Monitoring olive oils quality and oxidative resistance during storage using an electronic tongue

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ABSTRACT

Monitoring olive oils oxidative stability and quality parameters (free acidity, peroxide values, K_{232} and K_{270} extinction coefficients) is needed to guarantee that, during storage, their levels remain within the legal thresholds enabling their commercialization as high-value extra-virgin olive oils. Physicochemical levels are assessed using time-consuming routine analytical reference techniques. In this work, the feasibility of a novel approach that merges an electronic tongue and chemometric tools, for monitoring extra-virgin olive oils' quality along one year of storage at dark or exposed to light is discussed. The results confirmed that physicochemical parameters varied with the storage lighting conditions and more significantly with time. Also, multiple linear regression models, using sub-sets of 22–28 sensors selected with a meta-heuristic simulated annealing algorithm, allow evaluating the storage time-evolution of olive oils' peroxide values, extinction coefficients and oxidative stabilities with satisfactory accuracy ($R^2 \geq 0.98$ and ≥ 0.96, for leave-one-out and repeated K-fold cross-validation procedures, respectively). The capability of monitoring, in a single electrochemical assay, legal required quality parameters of olive oils, decreases considerable the analysis time and cost, allowing checking the compliance of extra-virgin olive oil quality with labeling. So, the use of electronic tongues for extra-virgin olive oil shelf-life assessment could be envisaged.

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1. Introduction

Extra-virgin olive oils (EVOO) are quite appreciated by consumers due to their quality, sensory attributes and health benefits. So, as pointed out in the literature, there still is a commercial need to develop fast, portable and low-cost analytical methods for guaranteeing olive oil commercial category namely to distinguishing EVOO from virgin and lampante olive oils (VOO and LOO, respectively). Olive oils physicochemical parameters have been shown as important markers for quality assessment and olive oil grade discrimination, minimizing the risk of incorrect or abusive

olive oils labeling (Garcia, Martins, & Cabrita, 2013; Sinelli, Cerretani, Di Egidio, Bendini, & Casiraghi, 2010). However, besides olive cultivar, edapho-climatic conditions, harvesting and technological procedures, EVOO's physicochemical quality is also greatly influenced by storage conditions, namely time, temperature, type of packing material, exposition to air and/or to light (Abbadi et al., 2014; Ayyad et al., 2015; Ben-Hassine et al., 2013; Bubola, Koprivnjak, Sladonja, & Belobrajić, 2014; Caponio et al., 2013; Cossignani, Luneia, & Damiani, 2007; Fadda et al., 2012; Gómez-Alonso, Mancebo-Campos, Salvador, & Fregapane, 2007; Jabeur, Zribi, Abdelhedi, & Bouaziz, 2015; Pristouri, Badeka, & Kontominas, 2010). Indeed, the levels of olive oils physicochemical quality parameters, such as the ultra-violet light absorption extinction coefficients (K_{232} and K_{270}), free acidity (FA) and peroxide value (PV) may significantly increase during storage

