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Design and in-house validation of a portable system for the determination of free acidity in virgin olive oil

1 **Design and in-house validation of a portable system for the determination**

2 **of free acidity in virgin olive oil**

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4 Marco Grossi[a], Rosa Palagano[b,*], Alessandra Bendini[b], Bruno Riccò[a],

5 Maurizio Servili[c], Diego Luis García-González[d], Tullia Gallina Toschi[b]

6

7 [a] Department of Electrical Energy and Information Engineering "Guglielmo Marconi" (DEI),

8 University of Bologna, Bologna, 40136, Italy

9 [b] Department of Agricultural and Food Sciences (DISTAL), University of Bologna, Cesena, 47521,

10 Italy

11 [c] Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia,

12 06121, Italy

13 [d] Instituto de la Grasa (CSIC), Sevilla, 41013, Spain

14

15 [*] Corresponding author: rosa.palagano@unibo.it (Rosa Palagano), Department of Agricultural and

16 Food Sciences (DISTAL) - University of Bologna, Piazza Goidanich 60, Cesena (FC), 47521, Italy,

17 Tel +39 0547 338121.

ABSTRACT

Nutritional and healthy values are well known properties of virgin olive oil (VOO). The product quality, in terms of belonging to a specific quality grade (extra virgin, virgin, lampante), is defined by a set of chemical-physical and sensory measurements. According to the official regulation of the European Union (EU Reg. 1348/2013) the free acidity is the first parameter that has to be determined by analysts; it gives information about the quality of the olives used to produce the VOO as well as the hydrolytic state of VOO just produced and stored. The official procedure is based on an acid-base titration that needs to be carried out in a chemical laboratory.

In this paper a portable battery-operated electronic system to measure olive oil free acidity is presented: the system can be used for quick "in situ" tests in a production environment (olive oil mills or packaging centers) by people without particular training. The working principles of the system is based on the creation of an emulsion between oil and a hydroalcoholic solution: the free acidity is estimated on the value of the emulsion electrical conductance.

This new system has been calibrated and in-house validated showing good results in terms of limit of detection and quantification, precision and accuracy, beyond a good correlation with free acidity data obtained applying the official method ($R^2_{adj} = 0.97$).

Abbreviations

EIS: electrical impedance spectroscopy, **EVOO:** extra virgin olive oil, **FA:** free acidity, **LOD:** limit of detection, **LOO:** lampante olive oil, **LOQ:** limit of quantification, **RSD:** relative standard deviation, **TAGs:** triglycerides, **VOO:** virgin olive oil.

1. Introduction

Virgin Olive Oil (VOO) is obtained from olives (the fruits of *Olea europaea L.*) applying only a mechanical-physical extraction process and represents a product highly appreciated for its beneficial effects on human health, mainly due to a high content of oleic acid and minor components such as phytosterols, carotenoids, tocopherols and hydrophilic phenols (Bendini et al., 2007). The European Commission Implementing Regulation 1348/2013 (EU Reg. No 1348/2013) defines a decision tree for verifying whether a VOO is consistent with the category declared, and the quality criteria that have to be checked by analysts are: free acidity (FA), peroxide value, specific extinctions in UV, sensory characteristics and ethyl esters of fatty acids. The first quality parameter in the above cited decision tree is, therefore, the determination of the free acidity of the oil sample; this is defined as the amount of free fatty acids, no longer linked to their parent triglyceride molecules (TAGs), and measured as percentage of oleic acid. Specifically, the top-quality product, Extra Virgin Olive Oil (EVOO), features a maximum FA of 0.8 g oleic acid/100 g oil, then the VOO features a maximum FA value of 2.0 g oleic acid/100 g oil and, finally, the Lampante Olive Oil (LOO), that is not suitable for the commercialization as it is, is characterized by a FA higher than 2.0 g oleic acid/100 g oil.

This parameter is especially affected by the quality of the olives used to produce the oil since free fatty acids arise from the separation of fatty acids from TAGs because of the action of enzymes, further stimulated by light, water, and heat. Thus, FA is an indicator of how fresh and how well handled the olives were before being milled (Tena, Wang, Aparicio-Ruiz, García-Gonzaléz, & Aparicio, 2015). To confirm this, different studies reported that geographic and environmental factors (Bustan et al., 2014) and the application of specific technological processes (such as filtration or a cooling treatment of olive paste) (Veneziani et al., 2018 a-b) do not affect significantly this parameter.

The official procedure to measure the oil free acidity is defined by the European regulations (EEC Reg. 2568/1991 and following amendments) and consists of an acid-base titration that, albeit simple and quick, must be carried out in a laboratory by trained personnel essentially due to the need of specific solvents and lab equipment.

However, in addition to official methods for the quality control of virgin olive oils, there is a strong need for simple, rapid and environmental friendly techniques, also suitable for on-site quality control even for new clients, who are "non-professional analytical skilled" end users (Inajeros-García, Gómez-Alonso, Fregapane, & Salvador, 2013; Valli et al., 2016). This represents an important issue in particular for the small oil mills and packaging centers that cannot afford the cost of external laboratory analysis.

The possibility of simple, quick and in-situ analysis for the food quality control (often implemented in the form of portable electronic systems) has been widely researched in the last years (Oujji et al., 2014; Grossi, Di Lecce, Arru, Gallina Toschi, & Riccò, 2015; Sture, Ruud Oye, Skavhaug, & Mathiassen, 2016; Arsalane et al., 2018). Concerning the olive oil free acidity determination, different innovative techniques have been presented in literature (Valli et al., 2016). One approach is based on Near-Infrared (NIR) spectroscopy, allowing to estimate acidity by optical spectroscopy analysis of the transmission spectra in the wavenumber range 4541 to 11726 cm^{-1} (Armenta, Garrigues, & de la Guardia, 2007). This solution has the advantage that the measurement can be carried out on the oil sample without any reagent, but it needs expensive instrumentation (optical spectrophotometer) and requires frequent re-calibration (different calibrations must be carried out depending on olives varieties or geographical origin etc.). A pH-metric procedure to measure oil acidity has been also proposed (Tur'yan, Berezin, Kuselman, & Shenhar, 1996): the oil sample is extracted with a chemical solvent (0.20 M triethanolamine in a 1:1 solution of water and isopropanol) and the pH measured. This technique is reported to be accurate, but the pH probe needs to be used with care and frequent cleaning and re-calibration are needed. Moreover, the used chemicals add costs for disposal after use.

