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RESEARCH BASED BOOK CHAPTER

IMPACT OF CURD WASHING AND BETA CYCLODEXTRIN ON THE REDUCTION OF CHOLESTEROL CONTENTS IN EWE´S MILK CHEESE

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Abstract

Beta-cyclodextrin (β-CD) is a cyclic oligosaccharide composed of seven glucose units. The utilization of β-cyclodextrin (β-CD) in the field of food research has been on the trend, primarily for its ability to reduce cholesterol levels. This can be attributed to β-CD's strong attraction to nonpolar compounds, including cholesterol. The objective of this study was to assess the impact of curd washing in ewe's milk cheese on the reduction of cholesterol by β-CD in pasteurized ewe's milk Manchego cheese, specifically focusing on the primary constituents of milk, lipids, and flavor characteristics. A significant reduction of approximately 98.45% in cholesterol levels was observed in the experimental cheeses subjected to treatment with β-CD. The remaining β-CD exhibited significant differences ($p \leq 0.05$) between the curd washing and non-curd washing conditions, with values of 0.51% and 0.27%, respectively. The physicochemical properties, specifically fat, moisture, and protein content, were not observed to be significantly affected by the process of curd washing, regardless of the presence or absence of β-CD. However, slight differences were observed in the levels of soluble nitrogen and non-protein nitrogen because of the treatment. The lipid fraction of fatty acids, triglycerides, and phospholipids exhibited comparable quantities in both the treated and untreated cheese samples, as influenced by curd washing and the presence or absence of β-CD. The influence of β-CD on flavor compounds and short chain free fatty acids was not found to be statistically significant for most compounds. However, 3 methyl butanal and ethanol were the only compounds that exhibited a statistically significant difference ($p \leq 0.05$). There were no statistically significant differences ($p \leq 0.05$) observed in the sensory attributes, including flavor, aroma, color, and acceptability, between the curd washing treatments with or without β-CD. However, a significant difference ($p \leq 0.05$) was observed in texture between the two treatments. The β-CD molecules are edible and nontoxic in nature, making them suitable for use in the safe processing of cholesterol removal in cheese manufacturing.

The reduction of residual β-CD can be improved by implementing curd washing, resulting in a 47% decrease. Hence, the current investigation points out that the implementation of curd washing proved to be an efficacious technique for the reduction of cholesterol in Manchego cheese while maintaining its inherent qualities. The methodology employed for the treatment of β-cyclodextrin (β-CD) was implemented.

Keywords

Beta Cyclodextrin, Ewe´s Milk, Curd Washing, Cheese, Manchego, Lipids

1. Introduction

Dairy products are considered nutrient-dense and healthy foods for all ages of consumers; however, dairy products such as butter, cream and cheeses possessed high fat contents. The occurrence of different metabolic diseases has been associated with lipids types and cholesterol level in the diet. During last decade, the American Heart Association and World Health Organization have recommended the consumers to follow the dietary guidelines regarding intake of saturated lipids, fatty acids and cholesterol to lower the risk of metabolic disorders and related coronary heart diseases. Therefore, the demand for dairy products with low cholesterol contents has been increasing rapidly because of health-conscious perceptions [1, 2]. In response, the dairy industry professionals and researchers are emphasizing to manufacture the dairy products with decreased level of cholesterol. Moreover, the high demand of cholesterol-reduced dairy products, coupled with the minimum production cost, is a salient feature that has been focused to aid dairy alternatives suppliers and manufacturers to target this dairy market.

Different strategies and methods to develop foods having reduced cholesterol contents are in practice on commercial, laboratory and industrial scales. Promising techniques for cholesterol reduction include blending in vegetable oils [3, 4], adsorption with digitonin and saponin [5, 6], extraction by crystallization and distillation [7, 8], removal by supercritical carbon dioxide extraction [9, 10] and assimilation of cholesterol by microbial enzymes [11, 12]. Several research and review published studies have described the use of β-CD in food [13 - 15]. β-CD molecule being non-toxic and non-digestible can be safely used as molecule to bind and remove cholesterol effectively from milk and dairy products [15]. From structural point of view, the β-CD molecule is a

cyclic oligosaccharide consisting of 7 glucose units. Majorly, enzyme cyclodextrin glycotransferases act on starch and produced β-CD molecule. This process involves breakdown of the polysaccharides chain and alternatively produced cyclic polysaccharide molecules. The doughnut shaped β-CD molecule has central portion with characteristics circular hydrophobic space and has similarity with cholesterol molecule diameter which provides the unique opportunity to β-CD molecule for its affinity to non-polar molecules such as cholesterol [16, 17].

The research investigations have concluded the feasibility of β-CD molecule to be applied as excellent substance for cholesterol removing from foods and dairy products including milk, however, up to date limited information is available which explained the impact of β-CD application for cholesterol reduction in ewe´s milk Manchego cheese and physico-chemical properties. Region wise, the Manchego cheese is considered one of the most famous representatives of the Spanish hard cheeses. Manchego cheese is commercially manufactured in the Spanish region of La Castilla la Mancha and substrate used for production is pure ewe´s milk which is collected from local herds under suitable conditions regulated by an origin appellation [18]. Manchego cheese is abundant with fat contents which ranged higher than 50% in the dry cheese. Manchego is a rich flavor cheese which increases with the aging time. The main mandate of this study was to evaluate the impact of curd washing on the reduction of cholesterol in ewe´s milk cheese and β-CD retention in treated pasteurized ewe´s milk Manchego cheese. Moreover, investigation was focused for β-CD effect on the main components of milk, lipids, and flavor characteristics of regular Manchego cheese.

2. Materials and Methods

2.1. Experimental Chemicals

Sigma (St. Louis, MO) provided the chemicals such as a-cyclodextrin (a-CD), β-cyclodextrin (β-CD) and all other reagent grade for the lab experiments. Deionized water was obtained from water purification and filtration system (Millipore Co).

2.2. Manchego Manufacturing Process

The Alonso method [15] was followed to treat ewe's milk with 1% beta cyclodextrin. The method described by Fernández-Garca [19] was used to make Manchego cheese. The same amount of whey that was drained off was used to make two separate batches of curd washing with deionized water. Three months of storage were allowed for cheese to be matured and ripened.

2.3. Gross Chemical Composition

The recommended procedures were used to determine the composition of fat, moisture, protein and nitrogen fractions [20].

