Evaluation of Vegetable Oils: RP-HPLC vs. Voltammetry at Carbon Paste Electrodes

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Abstract

The quality of Italian extra virgin olive oils (EVOOs) was evaluated based on the profile and quantity of phenolic compounds (PPs), providing a specific fingerprint. A total of 68 EVOOs from 11 Italian regions were analysed using high-performance liquid chromatography coupled with a diode array detector and mass spectrometer. The obtained data were subjected to multivariate statistical methods. Further, PPs and lipophilic vitamins were analysed in vegetable oils of 6 different kinds using a square-wave voltammetry based on their anodic oxidation. Both analytical methods were compared and critically evaluated.

Key words: Vegetable oils, Phenolic compounds, Lipophilic vitamins, Multivariate statistical analysis, High-performance liquid chromatography, Carbon paste electrodes, Square-wave voltammetry.

Introduction

The composition and quality of extra virgin olive oils (EVOOs) is significantly influenced by the olive tree cultivar, the ripeness of the olive fruit, pedoclimatic conditions, and technological aspects of plant cultivation and oil processing (including also storage). Unfortunately, EVOOs are very often adulterated. Either good manufacturing practice is not followed or a false geographical origin is indicated. Therefore, many analytical methods have been developed for the differentiation and classification of EVOOs. The profile and composition of phenolic compounds (PPs) could help to distinguish the EVOOs authenticity. Reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with mass spectrometric (MS), spectrophotometric (UV), or electrochemical detection is frequently used technique for this purpose. However, the separation is usually very long and requires a sample pre-treatment, which also prolongs the experiment[1,2]. On the other hand, carbon paste electroactive electrodes (CPEEs) in combination with square-wave voltammetry (SWV) seems to be a useful tool for simple, efficient, and low-cost assessment of the quality of various vegetable oils (VOs) without any sample pre-treatments[3,4]. Hence, the aim of this work was to compare these two approaches.

Experimental

For SWV, different kinds of commercial available VOs, such as EVOOs, rapeseed, sunflower, linseed, pumpkin, and chia oils, were analysed without any sample pre-treatment. Voltammetric measurements were carried out in the three-electrode system, comprising a working CPEE, an Ag/AgCl (3 mol L^{-1} KCl) reference with salt bridge, and a Pt-sheet (auxiliary) electrodes. CPEE contained graphite powder (CR-2) together with mineral oil, and/or selected vegetable oil (20 %; *w/w*). The resulting carbon paste mixtures were packed into a cavity of electrode piston-holders and left for one day to self-homogenise under laboratory conditions. All experiments were realized in 0.1 mol L^{-1} acetate buffer with pH 4.5. The operating parameters of the SWV

ramp were as follows: deposition potential of −0.4 V, deposition time of 30 s, potential range from −0.4 to +0.6 V, potential step of 5 mV, pulse amplitude of 25 mV, and frequency of 40 Hz[3].

A total of 68 EVOOs coming from various Italian regions were chromatographically analysed. For statistical evaluation, the samples were divided into three supra-regions: northern Italy (samples from Lazio, Tuscany, Umbria, Lombardy, Veneto, Trentino-Alto Adige, and Liguria), southern Italy (samples from Apulia, Calabria, and Campania), and Sicily. The phenolic fraction was isolated by liquid-liquid extraction described in detail in the article of Klikarová et. al[2]. Separations were performed on an Ascentis Express C18 (150 × 4.6 mm; 2.7 μm) column with a mobile phase composed of 0.1% aqueous HCOOH (A) and 0.1% acetonitrilic HCOOH (B) with a flow rate of 1 mL/min and the following gradient program: 0 min, 10% B; 4 min, 35% B; 12 min, 47% B; 12.5 min, 60% B; 16 min, 75% B; 21 min, 100% B[1,2].

Results and Discussion
Electrochemical analysis of selected VOs was carried out using solid phase voltammetry (SPV) at CPEEs. It was found that the profile of the obtained voltametric records is more important for the evaluation of oil quality than monitoring of the content of individual electroactive organic compounds, representing mainly hydroxytyrosol, tocopherols, tocotrienols, carotenoids, and tyrosol. Such a comparison of voltammograms seems to allow the determination of VO types, and thus works analogously to a fingerprint in forensic analyses (Fig. 1). Compared to other samples, rapeseed oils together with chia oils were significantly richer in tocopherols and phenolic compounds. However, samples of EVOOs and pumpkin oils had higher amounts of carotenoids and tyrosol.
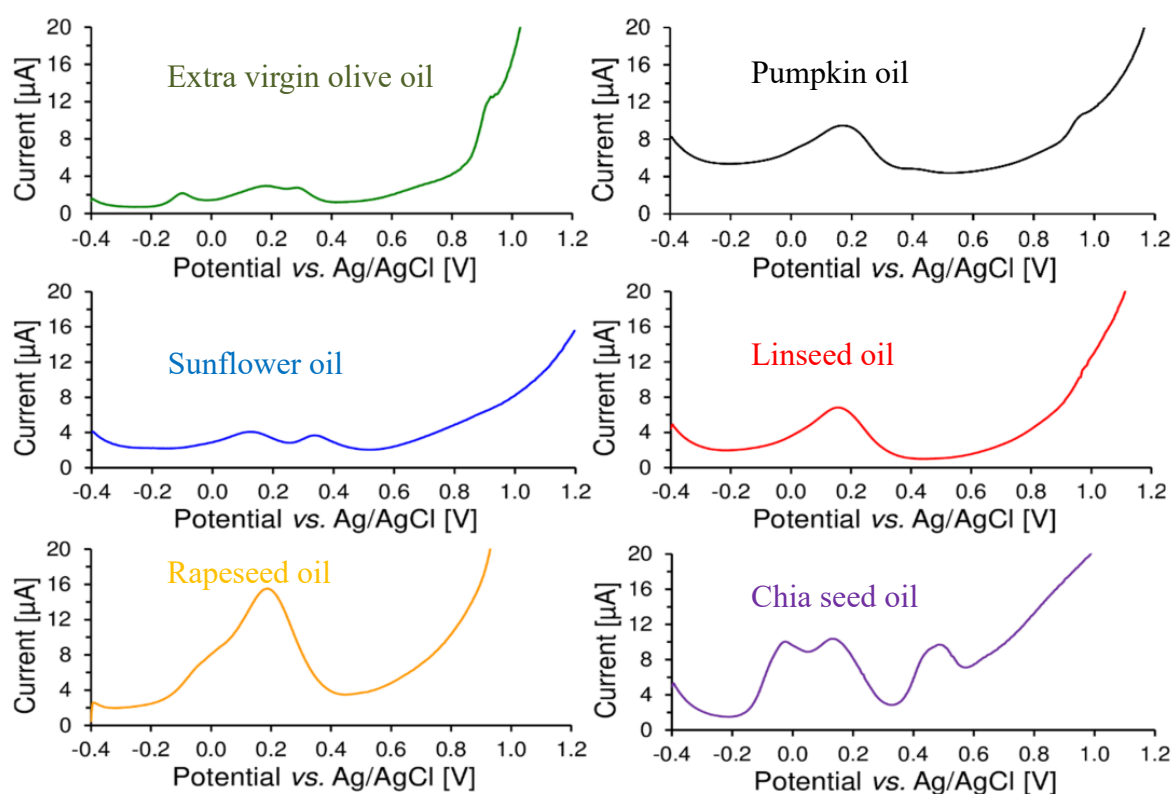

