First comprehensive characterization of volatile profile of north Moroccan olive oils: A geographic discriminant approach

Aadil Bajoub [a], Araceli Sánchez-Ortiz [b], El Amine Ajal [c], Noureddine Ouazzani [c], Alberto Fernández-Gutiérrez [a], Gabriel Beltrán [b], Alegría Carrasco-Pancorbo [a,*]

[a] Department of Analytical Chemistry, Faculty of Science, University of Granada, Ave. Fuentenueva s/n, 18071 Granada, Spain
[b] IFAPA Centro Venta del Llano Ctra. Bailén-Motril Km. 18.5. PO Box 50, 23620 Mengíbar, Jaén, Spain
[c] Agro-pôle Olivier, National School of Agriculture in Meknès, BP S/40 Meknès, Morocco

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ABSTRACT

Herewith we have evaluated the variability of the composition in terms of volatile compounds of monovarietal "*Picholine marocaine*" olive oils and checked the possible influence of their geographical origin. For this purpose, 92 olive samples were collected during the harvesting period 2012/2013 from 7 north Moroccan regions, and the analysis of the volatile profiles of the obtained oils was performed by using headspace solid-phase microextraction coupled to gas chromatography with flame ionization and mass spectrometry detectors (HS-SPME/GC-FID–MS). A total of 40 volatile compounds belonging to different chemical classes were identified and quantified. Significant differences in the concentration levels of volatile constituents from oils of different geographical origins were found. Furthermore, for testing the ability of the identified volatile compounds for the geographical origin discrimination of the investigated oils, a stepwise linear discriminant analysis (s-LDA) was applied. Results revealed a very satisfactory classification of the studied oils according their geographic origin.

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1. Introduction

Virgin olive oil (VOO) is a highly appreciated vegetable oil all over the world. In fact, besides being the main source of fats in Mediterranean countries where olive oil production is gathered, according to data from the International Olive Council (IOC), both the olive oil production and consumption are growing considerably in areas outside the Mediterranean region, mainly in the United States, Australia, Brazil, Japan and China (IOC, 2014). In both traditional and new olive oil consumer countries, VOO is consumed as a healthy and nutritious food with peculiar and delicate flavour.

Flavour is one of the most important attributes of VOO, and it is an important factor determining its acceptability and preference by consumers (Aparicio, Morales, & García-González, 2012; Kalua et al., 2007). The flavour perception of VOO results from a complex sensory interactions between taste and aroma compounds, and olfactory and taste human receptors (Bendini & Valli, 2012). Therefore, the characterization of VOO aroma and taste profiles has become an important issue over the last years. Within this context, the aroma profiles of various VOOs have been extensively studied (Angerosa, Basti, & Vito, 1999; Kaftan & Elmaci, 2011), and numerous volatile compounds, including

aldehydes, alcohols, esters, ketones and terpenes, etc...., have been identified and described as the main aroma compounds occurring in VOO (Gomes, Freitas, Cabrita, & Garcia, 2012).

Volatile compounds are considered as potential indicators of olive oil sensory quality. Both positive and negative olive oil sensory attributes can be associated with volatile substances. In this regard, it has been stated that whilst endogenous enzymes such as acyl hydrolase, lipoxygenase, hydroperoxide lyase, alcohol dehydrogenase, and alcohol acyltransferase, are responsible (through the lipoxygenase pathway (LOX)) for the biosynthesis of volatile compounds so-called "green odorants" related to the perception known as "Green Odour" notes in olive oil, chemical oxidation and exogenous enzymes usually derived from microbial activity are associated with sensory defects (Angerosa, Mostallino, Basti, & Raffaella, 2000; Angerosa et al., 2004; Morales, Luna, & Aparicio, 2005).

