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Original research article

Control of olive cultivar irrigation by front-face fluorescence excitation-emission matrices in combination with PARAFAC

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ABSTRACT

Due to their antioxidant properties, biophenolic compounds from vegetables and derived products are very demanded by the consumers. The olive fruit pulp is rich in these compounds, and, in this paper, the influence of irrigation on the levels of these compounds has been investigated. Methanolic extracts from olive paste samples submitted to different irrigation treatments were analyzed by front-face fluorescence. Excitation-emission matrices, recorded as a set of emission spectra in the range 290–450 nm, and in the excitation range of 240–290 nm, were analyzed by means of Parallel Factor Analysis (PARAFAC). The loadings and scores corresponding to three components were obtained. In the same samples, polyphenols were also analyzed by chromatography. High correlations were found between the first component PARAFAC scores and epicatechin (R = 0.856) and catechin plus epicatechin concentrations (R = 0.873), second component scores and oleuropein (R = 0.892, only when epicatechin concentration is lower than 0.55 mg/L) and the third component scores and vanillic acid concentrations (R = 0.877). The representation of the two first PARAFAC component scores allowed discriminate between the different irrigation treatments. Polyphenol concentrations obtained by both methods were analyzed statistically by ANOVA and Duncańs multiple test. The obtained results showed significant differences between the irrigation treatments.

1. Introduction

In our days, and due to their antioxidant properties, consumers are interested in biophenolic compounds from vegetables and derivate products (Difonzo et al., 2017; Talhauoi et al., 2015). Various studies suggest that these compounds are associated with beneficial effects, and they are related with a reduction in cancer colon (Bassani et al., 2016; Terzuoli et al., 2016). Olive fruits fresh pulp (*Olea europaea*) contains approximately a 3% (w/w) of hydrophilic (phenolic acids, phenolic alcohols, flavonoids and secoiridoids) and lipophilic (cresols and tocopherols) phenolic compounds.

In the last years, consumers consider the contents of biophenolic compounds as important quality markers (Servili et al., 2004). The concentration of biophenolic compounds and phenolic profile in olive fruits depend on the cultivar, agroclimatic conditions (rainfall and/or water stress), ripening stage, and agronomical techniques (Franco et al., 2014a; Tovar et al., 2002). Among the possible agronomical strategies to achieve a better quality, a cultivar-based tailor made irrigation treatment can improve the final olive oil quality (Machado et al., 2013). Furthermore, in super intensive olive orchard, and with the object to control the excessive vigor of olive trees, a regulated deficit irrigation should be controlled. In addition, the irrigation has influence in the productivity and quality of olive fruits (Alegre et al., 2000; Lavee et al., 2007).

The quality control of the fruit is the first step for the classification of the olives at the start of the production process, avoiding mixing of olives of different qualities (Guzmán et al., 2012). In most cases, quality control at factory reception is based on visual observation of the olives or information provided by the farmer. The implementation of online analytical control systems in the olive industries, at an initial stage of the production process, would provide for identification, quickly and accurately, the quality parameters of the raw material (intact olives) and, consequently, the final product (Salguero-Chaparro et al., 2013).

The quality control from harvesting and transformation to storage and the traceability and authentication of olive products are, nowadays, the main challenges for olives industry and control laboratories. In

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order to protect customers and producers against adulteration and false declarations, international organizations, such as European Union and International Olive Oil Council, have established rules and guidelines for the olive oil certification, including various physicochemical parameters and reference limits (Binetti et al., 2017). The improvement of product's quality continuously stimulates the search for new technologies. In addition, the olives industry has great interest on checking the quality using fast and reliable techniques. The inclusion of these data on the labels holds a potential added value, either in economic terms or in trade competitiveness (Cayuela and García, 2017). The reason is that from a consumer's perspective, there is a noticeable interest in information about food bioactive compounds content, among other substances.

Chromatography is the most popular analytical technique for olive oil individual phenolic compounds determination. However, in olive fruits this methodology is less common (Dagdelen et al., 2013; Machado et al., 2013; Pistarino et al., 2013). On the other hand, it is important to develop a rapid, cost-effective and simple sample treatment method. Among the possible alternative analytical methods, front-face fluorescence is a very useful technique because it allows handling samples practically without a pretreatment. Although front-face fluorescence has not yet been applied for the determination of biophenolic compounds in olive paste, numerous studies on the application of intrinsic fluorescence of olive oil, in combination with second order multivariate calibration techniques, such as PARAFAC and Unfolded Principal Component Analysis (UPCA), have been reported. In fact, the olive oil autofluorescence is associated to minor components, chemical species such as tocopherols, phenols and chlorophylls.

