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A Targeted Phospholipid Profiling Approach with PCA for Beans and Milk Using a Ready-to-Use MRM Method Package on LC/MS/MS

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❑ Introduction

Phospholipid profiling is usually performed by untargeted screening on GCMS and LC-TOF which involves extensive method development and data analysis work [1-2], due to complexity in structures. Shimadzu has developed an MRM method package for rapid profiling of phospholipids, covering 867 main components of various types of phospholipid with C14~C22 fatty acids. In this study, this ready-to-use MRM method package [3] was utilized for targeted screening of phospholipids in beans (*Glycine max*, *Vigna radiata*) and milk products without use of any standards successfully. By coupling with TQ-MS and PCA, this study aims to establish a rapid and highly sensitive phospholipid profiling approach on LC/MS/MS for food classification and authentication.

❑ Experimental

A mixed solvent of methanol and chloroform was utilized to cover extraction of a wide range of phospholipids from mung bean, soybean, soymilk, milk powder and fresh milk. Extraction was carried out and repeated two times to enhance recovery. Instead of time-consuming SPE, fast filtration was employed and proved to be sufficient for sample clean-up. Each sample was injected in triplicate (n=3) or 4 replicates (n=4).

Table 1. Analytical conditions on LCMS-8045

Column	: Kinetex Luna C8 column (150 x 2 mm, 5µm)
Mobile phase	: A: 20 mM ammonium formate in water B: acetonitrile:isopropanol (1:1)
Flow rate	: 0.3 mL/min
Elution mode	: Gradient elution, 20%B (0 – 1 min) → 40%B (2 min) → 92.5%B (2.01 – 25 min) → 100%B (26 min) → 100%B (26.01 – 35 min) → 20%B (35.10 min) → 20%B (35.11 – 38 min)
Column temp.	: 45 °C
Injection vol	: 5.0 µL
Interface & temp.	: Heated ESI, 300°C
MS mode	: MRM (+ and -)
Block temp.	: 400°C
DL temp.	: 250°C
Nebulizing gas flow	N_2, 2 L/min
Drying gas flow	N_2, 10 L/min

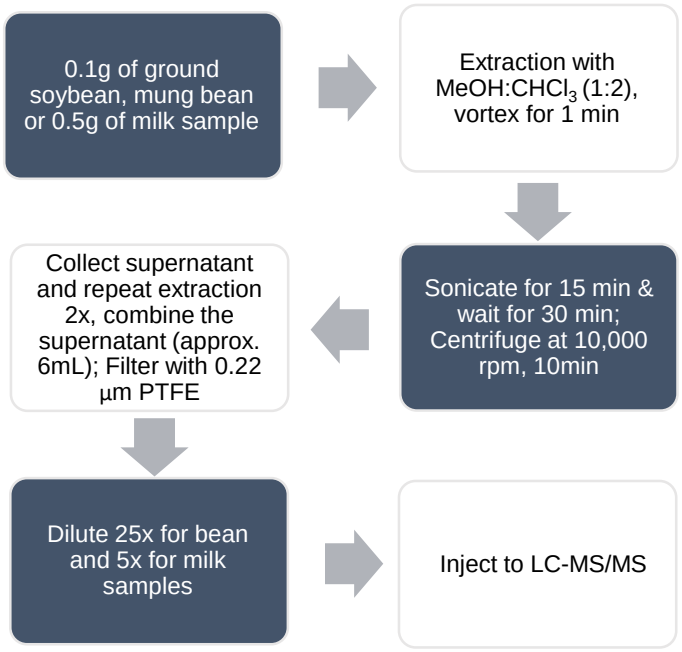

Figure 1. Flowchart of soybean, mung bean and milk sample preparation

❑ Results and Discussion

A. Determination of phospholipid ID class in bean and milk samples (Method 1)

The workflow of the phospholipid MRM method package is shown in **Figure 2**. Both bean and milk samples exhibited relatively similar profiles in the LCMS chromatograms (**Figure 3**). Based on method 1, PC, LPC, PE, LPE, LPG, PI, LPI, PS, LPS and SM were detected in both groups of samples (**Figure 4B** and **5B**). Phosphatidylglycerol (PG) is solely observed in bean samples. Expectedly, mung and soybean samples showed higher phospholipid abundance compared to milk samples.