Besides, volatile compounds have been found to play a main role in pattern recognition and classification studies, and are recognised as relevant VOO geographical and botanical markers (Cajka et al., 2010; Haddada et al., 2007; Luna, Morales, & Aparicio, 2006; Pizarro, Rodríguez-Tecedor, Pérez-del-Notario, & González-Sáiz, 2011; Pouliarekou et al., 2011). As a matter of fact, even if the composition and concentration of volatile compounds in olive oil are regulated by the enzymatic store which is genetically determined, they remain strongly affected by botanic origin (cultivar) as well as geographic

* Corresponding author.
  E-mail address: alegriac@ugr.es (A. Carrasco-Pancorbo).

origin factors such as climate, soil type, ripeness of the processed fruits and processing method (in particular, the malaxation time and temperature) (Aparicio & Morales, 1998; Brkić Bubola, Koprivnjak, & Sladonja, 2012; Kiralan et al., 2012; Sánchez-Ortiz, Romero-Segura, Sanz, & Pérez, 2012; Servili et al., 2008).

In Morocco, olive tree (*Olea europaea* L.) cultivation is a long and millenary tradition (Lenoir & Akerraz, 1984). Its favourable pedoclimatic conditions and the high adaptability of its main olive standard variety "*Picholine marocaine*" to different bioclimatic stages (Essadki, Ouazzani, Lumaret, & Moumni, 2006) contribute to the production of VOO with high quality. Therefore, the olive oil sector plays a very important role in the agricultural development policy pursued by Moroccan government over the last years. This strategy set (so called "Green Morocco Plan") includes, among others, olive area extension, improvement of farming practices and olive oil processing, storage and bottling techniques and olive oil chain value restructuration (MAPM, Moroccan Ministry of Agriculture and Marine Fisheries, 2013). In addition, to increase profitability and competitiveness of Moroccan VOOs in a growing and globalized olive oil market, the adopted development plan promotes and supports the establishment of geographical designations.

Even though the Moroccan legislative framework for the establishment and protection of geographical designations was adopted in 2008, there is still a lot of work to be done to develop methodological approaches to prove distinctiveness of Moroccan VOOs according to their geographical origin. Very few works can be cited to illustrate this point (Bajoub, Carrasco-Pancorbo, Ajal, Ouazzani, & Fernández-Gutiérrez, 2014; Haddi et al., 2013, 2011; Houlali et al., 2014), but they open up great expectations for testing the suitability of some olive oil compounds to be used as geographical markers of Moroccan VOOs.

The research presented herein constitutes a part of a pluriannual research program carried out by our group, aimed at evaluating the potential of using various olive oil compounds and multivariate analysis to discriminate north Moroccan virgin olive oils according to their geographical origin. It was designed to, firstly, determine the aroma profile of olive oils obtained from olive fruits of the standard variety "*Picholine marocaine*" grown in seven north Moroccan regions and, secondly, to evaluate the ability of the identified volatile compounds to trace the geographical origin of investigated olive oil samples. To the best of our knowledge, this is the first detailed and comprehensive characterization study of volatile compounds (evaluating their use for the geographical classification too) of Moroccan VOOs. Only a recent work characterizing the effect of storage on volatile compounds of eastern Moroccan olive oils (Tanouti, Serghini-Caid, Sindic, Wathelet, & Elamrani, 2012) can be found in the scientific literature.

2. Materials and methods

2.1. Sampling

A total of 92 "*Picholine marocaine*"*cv*. homogeneous olive fruit samples were collected, over the crop season 2012/2013, from seven north Moroccan olive growing areas: Chefchaouane, Fès, Meknès, Ouazzane, Sefrou, Taounate, and Taza. Only fresh and healthy olive fruits were hand-harvested, at breast height and circumference of olive trees randomly selected in representative olive orchards of the studied regions. Selected olive trees were mature trees (more than 30 year-old), spaced at 10 m × 10 m and conducted under non-irrigation conditions, which corresponds to the typical traditional olive orchard profile dominant in Morocco. The olive fruit samples (approximately one sample is 35 kg of olives) were collected from early November until mid-January, placed in rectangular plastic crates and immediately transported to the laboratory. At each sampling date, ripeness stage of collected olive fruits was determined according to the method proposed by the Agronomic Station of Mengíbar (Jaén) (Uceda & Frías, 1975), relying on assessment of the extent of the colour of olive fruit epidermis and mesocarp. Oil extraction was performed using an Oliomio laboratory mill (Oliomio,

