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Published Version:
Design and in-house validation of a portable system for the determination of free acidity in virgin olive oil / Grossi M.; Palagano R.; Bendini A.; Ricco B.; Servili M.; Garcia-Gonzalez D.L.; Gallina Toschi T.. - In: FOOD CONTROL. - ISSN 0956-7135. - ELETTRONICO. - 104:(2019), pp. 208-216. [10.1016/j.foodcont.2019.04.019]

This version is available at: https://hdl.handle.net/11585/700389 since: 2019-09-25

Published:

DOI: http://doi.org/10.1016/j.foodcont.2019.04.019

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FOOD CONTROL, Volume: 104, pages: 208-216

The final published version is available online at:
https://doi.org/10.1016/j.foodcont.2019.04.019

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

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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 principle 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.

The proposed system has been calibrated and in-house validated showing good results in terms of limit of detection and quantification, precision and accuracy. Moreover, a good correlation (R^2_{adj} = 0.97) with free acidity data obtained applying the official method on 30 olive oil samples belonging to different commercial categories (extra virgin, virgin and lampante olive oil) has been evidenced.

Keywords: portable system; free acidity; virgin olive oil; impedance spectroscopy; in-house validation; electrical conductance.

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

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, 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 ≥99.8%), ethanol (ACS reagent, purity ≥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 0.25% 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 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 hydroalcoholic 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 using a plastic graduated Pasteur pipette 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. In order to avoid error due to the eventual instability of the emulsion, the free acidity measurement should be realized within 30 minutes after the agitation. 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 concentration of the analyte and the slope of the calibration function (*m*) has been applied. The formulas used are:

$$LOD = 3\sigma/m \quad (1)$$

$$LOQ = 10\sigma/m \quad (2)$$

To evaluate the inter-day precision of the method, two virgin olive oil samples for each type of quality grade (samples EVOO_2 and EVOO_19 for EVOOs, samples VOO_1 and VOO_6 for VOOs and samples LOO_1 and LOO_3 for LOOs) were analyzed in triplicate for three different days and values were statistically evaluated by the Student's Test ($p<0.05$).

Accuracy and intra-day precision were studied analyzing the set of 30 olive oils with a different quality grade. Precision was expressed in terms of RSD (Relative Standard Deviation) measured for the free acidity values obtained with the portable system. For the accuracy, all the samples were also analyzed following the official method (acid-base titration) and the comparison between the values obtained by the two methods was performed (two-tailed paired t-test with $p < 0.05$).

3. Results and discussion

3.1 Design of the portable system

A portable battery-operated electronic system for in-situ measurements of olive oil free acidity has been built to allow olive oil quality assurance directly in the oil mills or packaging centers.

The dimensions of the instrument are 11 x 15 x 5 cm and some pictures are presented in Figure 2a. The instrument can be powered by USB port or using batteries (3 AAA alkaline batteries 1.5 V). The system is composed of an electronic board designed ad-hoc that performs all the operations to measure the free acidity, a 2 rows 16 columns LCD screen to output the measure results, four buttons for user interaction and the sensor previously described (see paragraph 2.3.2). The electronic board is based on the microcontroller STM32L152RCT6A and its schematic is presented in Figure 2b. The sensor used for the measurements is realized with electrodes of stainless steel, a material less affected by passivation than other materials. The same sensor could be used many

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

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

it is

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

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 modeled 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:

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

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:

$$G_m = \alpha + \beta \times \sqrt{FA} \quad (6)$$

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 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.

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

3.2 In-house method validation

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

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

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

It allows to obtain, directly on the display of the portable system, the free acidity value of the tested sample estimated starting from its electrical properties. Since this procedure is necessary to define the relation between the oil FA and the conductance, and, consequently, the mathematical function that has to be stored in the microcontroller non-volatile memory of the instrument, this step needs to be realized only when the instrument is built.

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

applying this new analytical approach in order to study its accuracy and intra-day precision (expressed in terms of RSD).

