FACULTY OF BIOSCIENCE ENGINEERING

Improved solvent extraction procedure and HPLC-ELSD analysis of polar lipids from dairy materials

THIEN TRUNG LE[a], TUYET MAI NGUYEN[a], JELENA MIOCINOVIC[ab], ROELAND ROMBAUT[c], JOHN VAN CAMP[a], AND KOEN DEWETTINCK[a]

[a]Department of Food Safety and Food Quality, Ghent University, 9000 Ghent, Belgium. [b]Institute of Food Technology and Biochemistry, University of Belgrade, Belgrade, Serbia. [c]CSM Innovation Centre Bakery Fats, 2170 Merksem, Belgium.

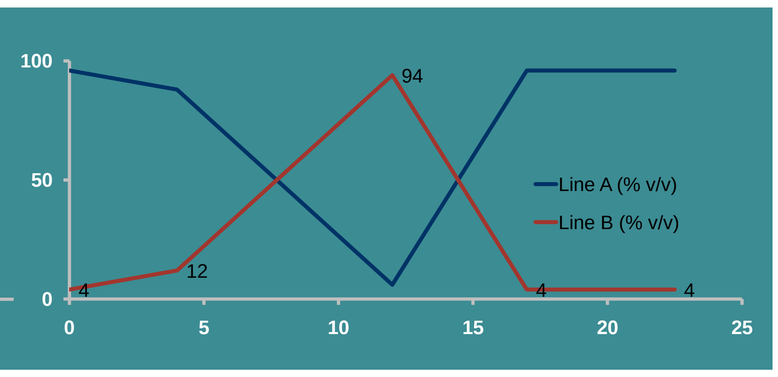
Content

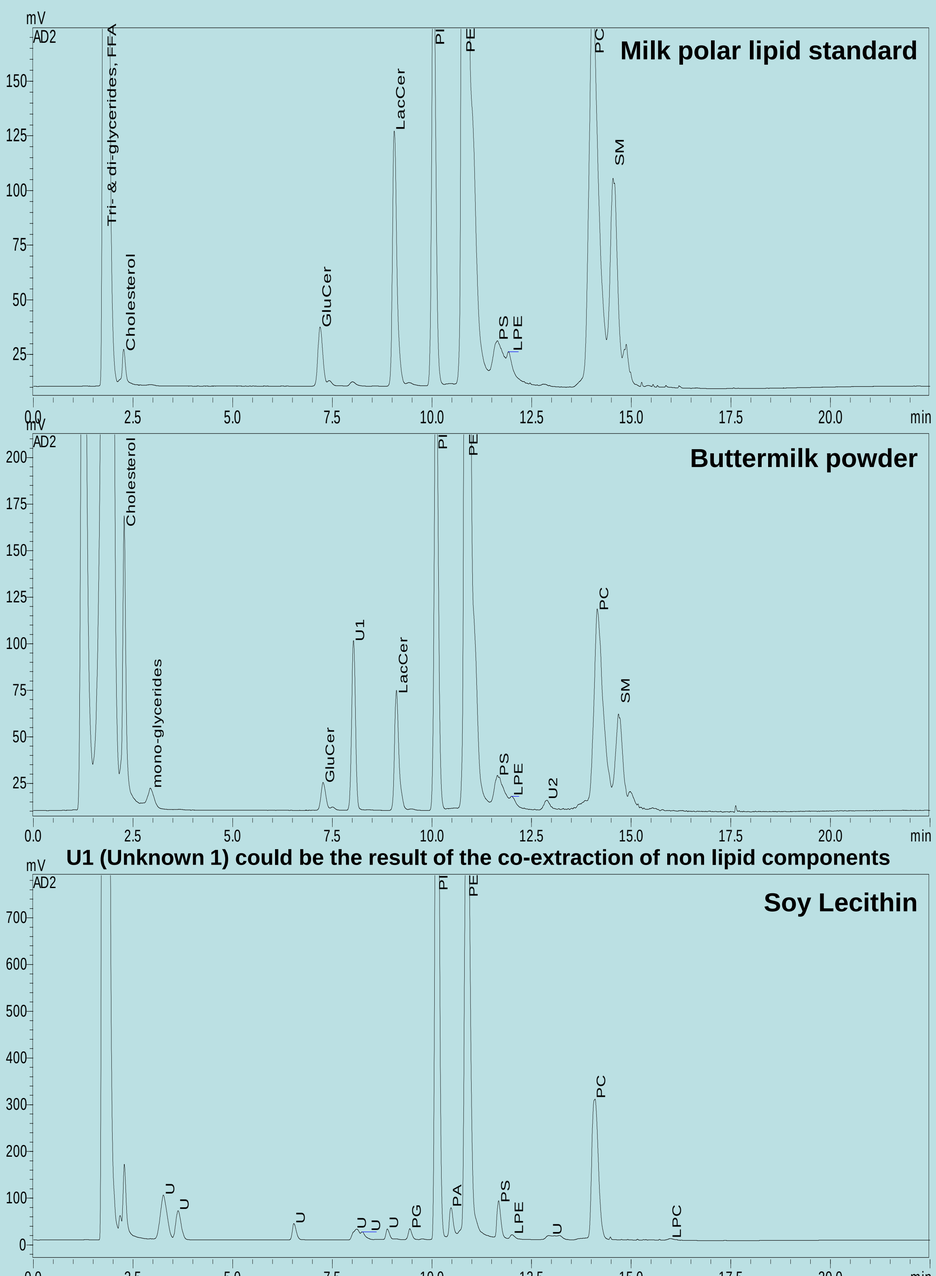
A normal phase HPLC-ELSD method employing dichloromethane, methanol, and acetic acid/triethylamine buffer as the mobile phase was developed based on Rombaut et al. (2005). J. Dairy Sci. 88:482-488 for analysis of polar lipids (PLs)

