

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

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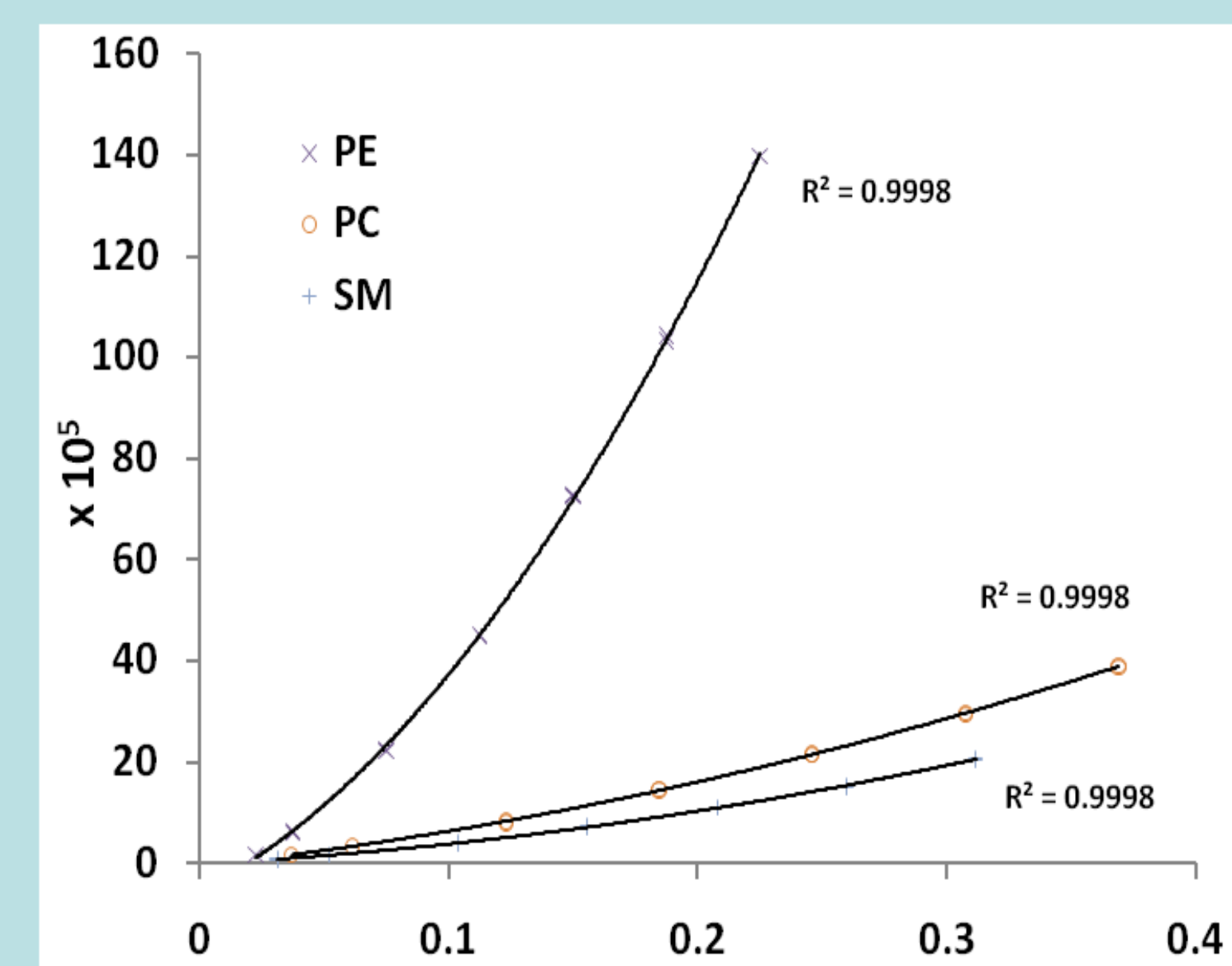