** Student from Nanyang Technological University, Singapore for internship training*

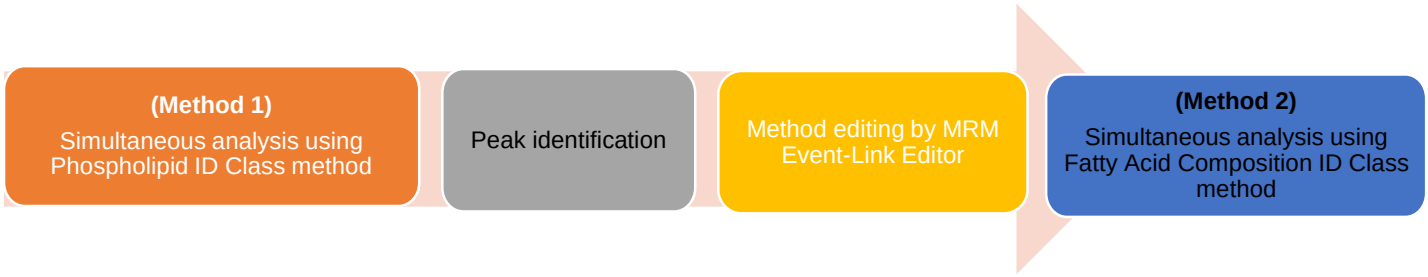

Figure 2. Flowchart of phospholipid MRM method package with Method 1 and Method 2 on LCMS-8045 with LabSolutions

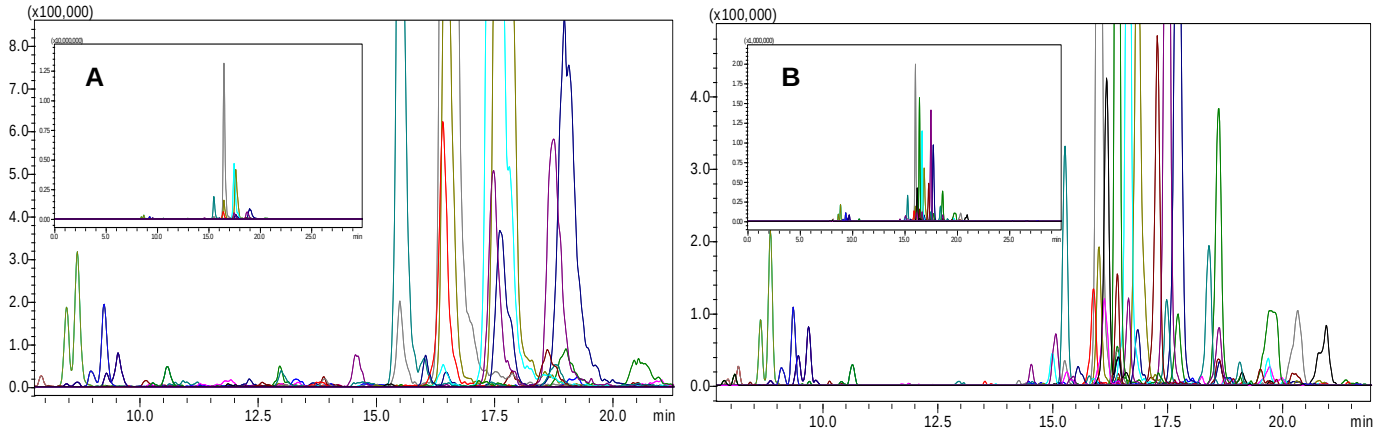

Figure 3. Overlaid MRM chromatograms of phospholipid profiles of (A) soybean and (B) soymilk

B. Phospholipid-based sample clustering by PCA

PCA was employed to emphasize the overview of all data trends. Bean samples (5 mung beans and 6 soybeans) obtained from different production areas (Southeast Asia, Australia, China, India) and cultivation types (organic and non-organic) were analysed.

PCA score plot (R^2X of 0.93) shows distinct separation between mung and soybeans by Principal Component 1 (**Figure 4**). Separation according to production area and cultivation type was also observed in standalone PCA of bean samples (data not shown).

Different milk samples (6 milk powder, 5 fresh milk, 5 soymilk) were also separated based on the account of phospholipid profiles (R^2X of 0.949) (**Figure 5**). Phosphatidylcholine (PC) was found to be dominant in soymilk and milk powder. Concurrently, separation of fresh milk from other samples was mainly attributed to phosphatidylserine (PS) and phosphatidylethanolamine (PE).