The liquid-liquid extraction method using solvents in the same reported work was modified to improve the efficiency for extraction of polar lipids (PLs) from dairy matrixes before HPLC analysis.

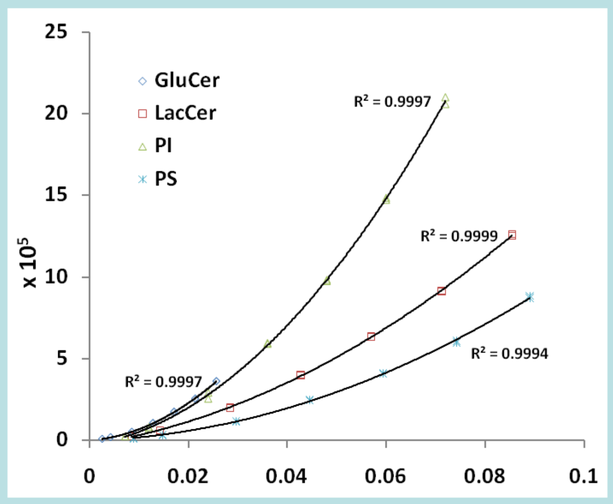
HPLC-ELSD Method

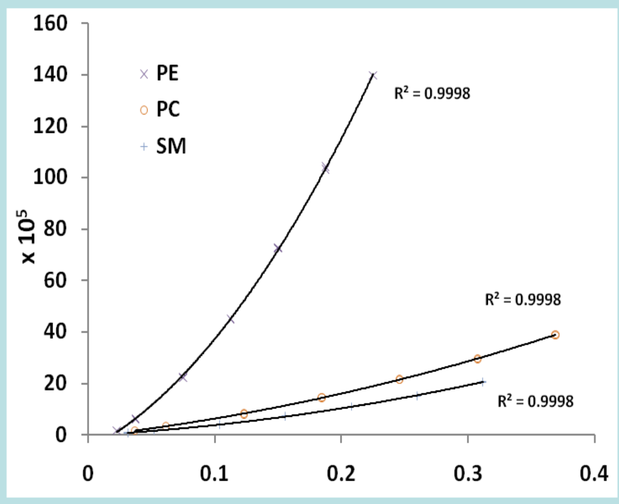
HPLC	Line A	Dichloromethane (stabilized by 0.1 % ethanol)
	Line B	Methanol and acetic acid/triethylamine buffer pH 4.5 at ratio 500/21 (v/v). The buffer = 7.2 mL acetic acid + 8.0 mL triethylamine + 118 mL HPLC water.
	Total flow rate	0.5 mL/min, Column oven set at 40 °C, sample chamber set at 20 °C
	Column	Precolumn Prevail silica 5u + Prevail silica 3u 150 x 3.0 mm (Grace Davison)
ELSD	Temperature: 65 °C, Nitrogen gas flow: 2.1 L/min, Gain: 1	





