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

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10

#### 11 Abstract

12 The quality of olive oil is essentially determined by the product free acidity and peroxide value, 13 while the total phenol content is also important for a high antioxidant capacity. Generally, these 14 parameters are measured with laboratory analysis, that are expensive and may require a few days. 15 Thus, a cheap and easy technique usable by untrained personnel, "on-site" and producing results "in 16 real time" during production is desirable, particularly as far as small olive oil mills and packaging 17 centers are concerned. This paper describes a technique to determine peroxide value and total 18 phenol content in olive oil, that is based on the measurement of optical density of an emulsion 19 between a suitable chemical reagent and a small quantity of the oil of interest. The optical density is 20 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 ( $R^2 =$ 21 22 0.883 and 0.895 for peroxide value and total phenol content, respectively) between data measured 23 with the standard methodology and the technique of this work, implemented also in the form of a 24 portable embedded system.

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26 *Keywords*: olive oil, peroxide value, total phenol content, sensor, optical density.

28 **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 35 36 characteristics. Quality parameters, such as free acidity, peroxide value, UV extinction coefficients, 37 fruity attribute, other sensory characteristics and defects, are strongly dependent on olives' ripeness 38 (Rotondi et al., 2004) and processing technology in the olive mills (Boselli et al., 2009). In addition, 39 the peroxide value, defined as milliequivalent of active oxygen per kilogram of oil (meq O<sub>2</sub>/kg oil) 40 and qualifying the oil primary oxidation, is also related to storage conditions (oxygen, light 41 exposure and temperature) after production. Another important quality parameter is the amount of 42 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 43 44 principally secoiridoids (oleuropein and ligstroside isomers) and their derivatives, such as tyrosol 45 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, 46 47 2014). Phenolic compounds are also largely responsible for the shelf-life of the oil (Lerma-Garcia et 48 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.

56 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 57 58 are desirable, in particular for small oil mills and packaging centers. For this reason, innovative 59 solutions have been proposed, such as: Near-InfraRed (NIR) spectroscopy (Armenta et al., 2007; 60 Ozdemir and Ozturk, 2007) to estimate acidity and peroxide value; Time Domain Reflectometry 61 (TDR) to determine water content (Ragni et al., 2012) and detect adulteration (Cataldo et al., 2012) 62 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. 63 64 However, all these techniques require expensive instrumentation and/or need frequent calibration 65 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 seconds) 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.

#### 79 **2. Materials and methods**

#### 80 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 sub-Section 3.3.

#### 88 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.

91 The sensor, depicted in Fig. 1 (a), consists of a cylindrical chamber (designed using Solid Edge by 92 Siemens Systems and fabricated with a MakerBot Replicator 3D printer) devoted to host the 25ml 93 polystyrene vial containing the emulsion between a suitable aqueous reagent (discussed in section 94 2.2) and the oil sample. The chamber features two diametrically opposed structures hosting a LED, 95 used as light source and a photodiode to detect the light transmitted through the sample. In the case 96 of peroxide value, the LED has a peak emission at 569 nm wavelength (biased with a 30 mA 97 current), while the photodiode is a BPW21R by Vishai (with wavelength peak sensitivity at 565 98 nm). In the case of total phenol content, instead, the LED has a peak emission at 835nm (biased 99 with a 80 mA current) and the photodiode is a OSD5-5T device by Centronic, with wavelength 100 peak sensitivity between 700 and 900 nm. As discussed in Section3, both the LED peak 101 wavelengths have been chosen by means of preliminary measurements on phenolic and peroxide 102 compounds using a SmartSpec 3000 spectrophotometer.

103 The experimental set-up is presented in Fig 1 (b). A DC power supply Agilent E3631A is used to 104 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.

#### 111 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 sulphate, sorbitol, sulphuric acid and xylenol orange, Sigma-Aldrich) (Cheeseman, 2006) in 7mL of distilled water. The reagent detects the peroxides concentration by oxidation of ferrous ions Fe<sup>2+</sup> to Fe<sup>3+</sup> according to the following reaction:

119 
$$Fe^{2+} + ROOH \rightarrow Fe^{3+} + RO + OH$$

Fe<sup>3+</sup> 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<sub>2</sub>CO<sub>3</sub>) 15% (i.e. 15g di sodium carbonate in 100mL of distilled water). As a consequence of the reaction with the phenolic compounds, the acids are reduced to tungsten and molybdenum oxides (W<sub>8</sub>O<sub>23</sub> and Mo<sub>8</sub>O<sub>23</sub>) featuring a typical blue colour.