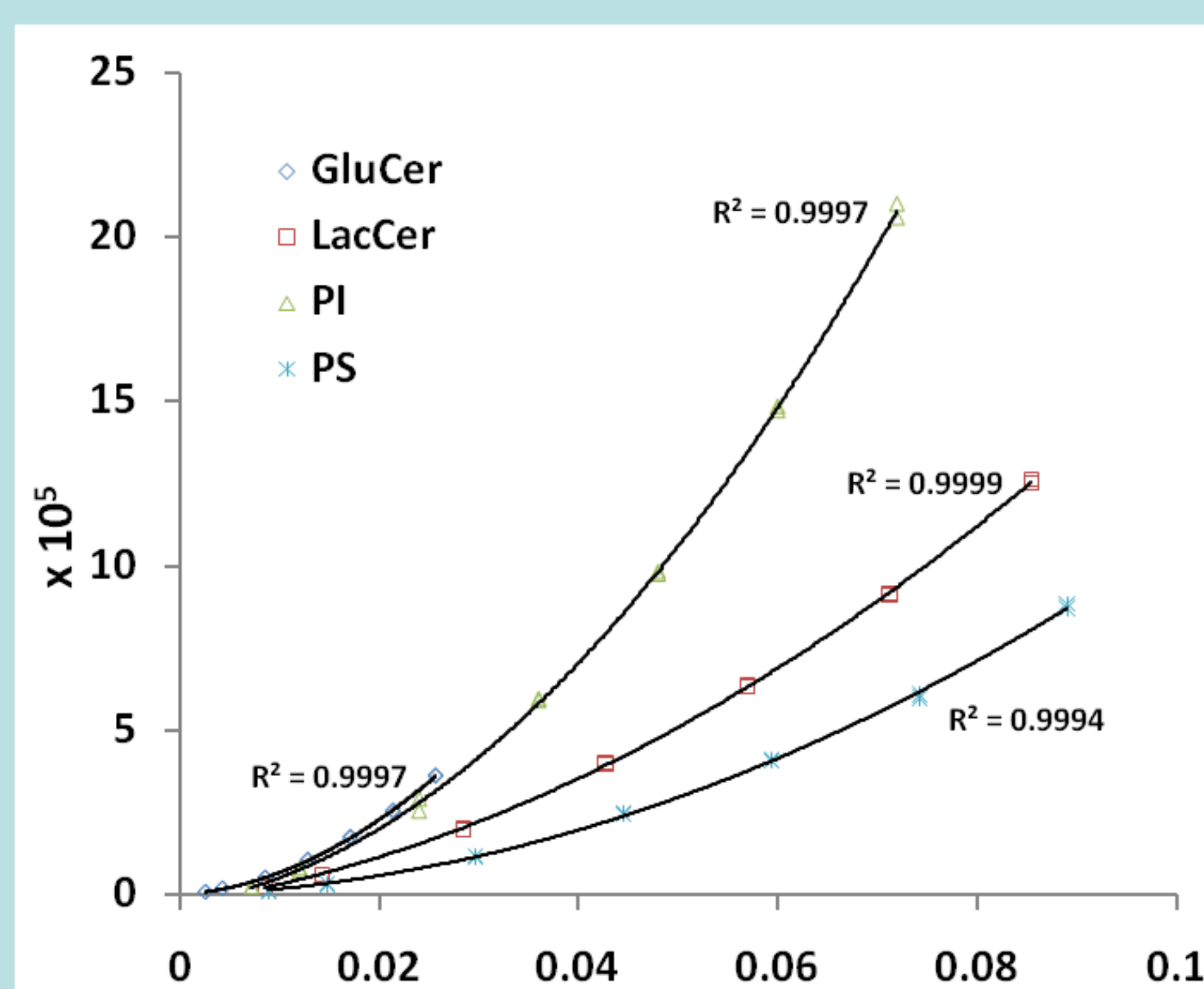
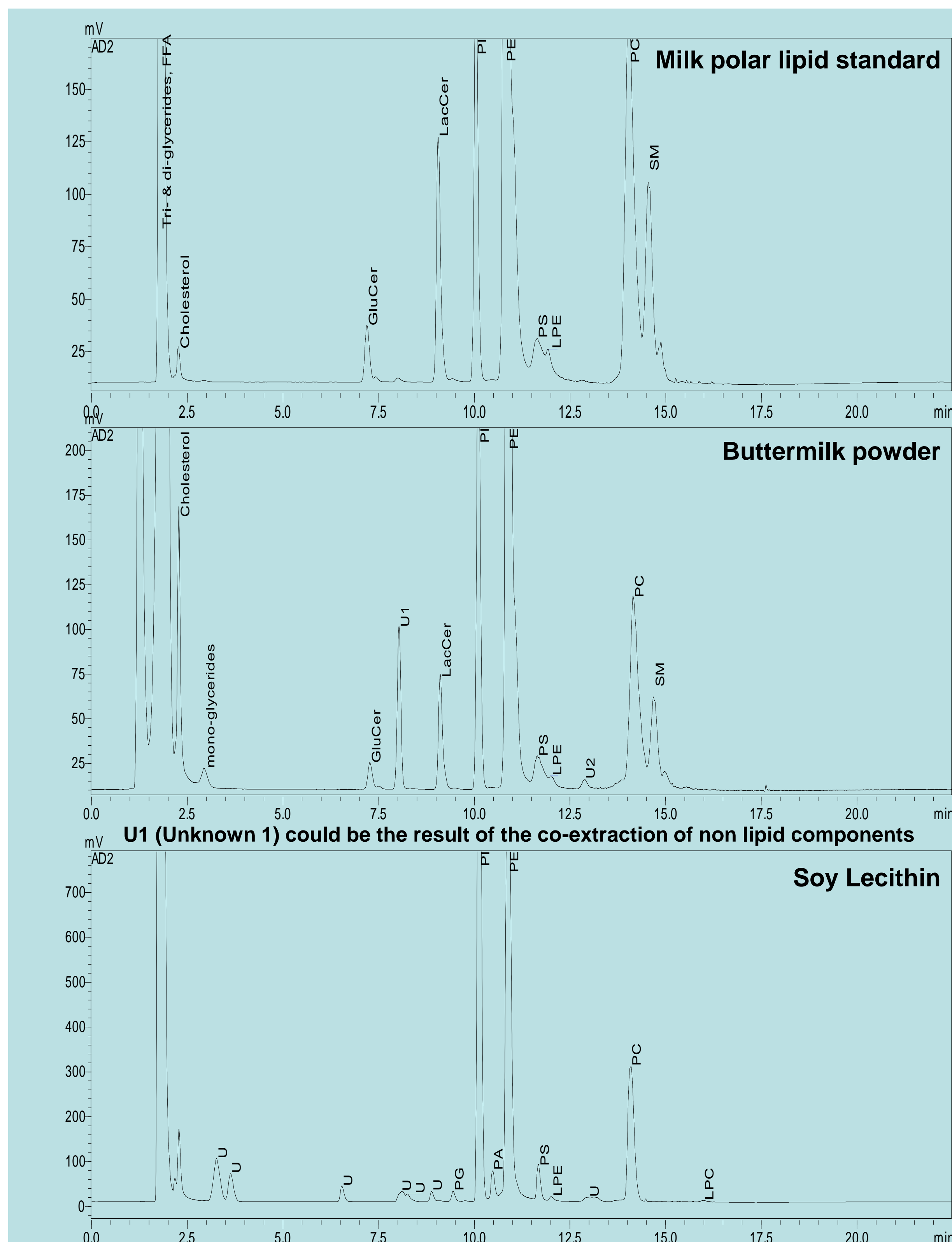