Two spectral ranges have been used for improving the explorative analysis of the excitation-emission fluorescence matrices (EEMs) of virgin and pure olive oils, by unfolded PCA and PARAFAC (Guimet et al., 2004). The first range was composed by chlorophylls, while the oxidation products and vitamin E were the predominant compounds in the second range. The application of unfolded PCA and PARAFAC to the EEMs allows distinguishing between virgin and (non-virgin) olive oils, mainly due to the contribution of the oxidation products. In fact, the differentiation between two types of oils was more efficient when the chlorophyll fluorescence region was excluded from the model. The potential of EEM fluorescence and three-way methods of analysis, to detect adulterations of pure olive oils from protected denomination of origin "Siurana", has been studied (Guimet et al., 2005). Unfolded-PCA and PARAFAC were applied for exploratory analysis. Linear discriminant analysis (LDA) and discriminant N-PLS regression, applied in training and validation sets, gave a classification rate close to 100%.

On the other hand, the quantification of chemical compounds of olive oil by means of fluorescence measures in combination with chemometric techniques has been also possible. In this content, the levels of chlorophylls *a* and *b* and pheophytins *a* and *b* of oils diluted in acetone have been determined, using a partial least square (PLS) calibration (Galeano Díaz et al., 2003). The tocopherols previously separated from olive oils were studied by means of a calibration of standards diluted in hexane using EEMs combined with PLS regression (Galeano Díaz et al., 2006).

The aim of this study was to develop a simple and fast fluorescence front-face method, in combination with PARAFAC, for the determination of different biophenolic compounds, in the paste of olives obtained from cultivars, with different irrigation treatments. The calculated concentration data were statistically compared with the results obtained by HPLC. For this statistical analysis, a 2-way factorial design was accomplished, in order to considerer the different factor effects between the methods (chemometric and chromatographic) and irrigation treatments.

2. Materials and methods

2.1. Samples

The study was carried out in a super-high-density olive orchard located in the area "Vegas Bajas del Guadiana", in a Junta de Extremadura property land, Solana de los Barros, Badajoz, Spain, (38°44′N, 6°38′W and an altitude of 263 m above sea level) and planted exclusively with Arbequina variety (*Olea europaea* L.).

The applied irrigation treatments consisted of watering with 75 (T1), 50 (T2) and 25% (T3) of the dose applied to the Control (C), respectively. Samples of olives were collected in November, with a ripeness index between 2 and 3 (spotted), using the subjective evaluation of color of the skin and flesh (Uceda and Frías, 1975). The olive sampling was carried out in the morning, and olives were randomly ripened in different sides of the olive tree, assuming a total of 10 kg per treatment in triplicate. After harvesting, the olives were immediately transported to the laboratory within 1 h, in ventilated storage at 4 °C trays, to avoid compositional changes.

2.2. Front-face fluorescence method

Fluorescence was determined using a Fluorescence Spectrophotometer (Varian Model Cary Eclipse Fluorescence Spectrophotometer, Agilent Technologies, Madrid, Spain), equipped with two Czerny-Turner monochromators (excitation and emission), a xenon lamp and two photomultiplier tubes as detector. The equipment was connected to a PC microcomputer via an IEEE 488 (GPIB) serial interface. The Cary Eclipse 1.0 software was used for data acquisitions. Measurements were carried out with a variable-angle front-face accessory, to ensure that reflected light, scattered radiation, and depolarization phenomena were minimized. The angle of incidence, defined as the angle between the excitation beam and the perpendicular to the cell surface, was set at 34°. Fluorescence measurements were recorded in a 10-mm quartz cell at 15 °C (room temperature). The slits of excitation and emission monochromators were set at 2.5 and 5 nm, respectively. EEMs were registered as a set of emission spectra with 0.5 nm of resolution. The photomultiplier tube sensitivity was 700 V and the scan rate was set at 300 nm min^{-1}. EEMs were recorded with an excitation range from 240 to 290 nm (each 5 nm), and emission from 290 to 450 nm (each 0.5 nm), according to Cabrera-Bañegil et al. (2017a). The total scanning time per sample was approximately 5 min. Measurements were performed by triplicate within a short period of time (2 days), to minimize the effects of instrumental fluctuation, mainly lamp intensity.

An EEM of a standard solution of each of the phenolic compounds present in olive paste was registered in the same front-face conditions.

2.3. Chemometric treatment

A PARAFAC model was built using the EEMs of a set of 5 olive samples grown with the highest water stress treatment (25%) (T3), 8 and 7 olive samples of 50% (T2) and 75% (T1) irrigation doses referred to control, respectively, and 10 olive samples of the Control group (the most irrigated group). The emission ranges of the data set were fixed at 302–450 nm, in order to reduce the Rayleigh signals, before the application of PARAFAC. In this way, the EEMs of 30 samples were arranged in a three-dimensional structure with a size of 30 × 296 × 11 (samples x number of emission wavelengths x number of excitation wavelengths). This array was decomposed by PARAFAC (Bro, 1997), applying the core consistency diagnostic tool for optimization of the number of components (Bro and Kiers, 2003). In all cases, non-negative constraints for the resolved profiles for all modes were applied with the purpose to obtain a realistic solution, because concentrations and spectral values are positive.