In both cases, the reagent was then mixed with 0.5 mL of the oil sample, all stirred for 30 seconds 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 theexperiments were purchased by local markets as well as olive oil mills.

#### 132 2.4 Reference methods

133 Olive oil peroxide value has been determined by European standard reference method with starch as 134 indicator and sodium thiosulphate  $(Na_2S_2O_3)$  as titrant, while total phenol content has been 135 determined according to spectrophotometric method proposed by Singleton and Rossi (Singleton and Rossi, 1965). Phenolic fraction has been extracted using about 4 g of virgin olive oil (VOO) 136 137 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. 138 139 The concentrated extract has been dissolved in 5 mL of aqueous methanol (50%), and filtered 140 through Minisart RC15 (0.2 µm) regenerated cellulose syringe filters (Sartorius AG, Göttingen, D). 141 Total phenol content has been determined using the Folin-Ciocalteau reagent (Sigma-Aldrich, St. 142 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 143 calibration curve  $R^2 = 0.993$ ). 144

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#### 146 **3. Results and discussion**

The reagents response was initially characterized with peroxide and phenolic compounds using a SmartSpec 3000 spectrophotometer. Then a set of 25 olive oil samples have been analyzed with the technique and the bench-top set-up described in Section 2.1. Finally an electronic board has been designed and fabricated to avoid the use of all bench-top instrumentation, thus demonstrating the feasibility of a simple and economical instrument for easy, fast and in-situ analysis of olive oil.

#### 152 *3.1. Spectral characterization of reagents response*

153 At first, the reagents were inoculated with known concentrations of peroxide and phenolic

154 compounds and the absorption spectra acquired with a SmartSpec 3000 spectrophotometer.

155 In the case of phenols, four different compounds were tested: three of these are typically present at 156 high concentration (oleuropein, hydroxytyrosol and tyrosol) while the forth one (*p*-coumaric acid) is present in small concentration. Fig. 2 (a) shows the absorption spectra in the case of different 157 158 concentrations of oleuropein in the 400 - 800 nm wavelength range. As can be seen the different concentrations of phenolic compound can be discriminated for wavelength > 500 nm with the 159 160 sensitivity increasing with the wavelength. In Fig. 2 (b) the absorbance measured at the wavelength 161 of 800 nm is plotted vs. the phenol content for all the tested compounds. Except for tyrosol for 162 which a higher sensitivity for concentrations below than 15 ppm is registred, in all cases a linear 163 relation between absorbance and concentration is found. The linear regression lines of the four 164 cases are calculated to estimate the compound sensitivity (Abs800/ppm) to the reagent with the 165 following results: 0.0199 (oleuropein), 0.1159 (hydroxytyrosol), 0.0248 (p-coumaric acid), while in 166 the case of tyrosol the sensitivity is 0.0755 for concentrations lower than 15 ppm and 0.019843 for 167 concentrations higher than 15 ppm. As depicted in Fig 2 (b), the intensity of blue color obtained by 168 reduction of tungsten and molybdenum oxides is proportional to the concentration of each phenol but shows a different response respect to the phenol structure which reacted. In fact Folin-Ciocalteu 169 170 detects the total reducing capacity that is different for different compounds (Apak et al., 2007). As 171 described in several paper, during the storage of VOO the trend of phenolic fraction, measured by 172 Folin-Ciocalteu assay showed a fluctuation due to the oxidation and lysis of phenolic compounds 173 disperse in the matrix. This result confirms the different response factor that the reaction mixture 174 presents towards individual phenols (Boselli et al., 2009; Fiori et al., 2014).