(Abbadi et al., 2014; Afaneh, Abbadi, Ayyad, Sultan, & Kanan, 2013; Fadda et al., 2012; Jabeur et al., 2015; Mendéz & Falqué, 2007; Rababah, Feng, Yang, Eriefej, & Al-Omoush, 2011; Stefanoudaki, Willians, & Harwood, 2010), those of the oxidative stability (OS) decrease (Stefanoudaki et al., 2010) and so, an olive oil classified as extra-virgin when bottled may suffer degradation during storage resulting in an inferior quality grade when purchase and consumed. In fact, olive oils quality changes are inevitable and start immediately after the olive oil extraction due to lipid oxidation, which may lead to rancidity (Ben-Hassine et al., 2013; Vacca, Del Caro, Poiana, & Piga, 2006) or to hydrolytic degradations causing partial loss of healthy minor constituents (Dabbou et al., 2011). Therefore, new analytical methods aiming to ensure the compliance of olive oil quality with labeling is of utmost relevance for olive oils producers and consumers (Abbadi et al., 2014). This aim could be accomplished by the development of simple, green, user-friendly and low-cost analytical devices that could provide fast assessment and monitoring of the physicochemical quality parameters of olive oils, which could be implemented as complementary or alternative methods to the time-consuming classical analytical reference techniques. For example, this need as motivate the development of simple, expeditious and economic techniques compared to the expensive and time consuming classical chromatographic techniques, like the use of Fourier transform infrared (FTIR) spectroscopy combined with chemometrics as a rapid tool to predict phenol content and antioxidant activity of olive fruits and oils (Machado et al., 2015) or the use of Ion Mobility Spectrometry (IMS) for assessing the stability and quality of single-variety EVOO over storage (Garrido-Delgado et al., 2015). Several sensor approaches, based on the use of electronic tongues, noses and/or eyes (E-tongues, E-noses and E-eyes, respectively), have been reported for olive oils qualitative and/or quantitative electrochemical characterization. These studies have proven the potential of single or fusion methodologies between E-tongues, E-noses and/or E-eyes regarding organoleptic characterization (Apetrei, Gutierez, Rodríguez-Méndez, & de Saja, 2007; Apetrei, Apetrei, Villanueva, de Saja, & Gutierrez-Rosales, 2010; Apetrei, Ghasemi-Varnamkhasti & Apetrei, 2016; Apetrei, Rodríguez-Méndez, Parra, Gutierrez, & de Saja, 2004; Rodríguez-Méndez, Apetrei, & de Saja, 2010; Veloso, Dias, Rodrigues, Pereira, & Peres, 2016), olive oil quality levels discrimination (Apetrei & Apetrei, 2013; Apetrei, Rodríguez-Méndez, & de Saja, 2005; Escuderos, Sánchez, & Jiménez, 2011, 2010; García-González & Aparicio, 2004; Oliveri, Baldo, Daniele, & Forina, 2009), olive oil geographical origin (Apetrei et al., 2010; Cosio, Ballabio, Benedetti, & Gigliotti, 2006; Haddi et al., 2013, 2011; Oliveri et al., 2009) or monovarietal olive oil classification according to olive cultivar (Cimato et al., 2006; Dias, Rodrigues, Veloso, Pereira, & Peres, 2016a; Dias et al., 2014). Moreover, the feasibility of applying electrochemical devices for evaluating polyphenolic contents in olive oils have been successfully reported (Apetrei & Apetrei, 2013; Rodríguez-Méndez, Apetrei, & de Saja, 2008) as well for the capability of E-noses and/or E-tongues to indirectly qualitatively assess the oxidation of EVOO at different storage periods and conditions, allowing differentiating olive oil samples stored under different light conditions and storage time-periods (Cosio, Ballabio, Benedetti, & Gigliotti, 2007). So, the implementation of cost-effective sensor-based devices for monitoring EVOO physicochemical quality along the commercialization line and storage, aiming to verify if their quality indexes still meet the required legal thresholds (FA < 0.8% oleic acid; PV < 20 mEq O_2/kg; K_{232} < 2.5 and K_{270} < 0.22 according to the Commission regulation (ECC) nº 2568/91) for being commercialized as high-value EVOO, is still an on-going exciting and challenging research topic. In this work, the feasibility of applying an E-tongue (with nonspecific cross-sensitivity lipid membranes) and multiple

linear regression (MLR) models, established using a simulated annealing (SA) variable selection algorithm, to simultaneously (i.e., in a single-run assay) quantify PV, K_{232} and K_{270} levels as well as oxidative stability (OS) of bottled EVOO, during one-year of storage (0, 3, 6, 9 and 12 months) and under different lighting conditions (kept at dark or exposed to light) aiming to simulate usual commercial storage of olive oils, is discussed. So, the work aimed to demonstrate the possibility of applying the E-tongue as a fast and cost-effective novel strategy for quantifying quality parameters and OS values of olive oils in a single-run, expanding the demonstrated capability of the electrochemical device for qualitative and semi-quantitative olive oils assessment, namely for monovarietal EVOO discrimination according to olive cultivar (Dias et al., 2016a, 2014; Peres, Veloso, Pereira, & Dias, 2014) or the classification according to sensory intensity perception of positive organoleptic sensations (Veloso et al., 2016).

