Food Chemistry 174 (2015) 226–232

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Discrimination and characterisation of extra virgin olive oils from three cultivars in different maturation stages using Fourier transform infrared spectroscopy in tandem with chemometrics

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ARTICLE INFO

*Article history:*
Received 8 September 2013
Received in revised form 19 September 2014
Accepted 6 November 2014
Available online 14 November 2014

*Keywords:*
Fourier transform infrared spectroscopy
Extra virgin olive oil
Maturation stages
Cultivars
Chemometrics
Chemical parameters

ABSTRACT

A methodology based on Fourier transform infrared (FTIR) spectroscopy, combined with multivariate analysis methods, was applied in order to monitor extra virgin olive oils produced from three distinct cultivars on different maturation stages. For the first time, this kind of methodology is used for the simultaneous discrimination of the maturation stage, and different cultivars.

Principal component analysis and discriminant analysis were utilised to create a model for the discrimination of olive oil samples. Partial least squares regression was employed to design calibration models for the determination of chemical parameters. The performance of these models was based on the multiple coefficient of determination ($R^2$), the root mean square error of calibration (RMSEC) and root mean square error of cross validation (RMSECV). The prediction models for the chemical parameters resulted in a $R^2$ ranged from 0.93 to 0.99, a RMSEC ranged from 1% to 4% and a RMSECV from 2% to 5%.

It has been shown that this kind of approach allows to distinguish the different cultivars, and to clearly discern the different maturation stages, in each one of these distinct cultivars.

Furthermore, the results demonstrated that FTIR spectroscopy in tandem with chemometric techniques allows the creation of viable and accurate models, suitable for correlating the data collected by FTIR spectroscopy, with the chemical composition of the EVOOs, obtained by standard methods.



## 1. Introduction

Extra virgin olive oil (EVOO) is a vegetable oil made from healthy and intact fruits of the olive tree (*Olea europaea* L.) only by mechanical means (crushing, malaxation and centrifugation) and can be directly consumed unrefined by humans. As no chemicals are used in this extraction process, the EVOO keeps the original characteristics and constituents which are lost in refined oils (Nieto, Hodaifa, & Peña, 2010). EVOO is one of the most significant food products in Mediterranean countries, and the olive tree counts among the oldest and most important oil-producing crops after the oil palm (Baldoni & Belaj, 2009).

The high demand for olive oil is associated with the Mediterranean culture based on dietary habits correlated with health benefits (Allalout et al., 2011). This has been correlated with the presence of high content of monounsaturated fatty acids, specifically oleic acid (60–80%) and its richness in minor components, including tocopherols and phenolic compounds, that other seed oils lack (Cicerale, Conlan, Sinclair, & Keast, 2009). These phenolic compounds have a great importance in biological systems once they act as natural antioxidants (Bendini, Cerretani, Carrasco-Pancorbo, et al., 2007; Bendini, Cerretani, Di virgilio, et al., 2007). Furthermore, they are also responsible for the stability and flavour

*Abbreviations:* FTIR, Fourier transform infrared spectroscopy; ATR, Attenuated Total Reflectance; PCA, principal component analysis; DA, discriminant analysis; PLS-R, partial least squares regression; PCR, Principal Component Regression; RI, Ripening Index; GAE, gallic acid; ABTS, 2,2-azino-bis(3-ethylbenzothiazoline)-6 sulphonic acid; $R^2$, multiple coefficient of determination; RMSEC, root mean square error of calibration; RMSECV, root mean square error of cross validation.

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http://dx.doi.org/10.1016/j.foodchem.2014.11.037
0308-8146/

of olive oil, and are associated to "pharmacological" properties, since these compounds have demonstrated some positive effects on certain physiological parameters (Bouskou, Tsimmidou, & Blekas, 2006). However, the quality of olive oil is influenced by a great number of factors and its phenolic composition and concentration depends on two of the most important ones, namely the nature of the cultivar and geographic origin (Bakhouche et al., 2013), and of the fruit ripening degree (Machado, Felizardo, Fernandes-Silva, Nunes, & Barros, 2013), where important chemical changes occur. There are other factors that affect the phenolic composition of olive oils such as irrigation regimes (Machado et al., 2013), oil extraction technology and the storage of the oil (Dabbou et al., 2011) and agricultural techniques used to cultivate olive fruit (Ayton, Mailer, Haigh, Tronson, & Conlan, 2007).

In this work, we studied olive oils from some cultivars that are cultivated in the main Portugal region for olive oil production (Alentejo) (INE, 2012). 'Cobrançosa' is a characteristic cultivar of Trás-os-Montes region. However, due to its interesting characteristics, it has been spread to other regions, namely in Alentejo. Galega, as Cobrançosa cultivar, is one of the most important cultivar used in Portugal, but there is little information available related to the study of its chemical composition. The other cultivar studied is Picual which is one of the most important cultivar grown in Andalusia, Spain, and characterised by having one of the highest content of phenolic compounds (Nieto et al., 2010).

The determination of total phenolic compounds, including *ortho*-diphenols and flavonoids, and antioxidant activity by colourimetric methods involves a pre-treatment of samples and consequently the destruction of the sample. Furthermore, these analyses are time consuming and require large amounts of reagents and solvents, which are quite expensive, and often toxic. To overcome these hurdles, spectroscopic methods have been used, such as Fourier-transform infrared spectroscopy (FTIR), which is an analytical technique, rapid, direct and simple to perform. It is non-destructive and does not require any sample preparation, particularly when used in conjunction with Attenuated Total Reflectance (ATR).

