LWT - Food Science and Technology 88 (2018) 103–108

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

# Nondestructive measurement of squalene in olive oil by near infrared spectroscopy

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ARTICLE INFO

*Article history:*
Received 18 May 2017
Received in revised form
25 September 2017
Accepted 30 September 2017
Available online 4 October 2017

*Keywords:*
Classification
Environment friendly
NIR
PLS model
Threshold

ABSTRACT

This study sets the basis for developing a rapid technique for measuring olive oil squalene, which is a healthy compound. This technique, based on near infrared spectroscopy, is environmentally friendly. The most suitable wavelength ranges were defined, studying the possible contribution from the visible spectra. For this purpose, Partial Least Squares analysis was independently set up using two optical arrangements, with wavelengths 350–2500 nm and 1100–2300 nm. Models from only near infrared wavelengths gave the best outcomes. The external validation exercise for estimating olive oil squalene was satisfactory, with $r^2$ 0.83 and residual predictive deviation 2.31. The results suggest the proposed technique is useful for estimating olive oil squalene content. A sorting test of olive oil in two classes according to its squalene content was carried out, with threshold in 5.0 g.kg$^{-1}$, using the model built. The success of this classification was 90%.



## 1. Introduction

Squalene (Fig. 1) is a triterpene aliphatic hydrocarbon, and it was named because of its profusion in shark liver oil, its richest source, where it reaches 900 g kg$^{-1}$. Shark liver oil has long been used as a traditional health food in Japan, with a particular benefit for vascular health (Hamadate et al., 2015). Squalene is widely distributed in nature, especially in vegetable oils such as olive oil, palm oil, wheat-germ oil, amaranth oil, or rice bran oil (Huang, Lin, & Fang, 2009). Therefore, by using different extraction methods, vegetables or marine animals can be suitable squalene sources (Vazquez, Torres, Fornari, Senorans, & Reglero, 2007).

The main part of virgin olive oil is the saponifiable fraction, a lipid matrix of triglycerides, diglycerides and monoglycerides accounting for 985–995 g kg$^{-1}$ (Civantos, 1999). Squalene is in relatively high quantities within the olive oil minor fraction. Eisner, Iverson, Mozingo, and Firestone (1965) stated that squalene makes up around 85–90% of the hydrocarbon fraction of olive oils. Besides, it makes up 60–75% of the olive oil unsaponifiable fraction, in concentrations between 0.2 and 7.5 g kg$^{-1}$ (Tiscornia & Evangelisti, 1982).

One of the most important differences between olive oil and plant seed oils is squalene. Compared to seed oils, olive oil is an important source of squalene. In other edible vegetable oils, squalene makes up only 0.02–0.3 g kg$^{-1}$ (Rao, Newmark, & Reddy, 1998). Thus, olive oil contains 7–300 fold more squalene than other vegetable oils and up to 5000 fold more than some vegetable foods (Liu, Ahrens, Schreibman, & Crouse, 1976). Therefore, virgin olive oil may be a part of the human diet especially rich in squalene.

Besides, the squalene content varies widely depending on the olive oil product with a range of 2–7 g kg$^{-1}$ (Rao et al., 1998). A significant difference between the extra virgin class (EVOO) and the virgin class (VOO) has been reported, with the latter having more squalene than the refined olive oils (Owen et al., 2000). Nergiz and Çelikkale (2011) showed that refining reduces the squalene content. Furthermore, they pointed out that the major decrease in squalene in vegetable oils within the refining steps occurs during oils' deodorization. Olive growing techniques (Psomiadou & Tsimidou, 1999), olive fruit variety (Nergiz & Ünal, 1990) and extraction (Nergiz & Ünal, 1990; Samaniego-Sánchez, Quesada-Granados, López-García de la Serrana, & López-Martínez, 2010) influence the level of squalene. Squalene acts as a weak antioxidant in olive oil (Owen et al., 2000). Thus, Psomiadou and Tsimidou (1999) proposed that squalene contributes to olive oil stability in a small quantity, even at low temperatures.

There is multiple scientific evidence on the beneficial effects

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https://doi.org/10.1016/j.lwt.2017.09.047
0023-6438/

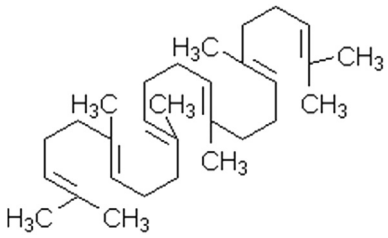


**Fig. 1.** Squalene structure.

that the intake of squalene from food has on health (Newmark, 1997; Lasekan, Clayton, Gendron, & Ney, 1990; Smith, 2000; Ostlund, Racette, & Stenson, 2002; Strandberg, Tilvis, & Miettinen, 1990; Smith, 2000). However, this feature of olive oil has received little attention in the market so far, since most consumers are unaware on this fact.

The olive oil industry has great interest on determining the quality of olive oil, using fast and reliable techniques. Besides, developing non-destructive techniques to reduce the expense of solvents and reagents is increasingly important in an international context of convergence towards environmental sustainability. Among the various non-destructive solutions to these needs, near-infrared spectroscopy (NIRS) has made major achievements. NIRS is based on multivariate models in which the spectral data correlate with the analyzed feature. It provides several important advantages, as NIRS needs no solvents or reagents, thus avoiding a major expense, while being environmentally friendly. Additionally, NIRS is a rapid, non-destructive, and potentially multi-parameter method.