In the case of peroxides, two different compounds were tested: hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and tertbutyl hydroperoxide (tBuOOH). The absorbance spectra in the wavelength range 400 - 800 nm are shown in Fig. 3 (a) for different tBuOOH concentrations. As expected, the reaction of  $Fe^{2+}$  ions with peroxide compounds results in the formation of  $Fe^{3+}$  ions that bind to xylenol orange producing an absorbance peak between 560 and 580 nm. The measured absorbance at 580 nm is plotted vs. compound concentration for both H<sub>2</sub>O<sub>2</sub> and tBuOOH in Fig. 3 (b). From the measured data, the absorbance can be empirically modelled as a linear function of the square root of the compound concentration. The results indicate that  $H_2O_2$  (y = 0.297 $\sqrt{x}$  + 0.436) is characterized by higher sensitivity than tBuOOH (y = 0.115 $\sqrt{x}$  + 0.265). In both cases a determination coefficient R<sup>2</sup> higher than 0.96 is achieved. The different response of FOX reagent to  $H_2O_2$  and tBuOOH can be due to the ability of sorbitol to scavenge hydroxyl radicals to yield peroxyl radicals which would propagate Fe<sup>2+</sup> oxidation (Jiang et al., 1990).

#### 187 *3.2. Experimental results*

188 A set of 25 olive oil samples have been tested using the technique and the experimental set-up 189 described in Section 2. The voltage  $V_{out}$  is acquired at time intervals of 5 seconds for a total of 1200 190 seconds for both tests (total phenol content and peroxide value).

In the case of total phenol content, the logarithm of Vout has been found to be linearly related with 191 the phenols concentration. The coefficient of determination  $R^2$  has been calculated after different 192 time intervals and plotted as function of time in Fig. 4 (a): the correlation between  $Log_{10}(V_{out}/V_M)$ 193 (where V<sub>M</sub> is the measured voltage in the absence of oil sample, i.e. due to the reagent only) and the 194 195 total phenol content increases with time, reaching a plateu after 600 seconds. Fig. 4 (b) shows the 196 measured values of  $Log_{10}(V_{out}/V_M)$  after 600 seconds plotted vs total phenol content as determined using the reference method. The linear regression line equation is  $Y = 0.0343 - 2.389 \cdot 10^{-3} \cdot X$  and the 197 198 coefficient of determination 0.895. The accuracy is slightly higher to what reported by Cerretani (Cerretani et al., 2010) when a  $R^2$  of 0.87 was obtained by FTIR attenuated total reflectance 199 spectroscopy using a wavelength range 3610 - 816 cm<sup>-1</sup> and a PLS chemometric analysis. The use 200 201 of an array of voltammetric sensors (Rodriguez-Mendez et al., 2008) reported an higher accuracy  $(R^2 = 0.986)$ , but the samples tested were only 6 olive oils and all above the 400 ppm; instead, in the 202 203 present work, 25 samples were analysed and the level of detection achieved was of 100 ppm.

In the case of peroxide value, a linear relation between the measured values of  $V_{out}/V_M$  and the peroxide value has been found. As shown in Fig. 4 (c), the coefficient  $R^2$  increases with time reaching a plateau after about 600 seconds . Fig. 4 (d) shows the measured values of  $V_{out}/V_M$  plotted

vs. the peroxide value as determined by the reference method. The linear regression line equation is 207  $Y = 0.6368 - 13.8 \cdot 10^{-3} \cdot X$  and the coefficient of determination 0.883. The dispersion between 208 209 measured data and the reference technique can be mainly associated with the property of the FOX 210 reagent that is able to determine all kinds of conjugated dienes including those without a peroxide 211 group, and not able to react with peroxides included in molecules without conjugated double bonds. 212 Furthermore the accuracy of the quantification can be also diminished by the presence of several 213 other compounds present in olive oil, such as carotenoids and chlorophyll, absorbing at 500-600 214 nm, and chain-breaking antioxidant able to reduce the color yield (Bou et al 2008). The obtained 215 accuracy is however higher than that reported in (Armenta et al., 2007) where a coefficient of determination  $R^2$  of 0.6558 resulted by NIR spectroscopy combined with PLS chemometric 216 217 analysis.

