Abu Bekr Belkaid Tlemcen University
Institute of Applied Sciences and Techniques -ISTA
1st year LMD,
Module: Analytical Tools of Biochemistry

PW N°03: Qualitative Analysis of Olive Oil (Application of Thin Layer Chromatography)

Olive oil has long been considered a source of health benefits. It is derived from raw materials and is composed of approximately 99% fat. The fat in olive oil consists of triglycerides, which are made up of various types of fatty acids. Their distribution is characteristic of olive oil and, at a more detailed level, of the different varieties or regions of production. It is rich in oleic acid (C18:1, n-9) and also contains linoleic acid (C18:2, n-6), linolenic acid (C18:3, n-3), as well as other fatty acids.

1. Principle

Thin layer chromatography (TLC) is a technique used for the separation of constituents. It is based on differences in the affinity of substances between two phases: one stationary (or fixed) and the other mobile. TLC is primarily carried out for the analysis of a mixture. The solid stationary phase is a plate, and the liquid mobile phase, called the eluent, is a solvent or a mixture of solvents. A small amount of the mixture to be separated is deposited onto the stationary phase, which is then brought into contact with the mobile phase. The mobile phase migrates from the bottom to the top by capillarity along the stationary phase, carrying the constituents of the mixture with it. This process, known as elution, allows for the separation of the mixture's components. Each constituent migrates to a certain height, which is characteristic of the substance and is referred to as the retention factor (Rf).

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front}}$$

Each spot corresponds to a constituent and is identified by comparing its Rf with that of a control (the same substance under identical operating conditions; same Rf).

2. Operating Protocol

a. Adsorbent: Glass or aluminum plates coated with silica gel.
b. Eluent: Migration solvent: petroleum ether / diethyl ether / acetic acid (85 / 15 / 2, v/v/v).
c. Chromatography Preparation and Development:

- Activate the TLC plate for 30 minutes at 105°C to remove all traces of moisture.
- Fill the chromatographic tank with the solvent mixture to a height of 0.5 cm.
- Cover the tank to allow the atmosphere to become saturated with eluent vapors.
- On the chromatographic plate, draw a horizontal line with a pencil 1 cm from the bottom edge. This line should not be immersed in the elution solvent in the tank.
- Apply a microdrop of each oil sample onto the line using a capillary tube or micropipette.
- Allow the spots to dry before elution.
- After elution, once the solvent reaches the upper front line, remove the plate and let it dry for a few minutes in an oven or with a hair dryer.

d. Revelation: The detection and visualization of the separated compounds are first carried out using a UV lamp (366 nm) to make the spots visible. This is followed by spraying with a specific reagent. In this case, revelation will be performed using iodine vapor.

3. Expression of Results

- Determination of the retention factor (Rf) and the color of each separated compound.
- Comparison of the positions of the different spots corresponding to the samples.
- Comparison of the composition of the two olive oil samples with data from the literature.
- What conclusions can be drawn from this analysis?