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Quality classification of Spanish olive oils by untargeted gas chromatography coupled to hybrid quadrupole-time of flight mass spectrometry with atmospheric pressure chemical ionization and metabolomics-based statistical approach

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ABSTRACT

The novel atmospheric pressure chemical ionization (APCI) source has been used in combination with gas chromatography (GC) coupled to hybrid quadrupole time-of-flight (QTOF) mass spectrometry (MS) for determination of volatile components of olive oil, enhancing its potential for classification of olive oil samples according to their quality using a metabolomics-based approach. The full-spectrum acquisition has allowed the detection of volatile organic compounds (VOCs) in olive oil samples, including Extra Virgin, Virgin and Lampante qualities. A dynamic headspace extraction with cartridge solvent elution was applied. The metabolomics strategy consisted of three different steps: a full mass spectral alignment of GC–MS data using MzMine 2.0, a multivariate analysis using Ez-Info and the creation of the statistical model with combinations of responses for molecular fragments. The model was finally validated using blind samples, obtaining an accuracy in oil classification of 70%, taking the official established method, "PANEL TEST", as reference.

1. Introduction

The determination of olive oil quality is typically performed by testers who establish if an olive oil must be labelled as Extra Virgin, Virgin or Lampante (not recommended for consumption) (Council, 2016). This strategy is known as "PANEL TEST", which classifies the oils according to two main properties: defects and positive attributes. The classification requirements are established by the International Council of Olive Oil (Council, 2016). Defects can be fusty, mouldy, sour and woody, and positive attributes can be fruity (green), bitter and spicy. According to the literature (Kalua et al., 2007; Luna, Morales, & Aparicio, 2006), the organic compounds responsible for olive oil flavour are typically esters, ketones, aldehydes, alcohols, terpenes, phenols and their derivatives, in concentrations ranging from a few ng L^{-1} to hundreds of mg L^{-1} and with different odour thresholds. PANEL TEST methodology could be considered slightly subjective, as the opinion of testers may vary. This may lead to misclassification of an oil, causing considerable economical losses, commercial problems and fraud. A more objective alternative could be based on the use of chromatographic techniques coupled to mass spectrometry (MS), which could allow the determination of chemical composition of the volatile fraction of olive oil samples, even at really low concentration levels. In this sense, the most by adequate analysis technique is gas chromatography coupled to mass spectrometry (GC–MS) (Angerosa et al., 2004; Flath, Forrey, & Guadagni, 1973).

According to the volatile characteristics of the compounds of interest, a specific extraction technique is also an important issue in order to perform a suitable separation from the matrix. From several studies on determination of VOCs in different matrices (Barco-Bonilla et al., 2011; Jiménez, Aguilera, Beltrán, & Uceda, 2006; Lam & Proctor, 2003; Salemi, Lacorte, Bagheri, & Barceló, 2006; Serrano, Beltrán, & Hernández, 2009), there is evidence for the extended use of trapping processes of the compounds in some kind of sorbent, either by forcing them to pass through the sorbent bed (P&T) (Barco-Bonilla et al., 2011; Salemi et al., 2006) or letting them to establish an equilibrium between the vapour phase and the adsorbent in a closed place (SPME) (Pouliarekou et al., 2011; Serrano et al., 2009). Other techniques also used for VOCs extraction include: direct headspace (HS) injection (Hu et al., 2014), stir bar sorptive extraction (SBSE) (Bicchi, Iori, Rubiolo, & Sandra, 2002), or liquid phase micro extraction (LPME) (Lee, Lee, Rasmussen, & Pedersen-Bjergaard, 2008).

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The determination of the chemical fingerprint of food samples is an interesting well-known approach for characterization of food products, for example in oil (Reboredo-Rodríguez et al., 2016; Reboredo-Rodríguez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2012) or tomato samples (Vaz-Freire, Da Silva, & Freitas, 2009). Chemical fingerprint, normally obtained using targeted analyses, can be further used to classify unknown samples, but due to the fact that a limited number of compounds must be selected *a priori*, some information regarding the samples is lost. In this context, metabolomics, which is defined as "the unbiased, global screening approach to classify samples based on metabolite patterns or fingerprints that change in response to disease, environmental or genetic perturbations with the ultimate goal to identify discriminating metabolites" (Cevallos-Cevallos, Reyes-De-Corcuera, Etxeberria, Danyluk, & Rodrick, 2009), can be an interesting approach to solve complex classification problems. One of the main drawbacks of metabolomics occurs when the relations or discrepancies between samples are just determined by a compound or group of compounds which are present at very low levels in complex matrices and can be easily dismissed. High resolution (HR) MS systems, such as time of flight (TOF) (Kind, Tolstikov, Fiehn, & Weiss, 2007) or magnetic sector (Kieken et al., 2009), enhance the detection of molecules in complex matrices at very low levels (Salihovic, Nilsson, Hagberg, & Lindström, 2013) due to their accurate mass measurements and extraordinary sensitivity, providing good results for the determination of non-target compounds. Furthermore, the use of atmospheric pressure chemical ionization (APCI), a very promising ionization source in GC, which is softer than the common electron ionization (EI), allowing the acquisition of high intensity peaks for the molecular ion ($M^{+\cdot}$) and/or the protonated molecule ($[M+H]^+$), depending on the nature of the compounds (Portolés, Sancho, Hernández, Newton, & Hancock, 2010). This information is really convenient as in some cases the molecular peak is absent in EI, due to its high fragmentation and in terms of sensitivity, the peak intensity can be reduced. GC coupled to hybrid quadrupole time of flight (QTOF) MS equipped with APCI allows the acquisition of accurate-mass full scan spectra at low and at high collision energy (MS^E mode) being a very useful tool for elucidation purposes.