2. Materials and methods

2.1. Olive oil samples

Thirty six dark amber glass bottles of blend EVOOs, from the same lot, produced at the north of Portugal (Mirandela region), were studied. The selected lot was an extra virgin olive oil with the Protected Designation of Origin (PDO) qualification "Azeite de Trás-os-Montes" PDO. These olive oils were obtained from olives (mainly from cultivars Cobrançosa and Verdeal Transmontana, with a 10% percentage of olives of cv Madural, according to the producer information) collected at the initial maturation indexes (1–3) and extracted at low temperatures (~22 °C). Four olive oil's different bottles were analyzed immediately after packing, regarding to physicochemical parameters (FA, PV, K_{232} and K_{270} extinction coefficients as well as ΔK values, and OS) as well as electrochemically. The other 32 bottles were stored in a laboratory at ambient temperature (during the one-year of storage the temperature varied in the range of 17 °C to 25 °C) in conditions that tried to mimic real-storage conditions of supermarkets, during a one-year storage period (3, 6, 9 and 12 months; being 8 bottles picked and analyzed at each time-period). Also, two different lighting conditions were studied: 16 bottles were stored at dark, protected from any exposition to daylight or artificial light from a lamp; and, the other 16 bottles were stored in lab open shelves exposed to natural daylight (that entered throw 3 windows but without any direct exposition to sun) and also to artificial lightness from 8 fluorescent lamps (lamps Phillips TL-D36W/840) that remained lit 14h per day, trying to mimic the environmental typical conditions of storage supermarket facilities. Each lamp provided a luminous flux of 3250 lm (according to the manufacturer information), which illuminated a 6×9 m^2 laboratory, corresponding to approximately 482 lux. At each storage period olive oils samples were also evaluated physicochemically and electrochemically. Throughout this work, lighting conditions will be coded as "Dark" and "Light" corresponding to olive oils stored in darkness and olive oil kept in shelf exposed to natural and artificial usual light. Concerning the storage date code T0 is used for fresh olive oil (not stored, analyzed just after being packed) and T3, T6, T9 and T12 for olive oils stored during 3, 6, 9 and 12 months.

2.2. Olive oils physicochemical quality parameters and oxidative stability evaluation

The olive oil's quality parameters assessed were the free acidity (FA), peroxide value (PV) and the specific coefficients of extinction at 232 and 270 nm (K_{232}, K_{270}, and ΔK). All the mentioned quality parameters were determined according to European Union

standard methods (Commission regulation (ECC) nº 2568/91). FA values are expressed in terms of oleic acid, since this acid is the major fatty acid found in olive oils (corresponding to 55%—83% of the total fatty acid content). The oxidative stability (OS) of each olive oil was also determined in a Rancimat 743 apparatus (Metrohm CH, Switzerland). For these assays, to 3.00 g of olive oil heated at 120.0 ± 1.6 °C a flow rate of 20 L/h of air (filtered, cleaned, and dried) was supplied. The resulting volatile compounds were collected in water, and the increasing water conductivity (µS/cm) was continuously measured. The time (in hours) taken to reach the conductivity inflection curve point was recorded, and corresponded to the OS value. All physicochemical assays were carried out in triplicate (i.e., 3 samples were collected from each olive oil bottle and analyzed).

2.3. E-tongue device

The E-tongue included the same two print-screen potentiometric arrays described by Dias et al. (2014). The electrochemical device contained 20 sensors (diameter: 3.6 mm; thickness: 0.3 mm) obtained from the combination of 4 different lipid additives (octadecylamine, oleyl alcohol, methyltrioctylammonium chloride and oleic acid; $\approx 3\%$); 5 different plasticizers (bis(1-butylpentyl) adipate, dibutyl sebacate, 2-nitrophenyl-octylether, tris(2-ethylhexyl)phosphate and dioctyl phenylphosphonate; $\approx 65\%$) and high molecular weight polyvinyl chloride (PVC; $\approx 32\%$). All reagents were from Fluka (minimum purity≥97%). The type of sensors and polymeric membrane compositions (relative percentage of additive, plasticizer and PVC) were selected based on a previous work (Dias et al., 2009) considering the satisfactory signal stability over time (%RSD < 5%) and repeatability (0.5% < % RSD < 15%) towards the basic standard taste compounds (sweet, acid, bitter, salty and umami). Lipid polymeric membranes were used since they promote interactions with taste substances via electrostatic or hydrophobic interactions (Kobayashi et al., 2010). Each sensor was identified with a letter S (for sensor) followed by a code for the sensor array (1: or 2:) and the number of the membrane (1—20, corresponding to different combinations of plasticizer and additive used) as previously reported (Dias et al., 2014).