The aim of this work is to present a portable electronic system to measure the free acidity in olive oil samples: the instrument is battery-operated and can be used for quick "in-situ" measurements in the oil production or bottling site. The working principle is based on the estimation of the oil FA from the electrical conductance measured by Electrical Impedance Spectroscopy (EIS) of the oil emulsion with a hydroalcoholic solution. EIS is used in a wide range of applications (Grossi, & Riccò, 2017a), such as to estimate the ripening degree of fruits (Harker, & Maindonald, 1994), to characterize plant tissues (Lin, Chen, & Chen, 2012; Ben Hamed, Zorrig, & Hichem Hamzaoui, 2016), to characterize and to detect the freezing end point of ice cream mixes (Grossi, Lanzoni, Lazzarini, & Riccò, 2012a), to estimate the water content in extra virgin olive oil (Ragni et al., 2013), to investigate the corrosion of metal surfaces exposed to acid electrolytes (Ansari, Quraishi, & Singh, 2015; Hussin, Rahim, Nasir, Ibrahim, & Brosse, 2016), to analyse human body composition (Khalil, Mohktar, & Ibrahim, 2014; Bera, 2014), to detect the end-point in a titration assay (Grossi, & Riccò, 2017b).

The system described in this paper represents a considerable improvement over previous studies (Grossi, Di Lecce, Gallina Toschi, & Riccò, 2014a-b) since it has been completely re-designed with focus on low-cost electronics and small dimensions. To make the system completely portable and reliable for on-site measurements, a temperature sensor has been included to compensate for conductance variations linked to changes in environmental temperature. Moreover, the presented

instrument has been in-house validated evaluating its performances in terms of correlation between the concentration of the analyte and the instrument response, limit of detection (LOD) and quantification (LOQ), precision and accuracy. The validation step, in fact, is one of the measures universally recognized as a necessary part of a comprehensive system of quality assurance in analytical chemistry and is an essential component of the measures that a laboratory should implement to allow it to produce reliable analytical data (Thompson, Ellison, & Wood, 2002).

2. Material and methods

2.1 Reagents and chemicals

Diethyl ether (ACS reagent, purity $\geq$99.8%), ethanol (ACS reagent, purity $\geq$96%) and phenolphtalein solution (indicator, 1% in ethanol) were supplied from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Sodium hydroxide 0.1 mol/L (N/10) was purchased from Carlo Erba Reagents S.r.l. (Milan, Italy).

Distilled water was produced by Elix Essential system (Millipore, Molsheim, France). Oleic acid (CAS Number 112-80-1, assay 90%) used as standard for building the calibration curve was supplied from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

2.2 Samples

In order to calibrate the system, 5 samples of refined sunflower oil (with free acidity and peroxide value under the limits for vegetables oils according to CODEX STAN 210-1999) added with oleic acid were prepared. To cover the range of free acidity of the different quality grades (EVOO, VOO, LOO) different concentrations of oleic acid (from 0% to 3.75%) were added (Table 1).

The in-house validation study was carried out on a set of 30 olive oil (numerically coded from 1 to 30) with a different quality grade: 20 EVOOs, 7 VOOs and 3 LOOs. An aliquot of 50 mL for each sample was stored in PET dark bottles at 11-12 °C before the analysis.

2.3 Determination of free acidity

2.3.1 Official method

The determination of FA by titration was applied according to the EU Reg. 2016/1227. An aliquot of the oil sample (the weight depends on the presumed free acidity) was dissolved in 100 mL of a mixture of diethyl ether and ethanol (1:1 v/v, previously neutralized) and the free fatty acids present were neutralized using sodium hydroxide solution (0.1 M). Phenolphthalein was used as indicator.

2.3.2 Portable system

The working principle of the portable system (Figure 1) is based on the measure of the electrical conductance of an emulsion between a hydroalcoholic solution and the oil sample. In presence of the hydroalcoholic solution, the free fatty acid molecules RCOOH, where R is the hydrocarbon

chain, dissociate in the ionic compounds H_3O^+ and RCO_2^- that contribute to the increase of the emulsion electrical conductance. Consequently, the higher the free fatty acid molecules concentration, the higher ions concentration and the higher the electrical conductance.

A 50 mL round bottom polypropylene tube (Falcon) modified to feature a couple of cap-shaped stainless-steel electrodes (6 mm in diameter, spaced by 12 mm one from the other) required for the electrical characterization is used as sensor to realize the measurement.

The first step to realize the measurement is a "reagent test" to check the electrical properties (electrical conductance) of the hydro-alcoholic solution in order to avoid an overestimation of the oil free acidity linked to the conductance of the solution. For this purpose, the tube (sensor) is filled with 9 mL of ethanol and 6 mL of distilled water and its conductance is measured. If the reagent passes the test and is suitable for the measure (conductance not higher than 0.6 μS), 1 mL of the oil sample to be tested is added and the obtained mixture is manually shaken vigorously for about 20 seconds to create an emulsion, whose conductance is measured to estimate the oil acidity. A video showing the procedure to realize the analysis is available in the online version of the paper.

