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

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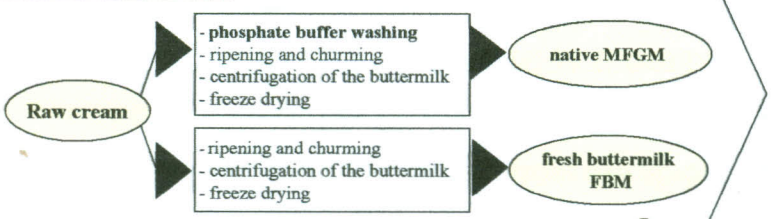
Introduction

Bovin milk contains around 4% fat dispersed in the serum phase in the form of spherical globules range from 0.2 to 15 µm of 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 for half of proteins, mainly enzymes, glycoproteins, and half of lipids. Within this lipid fraction, two-third 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; Miura 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



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 a 0.9% NaCl solution

Total lipids

3- Lipid fractionation by solid phase extraction (SPE)

- according to Vaghela and Kilara (1995) modified by this research
80 mg of extracted lipids is settled on 0.5g of aminopropyle powder and air dried. Sample is then packed to the top of a 2g conditionned aminopropyle column. The lipids fractionation into neutral lipids LN, free fatty acids FFA and polar lipids PL followed the elution phase proposed by Vaghela.

NL neutral lipids

FFA free fatty acid

PL polar lipids

4- Polar lipids analysis by HPLC-ELSD

according to a new method developped during this research: normal phase HPLC on a 150x4 mm Polaris silica Column equiped with a precolumn with the same packing and equilibrated at 40°C. Elution followes 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

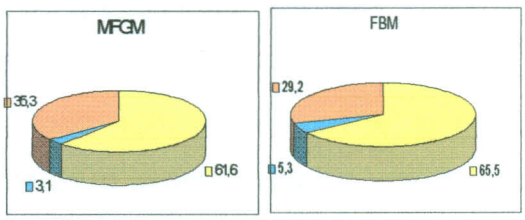
Proteins and lipids composition of MFGM and buttermilks

	MFGM	FBM
Proteins g / 100 g matter	36,8 +/- 0,3	31,0 +/- 0,1
Fat content g / 100 g matter	53,9 +/- 0,1	15,5 +/- 0,1
Phospholipids / Fat content %	32,0 +/- 0,8	24,2 +/- 0,2
Lactose and minerals g / 100 g matter	6,0	50,7
Dry matter	96,7 +/- 0,1	97,2 +/- 0,2

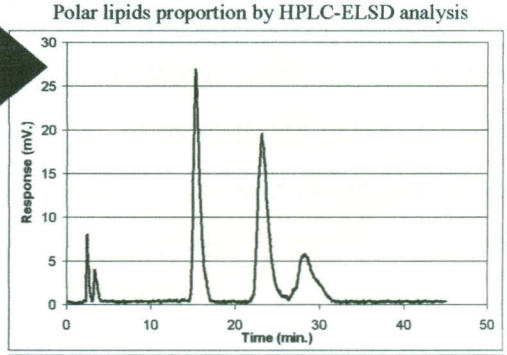
Lipid Extraction and fractionation yields

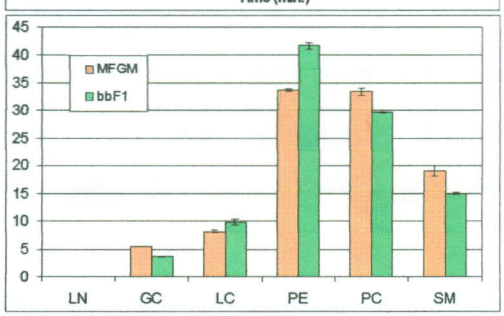
	MFGM	FBM
Extracted lipids g / 100 g matter	55,6 +/- 0,3	13,9 +/- 0,3
Shaikh Extraction Yield %	103,1	90
SPE Fractionation Yield %	98,9	92,2

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 procedure including the MFGM extraction from raw cream, purification steps of the polar lipid fraction and quantitative analysis of the differents ceramides, phospho- and sphingolipids. The analytical method consists first in a cold-extraction of total lipids to avoid oxydation 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 allowes quantitative determination of total polar lipids including ceramides. In consequence, this polar lipid content is systematicaly higher than phospholipids evaluation led by phosphorus analysis. The polar lipids fraction is analysed according to a quantitative 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 (glucosylceramide GL and lactosylceramide LC) are separated and quantified. Two membrane materials extracted from a raw cream were compared: a native MFGM and a experimental fresh buttermilk. 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.

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