2.4. Beta Cyclodextrin Determination

The analysis of β-CD was conducted using the Alonso method [21]. 10 g of cheese and 5 mg of a-CD was mixed in 1 mL water which served as the internal standard for quantitative analysis. Following a two-minute agitation period at a temperature of 40°C, the mixture underwent centrifugation at ambient temperature for a duration of thirty minutes, with a rotational speed of 40000 revolutions per minute (rpm). This process was conducted to eliminate the uppermost layer and subsequently filter it through a 0.45 m Millipore Co. membrane. A 30 µL portion of the supernatant, which had been spiked with the internal standard, was transferred to the autosampler. In the context of high-performance liquid chromatography (HPLC) analysis, a volumetric quantity of 10 µL of the supernatant aliquot was introduced onto the chromatographic column. Empower 2, a chromatographic data software manufactured by (Waters, Milford, MA) was used for the data collection and analysis processes. To complete the HPLC study and data acquisition, a Water (Alliance 2695 separation module) system was coupled to a 410 refractive index (RI) detector. A YMC ODS-AQ column manufactured by Teknochroma, USA was used in the separation process. The mobile phase was made by adding methanol to water at a 7:93 ratio. A steady flow of 1 mL/min of mobile phase was then injected into the system under isocratic conditions. The time required for elution was calculated using water standards. The concentration of β-CD in the

experimental sample was then quantified by comparing the peak area of the experimental sample containing β-CD to the internal standard (α-CD).

2.5. *Lipid Extraction Method*

The extraction of lipids from experimental samples was conducted according to the International Standard Method for Milk and Milk Products [22]. In summary, the experimental procedure adopted the addition of an ammonia-ethanol solution to the test component, subsequently followed by a lipid extraction technique utilizing hexane and diethyl ether as extraction solvents. The solvent underwent complete evaporation upon removal of the top layer. The lipid extracts were collected and subsequently stored in amber glass vials at a temperature of -20°C by following a nitrogen flushing procedure.

2.6. *Cholesterol Determination*

The technique employed for the analysis of cholesterol was capillary gas chromatography (GC), utilizing direct injection of milk fat, as outlined by Alonso [23]. For the GC analysis, a solution was prepared by dissolving approximately 30 mg of anhydrous milk fat and an internal standard of 5-cholestane (3.5 mg/mL in hexane) in 1 mL of hexane. The resulting solution (0.5 µL) was then injected into GC for analysis. The direct method for gas chromatography (GC) analysis of free cholesterol was applied using the Agilent Technology 6890 chromatograph (Palo Alto, CA). The GC apparatus was well equipped with a flame ionization detector and HP-5 fused silica capillary column. The dimensions of the column were 30m x 0.32 mm i.d. and 0.25 µm thickness. The experimental chromatography was conducted under the following conditions: The carrier gas was maintained at a head pressure of 17 psi. The column temperature was initially set at 280 °C and was held constant for 1 minute. Subsequently, the temperature was increased to 355 °C at a rate of 3 °C per minute. The injector temperature was set at 350 °C, while the detector temperature was set at 360 °C. The process of peak identification involved comparing the relative retention times of the peaks with the standard retention times. The quantification of cholesterol was conducted by evaluating the peak area of the sample in relation to the specific internal standard (5-

cholestane). The calculation of the cholesterol reduction percentage in milk fat was determined by employing the formula given below:

[(100 – cholesterol in milk fat) x 100]/ amount of cholesterol in untreated milk)

2.7. Triglycerides and Fatty Acids Analysis

Fatty acids methyl esters (FAMES) were produced by employing a solution of 2 N KOH in methanol to methanolized the extracted lipids. Alkaline catalysts were utilized in this process. The Fatty Acid Methyl Esters (FAMES) were analyzed using an Agilent Technology 6890 chromatograph (Palo Alto, California) well equipped with flame ionization detector (FID). The separation of fatty acids was conducted utilizing the Alonso [24] method on a CP-Sil 88 fused-silica capillary column (50 m x 0.25 mm i.d. x 0.2 m film thickness, Chrompack, California, USA). The triglycerides were assessed using gas chromatography (GC) with direct injection, utilizing a flame ionization detector and an Agilent gas chromatograph 6890 (Palo Alto, CA). The experiments were conducted following the Alonso method [24], by application of a WCOT fused silica capillary column with dimensions of 25 m x 0.25 mm x 0.1 m film thickness and coated with coated with OV 17 TRI (J.W. Scientific, Polson, CA, USA) [25].

2.8. Phospholipids Analysis

The extraction of cheese fat was conducted using an Accelerated Solid Extraction ASE-200 extractor (Dionex Corp., Sunnyvale, CA). A total of 2 g of freeze-dried cheese sample and 2 g of sea sand were utilized for the extraction process. The stainless-steel extraction cell employed in this study was coated with filters on both sides. A solvent mixture consisting of dichloromethane and methanol in a volumetric ratio of 2:1 was used, while a fixed condition of 10.3 MPa pressure was maintained to achieve the maximum yield of cheese fat [26]. The lipid classes were separated using a high-performance liquid chromatography (HPLC) system (model 1260; Agilent Technologies Inc.) connected to an evaporative light scattering detector (SEDEX 85 model; Sedere SAS, Alfortville Cedex, France). The nebulizing gas used was prefiltered compressed air, applied at a pressure of 350 kPa and a temperature of 60°C, with the gain was set to 3. In this study, two columns were applied in a sequential manner. The first column utilized

was with dimensions (250 × 4.5 mm Zorbax Rx-SIL column with 5-µm particle diameter; Agilent Technologies Inc.) while a second as precolumn with identical packing material was also utilized [26].

2.9. Volatile Compounds Analysis

The volatile fraction was analyzed using the headspace gas chromatographic-mass spectrometric (GC-MS) method as described by Alonso [27]. A total volume of 80 microliters of an aqueous solution containing propionic acid ethyl methyl ester at a concentration of 1.14 milligrams per milliliter was combined with 10 grammes of anhydrous sodium sulphate, which served the purpose of water retention. This mixture was then added to 10 grammes of pre-homogenized cheese. Before utilization, every individual standard dilution in an aqueous solution was prepared and placed in vials that were tightly sealed to prevent any external contamination. The vials were stored at a temperature of ¬20°C. The samples were subjected to a temperature of 80°C for a duration of 60 minutes to establish thermodynamic equilibrium between the sample and gaseous phase. Subsequently, the samples were analyzed using a static headspace apparatus (Model HSS 19395; Hewlett-Packard). The experimental setup was adjusted to apply pressure for a duration of 5 seconds, followed by a stabilization and filling period of 18 seconds, and finally an injection period lasting two minutes. Helium was employed as the carrier gas at a flow rate of 17.5 mL/min. The determination of volatile compounds involved the application of a Hewlett-Packard GC Model 5890 which was connected to a selective MS Model 5972. Polyethylene glycol was used as the injection medium in split mode (7:1 split ratio, 18 rate) for introducing samples into a capillary silica column (HP Innovas, 60m length, 0.25mm inner diameter, 0.25 m film thickness, manufactured by Hewlett Packard). The carrier gas used in the experiment was helium, with a flow velocity of 18 36.5 cm/s. The temperature protocol employed for the column involved an initial temperature of 33°C for a duration of 5 minutes, followed by a gradual increase of 1°C per minute until reaching 38°C. Subsequently, the temperature was further increased at a rate of 7°C per minute until reaching 210°C, where it was maintained for a period of 10 minutes. The interface line of the mass spectrometer (MS) was heated to a temperature of 280