The instrument is also equipped with a temperature sensor to measure the room environmental temperature during the analysis. The emulsion electrical conductance and the temperature are measured and these values are used to calculate the emulsion conductance at the calibration temperature (23.5 °C). Then the free acidity of the sample is estimated using the calibration function stored inside the microcontroller non-volatile memory.

2.4 In-house method validation

The following parameters were evaluated in order to ensure the method quality: limit of detection (LOD) and quantification (LOQ), intra-day and inter-day precision and accuracy.

Before the application of the portable system on olive oil samples and in order to verify the correlation between the concentration of the analyte and the response of the instrument, a calibration using samples of refined sunflower oil added with oleic acid previously described (see paragraph 2.2) was carried out. All the samples were analyzed in triplicate by both the official method to determine the reference value of FA, and by the portable system to measure the conductance value. Then, the values were plotted in order to study the correlation between the electrical conductance and the free acidity of the samples.

The limit of detection (LOD) and quantification (LOQ) were evaluated considering the linear relation between the conductance measured by the portable system and the square root of FA. The following formulas were applied:

$$LOD = 3\sigma/m \tag{1}$$

$$LOQ = 10\sigma/m \tag{2}$$

178 where σ is the standard deviation of the FA square root measured for the refined sunflower oil

179 without addition of oleic acid (sample A) and *m* is the slope of the curve.

180 To evaluate the inter-day precision of the method, two virgin olive oil samples for each type of

181 quality grade (samples EVOO_2 and EVOO_19 for EVOOs, samples VOO_1 and VOO_7 for

182 VOOs and samples LOO_1 and LOO_3 for LOOs) were analyzed in triplicate for three different

183 days and values were statistically evaluated by the Student's Test *(p<0.05)*.

184 Accuracy and intra-day precision were studied analyzing the set of 30 olive oils with a different

185 quality grade. Precision were expressed in terms of RSD (Relative Standard Deviation) measured

186 for the free acidity values obtained with the portable system. For the accuracy, all the samples were

187 also analyzed following the official method (acid-base titration) and the comparison between the

188 values obtained by the two methods was performed (two-tailed paired t-test with *p <0.05*).

189

190 **3. Results and discussion**

191 **3.1 Design of the portable system**

192 A portable battery-operated electronic system for in-situ measurements of olive oil free acidity has

193 been built to allow olive oil quality assurance directly in the oil mills or packaging centers.

194 The dimensions of the instrument are 11 x 15 x 5 cm and some pictures are presented in Figure 2a.

195 The instrument can be powered by USB port or using batteries (3 AAA alkaline batteries 1.5 V).

196 The system is composed of an electronic board designed ad-hoc that performs all the operations to

197 measure the free acidity, a 2 rows 16 columns LCD screen to output the measure results, four

198 buttons for user interaction and the sensor previously described (see paragraph 2.3.2). The

199 electronic board is based on the microcontroller STM32L152RCT6A and its schematic is presented

200 in Figure 2b.

201 To determine the FA of an oil sample the electrical conductance of the emulsion is measured by

202 Electrical Impedance Spectroscopy (EIS). In the proposed approach the sample under investigation

203 is stimulated with a 1 V 200 Hz sine-wave voltage signal $V_{in}(t)$ generated by the built-in 12-bits

204 DAC inside the microcontroller and applied to the sensor electrodes.

205 The current drawn by the sensor is converted to a sine-wave voltage $V_{out}(t)$ by means of a current-

206 to-voltage converter. Given

207 $V_{in}(t) = V_{M,in} \times sen(2\pi ft)$ (3)

208 it is

209 $V_{out}(t) = V_{M,out} \times sen(2\pi ft + \varphi)$ (4)

where $V_{M,in}$ and $V_{M,out}$ are the amplitude of the corresponding signals, φ is the phase difference between the current through the sample and $V_{in}(t)$, while f is the frequency of the test signal (200 Hz).

Both $V_{in}(t)$ and $V_{out}(t)$ are acquired by the built-in 12-bits ADC inside the microcontroller using a sampling frequency of 50 kHz and the sine-wave parameters are calculated using the algorithm previously presented by Grossi et al. (Grossi, Lanzoni, Lazzarini, & Riccò, 2012b).

The emulsion in direct contact with the electrodes can be modelled as the parallel of an electrical conductance (accounting for the conductance of the emulsion) and a capacitance (accounting for the emulsion dielectric properties): while the emulsion conductance (that dominates at low frequency) is affected by the sample acidity due to the variation of the ions concentration, the dielectric properties are almost independent.

The electrical conductance (G_m) is thus calculated as:

$$G_m = \frac{1}{R_F} \times \frac{V_{M,out}}{V_{M,in}} \times cos(\varphi) \tag{5}$$

where R_F is the feedback resistance (470 kΩ) of the current-to-voltage converter.

However, the relation between G_m and the free acidity is non-linear and it can be modelled with the function:

$$G_m = \alpha + \beta \times \sqrt{FA} \tag{6}$$

where α, β are empirical parameters that must be determined by a suitable calibration procedure and are also function of the calibration temperature.

Then, the sample free acidity can be estimated from the measured electrical conductance of the emulsion with the following formula:

$$FA = ((G_m - \alpha) / \beta)^2 \tag{7}$$

Since the system must be operated "in the field" and the environmental temperature is not a parameter under control, the environmental temperature (T) is measured by means of a MCP9700A analog temperature sensor integrated on the electronic board: the output voltage (that has a sensitivity of 10 mV/°C) is acquired by a channel of the microcontroller ADC and converted to the temperature value.

The system works as follows: the emulsion electrical conductance registered at a certain temperature ($G_{m,T}$) and the temperature (T) are measured and these values are used to calculate the emulsion conductance at the calibration temperature ($G_{m,Tcalib}$). Then the free acidity of the oil sample is estimated from the calculated value of $G_{m,Tcalib}$ using the calibration function stored inside the microcontroller non-volatile memory.

