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Assessing the varietal origin of extra-virgin olive oil using liquid chromatography fingerprints of phenolic compound, data fusion and chemometrics

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ABSTRACT

High Performance Liquid Chromatography (HPLC) with diode array (DAD) and fluorescence (FLD) detection was used to acquire the fingerprints of the phenolic fraction of monovarietal extra-virgin olive oils (extra-VOOs) collected over three consecutive crop seasons (2011/2012-2013/2014). The chromatographic fingerprints of 140 extra-VOO samples processed from olive fruits of seven olive varieties, were recorded and statistically treated for varietal authentication purposes. First, DAD and FLD chromatographic-fingerprint datasets were separately processed and, subsequently, were joined using "Low-level" and "Mid-Level" data fusion methods. After the preliminary examination by principal component analysis (PCA), three supervised pattern recognition techniques, Partial Least Squares Discriminant Analysis (PLS-DA), Soft Independent Modeling of Class Analogies (SIMCA) and K-Nearest Neighbors (k-NN) were applied to the four chromatographic-fingerprinting matrices. The classification models built were very sensitive and selective, showing considerably good recognition and prediction abilities. The combination "chromatographic dataset + chemometric technique" allowing the most accurate classification for each monovarietal extra-VOO was highlighted.

1. Introduction

Virgin olive oil (VOO) is one of the most appreciated vegetable oils all over the world, although it is especially popular in the Mediterranean countries where the production of this commodity is a millenary tradition (Kapellakis, Tsagarakis, & Crowther, 2008). Likewise, VOO consumption has continuously attracted considerable interest, due to its beneficial effects on human health throughout its significant role in the prevention of some chronic diseases (Covas, 2007 and López-Miranda et al., 2010). These healthy properties of VOO have been attributed mainly to its chemical composition, particularly its high content in certain bioactive compounds (Bendini et al., 2007; Preedy & Watson, 2010 and Visioli & Galli, 2002). VOO chemical composition is very complex but its components can be divided in two fractions: the saponificable fraction comprises 98–99% of the total weight of the oil and is mainly formed by triacylglycerides; the minor components fraction (about 2% of the total oil weight) includes more than 230 chemical compounds, primarily pigments, aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile and phenolic compounds (Servili et al., 2013). This fraction, despite being a minority group, considerably contributes to some of the most relevant properties of this matrix.

Over the last years, producers have shown a keen interest in producing VOOs under differentiating systems as a market segmentation strategy, to become more competitive and try to deal with the effects of the globalization process in the olive oil sector (Lamani & Khadari, 2015 and Parra-López, Hinojosa-Rodríguez, Sayadi, & Carmona-Torres, 2015). This segmentation strategy can explain the gradual appearance of products with geographical indications (Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Specialty Guaranteed (TSG)) and monovarietal certifications into the olive oil market. These products are expected to be premium olive oils with peculiar and unique sensory characteristics that reflect the effects of a particular geographical origin (which includes environmental and human elements) and/or exclusive features of the processed olive

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variety. Thus, because of their unique characteristics, these products are commonly highly prized, and occasionally subjected to various types of adulteration, such as the addition of other non-certified olive oils to enlarge the volume of the most expensive certified geographical and/or monovarietal VOOs, increasing the economic profits. Hence, in the last decades, many pieces of research developing reliable analytical methods have been performed to ensure compliance of these certified products with labeling, through authenticating their geographical and/or varietal origin (Perri, Benincasa, & Muzzalupo, 2012).

Numerous attempts have been made to find suitable analytical approaches to differentiate monovarietal VOOs according to their cultivar origin (Cichelli & Pertesana, 2004; Gurdeniz, Ozen, & Tokatli, 2008 and Sinelli et al., 2010). These analytical methodologies can be divided into three main approaches: conventional, known as targeted analysis, profiling techniques and fingerprinting methods. Target analysis has been applied for a long time to olive oil varietal origin authentication and includes the determination of a small set of known olive oil compounds (targets) using an analytical technique suitable for the compounds of interest. Among the different targeted techniques, chromatography has been the most extensively utilized for the discrimination of the varietal origin of VOO (Montealegre, Marina Alegre, & García-Ruiz, 2010). In contrast to this approach, profiling techniques seek to provide the qualitative and/or quantitative determination of a larger set of compounds, which are related considering their chemical nature and/or biosynthesis pathway. In practice, the profiling of olive oil secondary metabolites -such as phenolic and volatile compounds- has shown great potential for the efficient differentiation of monovarietal VOOs from different cultivars (Cecchi & Alfei, 2013 and García-Villalba et al., 2011). The relevance of fingerprinting approaches is undeniable too; they offer attractive advantages over other strategies, regarding analysis simplicity, accuracy and rapidity. In this approach, a metabolic fingerprint of the sample under study is generated and compared to a large sample population screening for differences among the samples under study. The most widely used analytical techniques in fingerprinting analysis for the varietal authentication of VOO are spectroscopic techniques such as Infrared Spectroscopy in the near (NIR) and mid (MIR) regions (Sinelli et al., 2010), Fourier Transform Infrared (FTIR) Spectroscopy (Concha-Herrera, Lerma-García, Herrero-Martínez, & Simó-Alfonso, 2009 and Gurdeniz, Tokatli, & Ozen, 2007) and Nuclear Magnetic Resonance (NMR) Spectroscopy (Mannina & Segre, 2010).