Figure 6A displays PCA score plot of consolidated bean and milk samples (R^2X of 0.96). Interestingly, soymilk products were grouped with soybean samples while the rest of samples were noticeably separated accordingly. PC(36:3), PC(36:4), SM(40:2) and PE(36:3) were significant for soymilk and soybean (**Figure 6B** and **7**) and in accordance to the report by Li *et al* (4) regarding higher abundance of PC and PE in soymilk compared to goat and bovine milk.

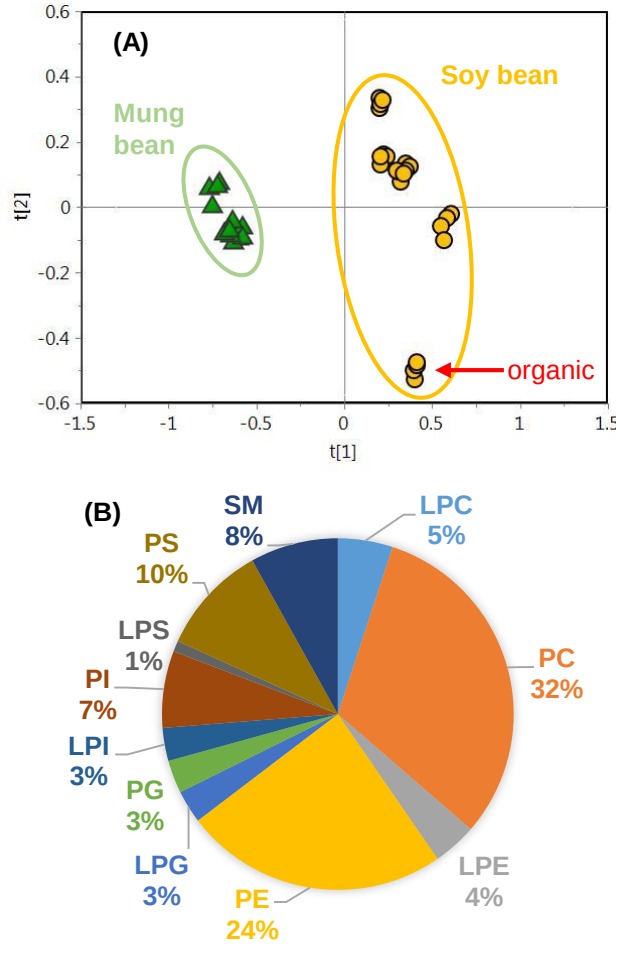

Figure 4. PCA score plot of 11 bean samples (*n*=4). Red arrow shows a cluster of organic soybean (A) and detected phospholipid in bean samples by Method 1 (B)

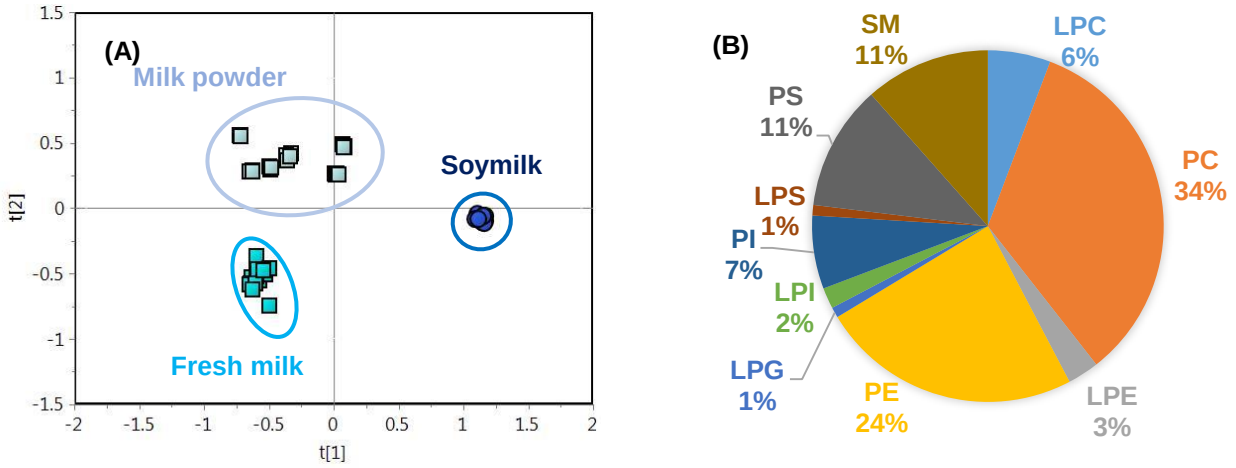

Figure 5. (A) PCA score plot of 16 milk samples (*n*=3, 4) and (B) detected phospholipid in milk samples by Method 1

