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Hyperspectral FTIR imaging of olive fruit for understanding ripening processes

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ABSTRACT

A combination of micro-FTIR hyperspectral imaging and chemometrics is proposed to better understand biochemical changes in olive fruit during the ripening process without the need for fractionation and related analytical methods. Principal component analysis was used for the study of the spatial distribution of vibrational spectroscopic signatures in flesh, hypodermis and epidermis tissues. Partial least squares discriminant analysis was then employed to highlight the main spectral changes related to ripening. Modifications in cell wall polysaccharides were identified as the major processes taking place during ripening in both the flesh and the hypodermis whereas olive oil accumulation was characteristic in the flesh of the fruit. Evidence of solubilization and depolymerization of cell wall polysaccharides as well as demethylation with the subsequent formation of carboxylate groups were found. Spectral features attributed to polyphenols and sugars also change during the ripening process. No clear temporal trends were observed in the epidermal tissues.

1. Introduction

Olive fruit provide the basis for olive oil extraction and table olives pickling production in the Mediterranean countries namely. The ripening stage of the fruit influences the quality and yields in both types of production (Borzillo et al., 2000). Thus, the texture of table olives is strongly affected by the firmness and ripening stage of the fruit. Oil quality progressively deteriorates with increased oxidative degradation and decreasing sensory quality, during fruit ripening (Borzillo et al., 2000; García et al., 1996). Firmness of the fruit also decreases and consequently the resistance to postharvest handling.

There is increasing interest for the development of methods for optimal picking date determination of olives, but most methods for evaluating maturity in olives rely almost exclusively on color changes (Lazzez et al., 2011). Recently, a machine vision system has been proposed to predict in a faster, automated and more objective manner the maturity index of fruit providing results in close agreement with a visually estimated maturity index (Guzmán et al., 2015). However, the optimal harvest time can be affected by many different aspects, including fruit retention force, fruit firmness, oil content, chemical composition, and sensory attributes. It is desirable therefore to rely on methods able to provide more information about the biochemical changes the fruit undergoes during the ripening process. In this context, Fourier transform infrared (FTIR) spectroscopy is an interesting alternative because, on the one hand, it provides information at the molecular level and, on the other hand, is rapid and does not need intensive sample preparation in contrast with analytical techniques involving the extraction, fractioning and isolation of the different compounds. The technique has proven very useful for the study of many vegetable materials, including wood (Moore and Owen, 2001), leaves (Ribeiro da Luz, 2006) and fruit cuticles (Johnson et al., 2007) as well as for the determination of antioxidants in diverse plant samples, such as grains, fruits and herbs (Cozzolino, 2015; Lu and Rasco, 2012). FTIR spectroscopy has been effectively employed to predict quality parameters of both olives and virgin olive oils (VOO) (Machado et al., 2015), to classify them according to geographical origin (Bendini et al., 2007; Tapp et al., 2003), to detect VOO adulteration (de la Mata et al., 2012; Rohman, 2017), to evaluate VOO freshness (Sinelli et al., 2007), to investigate processes of oxidation (Muik et al., 2007), as well as to discriminate between olive cultivars (Vergara-Barberán et al., 2015). With the advances in instrumentation and the coupling with microscopy, the technique has gained particular relevance in analyzing biological materials, because of the possibility of retrieving chemical and structural information at the tissue level (Salzer and Siesler, 2009). Hyperspectral imaging is now considered as an efficient combination between spectroscopy and conventional image processing to provide spatially discriminated chemical information about complex materials (Gowen et al., 2015; Lim et al., 2016). Each pixel of the acquired image corresponds to a spectrum, allowing analysis of the structure and details of samples (Amigo et al., 2015). In plant research, hyperspectral images

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in both the middle and near infrared region have gained interest for the comprehensive analysis of the spatial distribution patterns of plant constituents (Türker-Kaya and Huck, 2017). Seeds, leaves, kernels or whole fruit have been analyzed this way (André Weinstock et al., 2006; Cantarelli et al., 2009; Lorente et al., 2012; Serranti et al., 2013). For fruit, the detection of symptoms of disease caused by fungi (Folch-Fortuny et al., 2016) and insects (Rady et al., 2017), the classification of microbial agents (Teena et al., 2014), or the evaluation of fruit quality parameters (Li et al., 2018; Vetrekar et al., 2015), are interesting applications.

In this work, we propose the use of FTIR microscopy to monitor the changes during the maturation of the olive fruit in order to get a better understanding of the processes taking place and how they are reflected in the FTIR spectral characteristics of the different parts of the fruit. The use of an accessory of attenuated total reflection (ATR) to evaluate the changes at the macro level was previously tested in our laboratory (López-Sánchez et al., 2010). Oil accumulation in the fruit could be followed during the earlier stages of ripening, however, the changes in fruit texture with ripening lead to an anomalous behavior of the sample in contact with the ATR crystal and the strong interference of water hindered the observation of further spectral changes in the olive flesh. In this work, the combination of micro-FTIR hyperspectral imaging with appropriate chemometric tools were explored to investigate the main changes taking place during the ripening process of the olive fruit at the microscopic level. The identification of relevant spectroscopic signatures of such changes would facilitate the establishment of an alternative method much more precise than that based on color for optimizing harvest dates for olives.