In both cases there is a linear relation between the parameter measured with the experimental set-up ( $Log_{10}(V_{out}/V_M)$ ) for total phenol content and  $V_{out}/V_M$  for peroxide value) and the oil quality parameter measured with the reference methods. Thus, using the equation of the linear regression line, the oil quality parameters can be estimated.

#### 222 *3.3. Implementation as a portable embedded system*

To demonstrate that the technique proposed in this work can lead to a portable embedded system suitable for in-situ measurements, all the operations performed by the bench-top instrumentation and DAQ PC board have been implemented inside an electronic board designed "ad hoc", based on the µcontroller Dspic33ep512mu810, whose schematic is presented in Fig. 5 (a).

The LED is supplied with a square wave current (frequency 1 kHz) to allow removing the contribution of the environment radiation. In particular, a square wave voltage ranging from 0 to 830 mV is generated by the  $\mu$ controller and fed to the noninverting input of an operational amplifier. In turn, this latter drives a BJT transistor providing the LED with the supply current I<sub>LED</sub> (selectable with the value of the resistance R<sub>LED</sub>). The radiation transmitted through the sample is received by the photodiode that generates the current I<sub>photo</sub> proportional to the incident radiation. An 233 I/V converter generates a voltage V<sub>A</sub> proportional to I<sub>photo</sub>. A couple of programmable switches 234 allows to select two different feedback resistors (R<sub>F1</sub> or R<sub>F2</sub>). The voltage V<sub>A</sub> is then fed to a AC 235 coupling stage that generates a square wave voltage (V<sub>B</sub>) with mean value 2.5V. Such a voltage is 236 then fed to a synchronous rectifier (built with an AD8271 difference amplifier and an ADG733 triple single pole double throw SPDT switch) that provides a DC voltage (V<sub>C</sub>) equal to the high 237 level of V<sub>B</sub>. Then, a 18 bit ADC driver ADA4941 generates a differential voltage V<sub>OUT</sub> 238 (proportional to V<sub>C</sub>) that is fed to a 12 bit ADC (AD9220AR) providing the 12 bit digital 239 240 codification of  $V_{OUT}$  (D<sub>OUT</sub>) to be processed by the µcontroller. The measured data are sent to a 241 portable PC (via USB interface) to display the results and file the data. The system has been built by 242 using low-cost electronics and its total cost has been estimated in about 300 \$. All the software has 243 been developed using LabVIEW. Fig. 5 (b) and (c) present pictures of the sensor, while Fig. 5 (d) is 244 a photograph of the electronic board.

A significant subset of the olive oil samples have been tested using both the bench-top instrument set-up described in Section 2.1 and the embedded system presented above to compare the results.

Fig. 6 (a) shows a plot of  $\text{Log}_{10}(D_{\text{OUT}}/4096)$  vs. time (acquisitions every 5 seconds) for 3 samples featuring different values of total phenol content (the division by 4096 represents a normalization necessary for comparison with  $V_{\text{out}}/V_{\text{M}}$  of sub-Section 3.1). As can be seen, higher total phenol content results in lower values of measured  $D_{\text{OUT}}$ . Measurements on a set of 10 olive oil samples result in a linear correlation between  $\text{Log}_{10}(D_{\text{OUT}}/4096)$  and total phenol content ( $Y = -4.978 \cdot 10^{-4} \cdot X$  $-4.7 \cdot 10^{-3}$ ).

In Fig. 6 (b)  $D_{OUT}/4096$  is plotted vs. time for 3 samples featuring different values of peroxide value and, as can be seen, more oxidized samples exhibit lower values of  $D_{OUT}$ . Measurements on a set of 7 olive oil samples result in a linear correlation between  $D_{OUT}/4096$  and the peroxide value (Y = 8.532 $\cdot 10^{-3} \cdot X + 0.893$ ).

The linear regression equations have been used to compare the estimated values of both peroxide value and total phenol content with those obtained with the reference methods and the calculated determination coefficient  $R^2$  differs by less than 1.6% between the two measuring systems. In particular, in the case of peroxide value,  $R^2 = 0.901$  and 0.887 for the bench-top set-up and the embedded system, respectively; while with total phenol content  $R^2 = 0.932$  and 0.922 for the benchtop set-up and the embedded system, respectively. The agreement between the two measuring systems is very good.