Data processing, together with data acquisition, are the mainstays of metabolomics. The direct observation of sample chromatograms does not give significant information about the difference between sample quality, and thus specialized software is required to obtain chromatographic peaks and masses from raw data. In literature, metabolomics studies use different software to get the information needed from the chromatograms, such as XCMS package of R (Díaz, Pozo, Sancho, & Hernández, 2014), MetAlign (Tikunov et al., 2005) or MzMine 2.0 (Kind et al., 2007).

The aim of this work has been the development of a GC-(APCI) QTOF MS methodology to obtain the chemical profile/fingerprint of olive oil volatile compounds, in order to establish differences between virgin olive oil qualities using P&T extraction and through the use of metabolomics techniques, in order to give a more objective decision in their classification, compared with that provided by the "PANEL TEST".

2. Materials and methods

2.1. Chemicals and reagents

Internal standard triphenyl phosphate (TPP) $\geqslant$ 99% was purchased from Sigma Aldrich (Germany). Diethyl ether (residue analysis quality GC) and hexane (trace analysis quality (AT) GC) were provided by Scharlau (Barcelona, Spain).

Supelclean ENVI-Carb® SPE tubes 500 mg, volume 6 mL, 120–400 mesh, surface area 100 $m^2\,g^{-1}$, used as traps, were purchased from Supelco (Barcelona, Spain).

2.2. Olive oil samples

A total of 425 olive oil samples was provided by the "Interprofesional del Aceite de Oliva Español" Organization (INTERPRO, Spain), the "Agencia para el Aceite de oliva del Ministerio de Agricultura, Alimentación y Medio Ambiente" and the official control services from the "Consejería de Agricultura, Pesca y Desarrollo Rural de la Junta de Andalucía".

Oil samples were taken from different regions of Spain and included 300 quality characterized samples (120 Extra Virgin, 120 Virgin and 60 Lampante) and 125 blind samples (the quality was unknown during analysis). Samples were stored at −22 °C until use. Samples were characterized by means of pH measurements and physicochemical and organoleptic properties by the official participating laboratories (Laboratorio Arbitral Agroalimentario del MAGRAMA, Laboratorios Agroalimentarios de Córdoba y Atarfe de la Junta de Andalucía) and their corresponding certified "PANEL TESTs".

2.3. Sample treatment

Olive oil samples were allowed to defrost at room temperature before analysis. Then, they were aliquoted in 4 different 10-mL vials. One aliquot was used to perform the extraction and the remaining ones were stored at 4 °C.

Olive oil (5 g) was weighed into a 150-mL flask before inserting a magnetic stirrer. The flask was rapidly closed with a glass tap with a nitrogen entrance and the exit connected to the sorbent trap (Envi-Carb cartridge). The cartridge was conditioned with 2 × 5 mL of a mixture of hexane:diethyl ether (50/50; *v/v*) and vacuum dried for 10 min. Sample extraction was then carried out for 60 min at 40 °C with a nitrogen flow of 1 $L\,min^{-1}$ and with stirring at 300 rpm. After extraction, Envi-carb® cartridges were eluted by gravity with 5 mL of the hexane:diethyl ether mixture (50/50 *v/v*), into a glass tube previously weighed. A 50-μL aliquot of TPP solution at 5 $mg\,L^{-1}$ (in hexane) was added as internal standard. The entire extract was then concentrated under vacuum conditions with a MiVac Duo Concentrator (Genevac, Italy), until total removal of diethyl ether, i.e. until a final volume of approximately 0.5 mL. Finally, in order to adjust the final volume of the extract (0.5 mL of hexane), several drops of hexane were added until adjusted mass (0.3274 g). An aliquot of 100 μL of this extract was transferred to a 20-mL vial in order to generate a pool of extracts to prepare the quality control (QC) sample. The remaining extract was divided into two different vials with 200 μL inserts, sealed and stored in freezer at −20 °C until their analysis by GC-QTOFMS. In each extraction batch, 2 Extra Virgin, 2 Virgin and 2 Lampante oil samples were processed simultaneously.

2.4. GC–(APCI)QTOFMS

The chromatographic analyses were performed using an Agilent 7890A gas chromatograph, equipped with an Agilent 7693 autosampler, coupled to a quadrupole/time-of-flight mass spectrometer, Xevo G2 QTOFMS (Waters Corporation, Manchester, UK), with APCI source. The GC separation was performed using a fused silica HP-5MS capillary column with (30 m × 0.25 mm ID; film thickness 0.25 μm (J&W Scientific, Folsom, CA). The oven program was set as follows: 40 °C (3 min); 5 °C/min to 160 °C (1 min); 50 °C/min to 300 °C (2.2 min); total runtime 33 min. Injections of 1 μL of sample extracts were performed using pulsed splitless mode (50 psi) at a temperature of 270 °C with a pulse time of