degrees Celsius, and the injection process was carried out at a temperature of 200 degrees Celsius. The voltage of the photomultiplier was measured to be 18 V, while the electronic ionization energy was found to be 70 eV. Additionally, the electronic ionization energy was determined to be 1647 V.

2.10. Short Chain Free Fatty Acids Determination

To assess the existence of short chain free fatty acids (SCFFA), a 1g cheese sample was subjected to homogenization in 20 mL of distilled water. Subsequently, the resulting mixture was centrifuged at a speed of 10,000 revolutions per minute for a duration of 10 minutes. Finally, the supernatant was filtered using a 0.40 mm filter. The investigation was conducted utilizing a capillary silica column on a Hewlett-Packard model 5890 A gas chromatograph equipped with a flame ionization detector. The specific column used was an HP FFAP with dimensions of 30m length, 0.25mm internal diameter, and a 0.25 m film thickness, manufactured by Agilent Technologies J & W. The identification of each free fatty acid (FFA) was accomplished by utilizing the retention length of a standard, while quantitative analysis was conducted by comparing the peak areas of individual FFAs with the peak areas of 2-ethyl butanoic acid, which was employed as an internal standard.

2.11. Sensory Evaluation

A group of twenty-two expert sensory panelists conducted an evaluation of cheeses that were assigned random tags. The attributes of flavor, aroma, color, texture and acceptability were assessed using a five-point scale, with ratings ranging from 1 (poor) to 5 (excellent).

2.12. Statistical Analysis

SAS software (version 8.02, SAS Institute Inc, Cary, NC, USA) was used to run an analysis of variance (ANOVA) on the experimental data. A student t-test was used to analyze the data, and differences between treatments were declared significant at a P value of less than 0.05.

3. Results and Discussion

3.1. Gross Chemical Composition

The gross chemical composition and cholesterol removal rate are shown in Table 1 for four cheeses: control cheese without β-CD (CC), experimental cheese with 1% of β-CD (EC), washed control cheese without β-CD in milk (WCC) and washed experimental cheese with 1% of β-CD in milk (WEC). The fat, moisture, and protein content exhibited comparable ratios between the CC-EC and WCC-WEC, specifically ranging between 34.50% - 34.13% versus 32.51% - 31.53% for fat content, 37.79% - 38.10% versus 37.15% - 36.46% for moisture content, and 25.68% - 24.11% versus 25.10% - 24.96% for protein content, respectively. The cholesterol-reduced cheese is lower in fat than the control because less fat is integrated with casein through a fat protein network. This is probably owing to β-CD's effect on the casein matrix [28].

Comparisons of CC-EC and WCC-WEC showed statistically significant differences in soluble nitrogen (SN) and non-protein nitrogen (NPN) levels ($P \leq 0.05$). The percentages of protein content were found to be 25.68% and 24.11% for CC-EC, and 25.10% and 24.96% for WCC-WEC, respectively. Similarly, the percentages of protein content were 4.76% and 4.88% for CC-EC, and 5.79% and 6.01% for WCC-WEC, respectively. This phenomenon may be attributed to a slight elevation in proteolysis observed in EC cheeses, suggesting a potential rise in peptidase activity resulting from the influence of the β-CD [29]. Proteolysis occurs throughout the process of ripening, serving as a crucial metabolic process that regulates the sensory characteristics. Insoluble caseins undergo partial conversion into polypeptides and amino acids. The application of β-CD to milk, which serves as the precursor for cheese production, leads to alterations in the casein structure, thereby influencing the levels of soluble nitrogen (SN) and non-protein nitrogen (NPN). Additionally, this process may potentially expedite the maturation phase of the cheese.

The cholesterol removal rate in the WEC group, as compared to the WCC group, demonstrated a significant reduction of 98.45% versus 2.02%, respectively. This reduction was observed by comparing the cholesterol levels of 195.67 mg/100g fat to 191.71

mg/100g fat in the WEC group, and 1.37 mg/100g fat to 1.12 mg/100g fat in the WCC group. In a study conducted by Kwak [30], it was observed that cholesterol removal from Cheddar cheese exhibited similarities to the findings of Kin [31] in blue cheese, both of which utilized β-CD. There was a statistically significant difference ($p < 0.05$) observed in the remaining β-CD content between the EC and WEC samples, with values of 0.51% and 0.27%, respectively. This study provides confirmation that the removal of cholesterol using β-CD and curd washing does not significantly alter the primary chemical composition of Manchego ewe's milk cheese.

Table 1 Gross chemical composition for the experimental Manchego cheeses

Parameter	CC	EC	WCC	WEC
Fat (%)	34.50 ± 1.12^{a}	34.13 ± 1.25^{a}	32.51 ± 1.18^{a}	31.53 ± 1.13^{a}
Moisture (%)	37.79 ± 1.65^{a}	38.10 ± 1.80^{a}	37.15 ± 1.93^{a}	36.46 ± 1.70^{a}
Protein (%)	25.68 ± 1.04^{a}	24.11 ± 1.12^{a}	25.10 ± 1.16^{a}	24.96 ± 1.05^{a}
SN (% as protein)	4.76 ± 0.23^{a}	4.88 ± 0.29^{a}	5.79 ± 0.32^{b}	6.01 ± 0.35^{b}
NPN (% as protein)	2.41 ± 0.19^{a}	2.66 ± 0.22^{a}	3.95 ± 0.24^{b}	4.22 ± 0.41^{b}
pH	4.87 ± 0.15^{a}	5.28 ± 0.19^{a}	4.85 ± 0.25^{a}	5.30 ± 0.21^{a}
Cholesterol (mg/100g fat)	195.67 ± 6.03^{a}	191.71 ± 5.31^{a}	1.37 ± 0.19^{a}	1.12 ± 0.14^{a}
Cholesterol removal (% fat)	-	2.02 ± 0.25^{a}	97.29 ± 4.56^{b}	98.45 ± 5.12^{b}
Remain BCD (%)	-	-	0.51 ± 0.13^{a}	0.27 ± 0.11^{b}

CC: Control cheese; EC: experimental cheese (1% β-CD); WCC: Washed control cheese; WEC (1% β-CD): Washed experimental cheese. β-CD: Beta-cyclodextrin.