For these reasons, the application of FTIR in the study of olive oils has increased recently, mainly to evaluate the composition of fatty acids (Inarejos-Carcía, Gómez-Alonso, Fregapane, & Salvador, 2013), oxidised fatty acids (Lerma-García, Simó-Alfonso, Bendini, & Cerretani, 2011), peroxide value (Bendini, Cerretani, Carrasco-Pancorbo, et al., 2007; Bendini, Cerretani, Di virgilio, et al., 2007), acidity (Lerma-García et al., 2011), adulterations (Rohman & Che Man, 2012), sensory characteristics, phenolic and volatile compounds (Lerma-García et al., 2011), freshness (Sinelli, Cosio, Gigliotti, & Casiraghi, 2007) and authenticity (Lerma-Garcia, Ramis-Ramos, Herrero-Martinez, & Simo-Alfonso, 2010). Other authors described the use of FTIR–ATR for the simultaneous quantification of fatty acid composition, peroxide value and free acidity (Maggio et al., 2009). Despite some studies used FTIR–ATR–PLS tool to determine some analytical parameters (water content, phenolic content and antioxidant activity) in olive oils (Cerretani et al., 2010), the discrimination of varietal origin of olive oil and different maturation stages of the specific cultivars 'Cobrançosa', 'Galega' and 'Picual' growing in Alentejo region with resort to this technique, has not been undertaken yet.

The aim of this study was to use FTIR–ATR spectroscopy associated with chemometrics in order to differentiate EVOO's produced with olives from three cultivars on three different maturation stages. Discrimination was achieved using either an unsupervised method, principal component analysis (PCA), and a supervised method, factor discriminant analysis (FDA).

Furthermore, quantitative models to predict the chemical characteristics of EVOO's based on FTIR spectra measurements were developed using partial least square regression method (PLS-R) based on the Non-linear Iterative Partial Least Squares algorithm (NIPALS) algorithms.

## 2. Material and methods

### 2.1. Chemicals

Folin–Ciocalteu's reagent, 3,4,5-trihydroxybenzoic acid (gallic acid) and acetic acid, both extra pure (>99%) were purchased from Panreac (Panreac Química S.L.U., Barcelona, Spain). Sodium nitrate, aluminium chloride and sodium carbonate, all extra pure (>99%), were purchased from Merck (Merck Darmstadt, Germany). 2, 2-azino-bis(3-ethylbenzothiazoline)-6 sulphonic acid (ABTS), Trolox, and catechin, all of extra pure grade (>99%), were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium molybdate (99.5%) was purchased from Chem-Lab (Chem-Lab N.V., Zedelgem, Belgium). All other reagents used were of analytical grade. The water used in the experiments was deionised, obtained from a SGS water purification system.

### 2.2. Sampling

The present work was carried on monovarietal extra virgin olive oils from three cultivars (cv. 'Cobrançosa', 'Galega' and 'Picual'). The olive fruits were obtained from a certified olive orchard, at the National Plant Breeding Station, at Elvas (Portugal) during the crop season 2012/2013.

Only healthy olive fruits, without any kind of infection or physical damage, were collected from ten different trees of comparable age and vigour and located in distinct points of the same growing area. Thus differences in climate conditions, agricultural practices and geographical were excluded. Olives were handpicked at three ripening stages, except Picual cultivar olives that were picked during two harvesting periods. The harvesting dates are presented in Table 1. For the classification of the maturity index, the olives were evaluated according to their skin and pulp colour (Uceda & Hermoso, 1998). The ripeness index (RI) values range from 0 (100% intense green skin) to 7 (100% purple flesh and black skin).

After harvesting, the olive fruits were immediately transported to the laboratory mill where they were processed within 24 h. For the production of each olive oil, three kilos of fresh olive fruits were used using an Abencor system (INIA I.P., Elvas, Portugal) where olives were crushed with a hammer crusher and the past mixed at 25 °C for 30 min, centrifuged without addition of warm water and then filtered and transferred into dark glass bottles without headspace and stored in the dark at 4 °C until analysis. All samples were classified as extra virgin olive oils according to the EU regulations (EEC n.°2568/1991, 1991).

### 2.3. Extraction of the phenolic fraction

Three distinct aliquots were collected from each olive oil. For the extraction of polar phenolic compounds of each one of these

**Table 1**
Description of olive fruit at each sampling date.

| Cultivar | Maturity stage | Sample code | Harvest date | RI[a] |
|---|---|---|---|---|
| Cobrançosa | Green | Cob G | 02/10/2012 | 0.4 |
| | Semi-ripe | Cob SR | 12/10/2012 | 2.1 |
| | Ripe | Cob R | 08/11/2012 | 5.5 |
| Galega | Green | Gal G | 02/10/2012 | 0.4 |
| | Semi-ripe | Gal SR | 12/10/2012 | 2.1 |
| | Ripe | Gal R | 08/11/2012 | 5.5 |
| Picual | Green | Pic G | 02/10/2012 | 0.4 |
| | Ripe | Pic R | 08/11/2012 | 5.5 |

[a] Ripening Index.