Regardless of the approach selected, the obtained data are subsequently treated using appropriate chemometric tools, including unsupervised methods (mainly PCA and Hierarchical Cluster Analysis (HCA)), and supervised ones (such as Linear Discriminant Analysis (LDA), PLS-DA, SIMCA, k-NN, etc.) (Marini, Bucci, Magrì, & Magrì, 2010).

Previous studies focused on the development of analytical approaches for the varietal classification of VOOs have paid particular attention to phenolic compounds (Montealegre et al., 2010). Indeed, the phenolic composition of VOO is genetically determined, even if remains widely influenced by pedoclimatic conditions as well as agronomical and technological practices (Dabbou et al., 2009; Pérez et al., 2014; Romero-Segura, García-Rodríguez, Sánchez-Ortiz, Sanz, & Pérez, 2012 and Servili et al., 2004). Many publications dealing with the characterization of phenolic compounds in VOOs described the use of HPLC coupled to Mass Spectrometry (MS). Such coupling is not affordable for many laboratories due to its high cost. HPLC with DAD and/or FLD detection could be an advantageous and cheaper alternative for the high-throughput analysis of the VOO phenolic fraction. Certainly, the availability of these analytical platforms in most of the olive oil laboratories and research institutes could favor their application for routine analysis evaluating the varietal authenticity of certified monovarietal VOOs.

The aim of this study was, therefore, to ascertain the potential of using chromatographic fingerprints of phenolic compounds recorded by using DAD and FLD detectors (alone or combined) to discriminate among monovarietal VOOs, produced in northern Morocco, according to their cultivar of origin. The data matrices obtained (HPLC-DAD, HPLC-FLD and HPLC-DAD-FLD (using "Low-level" (LL) and "Mid-Level" (ML) data fusion strategies)) were evaluated applying different chemometric techniques (PCA, PLS-DA, SIMCA and k-NN). The classification and prediction performance of each combination "chromatographic dataset + chemometric technique" was assessed to identify which could lead to the most accurate varietal discrimination of oils under study.

2. Materials and methods

2.1. Olive fruit sampling

Olive trees in perfect sanitary conditions and conducted under the same agronomic conditions, in the olive experimental orchard of the Agro-pôle Olivier National School of Agriculture of Meknes (Morocco), were randomly selected for olive fruit sampling. 140 fresh and healthy olive fruits samples (approximately one sample was of about 35 kg of olives), at the same maturity stage (3.0–3.5), were handpicked over three consecutive crop seasons (2011/2012 (n = 47), 2012/2013 (n = 56) and 2013/2014 (n = 37)). The following olive varieties were considered in this study: **'Arbequina'** (2011/2012 (n = 4), 2012/2013 (n = 4) and 2013/2014 (n = 3)), **'Arbosana'** (2011/2012 (n = 5), 2012/2013 (n = 6) and 2013/2014 (n = 4)), **'Cornicabra'** (2011/2012 (n = 5), 2012/2013 (n = 6) and 2013/2014 (n = 5)), **'Frantoio'** (2011/2012 (n = 6), 2012/2013 (n = 9) and 2013/2014 (n = 6)), **'Picholine de Languedoc'** (2011/2012 (n = 10), 2012/2013 (n = 8) and 2013/2014 (n = 5)), **'Picholine Marocaine'** (2011/2012 (n = 9), 2012/2013 (n = 14) and 2013/2014 (n = 8)), and **'Picual'** (2011/2012 (n = 8), 2012/2013 (n = 9) and 2013/2014 (n = 6)). Olive samples were placed in rectangular plastic crates and immediately transported to the laboratory. Oil was extracted within 24 h of picking.

2.2. Oil extraction

Oil extraction was carried out in a laboratory instrument (Oliomio laboratory mill (Oliomio, Italy)). First, the olives were washed and defeated, then crushed using a crusher equipped with fix hole grid and groove knives impeller at a temperature of 25–27 °C. The paste produced falls into the malaxing part; malaxing was carried out for 45 min at 28–30 °C. The resulting olive paste was decanted at a temperature of 23–27 °C without the addition of any water. All the samples were subsequently filtered, placed in amber glass bottles (250 mL) at −18 °C, excluding any head space volume to assure their proper conservation against oxidation until the moment in which the chemical analyses were performed. Furthermore, physicochemical quality parameters were determined according to the analytical methods described in the European Union standard methods Regulations 2568/91 and the subsequent amendments (EC, 1991). The free acidity, expressed as percentage of oleic acid (%), peroxide value, expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2 kg^{-1}), K_{232} and K_{270} extinction coefficients (calculated from absorption at 232 and 270 nm, respectively) were determined in triplicate. Results allowed classifying all studied samples within the extra virgin category.