SN: Soluble nitrogen (% as protein); NNP: Non protein nitrogen (% as protein); β-CD: Beta-cyclodextrin.

$^{a, b}$ Different letters in the same row mean significant differences ($p \leq 0.05$). Mean standard deviation (n=3).

3.2. Lipid Characteristics

The average percentages of fatty acids in several cheese samples are presented in Table 2. These samples include control cheese made without β-CD in the milk (referred to as CC), experimental cheese made with 1% of β-CD (referred to as EC), washed control cheese made without β-CD in the milk (referred to as WCC), and washed experimental cheese made with 1% of β-CD in the milk (referred to as WEC). The amounts of specific fatty acids in fat derived from different sources did not change significantly ($p \leq 0.05$). There is a scarcity of literature regarding research conducted on the impact of β-CD on the lipid composition during the manufacturing process of low cholesterol cheeses. The fatty acid composition of the fractionated milk fat differed significantly from the control cheeses in a study by Chen [32] that used supercritical fluid extraction with carbon dioxide to remove cholesterol and fractionate milk fat. The authors found that the amounts of short and medium chain fatty acids in the extracted milk fat were reduced by 40% and 10%, respectively, when compared to the levels in the control milk fat. The results obtained by Gonzalez were consistent [9]. The purpose of this study was to compare the effects of β-CD treatment on cholesterol removal and the effects of β-CD treatment on the percentages of short-chain (C4-C8), medium-chain (C10-C12), and long-chain (C14-C18) fatty acids. Similar results were seen in a study on the use of beta cyclodextrin to lower cholesterol levels in milk fat by Alonso [15].

Triglyceride composition of washed control cheese (WCC) and washed experimental cheese (WEC) with 1% concentration of β-CD in milk are presented in Table 3, along with the control cheese (CC) that had no β-CD in the milk as a comparison. Fatty cheese's triglycerides were broken down into 15 categories, based on their carbon chain lengths (from C26 to C54). Each cluster stands for the accumulated existence of several triglyceride molecular variants sharing the same carbon-atom count. There were no significant differences between the CC-EC and WCC-WEC groups at the 0.05 level of significance. The short-term range (C24-C32) exhibits a slight difference in values between two intervals, specifically (8.81 - 8.93%) compared to (8.18 - 8.19%). Similarly, the medium-term range (C34-C48) shows a marginal variation in values,

namely (77.63 - 78.15%) versus (77.51 - 77.99%). Lastly, the long-term range (C50-C54) demonstrates a slight discrepancy in values, with (9.31 - 9.29%) in contrast to (9.95 - 9.98%). To date, there is a limited of research examining the triglyceride composition of cheeses subjected to β-CD treatment for cholesterol removal. The potential variation in triglyceride composition may have arisen from the differential extraction efficiency of solvents employed in the extraction process, as well as the utilization of supercritical fluid extraction techniques by the researchers.

Table 2 Fatty acids composition (g/100 g fat) of the experimental Manchego cheeses

Fatty acid	CC	WCC	EC	WEC
C4:0	2.24 ± 0.19^{a}	2.18 ± 0.22^{a}	2.14 ± 0.26^{a}	2.16 ± 0.24^{a}
C6:0	1.74 ± 0.06^{a}	1.68 ± 0.08^{a}	1.68 ± 0.05^{a}	1.67 ± 0.05^{a}
C8:0	1.70 ± 0.05^{a}	1.66 ± 0.07^{a}	1.66 ± 0.08^{a}	1.64 ± 0.06^{a}
C10:0	5.02 ± 0.15^{a}	4.95 ± 0.18^{a}	4.95 ± 0.13^{a}	4.91 ± 0.16^{a}
C10:1	0.28 ± 0.03^{a}	0.25 ± 0.05^{a}	0.25 ± 0.07^{a}	0.24 ± 0.05^{a}
C12:0	3.19 ± 0.11^{a}	3.14 ± 0.16^{a}	3.14 ± 0.18^{a}	3.11 ± 0.14^{a}
C14:0	9.22 ± 0.84^{a}	9.21 ± 0.92^{a}	9.21 ± 0.51^{a}	9.14 ± 0.42^{a}
C14:1	0.90 ± 0.03^{a}	0.88 ± 0.08^{a}	0.86 ± 0.06^{a}	0.89 ± 0.04^{a}
C15:0	0.24 ± 0.02^{a}	0.25 ± 0.04^{a}	0.25 ± 0.05^{a}	0.23 ± 0.07^{a}
C16:0	27.16 ± 1.52^{a}	27.41 ± 1.13^{a}	27.41 ± 1.18^{a}	27.21 ± 1.11^{a}
C16:1	0.73 ± 0.12^{a}	0.77 ± 0.15^{a}	0.77 ± 0.17^{a}	0.71 ± 0.13^{a}
C17:0	0.54 ± 0.07^{a}	0.58 ± 0.09^{a}	0.58 ± 0.07^{a}	0.52 ± 0.05^{a}
C18:0	13.39 ± 0.55^{a}	13.59 ± 0.65^{a}	13.59 ± 0.52^{a}	13.56 ± 0.39^{a}
C18:1t	2.62 ± 1.13^{a}	2.65 ± 0.26^{a}	2.65 ± 0.23^{a}	2.78 ± 0.14^{a}
C18.1c	23.28 ± 0.35^{a}	22.93 ± 1.06^{a}	22.93 ± 1.16^{a}	22.78 ± 1.21^{a}
C18:2	3.39 ± 0.08^{a}	3.26 ± 0.31^{a}	3.26 ± 0.24^{a}	3.26 ± 0.18^{a}
C18:3	0.39 ± 0.08^{a}	0.40 ± 0.06^{a}	0.40 ± 0.05^{a}	0.39 ± 0.03^{a}
C18.2 (c9t11)	0.96 ± 0.06^{a}	0.97 ± 0.08^{a}	0.97 ± 0.06^{a}	0.97 ± 0.05^{a}

CC: Control cheese; EC: experimental cheese (1% β-CD); WCC: Washed control cheese; WEC (1% β-CD): Washed experimental cheese. β-CD: Beta-cyclodextrin.