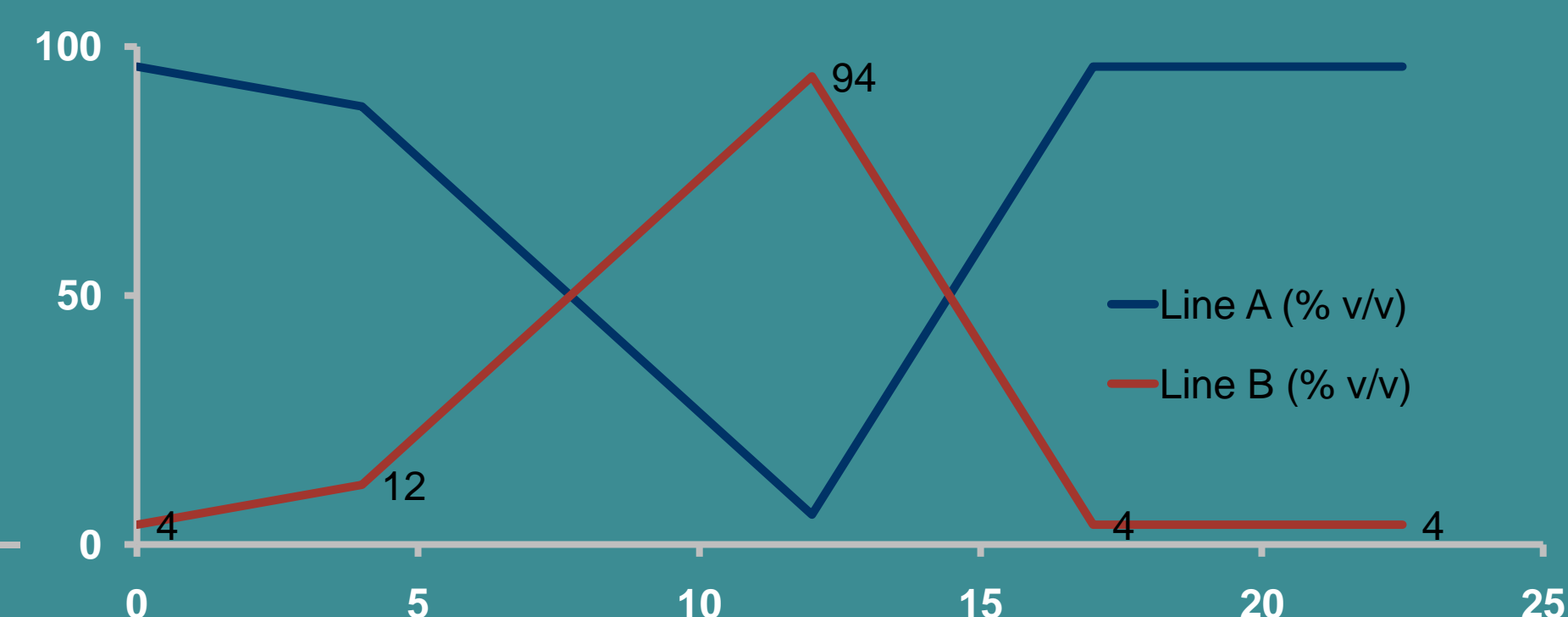
## Content