Several articles on the use of NIRS and chemometrics for the analysis of different olive oil features have been published in the recent years (Nenadis & Tsimidou, 2017). Stand out studies directed to characterizing intact olives and olive paste for optimizing the milling process (Giovenzana et al., 2017), to control the quality of olive pomace oil blended with palm oil used for deep-frying (Ben Hammouda, Zribi, Ben Mansour, Matthaus, & Bouaziz, 2017), as well as for the authentication and detection of fraud (Karunathilaka, Kia, Srigley, Chung, & Mossoba, 2016). Sorting olive oil based on alpha-tocopherol and total tocopherol content using NIRS has been recently reported (Cayuela & García, 2017). The NIRS ability to analyze the major olive oil quality features has been the subject of several studies (Armenta, Garrigues, & De la Guardia, 2007; Bendini et al., 2007; Cayuela, Moreda, & García, 2013; Conte et al., 2003; Costa et al., 2008). In fact, NIRS techniques are methods for these routine analyses in a growing number of laboratories. However, the possibility of measuring squalene in olive oil by NIRS has never been reported up to date. Besides, squalene NIR absorption bands have not yet been described, to the best of our knowledge.

Since the concentration of squalene varies widely among different olive oils, there is an interest in the development of rapid techniques to distinguish olive oils according to its content. In fact, the industry might have an interest in separating olive oils according to different squalene contents. The traditional method for the analysis of squalene in olive oil is GC. However, it is not usually performed in the olive oil industry, since squalene is not considered in the regulation to characterize the quality or purity of olive oil (European Commission, 1991). Therefore, there is a challenge on characterizing olive oil regarding squalene. This work sets up the basis for developing new rapid NIRS techniques for measuring olive oil squalene content. It was convenient to clarify if there are any regions from the olive oil's visible spectrum contributing to model performance, since pure squalene is a pale yellow liquid. The wavelengths that contribute to predictive models have been defined.

## 2. Material and methods

### 2.1. Olive oils

A total set of 180 olive oil samples was made up from different origins. High quality Extra Virgin Olive Oils (EVOO) were bought at olive oil specialized shops; this group contributed with 32 samples, of which 27 were varietal and the remaining 5 were mixtures from different varieties. These EVOO were used to elaborate 17 additional coupage samples. Olive oils normally found in the market were also used; this group was composed of 10 EVOOs, 40 Current Olive Oils and 25 Pomace Olive Oils. Olive oil samples were provided also from a collaborator industry, contributing with 14 EVOOs, 25 Virgin Olive Oils and 14 Lampante Olive Oils. The characteristics of the olive oil samples are shown in Table S1.

### 2.2. Reference analysis

Squalene analysis were carried out by Gas Chromatography (GC) according to Lanzón, Albi, Cert, and Gracián (1994), modified according to Moreda, Pérez-Camino, and Cert (2004), at the Instituto de la Grasa (CSIC). Briefly, 0.1 mg of olive oil sample was disposed in a 4 mL screw vial, adding 1 mL of squalane 5 mg $mL^{-1}$ as the internal pattern. This was dissolved in heptane to complete a volume of 3 mL and shaked gently by hand. Then 200 μL of methanolic 2 mol $L^{-1}$ KOH was added, separating the aqueous and lipid phases. The upper phase was collected into a 2 mL chromatography vial and then injected into the GC instrument. A GC HP-5890 (Hewlet Packard Enterprise, Palo Alto, USA) equipped with a split/splitless injection system was used with a SP-5 capillary column 5% phenylmethylsilicone fused silica, 30 m long, 0.25 mm internal diameter and 0.25 μm phase thickness, (Merck, Darmstadt, Germany). Flame ionization detector (FID) and software Chem Station for the recording and processing of data were used. The analyses were conducted with two replicates. The results were given with one significant digit.

### 2.3. Near infra-red spectroscopy

Optical arrangements NIRS and VIS/NIRS were used for defining the wavelengths contributing to the predictive models, especially for clarifying the contribution from visible spectra. Besides, using two different instruments allowed checking their results, beyond their comparison.

The samples' spectra were recorded directly from olive oils without any other treatment. The temperature of a body has an important influence on NIR radiation. Therefore, the samples were taken from 4 °C storage and placed at room temperature in the laboratory 18 h before processing. A thermostatic bath fixed at 33 °C for 30 min held the 20 mL sample containers to ensure temperature stability. The averaged spectrum from two measurements of 50 spectra each was registered, with each sample. The same procedure was used with both optical configurations.

For NIRS, the measuring mode was post dispersive transflectance. A Luminar (Brimrose Inc., Maryland, USA) spectrometer was used. This instrument consists of an acousto-optic tunable filter (AOTF) with InGaAs detector (1100–2300 nm). The reference is automatically taken, the scanning speed is 60 ms. The spectrometer is composed of a hand-held unit, equipped with a base for laboratory use. A transflectance probe accessory was used. The probe is in stainless steel, with threaded interchangeable optical path. The spectra were registered as a whole, the spectral variables