$^{a, b}$ Different letters in the same row mean significant differences ($p \leq 0.05$). Mean standard deviation (n=3).

Table 3 Triglycerides composition (g/ 100 g fat) of the experimental Manchego cheeses

Fatty acid	CC	EC	WCC	WEC
C24	0.33 ± 0.06[a]	0.35 ± 0.04[a]	0.32 ± 0.08[a]	0.31 ± 0.06[a]
C26	0.88 ± 0.09[a]	0.81 ± 0.08[a]	0.80 ± 0.05[a]	0.77 ± 0.08[a]
C28	1.64 ± 0.15[a]	1.53 ± 0.17[a]	1.44 ± 0.13[a]	1.42 ± 0.17[a]
C30	2.42 ± 0.23[a]	2.58 ± 0.27[a]	2.47 ± 0.21[a]	2.50 ± 0.29[a]
C32	3.54 ± 0.40[a]	3.66 ± 0.46[a]	3.15 ± 0.39[b]	3.01 ± 0.45[b]
C34	4.89 ± 0.38[a]	4.88 ± 0.41[a]	5.04 ± 0.48[a]	5.09 ± 0.56[a]
C36	7.21 ± 0.66[a]	7.35 ± 0.52[a]	7.04 ± 0.54[a]	7.18 ± 0.50[a]
C38	10.66 ± 1.11[a]	10.51 ± 1.19[a]	10.65 ± 1.30[a]	11.01 ± 1.39[a]
C40	17.35 ± 1.32[a]	17.60 ± 1.42[a]	17.89 ± 1.32[a]	17.15 ± 1.42[a]
C42	16.02 ± 1.40[a]	16.32 ± 1.35[a]	16.17 ± 1.50[a]	16.21 ± 1.68[a]
C44	8.83 ± 0.77[a]	8.66 ± 0.71[a]	7.44 ± 0.66[a]	8.89 ± 0.51[a]
C46	7.14 ± 0.62[a]	7.21 ± 0.63[a]	7.04 ± 0.52[a]	7.16 ± 0.59[a]
C48	5.35 ± 0.55[a]	5.62 ± 0.59[a]	5.71 ± 0.49[a]	5.30 ± 0.54[a]
C50	4.28 ± 0.35[a]	4.35 ± 0.42[a]	4.39 ± 0.51[a]	4.55 ± 0.62[a]
C52	4.57 ± 0.39[a]	4.44 ± 0.44[a]	4.31 ± 0.56[a]	4.24 ± 1.57[a]
C54	4.78 ± 0.43[a]	4.66 ± 0.36[a]	4.58 ± 0.45[a]	4.60 ± 0.41[a]

CC: Control cheese; EC: experimental cheese (1% β-CD); WCC: Washed control cheese; WEC (1% β-CD): Washed experimental cheese. β-CD:Beta-cyclodextrin.

[a, b] Different letters in the same row mean significant differences ($p \leq 0.05$). Mean standard deviation (n=3).

Phospholipid profiles for four types of cheese as shown in Table 4 were control cheese made with milk that lacked β-CD (CC), experimental cheese made with 1% β-CD (EC), washed control cheese made with milk that lacked β-CD (WCC) and washed experimental cheese made with 1% β-CD (WEC). No statistically significant difference ($p \leq 0.05$) in the relative composition of the different phospholipid classes in relation to total phospholipids (PL) was found between the groups (CC-EC vs WCC-WEC) of cheeses using analysis of variance. Phosphatidylethanolamine (PE) exhibited the highest abundance among the phospholipids, with a range of 42.42% to 45.72% as a proportion of total phospholipids (PL). This was followed by phosphatidylcholine (PC), which ranged from 27.23% to 32.04% as a proportion of total PL. Sphingomyelin (SM) had a comparatively lower abundance, ranging from 26.70% to 27.84% as a proportion

of total PL. Alonso (15) conducted a study investigating the impact of β-CD on phospholipids found in pasteurized milk and obtained comparable findings.

The collective proportion of these three distinct phospholipid species exceeded 80% of the overall phospholipid content found in dairy products. One possible explanation for the lack of impact of β-CD on these constituents of milk fat may be attributed to the formation of an inclusion complex between β-CD and cholesterol. The hydrophobic nature of the core cavity of β-CD is responsible for its affinity towards nonpolar substances such as cholesterol. The dimensions of the cavity are such that it closely accommodates a cholesterol molecule, highlighting the highly specific capability of β-cyclodextrin to create an inclusion complex with cholesterol. Consequently, β-CD can be readily accessed in the aqueous phase, where they form insoluble inclusion complexes that can be separated through centrifugation [15].

Table 4 Phospholipids composition of the experimental Manchego cheeses

Phospholipids	CC	WCC	EC	WEC
Total PLs	0.12 ± 0.03[a]	0.05 ± 0.01[a]	0.11 ± 0.03[a]	0.09 ± 0.01[a]
PE	42.42 ± 4.05[a]	45.72 ± 5.12[a]	38.25 ± 1.40[a]	40.61 ± 1.53[a]
PI	1.93 ± 1.31[a]	2.95 ± 0.30[a]	2.46 ± 0.62[a]	2.53 ± 1.51[a]
PS	1.75 ± 0.53[a]	1.68 ± 0.35[a]	3.21 ± 1.94[a]	2.10 ± 1.31[a]
PC	27.23 ± 0.74[a]	32.04 ± 1.40[a]	31.04 ± 2.21[a]	29.20 ±1.80[a]
SM	26.70 ± 5.32[a]	27.84 ± 3.83[a]	25.20 ± 1.53[a]	25.62 ± 3.22[a]

CC: Control cheese; EC: experimental cheese (1% β-CD); WCC: Washed control cheese; WEC (1% β-CD): Washed experimental cheese. β-CD; Beta-cyclodextrin.

PLs: Phospholipids; PE: Phosphatidylethanolamine; PI: Phosphatidylinositol; PS: Phosphatidylserina; PC: Phosphatidylcoline; SM: Sphyngomyelin.

[a, b] Different letters in the same row mean significant differences ($p \leq 0.05$). Mean standard deviation (n=3).