The portable instrument showed good precision results since all the obtained values were under the 15% that is considered as acceptable for the validation of a new method (Peters, Drummer, & Musshoff, 2007). All the samples were also analyzed following the official method (acid-base titration) and the comparison between the values obtained by the two methods was performed to measure the accuracy of the system (Table 2). The differences between the two series of results (official method *vs* portable system) were evaluated by using the two-tailed paired t-test ($p<0.05$). The two approaches did not give statistically differences for the mean values (t<t (critical value)). Moreover, the regression between the two series of values provided a coefficient R^2_{adj} of 0.97 (Figure 4) in 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 (Table 3), 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 come in contact with the hydroalcoholic solution, a dissociation occurs, leading to the formation of ions that produce an 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.

Funding

This work was supported by the Horizon 2020 European Research project OLEUM *"Advanced solutions for assuring the authenticity and quality of olive oil at a global scale"*, which has received funding from the European Commission within the Horizon 2020 Programme (2014–2020), grant agreement no. 635690. The information expressed in this article reflects the authors' views; the European Commission is not liable for the information contained herein.

Declaration of interest

None.

References

Ansari, K. R., Quraishi, M. A., Singh, A. (2015). Pyridine derivatives as corrosion inhibitors for N80 steel in 15% HCl: electrochemical, surface and quantum chemical studies. *Measurement, 76,* 136-147.

Armenta, S., Garrigues, S., de la Guardia, M. (2007). Determination of edible oil parameters by near infrared spectrometry. *Analytica Chimica Acta, 596,* 330-337.

Arsalane, A., El Barbri, N., Tabyaoui, A., Klilou, A., Rhofir, K., Halimi, A. (2018). An embedded

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gomez-Caravaca, A. M., Segura-Carretero, A., Fernandez-Gutierrez, A., Lercker, G. (2007). Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. *Molecules, 12,* 1679-1719.

Bera, T. K. (2014). Bioelectrical impedance methods for noninvasive health monitoring: a review. *Journal of Medical Engineering,* article ID 381251, 1-28.

Bustan, A., Kerem, Z., Yermiyahu, U., Ben-Gal, A., Lichter, A., Droby, S., Zchori-Fein, E., Orbach, D., Zipori, I., Dag, A. (2014). Preharvest circumstances leading to elevated oil acidity in 'Barnea' olives. *Scientia Horticulturae, 176,* 11-21.

Codex Alimentarius – CODEX STAN 210-1999 - Standard for named vegetable oils codex. Adopted in 1999. Revised in 2001-2003-2009-2017. Amended in 2005-2011-2013-2015.

Commission Implementing Regulation (EU) No 2016/1227 of 27 July 2016 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis.

Commission Implementing Regulation (EU) No 1348/2013 of 16 December 2013 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis.

Commission Regulation (EEC) No 2568/91 of 11 July 1991 on the characteristics of olive oil and on the relevant methods of analysis.

Grossi, M., Lanzoni, M., Lazzarini, R., Riccò, B. (2012b). Linear non-iterative sinusoidal fitting algorithm for microbial impedance biosensor. *Sensors & Transducers Journal, 137* (2), 235-244.

Grossi, M., Di Lecce, G., Gallina Toschi, T., Riccò, B. (2014a). A novel electrochemical method for olive oil acidity determination. *Microelectronics Journal, 45* (12), 1701-1707.

Grossi, M., Di Lecce, G., Gallina Toschi, T., Riccò, B. (2014b). Fast and accurate determination of olive oil acidity by electrochemical impedance spectroscopy. *IEEE Sensors Journal, 14* (9), 2947-2954.

Grossi, M., Di Lecce, G., Arru, M., Gallina Toschi, T., Riccò, B. (2015). An opto-electronic system for in-situ determination of peroxide value and total phenol content in olive oil. *Journal of Food Engineering, 146,* 1-7.

Grossi, M., Riccò, B. (2017a). Electrical impedance spectroscopy (EIS) for biological analysis and food characterization: a review. *Journal of Sensors and Sensor Systems, 6,* 303-325.