242 A more detailed description of the system by an electronical and mathematical point of view is
243 presented in Appendix A, while a discussion on the influence of the environmental temperature on
244 the measured conductance and the method used for compensation is presented in Appendix B.

245 **3.2 In-house method validation**

246 Before being used with real olive oil samples, the portable instrument has been calibrated. For this
247 purpose, all the samples of refined sunflower oil added with oleic acid (samples A-E) were analyzed
248 by both the official method to determine the reference value of free acidity and by the portable
249 system to measure the conductance value. The data obtained are shown in Table 1. Then the values
250 were plotted (Figure 3): in all cases the electrical conductance measured for the samples increases
251 with its free acidity with a non-linear relation, confirming what previously presented (Grossi, Di
252 Lecce, Gallina Toschi, & Riccò, 2014b). Starting from these data and applying a nonlinear
253 regression, the equation of the calibration curve was defined as follow:

254
$$FA = \left(\frac{G_{m,23.5°C} + 0.0678}{2.7877} \right)^2 \qquad (8)$$

255 where $G_{m,23.5°C}$ is the emulsion electrical conductance at the calibration temperature of 23.5 °C.

256 It allows to obtain, directly on the display of the portable system, the free acidity value of the tested
257 sample estimated starting from its electrical properties.

258 Subsequently, some parameters for the in-house validation of the instrument were evaluated in
259 order to check its performances and applicability on real olive oil samples.

260 LOD and LOQ were measured considering the FA of the refined sunflower oil and the slope of the
261 linear correlation between the free acidity square root and the conductance. The values obtained
262 were 0.02% and 0.06% of oleic acid for LOD and LOQ respectively.

263 Next, a set of 30 olive oil samples with a different quality grade (20 Extra Virgin Olive Oils –
264 EVOO; 7 Virgin Olive Oils – VOO; 3 Lampante Olive Oils – LOO) were analyzed in triplicate
265 applying this new analytical approach in order to study its accuracy and intra-day precision
266 (expressed in terms of RSD).

267 The portable instrument showed good precision results since all the obtained values were under the
268 15%. All the samples were also analyzed following the official method (acid-base titration) and the
269 comparison between the values obtained by the two methods was performed to measure the
270 accuracy of the system. The differences between the two series of results (official method *vs*
271 portable system) were evaluated by using the two-tailed paired t-test (*p<0.05*). The two approaches
272 did not give statistically differences for the mean values (t<t (critical value)). Moreover, the
273 regression between the two series of values provided a coefficient R^2_{adj} of 0.97 (Figure 4) in
274 agreement with data previously presented by Grossi et al. (Grossi, Di Lecce, Gallina Toschi, &

Riccò, 2014). Considering the commercial categories of the samples analyzed, all of them, with the only exception of the sample VOO_7, were classified in the same way by both approaches. Finally, considering the inter-day precision of the instrument, no significative differences (Student's Test, *p<0.05*) were found among the results obtained for each analyzed sample in the three different days.

4. Conclusions

The design and in-house validation of a portable battery-operated electronic system suitable for in-situ measurements of olive oil free acidity has been presented. The system is built with low cost electronics and embeds a temperature sensor to compensate variations of the measured electrical parameters with the environmental temperature, thus making it suitable for on-site free acidity measurements outside a laboratory. Its working principle is based on the estimation of the olive oil FA from the measure of the conductance of an emulsion between a hydro-alcoholic solution and the sample to be tested. When the free fatty acids present in the sample comes in contact with the hydro-alcoholic solution, a dissociation occurs, leading to the formation of ions that produce an increase of the measured electrical conductance.

The system has been calibrated and in-house validated. The data obtained showed good performances of the instrument in terms of LOD and LOQ, intra-day and inter-day precision. Moreover, it showed a good correlation ($R^2_{adj} = 0.97$) with the FA evaluated applying the official method demonstrating a satisfactory accuracy.

The procedure to realize the analysis is very quick and easy. This make the system suitable also for people without specific training. The application of this analytical device is addressed, in particular, to the estimation of free acidity of just produced virgin olive oils in oil mills. A next study will be focused on the evaluation of the performance of this portable system when applied to bottles stored for several months in dark/light conditions simulating possible different commercial conservation/exposure on shelves of groceries. This study will give indications regarding the maximum oxidation state of olive oil sample tolerable to use this portable system as accurate and reliable.

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Declaration of interest

None.