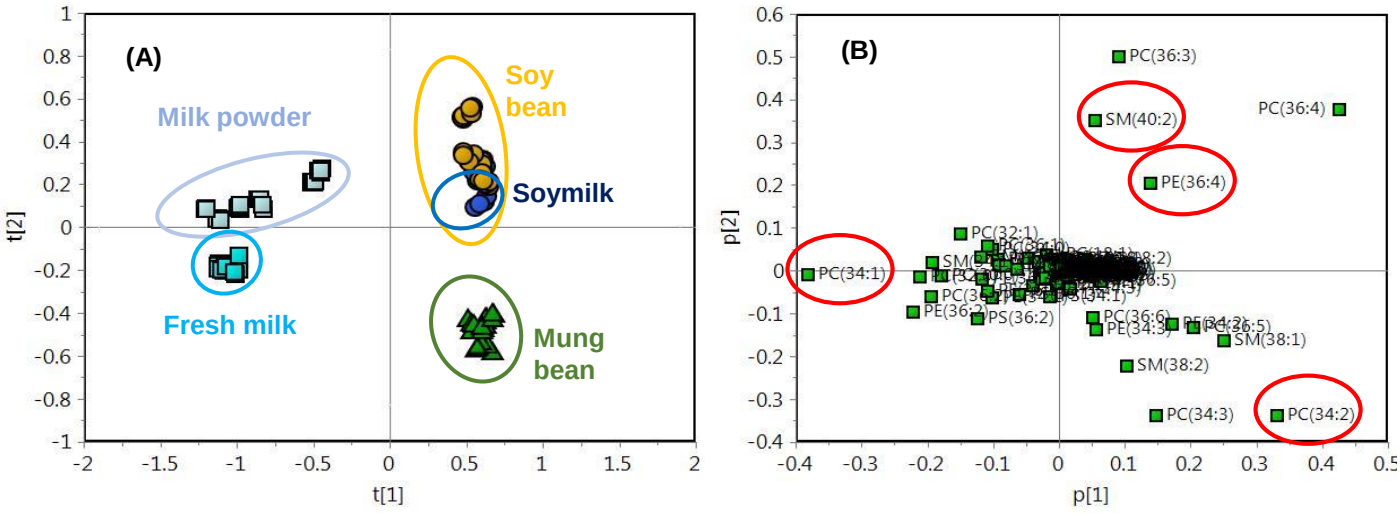

Figure 6. PCA score (A) and loading plot (B) of consolidated bean and milk samples. Determination of fatty acid composition was performed on highlighted phospholipids (red ellipse).

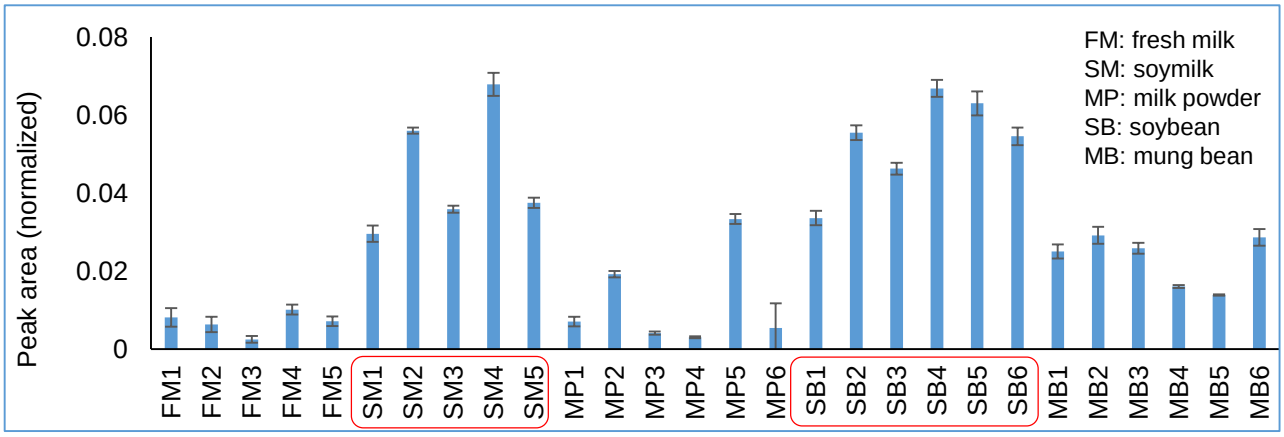

Figure 7. Peak area of significant phospholipids in soybean and soymilk, PE(36:4), (*n*=3 or *n*=4) in different samples. Error bar indicates standard deviation of injection replicates.

