Voltammetric fingerprinting of oils and its combination with chemometrics for the detection of extra virgin olive oil adulteration

Fotios Tsopelas [a], *, Dimitris Konstantopoulos [a], Anna Tsantili Kakoulidou [b]

[a] Laboratory of Inorganic and Analytical Chemistry, School of Chemical Engineering, National Technical University of Athens, Iroon Polytechniou 9, 157 80 Athens, Greece
[b] Laboratory of Pharmaceutical Chemistry, School of Pharmacy, University of Athens, Panepistimiopolis, Zografou, 157 71 Athens, Greece

HIGHLIGHTS

- Voltammetric fingerprinting for differentiation of olive oils and seed oils.
- Discrimination of virgin/extra virgin olive oils from plain olive oils.
- Successful prediction of unknown oil samples using PLS-DA and SIMCA.
- Quantification of adulteration of extra virgin olive oil using PLS.

GRAPHICAL ABSTRACT
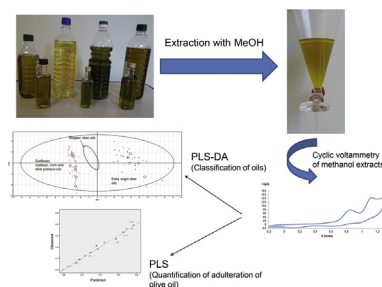
ABSTRACT

In the present work, two approaches for the voltammetric fingerprinting of oils and their combination with chemometrics were investigated in order to detect the adulteration of extra virgin olive oil with olive pomace oil as well as the most common seed oils, namely sunflower, soybean and corn oil. In particular, cyclic voltammograms of diluted extra virgin olive oils, regular (pure) olive oils (blends of refined olive oils with virgin olive oils), olive pomace oils and seed oils in presence of dichloromethane and 0.1 M of $LiClO_4$ in EtOH as electrolyte were recorded at a glassy carbon working electrode. Cyclic voltammetry was also employed in methanolic extracts of olive and seed oils. Datapoints of cyclic voltammograms were exported and submitted to Principal Component Analysis (PCA), Partial Least Square-Discriminant Analysis (PLS-DA) and soft independent modeling of class analogy (SIMCA). In diluted oils, PLS-DA provided a clear discrimination between olive oils (extra virgin and regular) and olive pomace/seed oils, while SIMCA showed a clear discrimination of extra virgin olive oil in regard to all other samples. Using methanolic extracts and considering datapoints recorded between 0.6 and 1.3 V, PLS-DA provided more information, resulting in three clusters-extra virgin olive oils, regular olive oils and seed/olive pomace oils-while SIMCA showed inferior performance. For the quantification of extra virgin olive oil adulteration with olive pomace oil or seed oils, a model based on Partial Least Square (PLS) analysis was developed. Detection limit of adulteration in olive oil was found to be 2% (v/v) and the linearity range up to 33% (v/v). Validation and applicability of all models was proved using a suitable test set. In the case of PLS, synthetic oil mixtures with 4 known adulteration levels in the range of 4—26% were also employed as a blind test set.

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* Corresponding author.
E-mail address: ftsop@central.ntua.gr (F. Tsopelas).

1. Introduction

Olive oil is obtained from the fruit of olive tree (Olea europea L.), the oldest known cultivated tree in history [1]. Olive oil is widely appreciated for its nutritional, health and sensory properties and it constitutes a principal ingredient of the so called Mediterranean dietary pattern [2,3]. Especially, extra virgin olive oil possesses the best reputation and nutritional characteristics due to its high content in monounsaturated fatty acids, vitamins and antioxidants [4]. In order to protect and highlight the value of its specific features, olive oil is included in designated origin/production ("protected designation of origin" (PDO)/"protected geographical indication" (PGI)) labeling of European Commission (EEC Regulation No. 2082/92). As olive oil is normally sold at a higher price than other vegetable oils, it is often adulterated with seed oils and olive oils of lower grade [5]. The verification of olive oil authenticity is of paramount importance to preserve the image of olive oil, to improve its competitiveness and increase the consumers' trust as stated in the last Horizon 2020 call [6].

The detection of virgin olive oil adulteration is a complex analysis and it can be mainly achieved by obtaining its fingerprint, which reflects its complex chemical composition and exploits the variability caused by differences of samples using chemometric techniques [7]. For this purpose, two different approaches can be followed. The first is based on specific chemical analysis, including quantification of fatty acids, sterols and triterpenic alcohols. In this case, gas and liquid chromatography are the methods of choice [7—12]. The main drawback of this approach is the necessity of sample pre-treatments, often resulting in a lengthy turnaround time [13]. The alternative approach relies on the implementation of instrumental methods not to verify or to quantify specific compounds, but to obtain a comprehensive and multivariate description of the chemical composition of the sample [13]. These nonspecific fingerprints can be obtained by Fourier transform infrared spectroscopy (FT-IR) [4,14], mid-infrared spectroscopy (MIR) [14,15], Raman spectrometry [14,16,17], nuclear magnetic resonance (NMR) [18] and differential scanning calorimetry [19]. Most of such approaches require extensive analytical resources and they can hardly be used for rapid analyses under field conditions.