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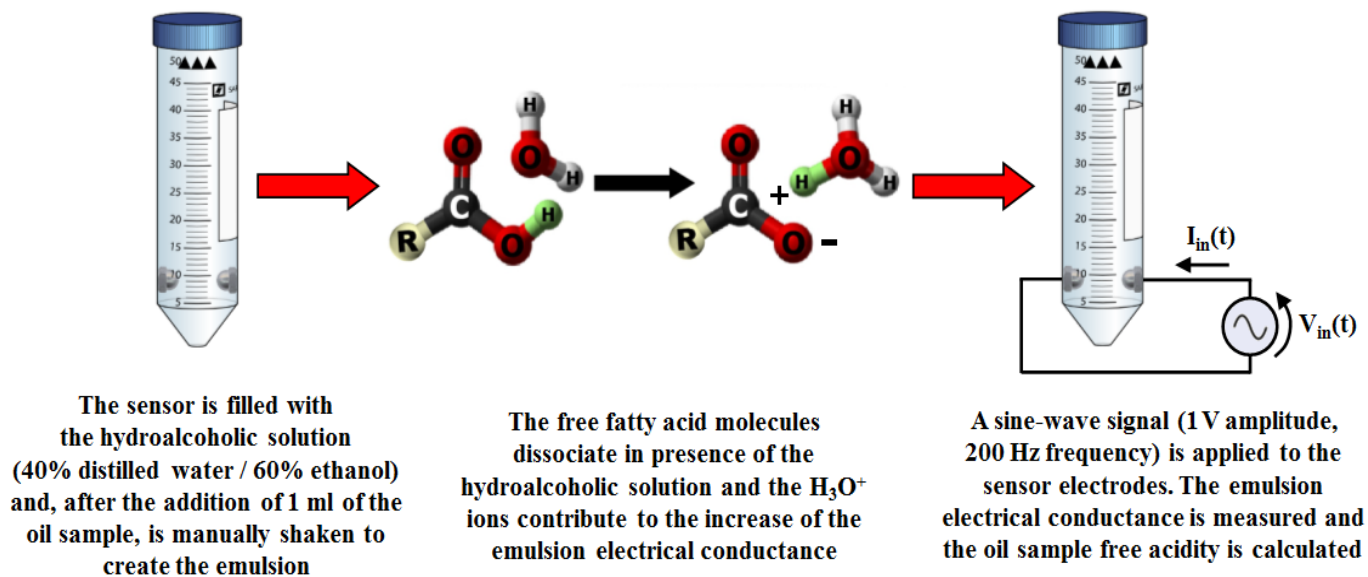
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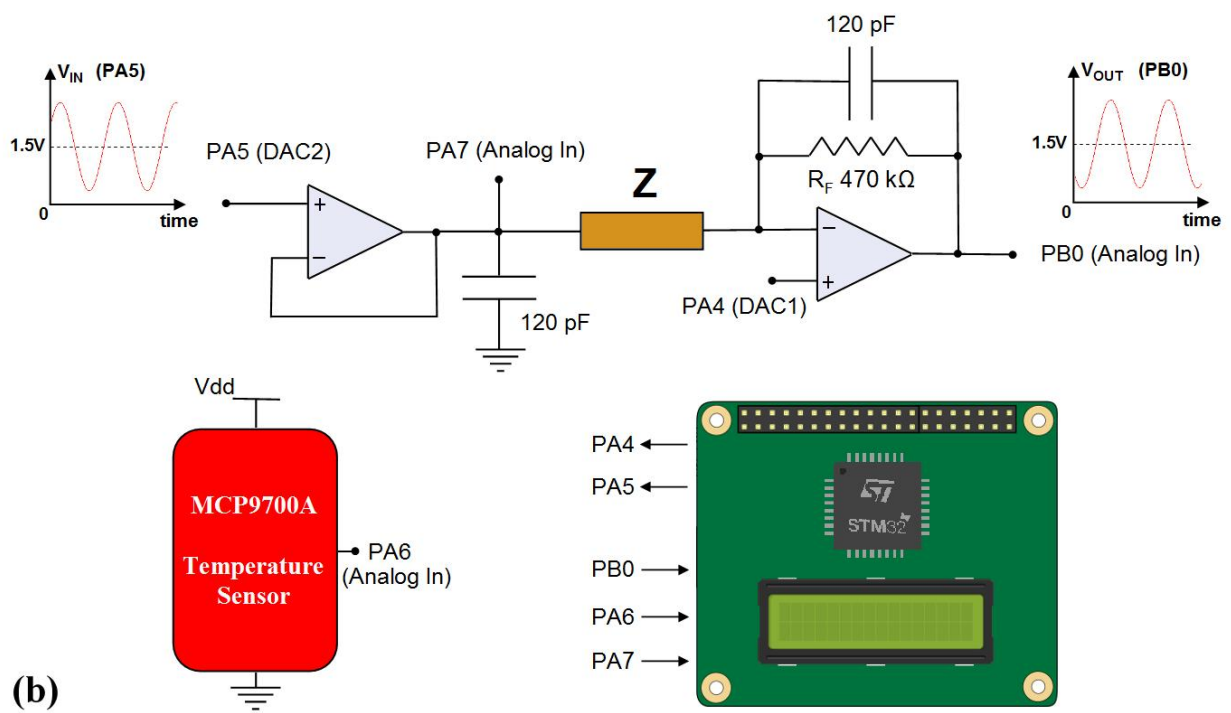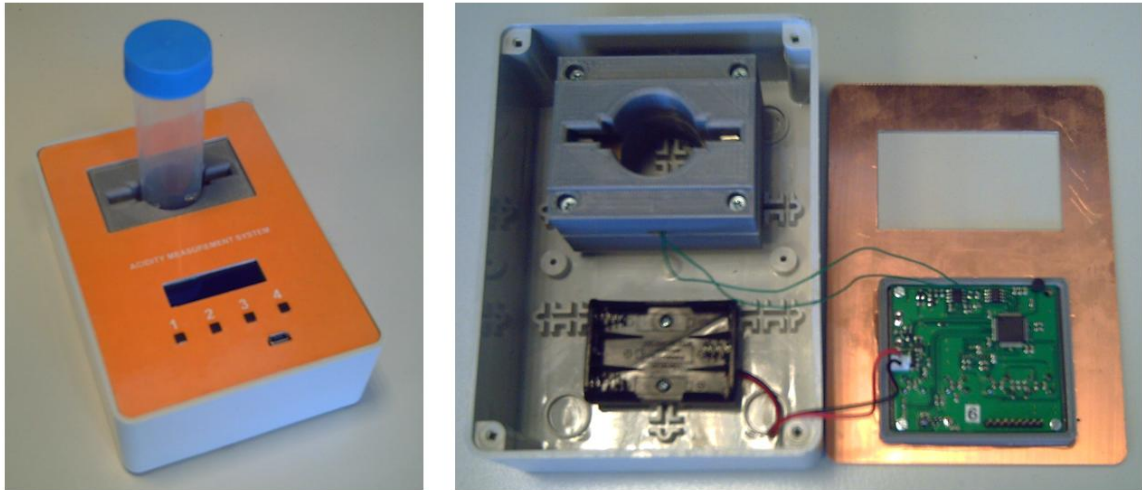
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The sensor is filled with the hydroalcoholic solution (40% distilled water / 60% ethanol) and, after the addition of 1 ml of the oil sample, is manually shaken to create the emulsion