2.4. E-tongue analysis: sample preparation and potentiometric assays

Olive oils were extracted using water-ethanol solutions (80:20 v/v), to overcome the difficulty of carrying out electrochemical assays in viscous non-conductive liquids (Apetrei et al., 2010) and electrochemically analyzed as previously described (Dias et al., 2014). Ethanol was of analytical grade (Panreac, Barcelona) and deionized type II water was used in all electrochemical assays. For the electrochemical assays, samples were withdrawn from each olive oil bottle, which was previously smoothly shaken, and extracted with a solution of deionized water and ethanol (p.a.). In each assay, 10.00 g of olive oil were mixed to 100 mL of hydroethanolic solution during 5—10 min under strong agitation. This process allowed the extraction of polar compounds which are related to sensory sensations of olive oils (Veloso et al., 2016). The mixture was left at ambient temperature during 60 min, after which, 40.0 mL ($\times 2$) of the supernatant solution was carefully removed and immediately analyzed with the E-tongue, during 5 min enabling to carried out several electrochemical scans, being usually retained the last one, which would correspond a pseudo-equilibrium overall signal. To minimize the risk of overoptimistic prediction performance of multivariate models, the data split procedure used to set the training and validation sets, was carried using only one electrochemical "average" signal profile per olive oil (assumed as the olive oil specific fingerprint) in order to avoid that results from assays and replicas of the same olive oil could belong to both training and validation sets. Since assays were carried out during one year (0, 3, 6, 9 and 12 months) to control the potentiometric signal drifts of the E-tongue sensors, a calibration standard hydroethanolic (H_2O:EtOH: 80:20 v/v) solution containing gallic acid monohydrate ($\sim 1 \times 10^{-3}$ mol/L, purchased from Sigma-Aldrich with a minimum purity $\geq 99\%$) was analyzed before and after each olive oil measurement series. Signal drifts were solved by subtracting the signal profile recorded by the E-tongue device during the analysis of each olive oil sample by the average signal profile recorded for the gallic acid standard solution.

2.5. Statistical analysis

The possible interaction effect between the two main effects (i.e, lighting conditions $\times$ storage date) was assessed and interpreted using graphs of estimated marginal means for each parameter studied, instead of applying a two-way ANOVA, since the experimental design was unbalanced (i.e., for T0, the first level of storage date effect corresponding to just bottled olive oils, no sub-level of the lighting conditions effect could be considered). Based on the plots the significance of the interaction effect as well as the type of interaction (additive or non-additive/disordinal effect) was qualitatively evaluated: plots with parallel or non-parallel lines would indicate a non-significant or a significant interaction effect, respectively. In the latter case, non-crossing lines or crossing lines would point to additive or non-additive (disordinal) interaction effect. In the first situation (additive effect) main effects may be discussed separately. In the second case (non-additive effect) main effects cannot be individually interpreted. Generally speaking, main effects should not be discussed in the presence of a significant disordinal non-additive interaction (Field, 2009; Winer, Brown, & Michels, 1991). When possible, the effects of storage conditions (lighting conditions or storage date) on EVOO's physicochemical parameters and sensorial sensations were evaluated separately by means of a t-Student test (for comparing lighting versus darkness stored conditions for each storage date) and by means of an one-way ANOVA followed, when appropriate, by the Tukey's post-hoc multi-comparison test, for assessing the effect of the storage date for olive oils kept in dark or exposed to light. Linear Pearson correlation coefficient (R-Pearson) was applied to evaluate the existence of bivariate correlations within the olive oils' physicochemical parameters. The potential of the E-tongue device to quantitatively estimate the physicochemical quality parameters of olive oils, periodically analyzed during one-year of storage and under different lighting conditions, was evaluated using multiple linear regression (MLR), principal components regression (PCR) and partial least-squares (PLS) models. Detailed information regarding multivariate statistical tools can be found in the literature (Izenman, 2008; Miller & Miller, 2010). For the MLR models, the best subsets of K independent predictors among the 40 E-tongue potentiometric signals recorded were chosen using a meta-heuristic simulated annealing (SA) variable selection algorithm (Bertsimas & Tsitsiklis, 1993; Cadima, Cerdeira, & Minhoto, 2004; Kirkpatrick, Gelatt, & Vecchi, 1983) allowing to find optimal MLR-SA models. The SA algorithm searches, iteratively, for a global minimum that optimizes a system with k ($\subseteq K$) variables. The solutions of the current and the new subsets of k variables are compared, using the tau2 quality criterion, which is a measure of the goodness of fitting. A new solution is randomly selected in the neighborhood of the current solution, being chosen if a better result is obtained. Usually, 10,000 attempts are used to select the best subset of variables (best model), starting the process of selecting the best subsets of variables on each trial, thus ensuring a greater

