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Effect of β-Cyclodextrin on Phospholipids and Cholesterol of the Milk Fat Globule Membrane

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Abstract

The aim of this study was to evaluate the effect of β-cyclodextrin (β-CD) on phospholipids and cholesterol in raw pasteurized milk with the purpuse on removal cholesterol from the milk fat. Total phospholipids decrease slightly in β-CD treated milk but were not signinificantly affected by the effect of the β-CD. β-CD is an effective oligosacharide for cholesterol removal from milk with more than 97% of reduction and does not affect significantly the individual phospholipids (phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, phosphatidylinositol and phosphatidylserina) of the milk fat globule membrane.

Keywords: Milk fat; Phospholipids; Cholesterol; β-Cyclodextrin

Introduction

Milk contains approximately 3.4% total fat. Recent studies have given considerable evidence that phospholipids can have a positive nutritional effect on human health, such as reduction of risk of cardiovascular disease [1-5]. In the food industry, phospholipids are used as emulsifiers or emulsion stabilizers when they are complex with protein [6]. Five major classes of phospholipids are found in milk fat, and their approximate percentages are phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, phosphatidylinositol and phosphatidylserina [7]. Phospholipids are located on the milk globule membrane with the cholesterol. They have both lipophilic and hydrophilic properties, and therefore, contribute significantly to the emulsification role of the membrane [8]. Today there is a growing interest in the manufacture of cholesterol reduced dairy products. Many methods for reducing cholesterol in foods have been developed, including blending in vegetable oils [9-11], extraction by distillation and crystallization [12], adsorption with saponin and digitonin [12], and removal by supercritical carbon dioxide extraction [13]. Nowdays, a number of studies have indicated that cholesterol removal from dairy product was most effectively achieved by beta-cyclodextrin β-CD [14,15]. The main objective of this study was to investigate the effect of the β-CD on phospholipids composition of the the milk fat globule membrane when the milk is treated with β-CD with the purpose on removal cholesterol from the milk fat.

Materials and Methods

Materials

Raw milk was obtained from Corporacion Alimentaria Peñasanta S.A. (Asturias, Spain). Milli-Q water was obtained with a Milli-Q Plus ultra pure water system from Millipore (Milford, MA, USA). β-CD and all solvents and reagents were of analytical grade from Sigma Chemical Co., (St. Louis, MO).

Standards

Phospholipids standards, phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, phosphatidylinositol, phosphatidylserina and cholesterol were purchased from Sigma Chemical Co., (St. Louis, MO).

Lipid extraction

Fat was extracted following a procedure described by International Standard Method for milk [16] using n-pentane and diethyl ether after first adding an ammonium hydroxide solution to the milk. The lipid extract obtained was stored in amber vials, exposed to a stream of N2 and frozen at -20°C until analysis.

Solid phase extraction (SPE) of PL

Lipid sample (500 mg) was dissolved in ml of chloroform-methanol (2:1, v/v). 1 ml of the fat solution was applied to different SPE cartridges. A silica gel bonded column (Supelclean LC-SI, 6 ml volume, 1 g sorbents, Supelco Bellfonte YSA) was used. After conditionning with hexane, the non-polar lipids were eluted with 5 ml of hexane-diethyl ether (8:2, v/v) and 5 ml hexane-diethyl ether (1:1, v/v). The recovery of phospholipids was performed by using two differents conditions: the first with 4 ml of methanol plus and the second with 2 ml of methanol plus 2 ml of chloroform-methanol-water (3:5.2, v/v/v). The recovered fraction was dried under a gentle stream of nitrogen and it was redissolved in 0.3 ml of chloroform-methanol (2:1, v/v) before injecting into the HPLC system.

HPLC analysis of PL

The HPLC analysis of phospholipids was performed using a Waters System (Alliance HPLC System 2695 separation module) coupled to a 2424 evaporative light scattering detector, data acquisitions and analysis were performed with a computer using the software version Empower 2 (Waters, Milford, MA). Separation was carried out on an Extrasil silica (150 × 4.0 mm I.D., 3 µm particle size) with a precolumn

(2 × 4.0 mm) from Tracer Analitica (Teknokroma, USA). Phospholipids were separated by chromatography with isocratic elution with isopropanol-hexane-water (55:37:8, v/v/v). The flow rate of the eluent was 1 ml/min and the column temperature was 35°C. The volume of sample injected was 20 µl. The temperature of the detector was 80°C and the gas flow was 10 ml/min. Compounds were identified by comparing the retention times of the sample peaks with those of the phospholipid standards.