The free fatty acid molecules dissociate in presence of the hydroalcoholic solution and the H_3O^+ ions contribute to the increase of the emulsion electrical conductance

A sine-wave signal (1 V amplitude, 200 Hz frequency) is applied to the sensor electrodes. The emulsion electrical conductance is measured and the oil sample free acidity is calculated

434
435

Figure 1

Figure 2
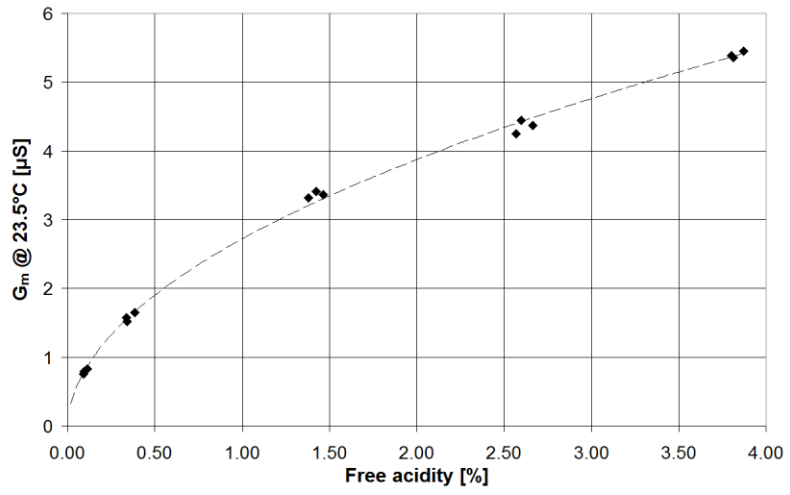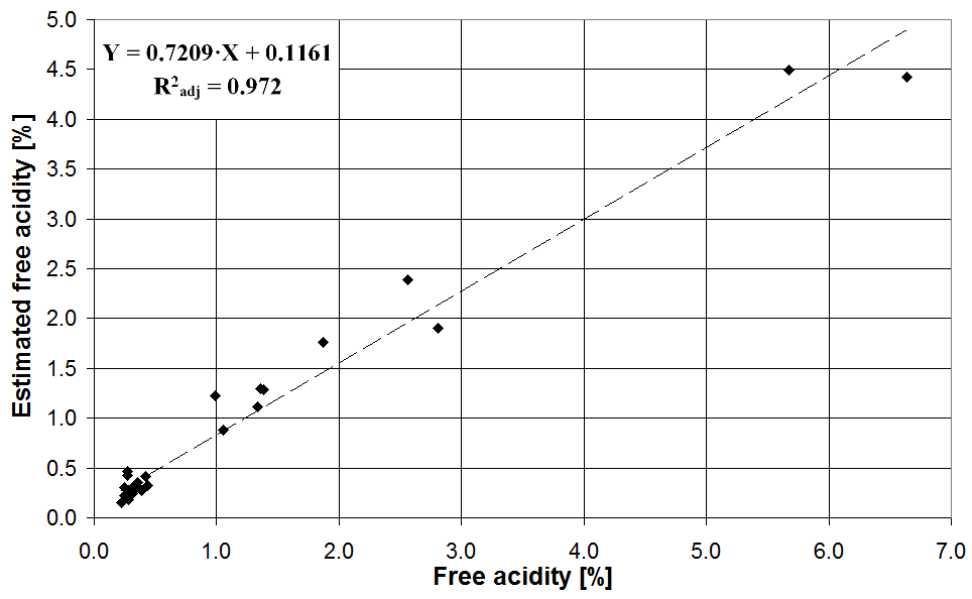
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Figure 3

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Figure 4

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Figure captions

445 **Figure 1.** Scheme of the working principle of the proposed electronic system for the determination

446 of free acidity in virgin olive oil (Grossi, & Riccò, 2017a).

447 **Figure 2.** Pictures of the outside and inside of the electronic system and the sensor for olive oil free

448 acidity analysis (a); schematic of the system hardware and of its electronical functioning (b).

449 **Figure 3.** Measured electrical conductance (G_m) at 23.5°C *vs* free acidity for the sunflower oil

450 calibration set.

451 **Figure 4.** Scatter plot of the estimated free acidity vs the free acidity measured by titration for a set

452 of 30 olive oil samples.

453 **Table 1**

454 Codes and description of the samples used for the calibration of the system, data of free acidity

455 determined by the official method and conductance measured with the portable system. The values

456 are mean of three replicates.

Sample code	Sample description	Free acidity (% oleic acid)	Conductance (µS)
A	Refined sunflower oil (without addition of oleic acid)	0.10 ± 0.01	0.79 ± 0.03
B	Refined sunflower oil + 0.25% oleic acid	0.36 ± 0.03	1.58 ± 0.06
C	Refined sunflower oil + 1.25% oleic acid	1.42 ± 0.04	3.36 ± 0.04
D	Refined sunflower oil + 2.50% oleic acid	2.61 ± 0.05	4.35 ± 0.10
E	Refined sunflower oil + 3.75% oleic acid	3.83 ± 0.04	5.40 ± 0.05

457

Table 2. Values of free acidity for all samples measured by the portable system and the official method. Results are expressed as % of oleic acid.