confidence in finding a true optimal solution. In the present study, for each sub-set of sensors under evaluation (possible combinations of 2–39 sensors), the set of sensors chosen was the one that maximized tau2 value (Cadima et al., 2004). For PCR and PLS models the signals of the 40 E-tongue sensors were used to establish the optimal number of principal components (PCs) in each model, which was set equal to the number of PCs that would minimize the root mean square error (RMSE). The predictive performances of the MLR-SA, PCR and PLS models established were compared based on the leave-one-out cross-validation (LOO-CV) procedure. The approach that showed the better prediction capability was further evaluated using the repeated K-fold cross-validation (K-fold-CV) procedure. This strategy was used aiming to minimize the risk of over-optimistic results usually reported with the LOO-CV (Dias et al., 2016a; Dias, Zelda, Veloso, & Peres, 2016b). For the second cross-validation procedure, data was divided into K subsets that allowed obtaining K models, each one fitted considering K-1 subsets, as the training set, leaving out one of the subsets for the internal validation, to compute the predictive error for the obtained model (Venables & Ripley, 2002). The number of K-folds was set equal to 7, enabling the formation of testing subsets with 15% of the initial data (i.e., at least 5 olive oil independent samples/bottles for the present work) thus allowing bias reduction. Also, by applying a repeated K-fold-CV procedure (with 10 repetitions), the uncertainty of the estimates could be significantly reduced. To normalize the weight of each variable in the final linear classification model, variable scaling and centering procedures were applied. The possibility of using the E-tongue method as tool for quantifying the classical physicochemical olive oil quality parameters was further checked by testing if the slope and intercept values for LOO-CV or repeated K-fold-CV procedures were equal to the theoretical expected values (one and zero, respectively), from a statistical point of view (Cadima et al., 2004), when representing the predicted values, estimated by the regression models versus the experimental data. All statistical analysis were performed using the Subselect (Cadima et al., 2004; Cerdeira, Silva, Cadima, & Minhoto, 2012) and MASS (Venables & Ripley, 2002) packages of the open source statistical program R (version 2.15.1) at a significance level of 5%.

3. Results and discussion

3.1. Trend of EVOO's physicochemical quality parameters and positive sensorial attributes during storage

Physicochemical quality parameters (FA, PV, K_{232}, K_{270}, ΔK, and OS) of olive oils, stored at light or dark conditions, were evaluated quarterly, during 12 months, and further compared with the starting parameters of just bottled olive oils. As mentioned, since the experimental design was unbalanced, the existence of a statistical significant interaction effect (lighting conditions × storage date) was assessed and interpreted using graphs of means instead. Since ΔK values kept almost constant (varying from 0.00 to 0.01) regardless the storage date and lighting storage conditions, this parameter was not statistically analyzed. The results (data not shown) pointed out that: (i) for FA there was no significant interaction effect between the main effects (parallel lines in the graph of estimated marginal means); (ii) for PV, K_{232}, K_{270} and OS the interaction effects were statistically significant (non-parallel lines) but, with the exception of the latter parameter, the interaction effects were additive (non-crossing lines) indicating that in those cases each main effect could be evaluated separately. Therefore, the influence of the storage date on EVOO's physicochemical parameters, regardless the lighting conditions, was further assessed using one-way ANOVA and the Tukey's test. The possible effect of the

storage lighting conditions, at each storage date, was investigated using a t-Student test. The existence of similar time-evolution trends were discussed using R-Pearson linear correlation coefficients.