3.3. Flavor Characteristics

Table 5 shows the extracted flavor compounds from four cheeses: control cheese (CC) made without the addition of β-CD in milk; experimental cheese (EC) made with 1% β-CD in milk; washed control cheese (WCC) made without β-CD; and washed experimental cheese (WEC) made with 1% β-CD in milk. After conducting a thorough examination, scientists were able to isolate 13 individual flavor compounds from both cheese samples, with some of those chemicals being more prevalent in one or the other. There was a total of 13 different flavor compounds found in cheeses, including 5 ketones, 3 aldehydes, and 6 alcohols. There were no statistically significant differences ($p \leq 0.05$) between the CC-EC and WCC-WEC cheeses when comparing the total amounts of ketones [(2567.00 - 2348.54) vs (2307.91 - 2141.87 ppm)], aldehydes [(1139.63 - 1062.10 ppm) vs (1377.45 - 1472.90 ppm)] and alcohols [(4217.77 - 4200.14 ppm) vs (4516.80 - 4590.10 ppm)] between (CC-EC vs WCC-WEC) cheeses. The levels of 3 methyl butanal in the CC-EC cheese ranged from 1121.42 to 1045.60 ppm, while the levels in the WCC-WEC cheese ranged from 4385.30 to 4456.02 ppm, representing a statistically significant difference ($p \leq 0.05$). Notably, among the evaluated flavor components, ethanol generation was highest, correlating with the results obtained by Kwak [30] in Cheddar cheese treated with β-CD. Researchers [33] found that the overall flavor components of cream cheese were not significantly different after the cholesterol was removed using β-CD. Odd-numbered-carbon ketones have low sensory thresholds and distinctive smell characteristics. Fatty acids undergo beta-oxidation and decarboxylation to produce the compounds. Aldehydes are quickly converted into alcohols or their equivalent acids, hence they are not the principal ingredients in cheeses. An aminotransferase catabolizes branched-chain amino acids, resulting in branched-chain aldehydes such 3-methyl butanal [34]. When compared to CC cheese and ethanol, this chemical showed statistical significance ($p \leq 0.05$) in the setting of EC.

Table 6 displays the levels of SCFFAs (acetic acid, propionic acid, butyric acid, and caproic acid) in a variety of cheeses. The samples include control cheese without the addition of cyclodextrin (β-CD) referred to as CC, experimental cheese containing 1% cyclodextrin referred to as EC, control cheese that underwent a washing process

without the addition of cyclodextrin referred to as WCC, and experimental cheese that underwent a washing process with 1% cyclodextrin referred to as WEC. The cheese samples were obtained from milk. After a three-month maturation period, the total short-chain fatty acid (SCFFA) levels in the matured cheeses did not exhibit a significant difference ($p \leq 0.05$). The average SCFFA levels were found to be 149.14 - 147.13 ppm and 151.77 - 158.43 ppm, respectively. Additionally, the individual levels of SCFFAs did not show any significant variation. Similar findings were reported by [30, 35] regarding the levels of short-chain fatty acids (SCFFAs) generated in both the control and cholesterol-reduced procedures, as well as in cheddar cheese manufactured using β-CD. During the three-month ripening period, the release of butyric and caproic acid occurs, contributing to the development of the characteristic flavour profile of Manchego cheese.

Table 7 presents the sensory characteristics of four cheese samples: control cheese without β-CD (CC) in milk, experimental cheese with 1% of β-CD (EC), washed control cheese without β-CD (WCC), and washed experimental cheese with 1% of β-CD (WEC). The sensory attributes were evaluated on a scale of 1 to 5. There were no statistically significant differences ($p \leq 0.05$) observed in the flavour [(3.32 - 3.16) vs (2.97 - 2.86)], aroma [(3.59 - 3.12) vs (2.88 - 2.80)], colour [(3.69 - 3.61) vs (3.49 - 3.37)], and acceptability [(3.45 - 3.39) vs (3.22 - 3.18)] between the CC-EC and WCC-WEC cheeses. The texture of the cheeses (CC-EC vs WCC-WEC) exhibited a significant difference ($p \leq 0.05$), with values of (3.45 - 3.39) and (3.22 - 3.18), respectively. This disparity may be attributed to the higher proteolysis observed in the EC and WEC cheeses resulting from the treatment with β-CD. Additionally, a slight increase in moisture content was observed in the cheese treated with β-CD, leading to a slower drainage process [36]. During a three-month ripening period, there was consistent adherence to the overall preference, with no observed differences in terms of flavor, aroma, color, or acceptability between the (CC - EC) and (WCC - WEC) groups. The present study revealed that, although there were slight differences, most sensory characteristics and overall preferences exhibited similar patterns between the control group and the curd washed with β-CD for a duration of three months. Consequently, it is possible to

hypothesize about the potential to produce cholesterol-reduced Manchego cheese through the implementation of β-CD and curd washing techniques.

Table 5 Volatile compounds (ppm) of the experimental Manchego cheeses

Compounds	CC	EC	WCC	WEC
Ketones				
2 Propanone	420.38 ± 32.39^{a}	401.66 ± 28.36^{a}	381.05 ± 26.89^{a}	369.96 ± 25.32^{a}
2 Butanone	27.65 ± 4.51^{a}	23.32 ± 4.16^{a}	20.16 ± 4.21^{a}	21.45 ± 4.69^{a}
2,3 Butanodione	1271.54 ± 48.45^{a}	1123.60 ± 53.75^{a}	1145.81 ± 56.38^{a}	1041.65 ± 59.98^{a}
2 Heptanone	562.30 ± 29.49^{a}	535.42 ± 25.12^{a}	512.18 ± 22.78^{a}	496.21 ± 30.21^{a}
3 Hydroxy 2 butanone	286.12 ± 18.66^{a}	264.54 ± 17.65^{a}	248.70 ± 20.09^{a}	212.60 ± 24.60^{a}
Aldehydes				
3 Methyl butanal	1121.42 ± 48.32^{a}	1045.69 ± 65.12^{a}	1358. 96 ± 70.32^{b}	1452.95 ± 77.80^{b}
Hexanal	14.16 ± 6.50^{a}	12.56 ± 3.89^{a}	13.54 ± 4.09^{a}	15.74 ± 4.96^{a}
Nonanal	4.05 ± 1.21^{a}	3.85 ± 1.09^{a}	4.95 ± 1.19^{a}	4.21 ± 1.29^{a}
Alcohols				
2 Propanol	14.50 ± 3.56^{a}	12.96 ± 4.39^{a}	10.64 ± 3.70^{a}	9.65± 3.96^{a}
Ethanol	4107.60 ± 62.30^{a}	4085.50 ± 89.60^{a}	4385.30 ± 95.79^{b}	4456.02 ± 109.10^{b}
2 Methyl 1 propanol	43.18 ± 7.11^{a}	46.13 ± 6.45^{a}	55.66 ± 7.80^{a}	58.69 ± 7.02^{a}
2 Butanol	29.31 ± 6.85^{a}	22.45 ± 5.02^{a}	25.69 ± 5.56^{a}	23.20 ± 5.04^{a}
2 Heptanol	36.18 ± 6.04^{a}	33.10 ± 7.32^{a}	39.57 ± 6.12^{a}	42.54 ± 5.52^{a}

CC: Control cheese; EC: experimental cheese (1% β-CD); WCC: Washed control cheese; WEC (1% β-CD): Washed experimental cheese. β-CD: Beta-cyclodextrin.