Sample code	Free acidity (portable system)	Free acidity (official method)
EVOO_1	0.18	0.25
EVOO_2	0.31	0.34
EVOO_3	0.25	0.28
EVOO_4	0.43	0.27
EVOO_5	0.30	0.25
EVOO_6	0.22	0.25
EVOO_7	0.18	0.25
EVOO_8	0.29	0.37
EVOO_9	0.29	0.34
EVOO_10	0.47	0.27
EVOO_11	0.32	0.33
EVOO_12	0.41	0.42
EVOO_13	0.24	0.28
EVOO_14	0.27	0.28
EVOO_15	0.32	0.44
EVOO_16	0.35	0.35
EVOO_17	0.18	0.28
EVOO_18	0.15	0.22
EVOO_19	0.27	0.39
EVOO_20	0.24	0.31
VOO_1	1.30	1.36
VOO_2	1.29	1.38
VOO_3	0.88	1.05
VOO_4	1.11	1.33
VOO_5	1.22	0.99
VOO_6	1.76	1.87
VOO_7	1.90	2.81
LOO_1	2.39	2.56
LOO_2	4.42	6.64
LOO_3	4.49	5.67

Appendix A

According to the working principle of the portable system, a 50 mL polypropylene tube (Falcon) vial modified to feature a couple of stainless-steel electrodes to measure the emulsion conductance (hereafter the sensor) is filled with 15 mL of hydro-alcoholic solution (40% distilled water/60% ethanol), then 1 mL of the olive oil sample is added and all is stirred to create an emulsion.

In presence of the hydroalcoholic solution, the free fatty acid molecule RCOOH, where R is the hydrocarbon chain, dissociates in the ionic compounds H_3O^+ and RCO_2^- that contribute to the increase of the emulsion electrical conductance. In the end, the higher the free fatty acid molecules concentration, the higher ions concentration and the higher the electrical conductance.

The emulsion electrical conductance is measured by Electrical Impedance Spectroscopy (EIS).

In the proposed approach the sample under investigation is stimulated with a sine-wave voltage signal $V_{in}(t)$:

$$V_{in}(t) = V_{M,in} \times sin\ (2\pi ft) \tag{A1}$$

and the current $I_{in}(t)$ through the sample is measured:

$$I_{in}(t) = I_{M,in} \times sin\ (2\pi ft + \varphi) \tag{A2}$$

where $V_{M,in}$ and $I_{M,in}$ are the amplitudes of the corresponding signals, f is the frequency of the test signal and φ is the phase difference between $I_{in}(t)$ and $V_{in}(t)$.

The sample electrical admittance is then expressed as:

$$Y = \frac{I_{in}(j2\pi f)}{V_{in}(j2\pi f)} = \frac{I_{M,in}}{V_{M,in}} \times (cos\ \phi + j \times sin\ \phi) = Re(Y) + j \times Im(Y) \tag{A3}$$

The emulsion in direct contact with the electrodes can be modelled as the parallel of an electrical conductance (accounting for the conductance of the emulsion) and a capacitance (accounting for the emulsion dielectric properties): while the emulsion conductance (that dominates at low frequency) is affected by the sample acidity due to the variation of the ions concentration, the dielectric properties are almost independent. Thus, the emulsion electrical conductance G_m can be estimated with the real component of the emulsion admittance $Re(Y) = |Y| \cdot cos(\varphi)$ where $|Y|$ is the admittance modulus.

In Figure A1 (a) the admittance modulus (measured with the commercial LCR meter Agilent E4980A) is plotted vs. the frequency of the applied test signal for different samples featuring different acidity in the frequency range 20 Hz – 2 MHz. As can be seen, $|Y|$ increases with sample acidity for test signals with a frequency lower than 20 kHz while it is almost independent on sample acidity for higher frequencies. This is the reason why the designed electronic system measures G_m with a single frequency measurement at 200 Hz.

The relation between G_m and the free acidity is non-linear and it can be modelled with the function:

22

494     $G_m = \alpha + \beta \times \sqrt{FA} + \gamma \times \sqrt[4]{FA^3}$ (A4)

495   where α, β and γ are empirical parameters that must be determined by a suitable calibration

496   procedure and are also function of the calibration temperature.

497   In Figure A1 (b) the qualitative plot of G_m vs FA is shown: as can be seen, the non-linear function

498   results in better accuracy for the estimated free acidity for lower acidity levels. This has been taken

499   in account by using a suitable amount of sample to create the emulsion to obtain good accuracy in

500   the acidity range of interest.

501

502
(a)   **(b)**
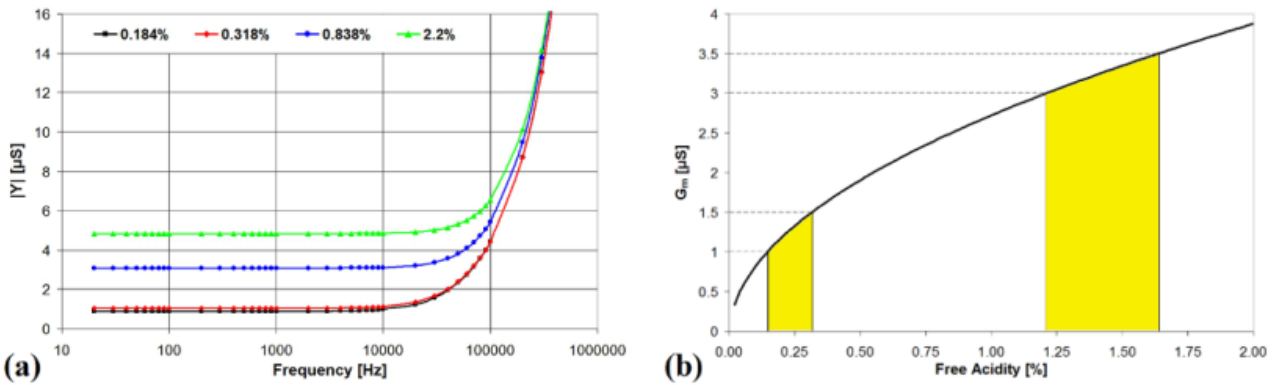
503   **Figure A1** (a) admittance modulus plotted *vs* frequency for olive oil samples featuring different free

504   acidity; (b) qualitative plot of the electrical conductance as function of sample acidity.