One-way ANOVA showed that, for each lighting storage condition, the storage date significantly affected, at a 5% significance level, FA, PV, K_{232}, K_{270} and OS values (P-values < 0.0001). Also, except for K_{270} coefficient, statistically similar linear time-evolution trends were found during storage time ($0.89 \leq R$-Pearson ≤ 0.999), regardless the lighting exposition. Based on Fig. 1 and on the results of the Tukey's test it can be stated that, in general, FA, K_{232}, K_{270} levels of olive oils stored at light or dark significantly increase with the storage time, being more evident after 9 months of storage. The increase of FA, K_{270} and PV over time is in accordance with previous studies (Afaneh et al., 2013; Ben-Hassine et al., 2013; Dabbou et al. 2011; Fadda et al., 2012; Rababah et al., 2011). K_{270} increase may be attributed to the formation of secondary products due to auto-oxidation. PV increased during the first 3 months of storage and then remained almost constant, which can be due to the primary oxidation that occurs in the presence of air in the bottle headspace (and enhanced by the exposition to light) leading to the production of peroxides until an equilibrium is reached between the production and decomposition of peroxides to secondary products. On the other hand, the OS values of olive oils decrease during the storage period but only slightly, which may be attributed to their expected high total phenolic contents, promoted by the low maturation indexes (1–3) of the olives used in the production, the low extraction temperature (~22 °C), and the olive cultivars used (cvs. Cobrançosa, Verdeal Transmontana and Madural (~10%)), which confer high resistance to oxidation (Sousa, Malheiro, Casal, Bento, & Pereira, 2014, 2015). After one-year of storage, independently of the lighting conditions, the maximum mean FA and PV levels ($0.28 \pm 0.00\%$ oleic acid and 7 ± 2 mEq O_2/kg, respectively) of olive oils did not exceed the legal limits (0.8% oleic acid and 20 mEq O_2/kg, respectively; ECC regulation n°2568/91), which could be tentatively attributed to the expected initial high contents in phenolic compounds considering the specific olive cultivars used (Sousa et al., 2014, 2015). Contrary, and as reported in the literature (Afaneh et al., 2013; Ben-Hassine et al., 2013), after 9–12 months, olive oils stored at dark (mean K_{232} values equal to 2.9 ± 0.7) or exposed to light (mean K_{270} values $\geq 0.26 \pm 0.05$) exceeded the legal thresholds for EVOO classification (2.5 and 0.22, respectively; ECC regulation n°2568/91). The results of the t-Student analysis also pointed out that lighting storage conditions (i.e., dark or natural/artificial light exposition) did not significantly affect olive oils' FA or OS along the one-year of storage. The similar OS found could be due to the use of dark amber glass bottles, which are known to minimize olive oils' quality degradation during storage and, also, because the light exposition was not intensive since it was achieved using fluorescent lamps (~482 lux) that mimic normal supermarket storage environment. Contrary, PV, K_{232} and K_{270} levels of olive oils with the same storage time, were significantly affected by the lighting storage conditions. Unexpectedly, PV of olive oils stored at light were statistically greater than those of olive oils stored at dark although the absence of light should delayed peroxide decomposition (Afaneh et al., 2013; Fadda et al., 2012). Similarly, the exposition of olive oils to light significantly increased K_{270} levels, oppositely to the findings of Ben-Hassine et al. (2013). Finally, as expected, olive oils stored under dark conditions showed significantly greater K_{232} values than those exposed to light.

Moreover, statistically significant linear correlations (P-value < 0.05) were found between some physicochemical quality parameters and the OS values, corresponding to changes that occurred during storage time under different lighting conditions. During storage (0–12 months), as previously reported (Rotondi
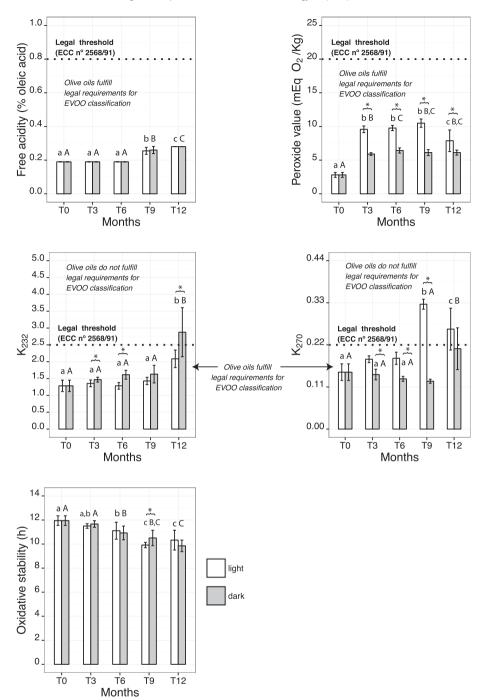
Fig. 1. Time evolution of olive oils' physicochemical parameters during storage. Dashed lined: legal thresholds (Commission regulation (ECC) n° 2568/91). Storage time: one-way ANOVA plus Tukey's test (different lowercase or uppercase letters indicate a significant difference, 5% significance level). Lighting storage conditions: t-Student test (asterisk indicate a significant statistical difference, 5% significance level).

et al., 2004), it was also found a significant negative correlation ($-0.97 < R$-Pearson < -0.86) of OS with FA (for light and dark conditions), with K_{232} extinction coefficient (for dark condition) and for K_{270} values (for light condition), i.e., olive oils with greater FA had lower OS, K_{232} and K_{270} levels.

Finally, it should be emphasized, as previously discussed, that based on the physicochemical quality parameters the initial high-quality blend EVOOs do not comply "extra-virgin" label re-quirements after 9–12 months of storage, since at least two pa-rameters (K_{232} and K_{270}) did not meet the required legal limits.