2. Materials and methods

2.1. Fruit samples

Twelve 'Picual' olive fruits (*Olea europaea* L.) without any sort of infection or physical damage were collected every week in the period from September 2015 to January 2016. This period corresponds to 19–36 weeks after full bloom (WAFB), which usually takes place around middle May. The beginning of the sampling period corresponded to fully developed olives that are still unripe. The olives were initially green but were black at the end of the sampling period. The samples were washed with distilled water to remove dust from the surface and cryopreserved at -18 °C. Two fruits representative of each week (in terms of color) were selected for further sample preparation and analysis.

2.2. Sample preparation

Longitudinal slices showing both flesh (mesocarp) and skin (exocarp) were cut using a cryostat CM 1950 Leica equipped with an encapsulated microtome and its own integrated cooling system. Slices of less than 40 µm were cut with a metal blade at low speed (50 strokes/min), deposited on calcium fluoride (CaF_2) slides and left dry at ambient temperature.

2.3. FTIR- hyperspectral imaging acquisition

Infrared spectra in the region 4000-750 cm^{-1} were recorded in transmission mode with a FTIR 6300 spectrometer coupled to an FTIR IRT-7000 microscopy with a mercury cadmium telluride (MCT) detector from JASCO (Tokyo, Japan). The objective used was a 16X Cassegrain with 20 mm working distance. The background was acquired in a clean area of the calcium fluoride slide. Hyperspectral point scanning imaging measurement, in which a spectrum of each small pixel of the sample is acquired, has been used (Grahn and Geladi, 2007). Scan number and aperture of the measurements were optimized to 200 scans and 50 µm x 50 µm, respectively, with a good signal-to-noise ratio. In each mapping, a specific area of the sample containing both exocarp and flesh tissues was chosen. A total of 34 hyperspectral images were considered (reaching, on average, between 100–120 spectra and a time consumption of 70 min per map).

2.4. Chemometrics analysis

Before analysis, the spectra were pre-processed by area-normalization and truncated (3rd order spline truncation) to reduce the spectral range to 2000-820 cm^{-1}. Hyperspectral images were analyzed by using PCA Model Editor 2.12.00 from Spectra Manager version 2 from JASCO. PLS-DA models were built in MATLAB 7.10.0 (R2010a The MathWorks Inc., Natick, MA, USA) employing the PLS-toolbox (Eigenvector Research Inc., Wenatchee, WA). To evaluate the performance of the PLS-DA models, a cross-validation approach based on Venetian blind method with ten data splits was used. In this method, ten sub-validation experiments are performed with one-tenth of the total number of spectra as a test dataset (that is 27, 11 and 18 for the flesh, hypodermis, and exocarp PLS-DA models respectively). Sensitivity values were calculated as the number of samples predicted in a certain class divided by the real number of samples in this class. Specificity values represent the number of samples predicted as "not in the class" divided by the actual number of samples not belonging to this class.

3. Results and discussion

3.1. Infrared spectra of the olive fruit

The olive is a drupe in three parts, the endocarp (pit), the mesocarp (flesh) and the exocarp (skin). During the early stages of development, the pit that surrounds the seed of the fruit, gets a hard structure of lignified cells. Oil accumulation then occurs. Once fully developed, the flesh forms 90% of the fruit weight, made up primarily of water, oil, reducing sugars such as glucose, fructose and sucrose, phenolic compounds, proteins and pectic substances. Lipids predominate in the exocarp composition (Fernández-Escobar et al., 2001). Typical infrared spectra of olive fruit exocarp and flesh are shown in Fig. 1.

A tentative assignment of the different bands observed based on literature is presented in Table 1. The FTIR spectra of the flesh are dominated by bands attributable to lipids, namely the C—H stretching bands in the region 2900-3010 cm^{-1} and the C═O stretching band of ester bonds at 1743 cm^{-1}, typical of triglycerides (López-Sánchez et al., 2010). The strong bands at 2854 cm^{-1} (CH_2 symmetric stretch) and 2924 cm^{-1} (CH_2 antisymmetric stretch) are typical of the long aliphatic chains of fatty acids (de la Mata et al., 2012). Furthermore, the broadband centered at about 3300 cm^{-1} is due to the OH stretching of polysaccharides and phenolic compounds (Heredia-Guerrero et al., 2014). In addition, a complex pattern is observed in the fingerprint region due to the overlapping of absorption bands from both lipids and polysaccharides (Schulz and Baranska, 2007). Cellulosic, hemicellulosic and pectic polysaccharides of the cell walls contribute to the complex pattern observed in the 900-1200 cm^{-1} region (Kačuráková et al., 2000). The most distinct features of the FTIR spectrum of the exocarp in comparison with the flesh appear at 1737, 1730, 1713 and 1689 cm^{-1} and can be related to the components of the plant cuticle, the external membrane covering the epidermal cells of different parts of vegetables, as leaves, petals, and fruit (Heredia-Guerrero et al., 2014). The bands observed at 1737 and 1730 cm^{-1} correspond to the C═O stretching of ester moieties in cutin, a polyester formed by polyhydroxy fatty acids, which is the main constituent of the plant cuticle; and cutan, a more hydrophobic polymer that can partially substitute cutin in the cuticle matrix, respectively (Heredia-Guerrero et al., 2014). The shoulder observed at 1713 cm^{-1} is associated with hydrogen-bonded ester groups (Shechter and Chefetz, 2008). Bands around 1689 cm^{-1} have been assigned to carboxylic acid groups interacting by H-bonds (Johnson