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### 265 **4. Conclusions**

A novel technique to measure peroxide value and total phenol content in olive oil has been presented that is based on optical density measurements of a suitable reagent inoculated with the olive oil of interest. The technique, suitable to be realized in the form of a low-cost, embedded electronic system, has been tested using an experimental set-up built with bench-top instrumentation and the results show that it can estimate with good accuracy the peroxide value ( $\mathbb{R}^2$ = 0.883) and the total phenol content ( $\mathbb{R}^2$  = 0.895) of olive oil in less than 10 minutes.

The proposed technique has been implemented in an electronic board to realize a portable embedded system; the results obtained with such an instrument have been compared with those coming from the bench-top set-up and an excellent agreement has been found.

The portable embedded system could be applied for fast and *in-situ* olive oil quality control and to assess the shelf-life without the need of trained personnel. Furthermore, it could be useful for a first evaluation of the total phenol content of virgin olive oils in case of interest of a productor to report on the lable the oilive oil claim, according with the EU Regulation 432/2012, stating that "Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress", but admitted only if at least 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) are contained in 20 g of olive oil.

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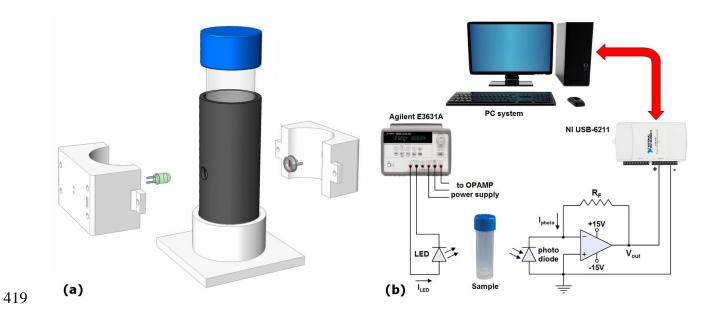
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- 421 Fig. 1. The sensor used for the experiments of the present work (a) and the experimental set-up built
- 422 with bench-top instrumentation used for the measurements (b).

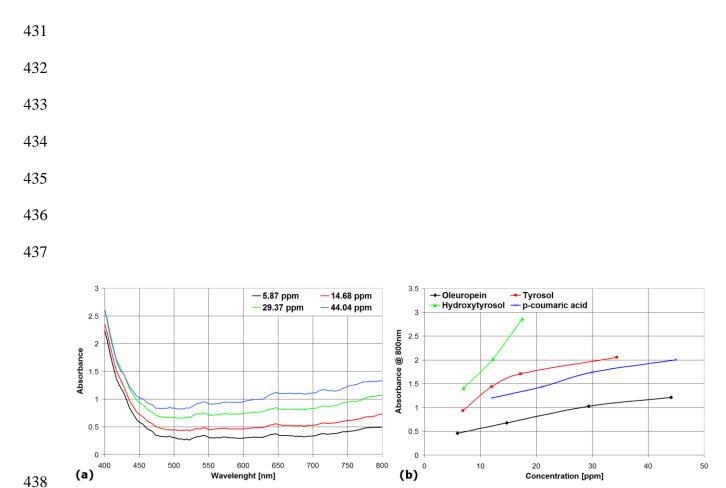
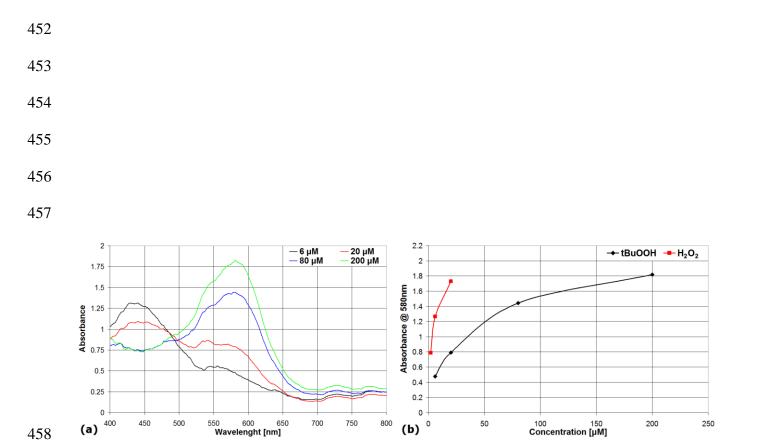