505

506   However, eq. A4 needs a computation intensive iterative algorithm to extract the estimated acidity

507   from the measured value of G_m as well as manual input of starting point to avoid failing in

508   algorithm convergence. Thus, a simpler model has been chosen (obtained by neglecting the molar

509   conductivity dependence on the H_3O^+ ions concentration) that is much more suitable to be

510   implemented in a low-cost microcontroller and can be described by the following function:

511     $G_m = \alpha + \beta \times \sqrt{FA}$ (A5)

512   The two models of eq. A4 and A5 have been tested with the data set of Grossi et al. (Grossi et al.,

513   2014b) and the results have shown how the accuracy in estimating free acidity is only marginal

514   higher for model A4 than model A5.

515   The sample free acidity can thus be estimated from the measured electrical conductance of the

516   emulsion with the following formula:

517     $FA = \left(\dfrac{G_m - \alpha}{\beta}\right)^2$ (A6)

518

519   **Appendix B**

520   The oil sample free acidity can be estimated by measuring the emulsion electrical conductance at

521   the temperature of calibration and then calculating the free acidity using equation A6. However,

522   since the system must be operated "in the field" and the environmental temperature is not a

523   parameter under control, there is the need to investigate how the emulsion conductance varies with

524   the temperature so that the oil free acidity can be estimated by the measure of the emulsion

525   conductance and the temperature.

526   Four different olive oil samples featuring different free acidity values (0.06% sample A, 0.37%

527   sample B, 0.81% sample C and 3.7% sample D) were tested inside a Binder APT KB 53 thermal

528   incubator for different temperatures between 15°C and 35°C.

529   In Figure B1 (a) the measured emulsion conductance is plotted vs the incubation temperature for

530   each sample. In all cases the G_m is a linear function of the temperature with determination

531   coefficients $R^2 > 0.99$. The calculated linear regression lines allow to determine the conductance

532   variation with temperature (i.e. $\partial G_m/\partial T$) for all samples: 0.0196 for sample A, 0.0495 for sample B,

533   0.0579 for sample C and 0.1417 for sample D. $\partial G_m/\partial T$ is thus found to increase with the sample

534   free acidity. Since the sample free acidity is also a function of the emulsion electrical conductance,

535   the relation between $\partial G_m/\partial T$ and $G_{m,23.5°C}$ has been plotted in Figure B1 (b).

536
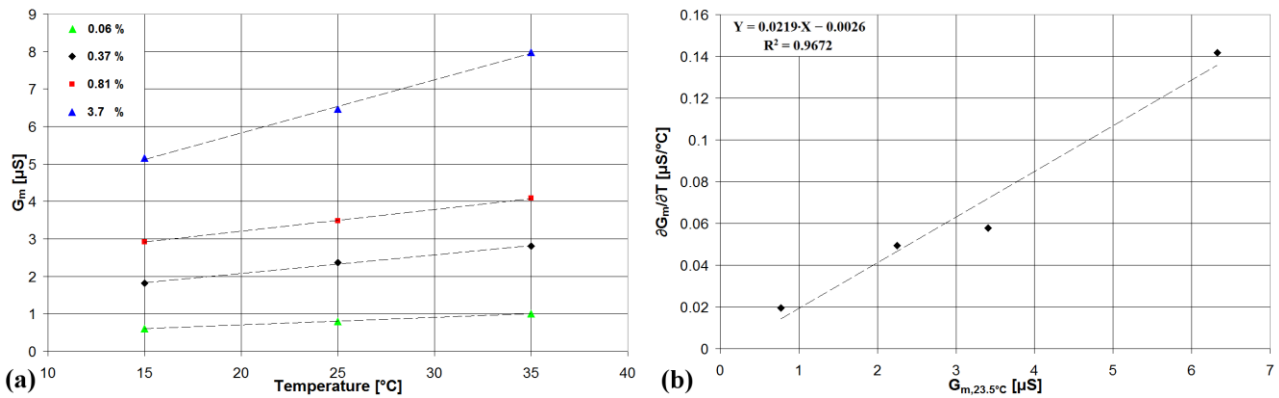
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538   **Figure B1** (a) measured emulsion conductance vs the incubation temperature for olive oil samples

539   featuring different free acidity; (b) $\partial G_m/\partial T$ plotted vs the electrical conductance at 23.5 °C.

540

541   As can be seen, a linear relation gives a good approximation ($R^2 = 0.9672$) of the function between

542    $\partial G_m/\partial T$ and $G_{m,23.5°C}$ thus:

543    $$\frac{\partial G_m}{\partial T} = 0.0219 \times G_{m,23.5°C} - 0.0026 \tag{B1}$$

544   where $\partial G_m/\partial T$ and $G_{m,23.5°C}$ are expressed as µS/°C and µS respectively. The emulsion electrical

545   conductance at the environmental temperature T can thus be expressed as:

546    $$G_{m,T} = G_{m,23.5°C} + \frac{\partial G_m}{\partial T} \times (T\text{-}23.5) \tag{B2}$$

547   and

548    $$G_{m,T} = G_{m,23.5°C} + \left(0.0219 \times G_{m,23.5°C}\text{-}0.0026\right) \times \left(T\text{-}23.5\right)$$ (B3)

549   The emulsion electrical conductance at $T_{calib} = 23.5°C$ can thus be estimated from the electrical

550   conductance at temperature T and the measured value of T using the following formula:

551    $$G_{m,23.5°C} = \frac{G_{m,T}+0.0026 \times (T\text{-}23.5)}{1+0.0219 \times (T\text{-}23.5)}$$ (B4)

552   Thus, by measuring $G_{m,T}$ and T, the value of $G_{m,23.5°C}$ can be calculated using equation B4 and, from

553   this value, the sample acidity can be estimated using equation A6.

Video

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