SN: Soluble nitrogen (% as protein); NNP: Non protein nitrogen (% as protein); β-CD: Beta-cyclodextrin.

$^{a, b}$ Different letters in the same row mean significant differences ($p \leq 0.05$). Mean standard deviation (n=3).

Table 6 Short chain free fatty acids (SCFFA) (ppm) of the experimental Manchego cheeses

SCFA	CC	EC	WCC	WEC
Acetic acid	92.91 ± 7.19[a]	93.06 ± 7.68[a]	193.06 ± 6.19[a]	129.53 ± 8.96[a]
Propionic acid	35.28 ± 5.65[a]	34.64 ± 4.56[a]	35.36 ± 4.96[a]	36.02 ± 4.90[a]
Butyric acid	17.10 ± 3.96[a]	16.13 ± 3.06[a]	17.32 ± 3.60[a]	21.16 ± 3.93[a]
Caproic acid	3.85 ± 2.52[a]	3.30 ± 2.80a	3.96 ± 3.12[a]	3.72 ± 3.61[a]

CC: Control cheese; EC: experimental cheese (1% β-CD); WCC: Washed control cheese; WEC (1% β-CD): Washed experimental cheese. β-CD: Beta-cyclodextrin.

SN: Soluble nitrogen (% as protein); NNP: Non protein nitrogen (% as protein); β-CD: Beta-cyclodextrin.

[a, b] Different letters in the same row mean significant differences ($p \leq 0.05$). Mean standard deviation (n=3).

Table 7 Sensory analysis of the experimental Manchego cheeses

Attribute	CC	EC	WCC	WEC
Flavour	3.32 ± 0.44a	3.16 ± 0.67a	2.97 ± 0.89a	2.86 ± 0.71a
Aroma	3.59 ± 0.49a	3.12 ± 0.57a	2.88 ± 0.83a	2.80 ± 0.81a
Color	3.69 ± 0.68a	3.61 ± 0.63a	3.49 ± 0.73a	3.37 ± 0.86a
Texture	3.70 ± 0.57a	3.43 ± 0.51a	3.29 ± 0.72b	3.03 ± 0.83b
Acceptability	3.45 ± 0.60a	3.39 ± 0.73a	3.22 ± 0.76a	3.18 ± 0.75a

CC: Control cheese; EC: experimental cheese (1% β-CD); WCC: Washed control cheese; WEC (1% β-CD): Washed experimental cheese. β-CD: Beta-cyclodextrin.

SN: Soluble nitrogen (% as protein); NNP: Non protein nitrogen (% as protein); β-CD: Beta-cyclodextrin.

[a, b] Different letters in the same row mean significant differences ($p \leq 0.05$). Mean standard deviation (n=3).

4. Conclusions

The goal of this research was to examine the effects of curd washing on the essential components of milk, lipids, and flavor characteristics of a regular Manchego cheese after cholesterol was reduced by β-CD in pasteurized ewe's milk Manchego cheese. Cholesterol was lowered by about 98.45 percent in β-CD-treated experimental cheeses. Curd washing had a statistically significant impact on the residual β-CD (0.51 vs. 0.27%, $p \leq 0.05$). Fat, moisture, and protein were not affected by curd washing with or without β-CD, and only soluble nitrogen and nonprotein nitrogen showed minor modifications by treatment. Curd washing and the presence or absence of β-CD had no discernible effect on the relative levels of fatty acids, triglycerides, and phospholipids in the lipid fraction of the treated and untreated cheese. Flavoring chemicals and free fatty acids with a short chain length were unaffected by the β-CD. The only two substances that showed statistical significance ($p \leq 0.05$) were 3-methylbutanal and ethanol. Sensory attributes (taste, smell, color, and acceptability) were not affected by curd washing with or without β-CD, but texture was (p 0.05). The β-CD molecules can be used safely as a cholesterol elimination processing during cheese production, lowering the amounts of residual β-CD, even though they are edible and harmless. As a result, the current investigation revealed that the treatment of β-CD by the action of curd washing was an effective procedure for removing cholesterol while keeping the qualities of Manchego cheese.

Author Contributions

Analysis design, L.A. and J.F.; performing the experiment, L.A., M.V.C., and J.F.; writing, L.A and J.F; review and editing, L.A. M.V.C., and J.F.

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Conflicts of Interest

The authors declare no conflict of interest.

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References

1. Hansel, B.; Nicolle, C; Lalanne, F.F.; Brucket, E. Effect of low-fat, fermented milk enriched with plant sterols on serum lipid profile and oxidative stress in moderate hypercholesterolemia. Am. J. Clin. Nutr. 2007, 86, 790-796.

2. Alonso, L. Derivados lácteos sin colesterol utilizando beta ciclodextrina. Technifood, 2013, 85,3, 121-123.

3. Hariharan, K.; Soma Kurien, K.; Venkat Rao, S. Effect of supplementation of milk fat with peanut oil on blood lipids and lipoproteins in infants. Inter. J. Food Sci Nut. 1995, 46, 309-317.

4. Krause, A.J.; Lopetcharat, K.; Drake, M.A. Identification of the characteristics that drive consumer liking of butter. 2007, J. Dairy Sci. 90, 2091-2102.

5. Micich, T.J. Behaviours of polymers supported digitonin with cholesterol in the absence and presence of butter oil. J. Agri. Food Chem. 1990, 38, 1839-1843.

6. Micich, T.J.; T.A. Foglia, T.A.; Holsinger, V.H. In vitro studies on saponin-vitamin complexation. J. Agri. Food Chem. 1992, 40, 1321-1325.

7. Arul, J., Boudreau, A.; Makhlouf, J.; Tardif, R.; Bellavia. T. fractionation of anhydrous milk fat by short path destillation. J. American Oil Chem. Soc. 1988a, 65, 1642-1646.

