Analysis of polar lipids from Milk Fat Globule Membrane (MFGM) by SPE and HPLC-ELSD

Introduction

Bovine milk contains around 4% fat dispersed in the serum phase in the form of spherical globules ranging from 0.2 to 15 µm in diameter. This fat in water emulsion is stabilized by the membrane surrounding fat globules, the MFGM. This membrane is originated from the secretory cells of the mammary gland (Singh, 2005). The MFGM composition is a complex mixture composed of half of proteins, mainly enzymes, glycoproteins, and half of lipids. Within this lipid fraction, two-thirds is neutral lipids, triacylglycerides from the lipid core and cholesterol (3%), and one-third is polar lipids including in majority phospho- and sphingolipids and minor components such as glycolipids (Danthine et al., 2000; Muira et al., 2004). With their amphiphilic structures, these polar lipid compounds play an important role in emulsifying and stabilizing properties. Polar lipid analysis is a preliminary and necessary step for a better understanding of fundamental properties of this natural emulsifying system. This research describes a global method of extraction, purification and selective detection of the polar lipids of the MFGM material extracted from a raw cream.

Experimental strategy

1 - MFGM extraction

- phosphate buffer washing
- ripening and shaking
- centrifugation of the buttermilk
- freeze drying
- native MFGM

2 - Total lipid extraction

- according to Shaikh method modified by Rombaut et al. (2005)
- 5 g of dried sample is diluted with 20 ml deionized water. Lipids are extracted with CHCl3 / CH3OH in 4 steps and chloroform phase is washed with 0.9% NaCl solution

3 - Lipid fractionation by solid phase extraction (SPE)

- according to Vaghela and Kilara (1995) modified by this research
- 1 ml of lipids is placed onto aminopropyle phase, the lipids fractionation into neutral lipids LN, free fatty acids FFA and polar lipids PL followed the elution phase proposed by Vaghela

4 - Polar lipids analysis by HPLC-ELSD

- according to a new method developed during this research: normal phase HPLC on a 150x4 mm Polaris silica Column equipped with a precolumn with the same packing and equilibrated at 40°C. Elution follows a linear gradient of the mobile phase CHCl3 / CH3OH / formic acid / water at pH 3. The Alltech ELSD HP 1050 use compressed air at 115°C with a flow rate of 2.3 L/min. The analysis time is 45 minutes including regeneration step.

Results

Protocols and composition of MFGM and buttermilk

<table>
<thead>
<tr>
<th>Proteins g / 100 g matter</th>
<th>MFGM</th>
<th>FBM</th>
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<tbody>
<tr>
<td>36.8 +/- 0.3</td>
<td>31.0 +/- 0.1</td>
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<tr>
<td>Fat content g / 100 g matter</td>
<td>53.9 +/- 0.1</td>
<td>15.6 +/- 0.1</td>
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<tr>
<td>Phospholipids / Fat content %</td>
<td>32.0 +/- 0.8</td>
<td>24.2 +/- 0.2</td>
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<tr>
<td>Lactose and minerals g / 100 g matter</td>
<td>6.0</td>
<td>60.7</td>
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<tr>
<td>Dry matter %</td>
<td>96.7 +/- 0.1</td>
<td>97.2 +/- 0.2</td>
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Lipid Extraction and fractionation yields

<table>
<thead>
<tr>
<th>MFGM</th>
<th>FBM</th>
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<tr>
<td>Extracted lipids g / 100 g matter</td>
<td>56.6 +/- 0.3</td>
</tr>
<tr>
<td>Shaikh Extraction Yield %</td>
<td>103.1</td>
</tr>
<tr>
<td>SPE Fractionation Yield %</td>
<td>90.9</td>
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Proportion of three lipidic fractions by SPE

Polar lipids proportion by HPLC-ELSD analysis

Conclusion

The study of the complex proteins/lipids matrix which constitutes MFGM has claimed for developing a specific protocol including the MFGM extraction from raw cream, purification and fractionation of the polar lipid fraction and quantitative analysis of the different ceramides, phospho- and sphingolipids. The analytical method consists first in a cold-extraction of total lipids to avoid oxidation and lipolysis risks. The extraction yield is around 100% compared with lipid content measurement by Mojonnier method. Next, lipid fractionation is set using a solid phase extraction on aminopropyle phase according to the modified Vaghela method. This separation step results in three purified fractions and allows quantitative determination of total polar lipids including ceramides. In consequence, this polar lipid content is systematically higher than phospholipids evaluation led by phosphorus analysis. The polar lipids fraction is analysed according to a quantiative and qualitative HPLC-ELSD method. By a new method, the three main phospholipids of the MFGM (phosphatidylethanolamine PE, phosphatidylcholine PC and sphingomyelin SM), but also two ceramides present in smaller proportion (glucosylerceramide GL and lactosylerceramide LC) are separated and quantified. Two membrane materials extracted from a raw cream were compared: a native MFGM and a experimental fresh buttermilk. The native MFGM shows higher proportion of polar lipids than fresh buttermilk certainly due to triglyceride contamination. Proportions between PE, PC and SM change too: fresh buttermilk presents a majority of PE and less PC and SM than the native MFGM. These results emphasize the importance of the extraction conditions for the fundamental study of MFGM and the necessity of developing an efficient analytical procedure for polar lipids determination.

Bibliography:

- Vaghela M N and Kilara A, 1995. A rapid method for extraction of total lipids from whey protein concentrates and separation of lipid classes with solid phase extraction. JAOCs, 72 - 10, 1117-1121.