Grossi, M., Riccò, B. (2017b). An automatic titration system for oil concentration measurement in metalworking fluids. *Measurement, 97,* 8-14.

Harker, F. R., Maindonald, J. H. (1994). Ripening of nectarine fruit. *Plant Physiology, 106,* 165-171.

Hussin, M. H., Rahim, A. A., Nasir, M., Ibrahim, M., Brosse, N. (2016). The capability of ultrafiltrated alkaline and organosolv oil palm (Elais guineensis) fronds lignin as green corrosion inhibitor for mild steel in 0.5 M HCl solution. *Measurement, 78,* 90-103.

Lin, C. M., Chen, L. H., Chen, T. M. (2012). The development and application of an electrical impedance spectroscopy measurement system for plant tissue. *Computers and Electronics in Agriculture, 82,* 96-99.

Oujji, N. B., Bakas, I., Istamboulié, G., Ait-Ichou, I., Ait-Addi, E., Rouillon, R., Noguer, T. (2014). A simple colorimetric enzymatic-assay, based on immobilization of acetylcholinesterase by adsorption, for sensitive detection of organophosphorus insecticides in olive oil. *Food Control, 46,* 75-80.

Peters, F. T., Drummer, O. H., Musshoff F. (2007). Validation of new methods. *Forensic Science International, 165,* 216–224.

Ragni, L., Iaccheri, E., Cevoli, C., Berardinelli, A., Bendini, A., Gallina Toschi, T. (2013). A capacitive technique to assess water content in extra virgin olive oils. *Journal of Food Engineering, 116* (1), 246-252.

Sture, O., Ruud Oye, E., Skavhaug, A., Mathiassen, J. R. (2016). A 3D machine vision system for quality grading of Atlantic salmon. *Computers and Electronics in Agriculture, 123,* 142-148.

Tena, N., Wang, S. C., Aparicio-Ruiz, R., García-Gonzaléz, D. L., Aparicio R. (2015). In-depth assessment of analytical methods for olive oil purity, safety, and quality characterization. *Journal of Agricultural and Food Chemistry, 63,* 4509−4526.

Thompson, M., Ellison, S. L., Wood, R. (2002). Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). *Pure and Applied Chemistry, 74* (5), 835-855.

Veneziani, G., Esposto, S., Taticchi, A., Urbani, S., Selvaggini, R., Sordini, B., Servili, M. (2018a). Characterization of phenolic and volatile composition of extra virgin olive oil extracted from six Italian cultivars using a cooling treatment of olive paste. *LWT – Food Science and Technology, 87,* 523-528.

Veneziani, G., Esposto, S., Minnocci, A., Taticchi, A., Urbani, S., Selvaggini, R., Sordini, B., Sebastiani, L., Servili, M. (2018b). Compositional differences between veiled and filtered virgin olive oils during a simulated shelf life. *LWT – Food Science and Technology, 94,* 87-95.