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.

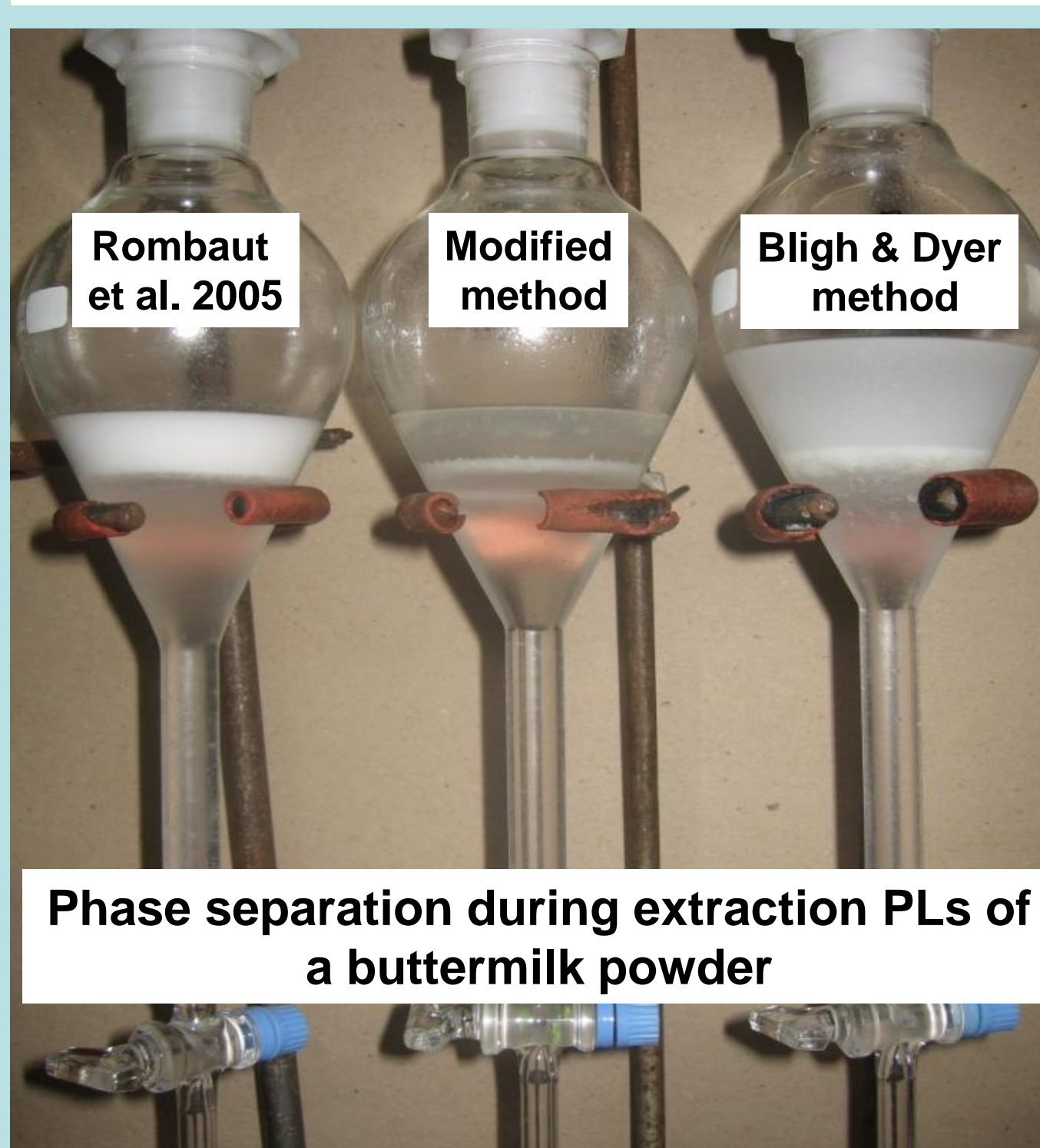
## HPLC-ELSD Method

HPLC	<b>Line A</b>	Dichloromethane (stabilized by 0.1 % ethanol)
	<b>Line B</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.
	<b>Total flow rate</b>	0.5 mL/min, Column oven set at 40 °C, sample chamber set at 20 °C
	<b>Column</b>	Precolumn Prevail silica 5u + Prevail silica 3u 150 x 3.0 mm (Grace Davison)
ELSD	<b>Temperature:</b> 65 °C, <b>Nitrogen gas flow:</b> 2.1 L/min, <b>Gain:</b> 1	



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

## Extraction Method



### Modified extraction method: three steps

- sample was added with 3 mL 10% (w/v) CaCl<sub>2</sub> solution
- diluted to 20 mL and extracted with 80 mL chloroform:methanol 2:1
- extracted two times more with 40 mL chloroform:methanol 20:1

## Conclusions

### HPLC-ELSD

- 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
- Total time of one run 22.5 min, obtained distinct separation of PL peaks
- Duplicate peak retention time variation ~ 0.01 min, duplicate RSD of peak area < 3%
- 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

- Addition of CaCl<sub>2</sub> destabilized the emulsion in the upper phase → higher extraction efficiency
- Addition of CaCl<sub>2</sub> increased the ionic strength → facilitated the phase separation → saved time and/or solvent use
- Addition of CaCl<sub>2</sub> was necessary for low mineral materials e.g. membrane-filtered products
- 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

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