Link 4: Extraction and analysis of polar lipids

Polar lipids such as phospholipids and partial acylglycerols possess unique properties due to its amphiphilicity. Such properties are beneficial especially for emulsification, dispersion, encapsulation and delivery of nutraceuticals and pharmaceuticals. Polar lipids are usually found in naturally occurring oils and fats in complex mixture with neutral lipids. Due to its high similarity with neutral lipids, the extraction process for highly pure polar lipids can be problematic. Most solvent extraction procedures extract both polar and neutral lipids at the same time. Degumming process; perhaps, is the only known large scale method used to separate phospholipids from neutral lipids. In degumming, water is added to hydrate the phospholipids. Upon hydration, the phospholipids will form heavy oil-insoluble sludge which can be easily separate through centrifugation [1]. Despite the high similarity in terms of properties between polar and neutral lipids, partial acylglycerols which have rather different evaporating temperatures can be separated from the neutral lipids by using short path distillation. In fact, the separation efficiency can be as high as up to 95% [2].

In order to analyze the content of polar lipids, it is necessary to firstly separate it from neutral lipids. This can be achieved through thin layer chromatography (TLC). For routine separation of polar lipids from neutral lipids, the oils or fats dissolved in folch solution are firstly spotted on the spotting line of the activated silica TLC plate. Following drying, the plate is then developed in a developing tank with mobile phase [3, 4]. A number of different mobile phases can be used for aforementioned purposes. Once the plate is sufficiently developed, it is removed from the developing tank, dried and visualized using a UV lamp. Polar lipids usually migrate lesser than neutral lipids and remains at the lower part of the silica TLC plate (Fig. 1) [4]. The separated individual bands can then be scraped and extracted using folch solution.

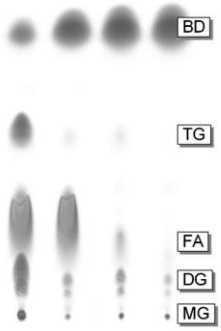

Fig. 1: TLC separation of polar lipids (MG and DG) from neutral lipids

Once the polar lipids have been separated from neutral lipids, further analysis can be done to examine its composition. This link will explore mainly on separation of phospholipids. For separation of partial acylglycerols, please refer to link 3.

There are several analytical methods available for analysis of phospholipids. TLC and high performance liquid chromatography (HPLC) can be used to analyze the different class of phospholipids. Similar to aforementioned described TLC procedure, mobile phase comprising of chloroform, methanol and water can be used to separate the different classes of phospholipids namely lysophosphatidylcholine (LPC), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and sphingomyeline. Similar separation can also be achieved by using HPLC which employed a silica column and mobile phase comprising of isopropanol, chloroform and water.

Aside from analysis of phospholipids of different classes, it has also become increasingly important to analyze the fatty acid composition of the phospholipids. This is mainly due to the growing interest in producing phospholipids with specific fatty acids for functional purposes. For example, structured phospholipids enriched with medium chain fatty acids were found to produce relatively more stable emulsions [5]. TLC and gas chromatography (GC) can be adopted to achieve the separation of phospholipids with different fatty acid compositions. By using a mobile phase comprising of chloroform, methanol and water, the long-chains PC, medium and long chains PC and medium-chains PC can be sufficiently separated (Fig. 2).

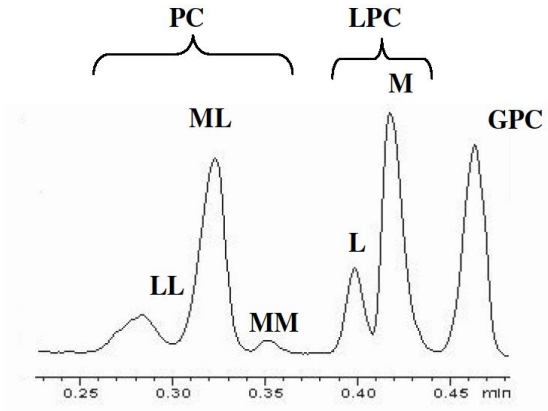

Fig. 2: Separation of PC with different chain length by using TLC [5]

In order to use GC to analyze the fatty acid composition of phospholipids, firstly the phospholipids must be transesterified to form fatty acid methyl esters. Base transesterification by using potassium hydroxide can be used to analyze the fatty acid composition of phospholipids. Please refer to link 1 for detail information about analysis of fatty acid composition.

The lipid lab of Aarhus University has long tradition working in extraction and analysis of polar lipids. Our lab is well equipped with TLC-FID, HPLC and GC to perform the required procedure.

References

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