Journal of Food Engineering 146 (2015) 1–7

Contents lists available at ScienceDirect

Journal of Food Engineering

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

An opto-electronic system for in-situ determination of peroxide value and total phenol content in olive oil

Marco Grossi [a,*], Giuseppe Di Lecce [b], Marco Arru [a], Tullia Gallina Toschi [c], Bruno Riccò [a]

[a] Department of Electrical Energy and Information Engineering "Guglielmo Marconi" (DEI), University of Bologna, Bologna, Italy
[b] Department of Food Chemistry, Eurofins Chemical Control, Cuneo, Italy
[c] Department of Agricultural and Food Sciences – DISTAL, University of Bologna, Cesena, Italy

ARTICLE INFO

Article history:
Received 15 May 2014
Received in revised form 21 July 2014
Accepted 21 August 2014
Available online 1 September 2014

Keywords:
Olive oil
Peroxide value
Total phenol content
Sensor
Optical density

ABSTRACT

The quality of olive oil is essentially determined by the product free acidity and peroxide value, while the total phenol content is also important for a high antioxidant capacity. Generally, these parameters are measured with laboratory analysis, that are expensive and may require a few days. Thus, a cheap and easy technique usable by untrained personnel, "on-site" and producing results "in real time" during production is desirable, particularly as far as small olive oil mills and packaging centers are concerned. This paper describes a technique to determine peroxide value and total phenol content in olive oil, that is based on the measurement of optical density of an emulsion between a suitable chemical reagent and a small quantity of the oil of interest. The optical density is measured by illuminating the sample with a LED with peak wavelength of 569 nm for peroxide value and 835 nm for total phenol content. The experimental results show good correlation (R^2 = 0.883 and 0.895 for peroxide value and total phenol content, respectively) between data measured with the standard methodology and the technique of this work, implemented also in the form of a portable embedded system.

1. Introduction

Olive oil is a vegetable lipid obtained by extraction process from olives (the fruits of *Olea europaea* L., family Oleaceae) highly appreciated for its beneficial effects on human health, mainly due to a high content of oleic acid and phenolic compounds (Tulipani et al., 2012). Clinical studies provide evidence that regular olive oil consumption reduces the risk of coronary heart diseases (Keys et al., 1986), oxidative damage to DNA and RNA (Machowetz et al., 2007) and Alzheimer disease (Abuznait et al., 2013; Monti et al., 2011).

Olive oil quality is related to its chemical composition, oxidative stability and sensory characteristics. Quality parameters, such as free acidity, peroxide value, UV extinction coefficients, fruity attribute, other sensory characteristics and defects, are strongly dependent on olives' ripeness (Rotondi et al., 2004) and processing technology in the olive mills (Boselli et al., 2009). In addition, the peroxide value, defined as milliequivalent of active oxygen per kilogram of oil (meq O_2/kg oil) and qualifying the oil primary oxidation, is also related to storage conditions (oxygen, light exposure and temperature) after production. Another important quality parameter is the amount of phenolic compounds that contribute to the oil sensory taste producing a distinctive bitter and a pungent perception (Gutierrez-Rosales et al., 2003). Phenolic compounds found in olive oil are principally secoiridoids (oleuropein and ligstroside isomers) and their derivatives, such as tyrosol and hydroxytyrosol, that exhibit a strong antioxidant activity: they act as free radicals traps protecting from heart disease and displaying anticancer activity (Notarnicola et al., 2011; Zanoni, 2014). Phenolic compounds are also largely responsible for the shelf-life of the oil (Lerma-Garcia et al., 2009).

The European Commission regulation No. 2568/91 and subsequent amendments define manual titration methods to measure acidity and peroxide value in olive oil (EEC 2568, 1991), to be carried out in a laboratory environment by trained personnel. Instead, no official determination is currently established for the total phenol content, usually determined using spectrophotometry or high performance liquid chromatography (HPLC), techniques requiring expensive instrumentation, a laboratory environment (IOC/T.20/Doc No 29, 2009; Tasioula-Margari and Okogeri, 2001) as well as preventive extraction of the polyphenols.

From the production point of view, the need to ship oil samples to a laboratory for analysis leads to high costs and long delays. Therefore, simple and fast techniques useable for on-site quality control are desirable, in particular for small oil mills and packaging

* Corresponding author. Tel.: +39 0512093082; fax: +39 0512093785.
E-mail address: marco.grossi8@unibo.it (M. Grossi).

http://dx.doi.org/10.1016/j.jfoodeng.2014.08.015
0260-8774/

centers. For this reason, innovative solutions have been proposed, such as: Near-InfraRed (NIR) spectroscopy (Armenta et al., 2007; Ozdemir and Ozturk, 2007) to estimate acidity and peroxide value; Time Domain Reflectometry (TDR) to determine water content (Ragni et al., 2012) and detect adulteration (Cataldo et al., 2012) in extra virgin olive oil; Rapid Fourier Transformed Infrared (FTIR) spectroscopy (Cerretani et al., 2010) and voltammetric sensors (Rodriguez-Mendez et al., 2008) to estimate total phenol content. However, all these techniques require expensive instrumentation and/or need frequent calibration for olives of different varieties, country of origin and harvest season.

As viable alternatives, amperometric and pH-metric techniques have been proposed to measure peroxide value (Kardash-Strochkova et al., 2001; Adhoum and Monser, 2008) and total phenol content (Capannesi et al., 2000), but these methods are still at research stage and have been validated only on small amounts of samples in laboratory environment. Moreover, some techniques use toxic compounds (such as chloroform) to increase oil solubility in reagents, unsuitable for use in normal working environment.