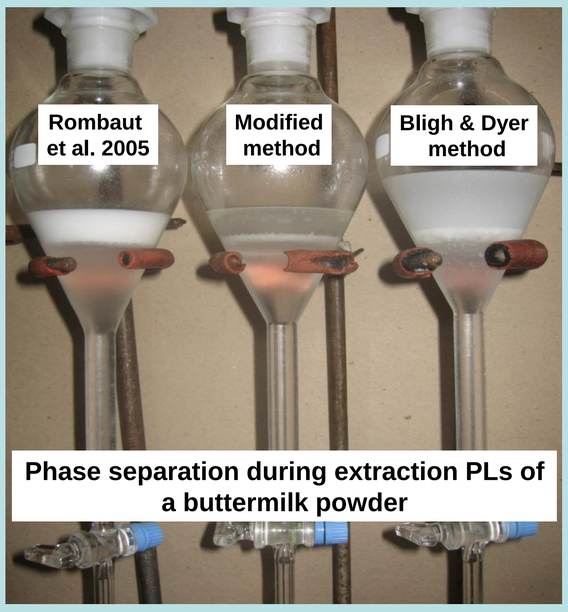
U1 (Unknown 1) could be the result of the co-extraction of non lipid components





Duplicate injection of standard PLs: Peak area vs. concentrations (mg/mL) at 10 μL injection, fitted with quadratic equations

Extraction Method



Phase separation during extraction PLs of a buttermilk powder

Modified extraction method: three steps

- **sample was added with 3 mL 10% (w/v) $CaCl_2$ solution**
- **diluted to 20 mL and extracted with 80 mL chroloform:methanol 2:1**
- **extracted two times more with 40 mL chloroform:methanol 20:1**

Conclusions

HPLC-ELSD	Extraction
The method used dichloromethane, a less toxic solvent compared to chloroform. Low pH of the buffer maintains the long service life of the column. Use of acetic acid resulted in narrower and more symmetric peaks than use of formic acid	Addition of $CaCl_2$ destabilized the emulsion in the upper phase → higher extraction efficiency
Total time of one run 22.5 min, obtained distinct separation of PL peaks	Addition of $CaCl_2$ increased the ionic strength → facilitated the phase separation → saved time and/or solvent use
Duplicate peak retention time variation ~ 0.01 min, duplicate RSD of peak area < 3%	Addition of $CaCl_2$ was necessary for low mineral materials e.g. membrane-filtered products
There is still a need to identify other minor peaks, separation can be further improved by changing mobile phase composition and/or elution gradient	Extraction recovery: ~ 100%

Batch RSD of PL concentrations < 5%, long-term RSD of PL concentrations < 5 % except for PS < 7.5%

Reference

Le, T. T., J. Miocinovic, T. M. Nguyen, R. Rombaut, J. Van Camp, and K. Dewettinck. 2011. Improved solvent extraction procedure and HPLC-ELSD method for analysis of polar lipids from dairy materials. J. Agric. Food Chem. 59(19):10407-10413

Faculty of Bioscience Engineering – Laboratory of Food Technology and Engineering
Contact person: Thien Trung Le
Coupure links 653, 9000 Ghent, Belgium, tel. +32 9 264 61 62, fax +32 9 264 62 18

http://www.foodscience.ugent.be
Le.TrungThien@UGent.be