Italy) within 24 h from harvest. This system reproduces at laboratory scale the industrial process: olives were washed to eliminate any foreign material and poured into the receiving hopper, where a screw feeds the crusher that is 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 water and finally, olive oil was centrifuged. The obtained oils were filtered and stored in amber glass bottles at 4 °C in darkness without headspace until analysis.

2.2. Physico-chemical quality criteria

Free fatty acids (expressed as percentage of oleic acid (%)), peroxide value (expressed as milliequivalents of active oxygen per kilogramme of oil (meq O_2/kg)), and K_{232} and K_{270} extinction coefficients (calculated from absorption at 232 and 270 nm, respectively) were measured in accordance with the European Official Methods described in the European Union Standard Methods Regulations 2568/91 and the subsequent amendments (EC, European Community Commission, 1991). All parameters were determined in triplicate for each sample.

2.3. Volatile compound analysis

Volatile compounds in north Moroccan olive oil samples were analysed, in duplicate, by HS-SPME. The conditions associated with this extraction procedure were selected taking into account the results reported in previous research involving the analysis of VOO's volatile compounds (Cavalli, Fernandez, Lizzani-Cuvelier, & Loiseau, 2003; Sánchez-Ortiz et al., 2012; Vichi, Castellote, et al., 2003). Sections 2.3.1 to 2.3.4 describe the chemical and reagents, sample preparation protocol, and the methodology used for the analysis, as well asthe approach to identify and quantify the volatile compounds.

2.3.1. Chemicals and reagents

Chemical standards of (E)-2-pentenal, (E)-hex-2-en-1-yl acetate, (E)-hex-2-enal, (E)-hex-2-enol, (E)-hex-3-enol, (E)-pent-2-en-1-ol, (Z)-3-hexen-1-ol acetate, (Z)-hex-2-enol, (Z)-hex-3-enol, (Z)-pent-2-en-1-ol, 1-pentanol, 2-methyl-butanal, 3-methyl-butanol, acetic acid, ethyl acetate, ethyl hexanoate, hexan-1-ol, hexanal, methyl acetate, methyl hexanoate, nonanal, ocimene, pent-1-en-3-ol, pentan-2-one, and pentanal were purchased from Sigma Aldrich (St. Louis, MO, USA). All standards were of high-purity grade. Volatile compound standard stock solutions were first prepared by dissolving the appropriate amount of these compounds in redeodorized high-oleic sunflower oil and then diluted to working concentrations. All solutions were stored in a dark flask at −20 °C.

2.3.2. Headspace solid phase microextraction (HS-SPME) procedure

Olive oil samples were tempered at room temperature. Then, 1.000 ± 0.001 g of each oil sample was weighted and placed in a 10 mL glass vial obtained from Agilent Technologies (Palo Alto, CA, USA), tightly capped with polytetrafluoroethylene (PTFE) septum, and left for 10 min at 40 °C on a heating magnetic platform agitation to allow the equilibration of the volatile compounds in the headspace. After reaching the equilibration, volatile substances were adsorbed using a Supelco fibre holder (Bellefonte, PA, USA) and a 50/30 µm divinylbenzene–carboxen–polydimethylsiloxane (DVB–CAR–PDMS) fibre, preconditioned according to manufacturer's instructions. An exposition of 50 min with continued heating (40 °C) and agitation was applied for the adsorption of the volatile compounds existing in the headspace. Desorption of volatile compounds trapped in the SPME fibre was performed directly into the gas chromatograph (GC) injector port equipped with a 0.75 mm i.d. inlet liner for 1 min at 250 °C and operated in splitless mode. The type of fibre and the extraction conditions