Fig. 1 Square-wave voltammograms obtained at CPEEs prepared from various vegetable oils; adopted from[3].

In VOs, mainly tocopherols are thermolabile[5], which can be utilised to test the degradability of commercial products. The rapeseed oil was heated at 150 °C and an aliquot volume was analysed every 5 min. A typical decrease of the tocopherol anodic peak was observed. Thus, it can be argued that SPV at CPEEs can also detect the degradation of electroactive compounds in VOs, and therefore evaluate their freshness.

Unfortunately, the proposed electrochemical method is not able to detect specific features within oil of the same type and thus does not reveal more detailed information, such as its botanical and geographical origin. This was successfully achieved by RP-HPLC-DAD/MS method, where only EVOOs were investigated. The samples were extracted and analysed and the data obtained were subjected to factor analysis, principal component analysis, and linear discriminant analysis. Factor analysis, as well as principle component analysis divided the samples into two groups according to the total content of phenolic compounds present in the oils and the ripeness of olives used for production of the corresponding oil, while linear discriminant analysis clearly divided the EVOOs into three clusters without any outliers according to their geographical origin (Fig. 2). Thus, it can be claimed that the PPs fingerprint, together with multivariate statistical methods, can help to reveal non-compliance with good manufacturing practice and/or unauthorized use of certification labels (Protected Designation of Origin and Protected Geographical Indication) designating a higher quality product.
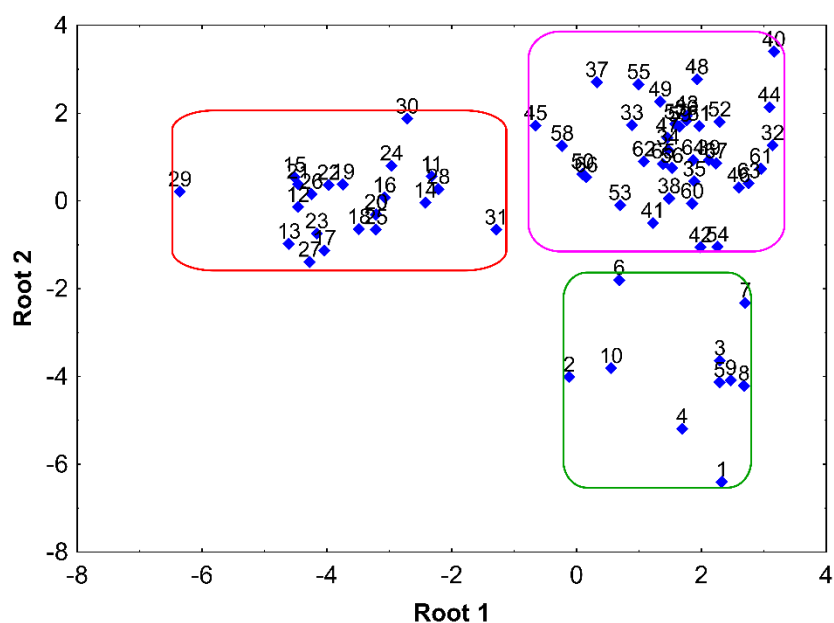

Fig. 2 Scatterplot of the linear discriminant score; adopted from[1].
Note: Northern Italy (purple), southern Italy (red), and Sicily (green).

Conclusion

The results show that electroactive organic compounds present in vegetable oils provided a spectrum of anodic signals, the number and overall shape of which were specific to a certain type of oil, and thus served as "electrochemical fingerprints". Thus, SPV at CPEEs using SWV is able to distinguish only individual types of vegetable oils, while the use of RP-HPLC allows to determine even the botanical and geographical origin within oil of the same type. It follows that the SPV-SWV developed can serve as a useful and rapid method for revealing of counterfeit vegetable oils, including indicative assessment of their actual freshness; however, HPLC is a more powerful tool for controlling good manufacturing practice.

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