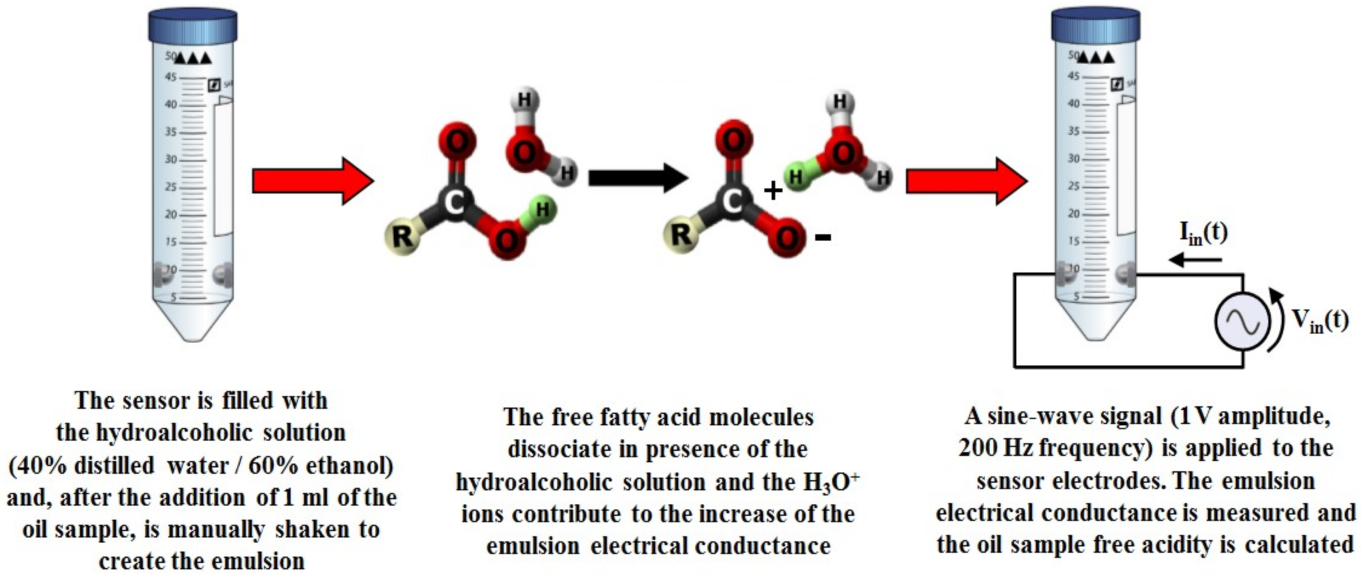

O
H
O
H
R
C
O
H
O
+
H
O
H
H
R
C
O
-
I_in(t)
V_in(t)
The sensor is filled with the hydroalcoholic solution (40% distilled water / 60% ethanol) and, after the addition of 1 ml of the oil sample, is manually shaken to create the emulsion
The free fatty acid molecules dissociate in presence of the hydroalcoholic solution and the H3O+ 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