8. Arul, J.; Boudreau, A.; Makhlouf, J.; Tardif, R.; Bellavia, T. Distribution of cholesterol in milk fat fractions. J. Dairy Res. 1988b, 55, 361-371.

9. Gonzalez-Hierro, M.T.; Ruiz-Sala, P.; Alonso, L.; Santamaria. G. Extraction of ewe's milk cream with supercritical carbon dioxide. Z. Lebens. Unters. Forsch. 1995, 200, 297-300.

10. Sangbin, L.; Mi-Kyung, J.; Hae-Soo, K. Cholesterol removal from milk fat by supercritical carbon dioxide extraction in coupled with adsorption. Korean J. Food Sci. Technol. 1998, 30: 574-580.

11. Gilliland, S.E.; Nelson, C.R.; Maxwell. C. Assimilation of cholesterol by Lactobacillus acidophilus. Appl. Environ. Microbiol. 1985, 49, 377-389.

12. Walkek, K.W.; Gilliland. S.E. Relationships among bile tolerance,bile salt deconjugation, and assimilation of cholesterol by Lactobacills acidophilus. J. Dairy Sci. 1993, 76, 956-961.

13. Kwak H.S.; Ahn, J. Optimization cholesterol removal in cream using β-cyclodextrin and response surface methodology. J. Food Sci. 1999, 64, 629-632.

14. Lee, D.K., Ahn, J.; S. Kwak, H. Cholesterol removal from homogenized milk with β-cyclodextrin. J. Dairy Sci. 1999, 82, 2327-2330.

15. Alonso, L.; Cuesta, P.; Fontecha, J.; Juárez.; Gilliland, S.E. Use of beta cyclodextrin to decrease the level of cholesterol in milk fat. J. Dairy Sci. 2009, 1941-1946.

16. Reineccius, T.A.; Reineccius, G.A.; Peppard, T. L. Potential for β-cyclodextrin as partial fat replacer in low-fat foods. J. Food Sci. 2004, 69, 334-341.

17. Szente, L.; Szejtli, J. Cyclodextrin as food ingredients. Trends Foods Sci. Tech. 2004, 15, 137-142.

18. Ministerio de Agricultura, Pesca y Alimentación. Boletín Oficial del Estado (Spain). 1985, 5, 267.

19. Fernández-García, E.; López-Fandiño, R.; Alonso, L.; Ramos, M. The use of lipolytic and proteolytic enzymes in the manufacture of Manchego cheese type cheese from ovine and bovine milk. J. Dairy Sci. 1994, 77, 2139-2149.

20. Alonso, L.; Juárez, M.; Ramos, M.; Martín Alvarez, P.J. Overall composition, nitrogen fraction and fat characteristics of Cabrales cheese during ripening. European Food Res. Tecnol., 1987, 185, 481-486.

21. Alonso, L.; Cuesta, P.; Gilliland, S.G. Development of a HPLC method for determining residual beta-cyclodextrin in milk, cream and butter as a novel prebiotic in dairy foods. Chromatographia, 2008, 69, 1089-1092.

22. ISO-IDF. Milk and milk products. Extraction methods for lipids and liposoluble compounds. ISO 14152; Int. Dairy Fed.: Brussels, Belgium; 2001, p 172.

23. Alonso, L., Lozada, L. Fontecha, J.; Juarez, M. Determination of cholesterol in milk fat by gas chromatography with direct injection and sample saponification. Chromatographia. 1995, 41, 23-25.

24. Alonso, L., Fontecha, J.; Lozada, L.; Fraga, M.J.; Juárez, M. Fatty acid composition of caprine milk: major, branched-chain and trans fatty acids. J. Dairy Sci. 1999, 82, 878-884.

25. Alonso, L. Capillary gas chromatography of some triglycerides in cheese using programmed temperature injection. Chromatographia. 1993, 35, 9-12.

26. Alonso, L.; Fontecha, J.; Juárez, M. Development of a headspace gas chromatographic mass spectrometric method for determining methyl-ketones and secondary alcohols in blue cheese. J. Chromatographic Sci. 1999, 37, 108-112.

27. Castro-Gómez, M.; Rodriguez Alcalá, L.M.; Calvo, M.V.; Romero, J.; Mendiola, J.A.; Ibáñez, E.; Fontecha, J. Total milk fat extraction and quantification of polar and neutral lipids of cow, goat, and ewe milk using a pressurized liquid system and chromatographic techniques. 2014, J. Dairy Sci. 97, 6719-6728.

28. Ibrahim, A.H.; Soryal. Effect of using cross linked β-cyclodextrin on physicochemical properties of Domiati goat´s cheese. J. Food Dairy Sci. 2014, 5, 581-600.

29. Smit, G.; Verheul.; Kranenburg, V; Ayad, E.; Siezen, R.; Engels, W. Cheese flavor development by enzymatic conversions of peptides and amino acids. Food Res. International. 2000, 33, 153-160.

30. Kwak, H.S.; Jung, C.S., Seok, J.S.; Ahn. J. Cholesterol removal and flavor development in cheddar cheese. Asian Aust. J. Anim. Sci. 2003, 16, 409-416.

31. Kim, H.Y.; Bae, H.Y.; Kwak, H.S. Development of cholesterol-reduced Blue cheese made by crosslinked β-cyclodextrin. Milchwiss. 2008, 63, 53-56.

32. Chen, H.; Schwartz, S.; Spanos, G.A. Fractionation of butter oil by supercritical carbon dioxide. J. Dairy Sci. 1992, 75:2659-2669.

33. Jeon, S.S.; Joo, S.; Ganesan, P.; Kwak, H.S. Comparative study of flavor, texture, and sensory in cream cheese and cholesterol removed cream cheese. 2012, Food Sci. Biotechnol. 21, 159-165.

34. Smit, G.; Smit, B.A.; Engel, J.M. Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. 2005, FEMS Microbiol. Reviews. 29, 591-610.

35. Kim, S.Y.; Park, S.Y.; Ahn, J.; Kwak, H.S. Properties of cholesterol reduced block type process cheese made by crosslinked β-CD-cyclodextrin. 2008, Korean J. Food Ani. Resour. 28, 463-469.

36. Metzge, N.S.; Mistry, V.V. A new approch using homogenization of cream in the manufacture of reduced Cheddar cheese. 1. Manufacture, composition, and yield. 1994, J. Dairy Sci. 77, 3506-3515.

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