In recent years, substantial efforts have been oriented towards the development of simplified, fast and inexpensive approaches with the possibility to be utilized in mobile analytical devices. In this aspect, electrochemical techniques are very attractive owing to their high sensitivity, inherent simplicity, miniaturization and low cost. These techniques can provide a non-specific fingerprint of oil samples, reflecting the redox properties of the electroactive species present in the oils. However, electroanalytical methodologies have been rarely applied to direct measurements in edible oils mainly due to the very poor conductivity of the matrix. The increase of conductivity by the addition of room temperature ionic liquids, such as tri-hexyl(tetradecyl)phosphonium bis (trifluoromethylsulfonyl) imide and trihexyl(tetradecyl)phosphnium decanoate has been described [20,21]. An alternative approach lies on the use of electrodes chemically modified with the oil of interest. Indeed, Apetrei et al. used oils as electroactive binder to prepare carbon paste electrodes (i.e. carbon paste electrodes chemically modified with olive oil) and recorded electroanalytical signal by immersing them in different aqueous electrolytic solutions [22,23].

However, the employment of voltammetric techniques to the detection of olive oil adulteration is very rarely described in the literature. A first attempt for edible oil discrimination according to their voltammetric response on chemically modified carbon paste electrodes was made by Apetrei et al. [22], using three virgin olive

oils of different quality, a refined olive oil and two seed oils. Based on their previous work on chemically modified electrodes [22,23], Apetrei and Apetrei developed also voltammetric e-tongues for the detection of olive oil adulteration with seed oils [24]. Oliveri et al. also achieved a discrimination of olive from maize oils as well as classification of olive oils according to their geographical origin [25]. However, more systematic investigations are still needed, especially using extended datasets (oil samples) in order to find the suitable conditions for the accurate quantification of olive oil adulteration, independently of the adulterant oil.

The aim of the present study was to investigate two approaches for the voltammetric fingerprinting of oils and their combination with chemometrics in order to detect the adulteration of extra virgin olive oil with olive pomace oil as well as the most common seed oils, namely sunflower, soybean and corn oil. For this purpose, cyclic voltammograms of olive, olive pomace oils and seed oils were recorded at a glassy electrode. Cyclic voltammetry was also employed in methanolic extracts of olive and seed oils. Datapoints of cyclic voltammograms were exported and submitted to principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) for discrimination of oils according to their origin. Class-modeling analysis by means of soft independent modeling of class analogy (SIMCA) was also employed to focus on the extra virgin olive oil samples. Finally, the potential of the developed electroanalytical approach to quantify extra virgin olive oil adulteration was investigated by submitting datapoints of cyclic voltammograms obtained for different adulteration levels to Partial Least Square (PLS) analysis.

2. Materials and methods

2.1. Reagents

All reagents used were of analytical grade unless otherwise is stated. Absolute ethanol, KCl ($\geq$99.5%) and MeOH ($\geq$99.9%, HPLC grade) were from Merck, CH_2Cl_2 (HPLC grade) from Fischer Chemical and $LiClO_4$ ($\geq$98.0%) from Sigma- Aldrich. All reagents were supplied by Chemilab (Athens, Greece).

2.2. Samples

90 samples of six kinds of edible vegetable oils, namely extra virgin oil, regular olive oil (mixtures of virgin and refined olive oil), olive pomace oil, sunflower oil, soybean oil and corn (maize) oil, were purchased from local markets and super-markets. The training set consisted of 75 oils, particularly 29 extra virgin olive oils, 5 regular olive oils, 10 olive pomace oils, 11 sunflower oils, 6 soybean oils and 14 corn oils. Blind test set for validation of PLS-DA, SIMCA and PLS models consisted of 15 oil samples; 5 extra virgin oils, 2 regular olive oils, 2 olive pomace oils as well as 2 soybean oils, 2 corn oils and 2 sunflower oils. These samples correspond almost to all commercially available edible vegetable oils in Greece. The origin of olive oil samples involves the four main olive oil production areas of Greece, namely Crete, Peloponnese, Ionian and Aegean islands and they correspond to the olive crop period of 2015—2016. Among the 29 extra virgin olive oils of the training set, 11 PDO/PGI and 4 organic ones were involved. It should be noted that especially PDO/PGI olive oils, apart from guaranteeing the geographical origin and cultivar of olives, are also considered as "standard" extra virgin olive oil samples due to the strict production rules during the whole processing cycle [26]. All oil samples were kept refrigerated (4 °C) until the analysis.

For quantification of olive oil adulteration, seed oil and olive