Figure 1

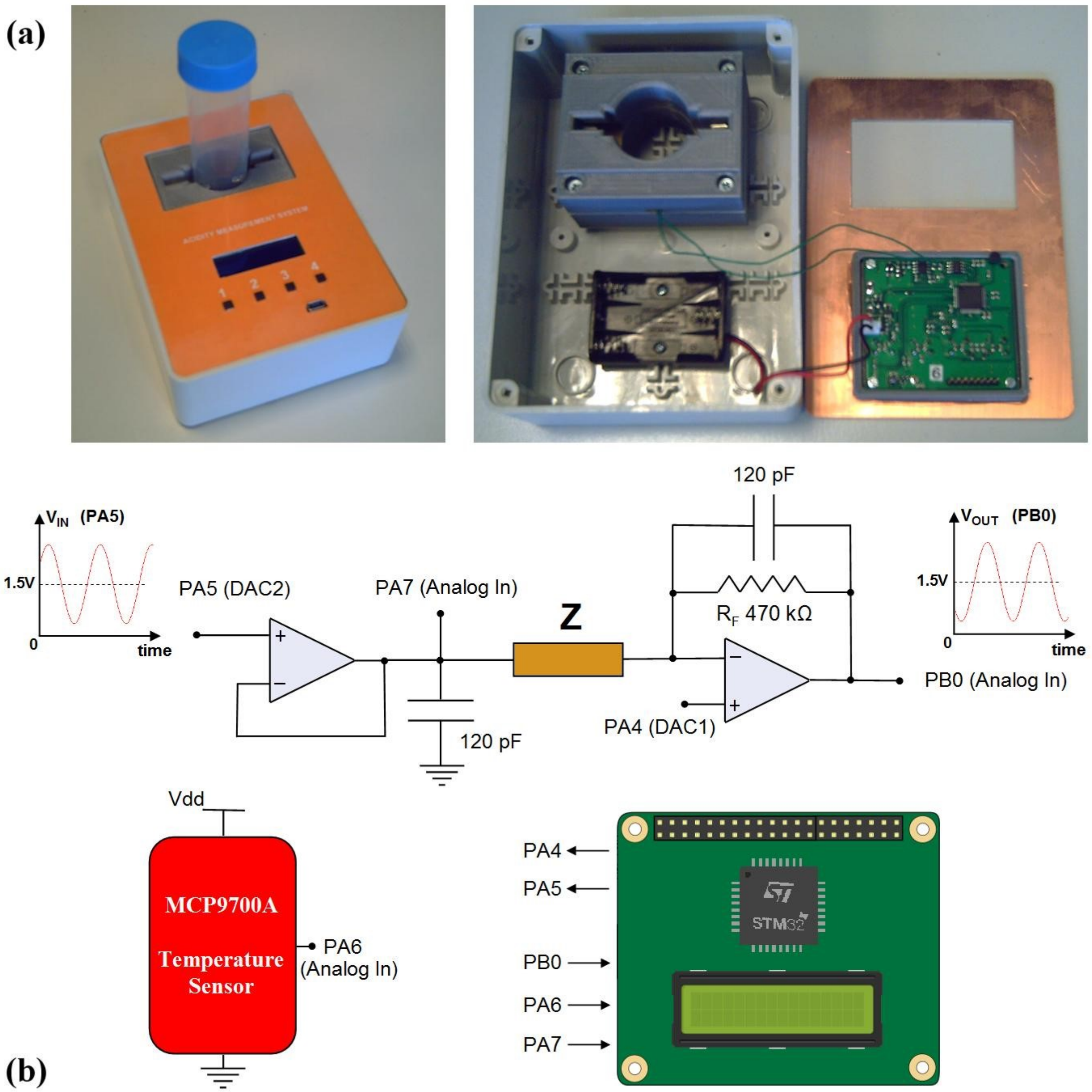
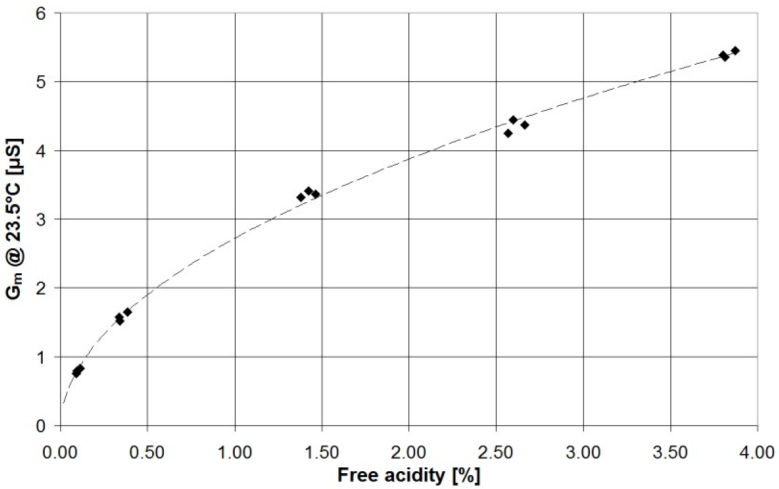
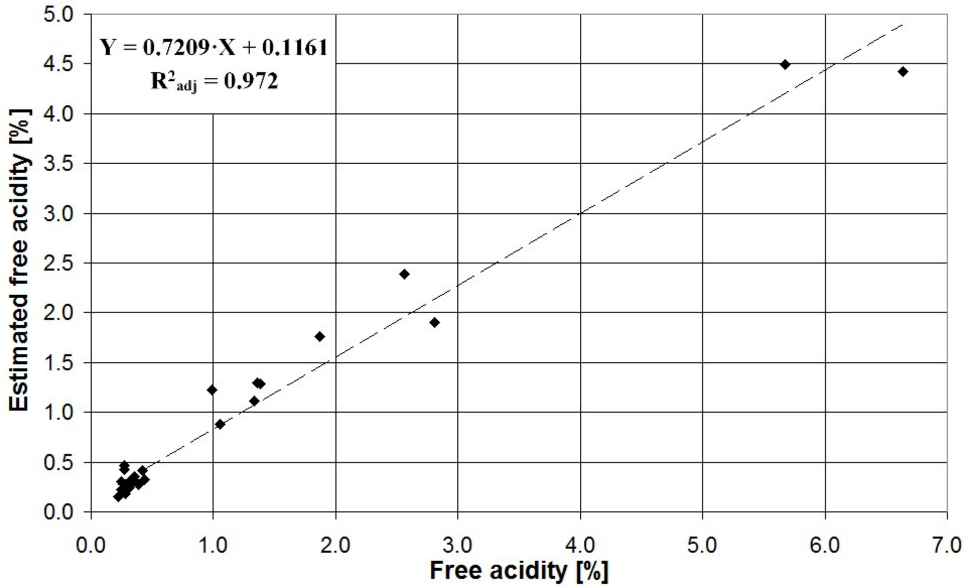