Indeed, the use of glass containers to store olive oil is not consensual in the literature, being described as suitable (Gómez-Alonso et al., 2007; Pristouri et al., 2010; Torres & Maestri, 2006) and inappropriate (Garrido-Delgado et al., 2015; Mendéz & Falqué, 2007; Samaniego-Sánchez, Oliveras-López, Quesada-Granados, Villalón-Mir, & Serrana, 2012; Vekiari, Papadopoulou, & Kiritsakis, 2007) packing material. This fact, show the perti-nence of developing electrochemical devices for indirectly assessing shelf-life of stored EVOOs, by monitoring physico-chemical quality parameters.
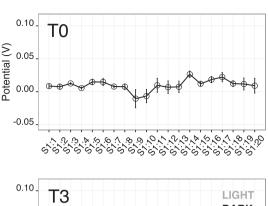
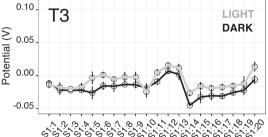## 3.2. E-tongue signal profiles of olive oils during storage

Each electrochemical analysis provided 40 potentiometric signals (for the 20 sensors and the respective replicas) varying from −0.25 V to +0.35 V, showing each pair of sensor/sensor-replica slight signal differences due to the slight variations of the membrane composition, transparency and porosity attributed to the drop-by-drop technique applied, which may lead to the formation of inhomogeneous membranes (Dias et al., 2014). The magnitude of the signal profiles recorded with each sensor membrane of the E-tongue could be tentatively attributed to the expected content in polar compounds of the hydroethanolic extracts (Veloso et al., 2016). Although the voltage signals were of similar magnitude for all sensors, to overcome possible undesired signal drift effects, considering the large analysis time interval (assays performed during a one-year period), the sensor signals recorded for each olive oil extract and time-period were corrected by subtracting the average signal (+0.04 V to +0.22 V) recorded for a standard solution of gallic acid (~1 × 10^{-3} mol/L) at each time-period by each sensor. The final corrected sensor signals varied between −0.34 V and 0.19 V. Fig. 2 exemplifies the E-tongue signal average profiles (together with the related standard deviations) recorded with one of the two multisensory array (which comprised 20 different lipidic-based sensors). As can be inferred, olive oils with different storage time periods and stored at different lighting conditions showed different electrochemical profiles and signal magnitudes. The overall signal variability, which can be tentatively attributed to the changes of the physicochemical and/or sensorial attributes of olive oils that naturally occur during storage, may be further used to verify the capability of the E-tongue for monitoring the time-evolution of the olive oils' physicochemical quality parameters.
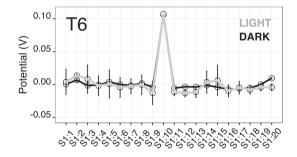
3.3. Estimation of EVOO's physicochemical levels using the E-tongue device
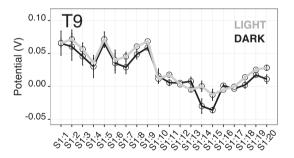
The potential of the E-tongue device to enable the quantification of quality physicochemical parameter levels during olive oil storage (namely PV, K_{232}, K_{270} and OS) was evaluated (FA level was not considered due to the narrow concentration interval found for the olive oils analyzed: from 0.19 to 0.28). Indeed, it was meant to perform a quantitative study in opposition to the usual qualitative approaches, such as the exploratory unsupervised (e.g., principal component analysis and cluster analysis) and supervised (e.g., linear discriminant analysis) multivariate techniques, which have already proven to be suitable for olive oil storage conditions evaluation using electrochemical techniques (Cosio et al., 2007). The feasibility of the aimed quantitative strategy would enable to envisage a possible application of E-tongue devices as a quality control on-line tool in the olive oil field.

For that purpose, MLR-SA models based on the most informative sub-sets of sensors selected using the SA algorithm (among the 40 lipid polymeric sensors comprised in the E-tongue device), PCR and PLS models using all the 40 E-tongue sensors and a number of PCs that minimizes the RMSE, were established and their performance assessed using LOO-CV procedure. The results (Table 1) showed that MLR-SA approach possessed the best predictive capability for quantifying the 4 physicochemical parameters evaluated. Indeed, as pointed out by the results of Table 1, the use of full-sensor multivariate statistical methods (PCR and PLS models) resulted in less reliable prediction models and could not minimize the possible noise effect of using more sensors (40 against 22–26 sensors). The predictive performances of the MLR-SA models were further assessed by means of the repeated K-fold-CV procedure. The results from the two internal cross-validation procedures implemented (Figs. 3–6) show that the MLR-SA models established using the
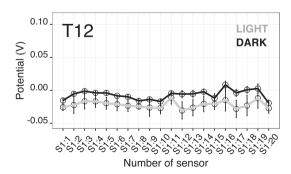

Fig. 2. E-tongue corrected mean signal profiles (error bars − related standard deviations) recorded during the potentiometric analysis of olive oils' hydroethanolic extracts along storage (storage time: T0, T3, T6, T9 and T12 − initial time and 3–12 months of storage; storage respectively; storage lighting conditions: dark − black full lines and light − grey full lines).

