact of lowering cholesterol on the chemical and physical characteristics as well as sensory testing in both genotypes, chemical analysis, and hardness tests were performed on both varieties of cheese.

Results from the proximate analysis, which took into account fat, protein, and moisture, showed that the ratios of (QCC) and (QEC) were similar (Table 3). This conclusion is consistent with [18]. They stated that the addition of β-CD had no influence on the chemical characteristics of milk, and the protein, fat, and moisture percentages stayed the same. Additionally, a texture examination was performed for both cheese varieties to compare the hardness of QCC and QEC.

Table 3: Proximate analyses of Queso Blanco control cheese (QCC), and experimental Queso Blanco cheese (QEC).

Test%	QCC	QEC
Moisture	62.53 ± 02a	62.30 ± 01a
Fat	23.33 ± 01a	23.66 ± 02a
Protein	20.41 ± 05a	20.44 ± 05a

Values are expressed in mean ± standard deviation (n=3). Means within the same row followed by the same superscript lowercase letters indicate no significant differences at $p<0.05$ by Tukey's multiple comparisons tests.

The (Table 4) shows the results of the texture analysis of QCC and QEC. Specifically, it compares the hardness of the two types of cheese. Hardness measures how firm the cheese is when it is applied. The harness value for QCC scored around 669.75 g, while QEC has a slightly higher hardness of around 676.75 g. The texture study verifies that the texture is unaffected by using 1% β-CD, and there was no significant difference ($p <0.05$) between QCC and QEC in terms of hardness. A similar finding was decelerated by [18].

The last investigation done for QCC and QEC was sensory evaluation. The (Table 5) shows the average sensory scores for each sensory attribute. The results show that the sensory properties (texture, color, and flavor) of Queso Blanco cheese are not significantly ($p<0.05$) affected by 1% β-CD treatment. This finding is in agreement with [19].

Overall, all of the results indicate that β-CD can reduce cholesterol in cheese without compromising its chemical, physical, and sensory properties.

Table 4: Texture analyses of Queso Blanco control cheese (QCC), and experimental Queso Blanco cheese (QEC).

Texture	QCC	QEC
Hardness (g)	669.75 ± 0.2a	676.75 ± 0.1a

Values are expressed in mean ± standard deviation (n=3). Means within the same row followed by the same superscript lowercase letters indicate no significant differences at $p<0.05$ by Tukey's multiple comparisons tests.

Table 5: Effect of β-CD treatment on the mean values of sensory evaluation of QCC and QEC.

Sensory properties	QCC	QEC
Texture	8.35 ± 0.05a	8.43 ± 0.02a
Color	8.78 ± 0.10a	8.91 ± 0.06a
Flavor	8.42 ± 0.50a	8.44 ± 0.05a

Values are expressed in mean ± standard deviation (n=3). Means within the same row followed by the same superscript lowercase letters indicate no significant differences at $p<0.05$ by Tukey's multiple comparisons tests.

Conclusion

It is possible to reduce cholesterol in bovine milk by up to 50% by adding 1% β-CD without sacrificing the milk's physical and chemical characteristics or its texture or flavor. The production of healthier dairy products, like Queso Blanco cheese, is thereby made possible by β-CD. Future research suggests using different concentrations of β-CD, and knowledge of the implications of these findings may lead to innovative dairy processing methods that enhance health benefits while also meeting consumer demand for functional foods. It was recommended that future studies look at β-CD treatment for large-scale production to reduce cholesterol in various dairy products without compromising their sensory qualities and quality.

Acknowledgment

We thank Dr. Abdol Al-Majeed from Baghdad University for his valuable input and advice, and we appreciate all his ideas.

Conflict of interest

The authors declare no conflict of interest.

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