(a)
(b)
120 pF
VIN (PA5)
1.5V
0
time
PA5 (DAC2)
PA7 (Analog In)
Z
RF 470 kΩ
VOUT (PB0)
1.5V
0
time
PB0 (Analog In)
120 pF
PA4 (DAC1)
Vdd
MCP9700A
Temperature Sensor
PA6
(Analog In)
PA4
PA5
PB0
PA6
PA7
STM32

Figure 2



6
5
4
3
2
1
0
Gm @ 23.5°C [µS]
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
Free acidity [%]

Figure 3



Y = 0.7209·X + 0.1161
R2adj = 0.972
Estimated free acidity [%]
Free acidity [%]
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0

Figure 4

Figure captions

Figure 1. Scheme of the working principle of the proposed electronic system for the determination of free acidity in virgin olive oil.

Figure 2. Pictures of the outside and inside of the electronic system and the sensor for olive oil free acidity analysis (a); system hardware and electrical scheme (b).

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

Figure 4. Scatter plot of the estimated free acidity *vs* the free acidity measured by titration for a set of 30 olive oil samples.

Table 1

Codes and description of the samples used for the calibration of the system, data of free acidity determined by the official method and conductance measured with the portable system.

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	1.4 ± 0.04	3.36 ± 0.04
D	Refined sunflower oil + 2.50% oleic acid	2.6 ± 0.05	4.35 ± 0.10
E	Refined sunflower oil + 3.75% oleic acid	3.8 ± 0.04	5.40 ± 0.05

The values are mean of three replicates. According to the EU Reg. 2016/1227, the FA values from 0 up to 1 (including 1) are reported with two decimal places and the FA values higher than 1 are reported with one decimal place.

Table 2. Values of free acidity (mean and standard deviation) for all samples measured by the portable system and the official method.

Sample code	Free acidity (portable system)	Free acidity (official method)
EVOO_1	0.18 ± 0.01	0.25 ± 0.01
EVOO_2	0.31 ± 0.03	0.34 ± 0.02
EVOO_3	0.25 ± 0.01	0.28 ± 0.01
EVOO_4	0.43 ± 0.01	0.27 ± 0.01
EVOO_5	0.30 ± 0.02	0.25 ± 0.01
EVOO_6	0.22 ± 0.01	0.25 ± 0.01
EVOO_7	0.18 ± 0.02	0.25 ± 0.01
EVOO_8	0.29 ± 0.04	0.37 ± 0.02
EVOO_9	0.29 ± 0.01	0.34 ± 0.01
EVOO_10	0.47 ± 0.01	0.27 ± 0.01
EVOO_11	0.32 ± 0.02	0.33 ± 0.01
EVOO_12	0.41 ± 0.02	0.42 ± 0.02
EVOO_13	0.24 ± 0.02	0.28 ± 0.01
EVOO_14	0.27 ± 0.03	0.28 ± 0.01
EVOO_15	0.32 ± 0.02	0.44 ± 0.02
EVOO_16	0.35 ± 0.02	0.35 ± 0.01
EVOO_17	0.18 ± 0.02	0.28 ± 0.01
EVOO_18	0.15 ± 0.01	0.22 ± 0.01
EVOO_19	0.27 ± 0.02	0.39 ± 0.02
EVOO_20	0.24 ± 0.00	0.31 ± 0.01
VOO_1	1.3 ± 0.09	1.4 ± 0.02
VOO_2	1.3 ± 0.02	1.4 ± 0.03
VOO_3	0.88 ± 0.07	1.1 ± 0.01
VOO_4	1.1 ± 0.05	1.3 ± 0.00
VOO_5	1.2 ± 0.05	0.99 ± 0.00
VOO_6	1.8 ± 0.12	1.9 ± 0.01
VOO_7	1.9 ± 0.07	2.8 ± 0.02
LOO_1	2.4 ± 0.32	2.6 ± 0.00
LOO_2	4.4 ± 0.04	6.6 ± 0.05
LOO_3	4.5 ± 0.17	5.7 ± 0.01