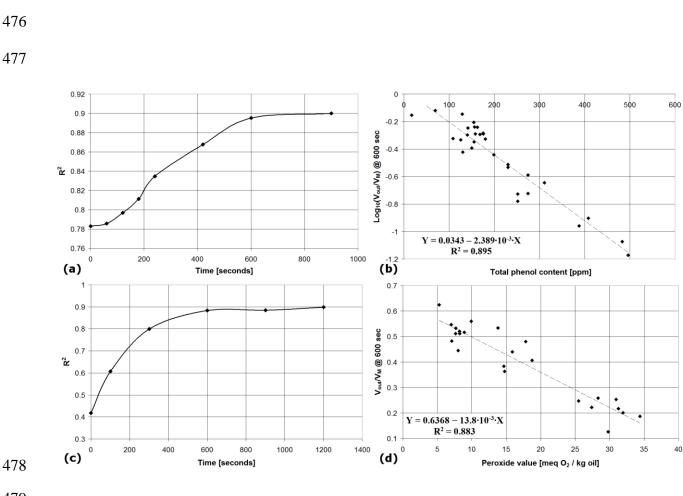


Fig. 2. Measured absorbance vs. wavelength in the range 400 - 800 nm for different concentrations of oleuropein (a) and measured absorbance at 800 nm plotted vs. phenol content for different phenolic compounds (b).

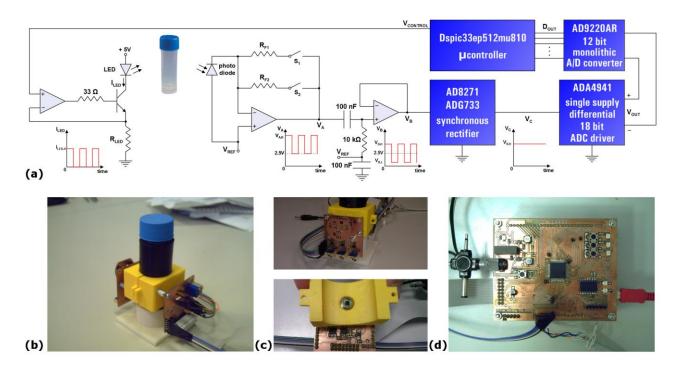




460 Fig. 3. Absorbance plotted vs. wavelength in the range 400 – 800 nm for different concentrations of
461 tert-butyl hydroperoxide (a) and measured absorbance at 580 nm plotted vs. concentration for tert462 butyl hydroperoxide as well as vs. hydrogen peroxide (b).



480 Fig. 4. Coefficient of determination R<sup>2</sup> plotted vs. time for the total phenol content (a) and peroxide
481 value (c). Scatter plot and linear regression line calculated for 600 seconds for total phenol content
482 (b) and peroxide value (d).

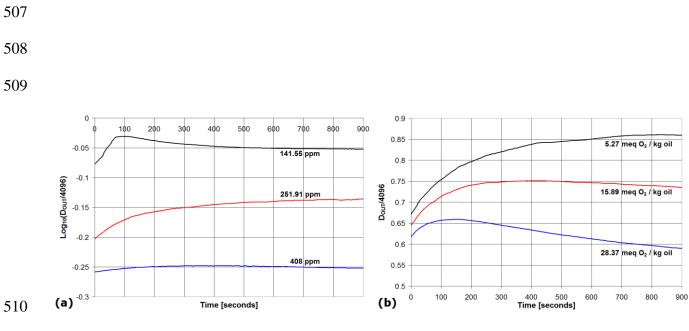






**Fig. 5**. Schematic of the electronic board of the embedded system realized in the present work (a);

495 pictures of the sensor (b) and (c); photograph of the electronic board (d).



512 Fig. 6. Data measured with the designed electronic board for different oil samples vs. time in the513 case of total phenol content (a) and peroxide value (b).