Determination Cholesterol by Gas Chromatography

The technique chosen for cholesterol determination was as described by Alonso (1995) using direct injection of milk fat by capillary gas chromatography (GC). Approximately 30 mg anhydrous milk fat and 0.1 ml 5-α-cholestane as internal standard (3.5 mg/mL in hexane) was dissolved in 1 mL of hexane; 0.5 µL of the resulting solution was injected for GC analysis. The GC analysis for free cholesterol by this direct method was on an Agilent Technology 6890 chromatograph (Palo Alto, CA) equipped with flame ionization detector. Analyses were performed using a HP-5 fused silica capillary column (30 m × 0.32 mm i.d. 0.25 µm thickness). Experimental chromatographic conditions were: He carrier gas at 17 psi head pressure; initial column temperature 280°C, held for 1 min, increased to 355°C at 3°C/min. Injector temperature 350°C and detector temperature was 360°C. Peak identification was done by comparison of relative retention times with retention times of standards. Quantification of cholesterol was conducted by comparing sample peak area with of the 5α-cholestane internal standard. The percentage of cholesterol reduction in milk fat was calculated by the formula [(100 – amount of cholesterol in milk fat) x 100]/amount of cholesterol in untreated milk).

Statistical Analysis

All experiments were carry out three times and each experiments was analysed two times. Experimental data were treated by analysis of variance (ANOVA) using the statistical software SAS (version 8.02, SAS Institute Inc, Cary, NC, USA). Differences among treatments were determined by statistical analysis using a Student t-test where ($P<0.05$) was considered statiscally significant.

Results and Discussion

In the last decade, evidence has being gathered to suggest that an excess of cholesterol in the diet might be deleterious. A number of studies have indicated the importance of cholesterol reduction in dairy products. Cholesterol can be removed from milk and dairy products by a β-CD a cyclic oligosacharide consisting of seven glucose unit.

There are not data in the literature concerning phospholipid compositions in milk treated with β-CD targeting in removal of cholesterol from the milk fat. In order to evaluate the efect of β-cyclodextrin on the phospholipids, Table 1 shows the phospholipid composition of control raw milk and raw milk treated with β-CD. Analysis of variance did not reveal a significant difference ($P<0.05$) in relative composition of the differents phospholipids classes among the control milk and β-cyclodextrin treated milk. Phosphatidylethanolamine is the most predominant phospholipid representing a mean value of 35.46 ± 0.28% in control milk and 34.12 ± 0.33% in treated milk. Followed by phosphatidylcholine 16.95 ± 0.67% and sphingomyelin 12.61 ± 0.42% in control milk and 15.62 ± 0.59% and 13.74 ± 0.61% in treated β-CD for those phospholipids. These three species of phospholipids represent more than 80% of the total phosphoplipids in the dairy samples [17-20].

Phospholipids							Cholesterol
Sample	PL (% of sample)	PE (% of PL)	PI (% of PL)	PS (% of PL)	PC (% of PL)	SM (% of PL)	CH (mg/100 g milk fat)
Control Milk	0.023 ± 0.001	35.46 ± 0.28	3.89 ± 0.19	5.38 ± 0.48	16.95 ± 0.67	12.61 ± 0.42	292.46 ± 12.63
0.6% β-CD Milk	0.022 ± 0.001	34.12 ± 0.33	3.96 ± 0.25	5.59 ± 0.56	15.62 ± 0.59	13.74 ± 0.61	8.93 ± 0.58

Table 1: Phospholipid (PL) composition, phosphatidylethanolamine (PE), phosphatidylinositol, (PI), phosphatidylserina (PS), phosphatidylcholine (PC), sphingomyelin (SM) and cholesterol of control raw milk and raw milk with 0.6% β-cyclodextrin.

One of the reasons why the β-CD did not affect the these components of the milk fat could be based on the fact that β-CD very specifically forms an inclusion complex with cholesterol. β-CD is a cyclic oligosacharide of seven glucose units, the molecule is doughnut shaped. The central cavity is hydrophobic, giving the molecule its affinity for non-polar molecules such cholesterol. The radious of the cavity is such as to accommodate a cholesterol molecule almost exactly, confering the highly specific nature of β-CD ability to form an inclusion complex with cholesterol. The cholesterol is amphiphilic and the cholesterol molecules in the oil phase of an oil in water emulsion as milk tend to concentrate at the oil water interface. They are therefore accesible to β-CD in the aqueous phase forming the insoluble inclusion complex which can be remove by centrifugation.

Conclusions

Results from the present study suggest that the treatment of whole pasteurized milk with β-CD did not affect the phospholipids compositions in the milk fat globule membrane. Therefore, this process can be applied to milk for making low cholesterol dairy products without altering any nutritional properties of phospholipids of the milk fat.

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