Results are expressed as % of oleic acid. According to the EU Reg. 2016/1227, the FA values from 0 up to 1 (including 1) are reported with two decimal places and the FA values higher than 1 are

Table 3. Values of free acidity (mean and standard deviation) measured in three different days to evaluate the inter-day precision.

Sample	Day 1	Day 2	Day 3
EVOO_2	0.31 ± 0.03	0.34 ± 0.01	0.32 ± 0.02
EVOO_19	0.27 ± 0.02	0.25 ± 0.01	0.27 ± 0.03
VOO_1	1.3 ± 0.09	1.3 ± 0.02	1.3 ± 0.01
VOO_6	1.8 ± 0.12	1.8 ± 0.08	1.8 ± 0.05
LOO_1	2.4 ± 0.32	2.5 ± 0.18	2.5 ± 0.11
LOO_3	4.5 ± 0.17	4.6 ± 0.25	4.4 ± 0.04

EVOO_2 and EVOO_19: extra virgin olive oils; VOO_1 and VOO_6: virgin olive oils; LOO_1 and LOO_3: lampante olive oils. According to the EU Reg. 2016/1227, the FA values from 0 up to 1 (including 1) are reported with two decimal places and the FA values higher than 1 are reported with one decimal place.

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 $V_{in}(t)$:

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

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

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

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:

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

The emulsion in direct contact with the electrodes can be modeled 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

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

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

In Figure A1 (b) the qualitative plot of G_m vs FA is shown: as can be seen, the non-linear function results in better accuracy for the estimated free acidity for lower acidity levels. This has been taken in account by using a suitable amount of sample to create the emulsion to obtain good accuracy in the acidity range of interest.

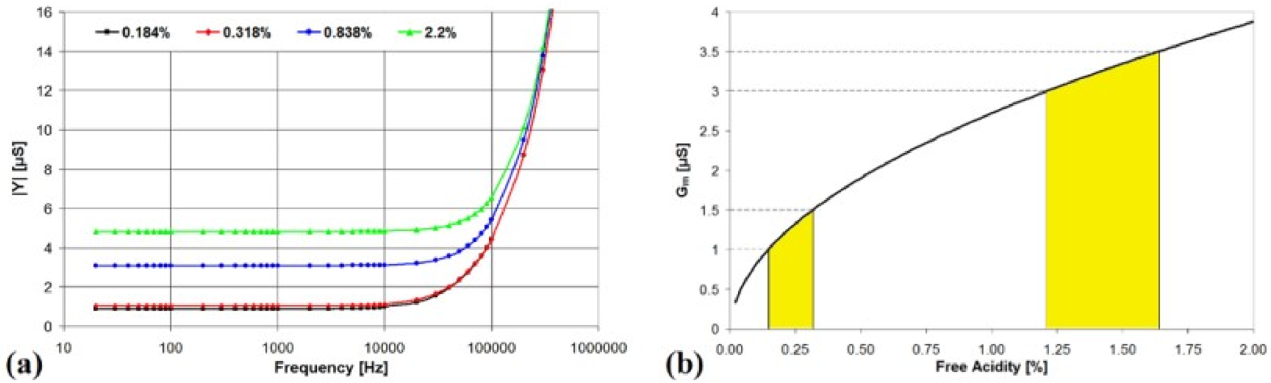


Figure A1 (a) admittance modulus plotted *vs* frequency for olive oil samples featuring different free acidity; (b) qualitative plot of the electrical conductance as function of sample acidity.

