Link 3: Analysis of glycerides and partial glycerides

Naturally occurring oils and fats such as coconut oil and butterfat are a complex mixture of glycerides and partial glycerides. Presence of a mixture of different glycerides and partial glycerides provides naturally occurring oils and fats with distinct physical properties such as wide range of melting and crystallization behavior, plasticity, mouthfeel and emulsifying capability. For example, palm oil which is rich in palmitic acid is a type of beta-prime tending oil frequently used for baking to impart desirable properties such as airy baked goods. Another worth mentioning example is the use of partial glycerides such as mono and diacylglycerol to enhance the plasticity of fats [1, 2]. Thus, it has become increasingly important to analyze and identify the different types of glycerides and partial glycerides in order to examine the link between glycerides, partial glycerides and physical properties.

Analysis of glycerides and partial glycerides can sometimes be a challenging and daunting task. To solve the mystery of glycerides and partial glycerides, reversed phase high performance liquid chromatography (RP-HPLC) or gas chromatography (GC) can be used. RP-HPLC separation of glycerides and partial glycerides provides easy sample preparation without prior need for sample derivatization. A C18 column is usually used as the stationary phase; meanwhile, a number of different solvent mixtures have been used as the mobile phase. Some of the more popular solvent combinations include acetone and acetonitrile [3-5], isopropanol, hexane and acetonitrile [6] and many more. The detection can be carried out by using a number of different detectors with the evaporative light scattering detector (ELSD), refractive index detector (RID) and photo diode array detector (PDA) being the most popular. Separation is generally based on the equivalent carbon number (ECN) with the molecule with smaller ECN being eluted first. For example, TAG with ECN 44 is eluted before TAG with ECN48 [4] (Fig. 1). Although not illustrated in Fig. 1, separation problem occurs especially among molecules possessing the same ECN number [3]. For example, both peaks 7 and 9 in Fig. 2 have a ECN number of 30 and thus co-eluted as not well resolved peaks. This can be resolved by separation of the molecules based on degree of unsaturation using either TLC or low pressure chromatography on silver nitrate impregnated silica gel. Another worth mentioning precautious for RP-HPLC separation is the difference in terms of detector response factors for glycerides species. In order to ensure accurate quantification, relative quantification with either internal or external standards can be used.

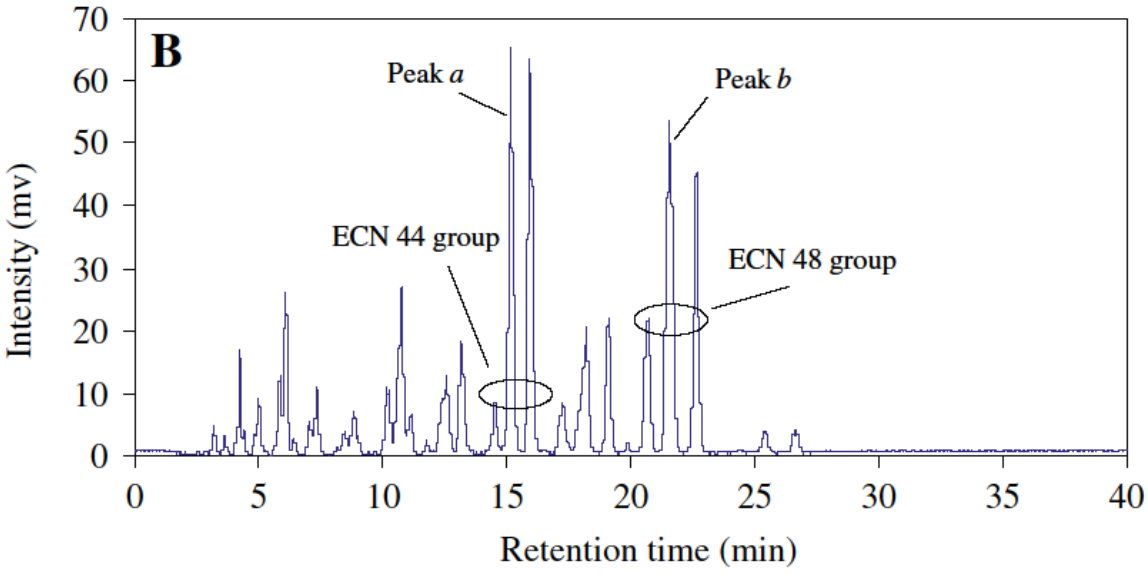

Fig. 1: HPLC chromatogram of TAG profiles of interesterified fat [4]