Recently, we have proposed a novel technique based on Electrical Impedance Spectroscopy to measure olive oil acidity that is fast (response time in about 30 s) and can be easily implemented in the form of a low-cost portable embedded system (Grossi et al., 2013).

To complete this work, we here present a simple and effective technique to measure peroxide value and total phenol content in olive oil that, as will be shown, is fast, accurate and can be implemented in the form of a low-cost embedded electronic system.

2. Materials and methods

2.1. Technique

The technique used in this work is based on the creation of an aqueous emulsion between the oil sample and a chemical reagent. The optical density (OD) of such an emulsion is determined by illuminating the sample with a LED and measuring the transmitted light through the sample with a photodiode. A large set of experimental results show a good correlation between the measured OD and the quality parameters determined by reference methods. The proposed technique is suitable to be implemented in the form of a portable instrument suitable for quick in-situ quality control, as will be discussed in Section 3.3.

2.2. Experimental set-up

In order to validate the technique used in this work, measurements on olive oil samples have been initially carried out using an ad-hoc experimental set-up of bench-top instruments.

The sensor, depicted in Fig. 1(a), consists of a cylindrical chamber (designed using Solid Edge by Siemens Systems and fabricated with a MakerBot Replicator 3D printer) devoted to host the 25 mL polystyrene vial containing the emulsion between a suitable aqueous reagent (discussed in Section 2.3) and the oil sample. The chamber features two diametrically opposed structures hosting a LED, used as light source and a photodiode to detect the light transmitted through the sample. In the case of peroxide value, the LED has a peak emission at 569 nm wavelength (biased with a 30 mA current), while the photodiode is a BPW21R by Vishai (with wavelength peak sensitivity at 565 nm). In the case of total phenol content, instead, the LED has a peak emission at 835 nm (biased with a 80 mA current) and the photodiode is a OSD5-5T device by Centronic, with wavelength peak sensitivity between 700 and 900 nm. As discussed in Section 3, both the LED peak wavelengths have been chosen by means of preliminary measurements on phenolic and peroxide compounds using a SmartSpec 3000 spectrophotometer.

The experimental set-up is presented in Fig. 1(b). A DC power supply Agilent E3631A is used to provide the LED operating current (I_{LED}) and the power supply for the operational amplifier. The photodiode current (I_{photo}), related to the detected light intensity, is converted into a voltage (V_{out}) by a current-to-voltage converter. The voltage V_{out} is acquired by a NI USB-6211 Data Acquisition (DAQ) board by National Instruments and transmitted to a PC for further analysis. All the software for DAQ control, analysis, data presentation and filing has been realized with LabVIEW (National Instruments). Statistical analysis on the experimental data has been carried out with Microsoft EXCEL.

2.3. Chemicals and media

Phenolic reference standards (oleuropein, tyrosol, hydroxytyrosol, *p*-coumaric acid) and peroxide compounds (hydrogen peroxide, H_2O_2, and tert-butyl hydroperoxide, tBuOOH) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The reagent for peroxide value determination was prepared diluting 8 mL of ferrous ion oxidation xylenol orange (FOX) reagent (an aqueous solution of ferrous ammonium sulfate, sorbitol, sulfuric acid and xylenol orange, Sigma–Aldrich) (Cheeseman, 2006) in 7 mL of distilled water. The reagent detects the peroxides concentration by oxidation of ferrous ions Fe^{2+} to Fe^{3+} according to the following reaction:

$$Fe^{2+} + ROOH \rightarrow Fe^{3+} + RO + OH^{-}$$

Fe^{3+} ions formed in the reaction are then detected using the dye xylenol orange which binds Fe^{3+} forming a complex that strongly absorbs in the wavelength range 540–580 nm.

For the total phenol content, instead, the reagent was prepared mixing: 13 mL of distilled water, 1 mL of Folin–Ciocalteu reagent (a mixture of phosphomolybdate acid $H_3PMo_{12}O_{40}$ and phosphotungstate $H_3PW_{12}O_{40}$) and 1 mL of sodium carbonate (Na_2CO_3) 15% (i.e. 15 g di sodium carbonate in 100 mL of distilled water). As a consequence of the reaction with the phenolic compounds, the acids are reduced to tungsten and molybdenum oxides (W_8O_{23} and Mo_8O_{23}) featuring a typical blue color.

In both cases, the reagent was then mixed with 0.5 mL of the oil sample, all stirred for 30 s to create the emulsion, then the vial is placed in the sensor for the measure.

All the chemicals used in the experiments are of analytical grade. The olive oil samples used in the experiments were purchased by local markets as well as olive oil mills.

2.4. Reference methods

Olive oil peroxide value has been determined by European standard reference method with starch as indicator and sodium thiosulphate ($Na_2S_2O_3$) as titrant, while total phenol content has been determined according to spectrophotometric method proposed by Singleton and Rossi (1965). Phenolic fraction has been extracted using about 4 g of virgin olive oil (VOO) with 5 mL of methanol:water (60:40). The extraction procedure has been repeated two time and the hydroalcoholic fractions have been combined and evaporated by rotavapor up to complete dryness. The concentrated extract has been dissolved in 5 mL of aqueous methanol (50%), and filtered through Minisart RC15 (0.2 μm) regenerated cellulose syringe filters (Sartorius AG, Göttingen, D). Total phenol content has been determined using the Folin–Ciocalteu reagent (Sigma–Aldrich, St. Louis, MO, USA) and measuring at 750 nm with a Shimadzu spectrophotometer UV–VIS 1204 (Kyoto, Japan). The results have been expressed as mg of gallic acid per kg of VOO (gallic acid calibration curve $R^2 = 0.993$).