(permission for publishing the Figure A1(a) obtained from Grossi et al., 2014. Microelectronics Journal, 45 (12), 1701-1707)

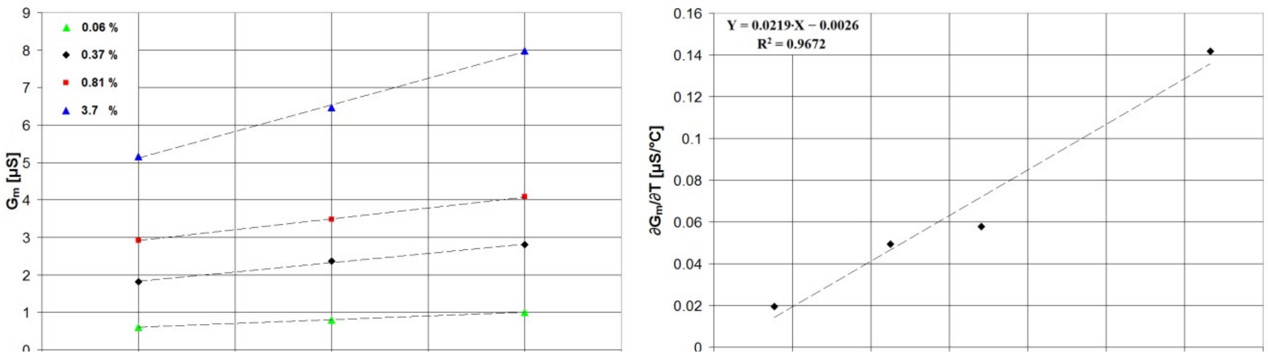
However, eq. A4 needs a computation intensive iterative algorithm to extract the estimated acidity from the measured value of G_m as well as manual input of starting point to avoid failing in algorithm convergence. Thus, a simpler model has been chosen (obtained by neglecting the molar conductivity dependence on the H_3O^+ ions concentration) that is much more suitable to be implemented in a low-cost microcontroller and can be described by the following function:

Appendix B

The oil sample free acidity can be estimated by measuring the emulsion electrical conductance at the temperature of calibration and then calculating the free acidity using equation A6. However, since the system must be operated "in the field" and the environmental temperature is not a parameter under control, there is the need to investigate how the emulsion conductance varies with the temperature so that the oil free acidity can be estimated by the measure of the emulsion conductance and the temperature.

Four different olive oil samples featuring different free acidity values (0.06% sample A, 0.37% sample B, 0.81% sample C and 3.7% sample D) were tested inside a Binder APT KB 53 thermal incubator for different temperatures between 15°C and 35°C.

In Figure B1 (a) the measured emulsion conductance is plotted vs the incubation temperature for each sample. In all cases the G_m is a linear function of the temperature with determination coefficients $R^2 > 0.99$. The calculated linear regression lines allow to determine the conductance variation with temperature (i.e. $\partial G_m/\partial T$) for all samples: 0.0196 for sample A, 0.0495 for sample B, 0.0579 for sample C and 0.1417 for sample D. $\partial G_m/\partial T$ is thus found to increase with the sample free acidity. Since the sample free acidity is also a function of the emulsion electrical conductance, the relation between $\partial G_m/\partial T$ and $G_{m,23.5°C}$ has been plotted in Figure B1 (b).



where $\partial G_m/\partial T$ and $G_{m,23.5°C}$ are expressed as μS/°C and μS respectively. The emulsion electrical conductance at the environmental temperature T can thus be expressed as:

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

and

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

The emulsion electrical conductance at $T_{calib} = 23.5°C$ can thus be estimated from the electrical conductance at temperature T and the measured value of T using the following formula:

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

Thus, by measuring $G_{m,T}$ and T, the value of $G_{m,23.5°C}$ can be calculated using equation B4 and, from this value, the sample acidity can be estimated using equation A6.