C. Determination of fatty acid composition for significant phospholipids (Method 2)

MRM parameters (Method 2) for determining fatty acid composition was acquired based on identification results of Method 1 by using MRM Event-Link Editor. Fatty acid composition was determined selectively for important phospholipids (**Figure 6B**), PE (36:4_18:1/18:3) or PE (36:4_18:2/18:2), SM (40:2_d18:1/22:1), PC (34:1_16:0/18:1), and PC (34:2_16:0/18:2). The MRM chromatograms of the fatty acids were obtained for several selected samples (**Figure 8**).

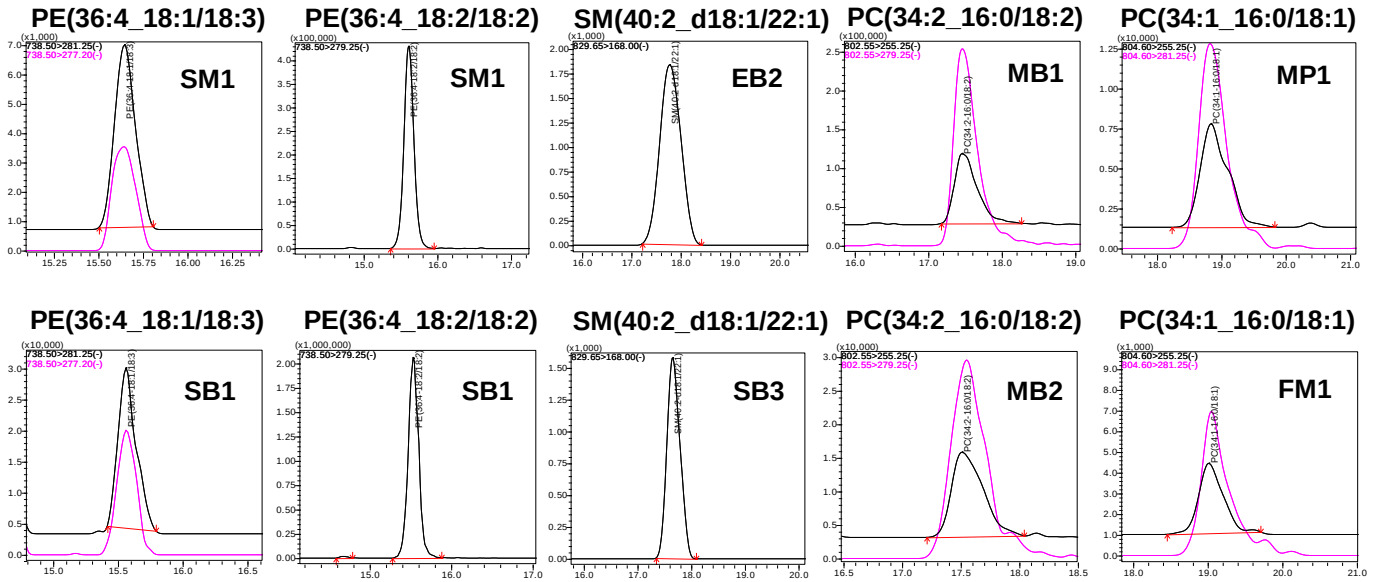

Figure 8. MRM chromatograms of selected phospholipids with fatty acid composition (label on top of each diagram) in different samples (detail of samples shown in **Figure 7**). Identification of fatty acid composition was performed according to 1 or 2 MRM transitions in Method 2 of the Method Package.

❑ Conclusion

A ready-to-use phospholipid MRM method package was applied for rapid profiling of beans and milk products. It is, therefore, not prerequisite to optimize separation conditions, MRM transitions or measurement parameters. More than 100 phospholipid classes were identified simultaneously without the need of chemical standard. PCA was performed based upon phospholipid profiles and successfully assembled soymilk together in a cluster with soybean samples in a consolidated bean and milk data. The results verify the applicability of the phospholipid MRM method package for food classification and authentication.

❑ References

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