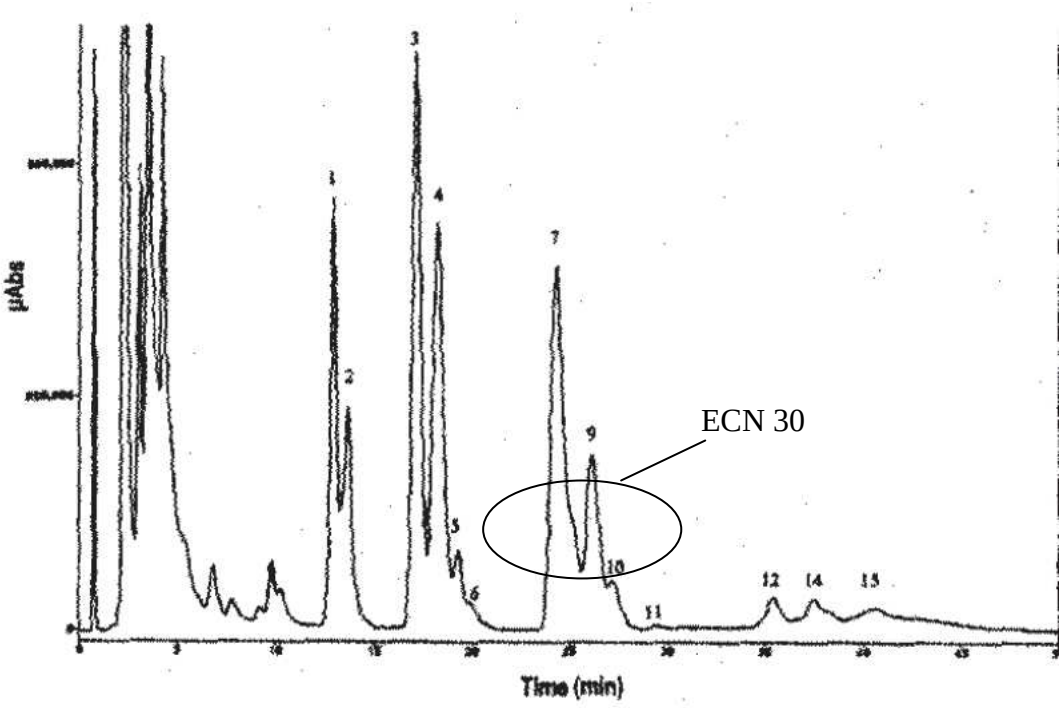

Fig. 2: HPLC chromatogram of DAG produced from soybean oil [3]

Despite the ease of glycerides analysis using RP-HPLC, sometimes GC is employed due to its high sensitivity and reproducibility. In GC separation of glycerides, the oils and fats must be firstly derivatized to improve its volatility. Some of the more commonly used derivatization agents include BSTFA [5], TMS ether and propionic anhydride. Following derivatization, the sample can be injected into the GC column for separation. Fused silica column is one of the more commonly used stationary phases; meanwhile, helium or inert gasses such as nitrogen is used as the mobile phase or carrier gas. Similar as the RP-HPLC, separation of the different glycerides and partial glycerides is also based on ECN with the TAG with smaller ECN number being firstly eluted (Fig. 3). Following separation, the different compounds are detected usually with flame ionization detector. As with the RP-HPLC, different compounds may have different response factors. Thus, relative quantification with either internal or external standards can be used to provide accurate quantification.

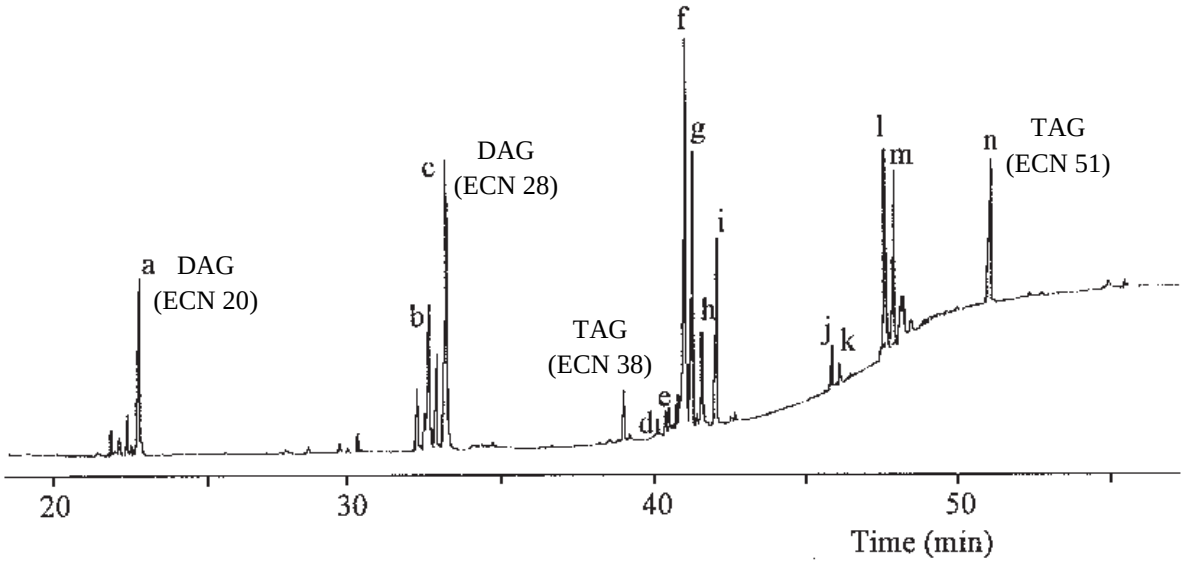

Fig. 3: GC chromatogram of partial glycerides and glycerides profiles of structured lipid

In the lipid lab of Department Engineering, Aarhus University, we are sufficiently equipped with HPLC, GC-FID and GC-MS for analysis of glycerides and partial glycerides composition. We have developed in-house methods for the aforementioned purposes.

References

1. Cheong, L.Z., et al., *Physicochemical, textural and viscoelastic properties of palm diacylglycerol bakery shortening during storage.* Journal of the Science of Food and Agriculture, 2010. **90**(13): p. 2310-2317.
2. Cheong, L.Z., et al., *Baking performance of palm diacylglycerol bakery fats and sensory evaluation of baked products.* European Journal of Lipid Science and Technology, 2011. **113**(2): p. 253-261.
3. Lo, S.K., et al., *Analysis of 1,2(2,3)- and 1,3-positional isomers of diacylglycerols from vegetable oils by reversed-phase high-performance liquid chromatography.* Journal of chromatographic science, 2004. **42**(3): p. 145-54.
4. Ibrahim, N., Z. Guo, and X. Xu, *Enzymatic Interesterification of Palm Stearin and Coconut Oil by a Dual Lipase System.* Journal of the American Oil Chemists' Society, 2008. **85**(1): p. 37-45.
5. Mu, H., et al., *Chromatographic methods in the monitoring of lipase-catalyzed interesterification.* European Journal of Lipid Science and Technology, 2000. **102**(3): p. 202-211.
6. Cheong, L.-Z., et al., *Physical Characterization of Lard Partial Acylglycerols and Their Effects on Melting and Crystallization Properties of Blends with Rapeseed Oil.* Journal of Agricultural and Food Chemistry, 2009. **57**(11): p. 5020-5027.