Table 1
Comparison of the predictive performances, for LOO-CV procedure, of the best MLR-SA, PCR and PLS models established based on the E-tongue signals profiles, for assessing peroxide values (PV, mEq O_2/kg), extinction coefficients (K_{232} and K_{270}) and oxidative stability (OS, h) of olive oils stored during one year under different lighting conditions.

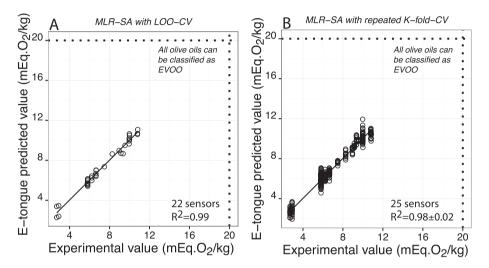| Physicochemical parameters	Multivariate statistical models (results for LOO-CV procedure)					
	MLR-SA		PCR		PLS	
	Nº sensors	R^2	Nº PCs	R^2	Nº PCs	R^2
PV	22	0.990	13	0.735	5	0.756
K_{232}	23	0.990	11	0.768	4	0.765
K_{270}	26	0.983	10	0.712	6	0.751
OS	26	0.987	8	0.762	5	0.793

PCs: principal components.

Fig. 3. Quantification of olive oils' *PV* using E-tongue-MLR-SA models: (A) leave-one-out cross-validation ($R^2_{LOO-CV} = 0.99$; 22 sensor signal profiles); (B) repeated K-folds cross-validation (K = 7 folds with 10 repeats; $R^2_{repeated\ K-folds} = 0.98 \pm 0.02$; 25 sensor signal profiles). Dashed lines: EVOOs' correct classifications or misclassifications according to legal thresholds (Commission regulation (ECC) nº 2568/91).

Fig. 4. Quantification of olive oils' K_{232} values using E-tongue-MLR-SA models: (A) leave-one-out cross-validation ($R^2_{LOO-CV} = 0.99$; 23 sensor signal profiles); (B) repeated K-folds cross-validation (K = 7 folds with 10 repeats; $R^2_{repeated\ K-folds} = 0.96 \pm 0.05$; 23 sensor signal profiles). Dashed lines: EVOOs' correct classifications or misclassifications according to legal thresholds (Commission regulation (ECC) nº 2568/91).
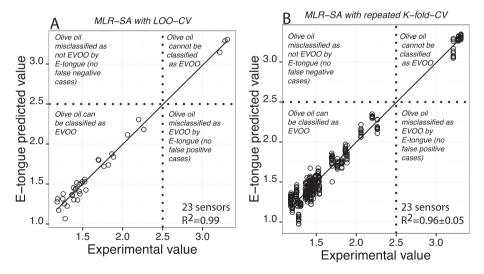
potentiometric signal profiles of 22–28 E-tongue sensors, depending on the parameter, enabled a satisfactory quantification of physicochemical quality parameters (LOO-CV: $R^2 > 0.98$; and, repeated K-fold-CV: $R^2 \geq 0.96$), pointing out the possibility of using the E-tongue as a practical monitoring tool for evaluating the changes of key olive oil's quality physicochemical parameters during storage. As previously discussed, the repeated K-fold-CV procedure was used to minimize the risk of data over-fitting (which could result in over-optimistic model performance) and for that, the olive oils dataset was split into 7 folds (K-folds = 7), meaning
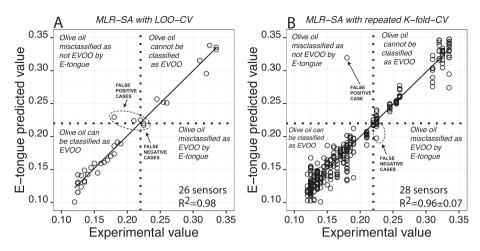
Fig. 5. Quantification of olive oils' K_{270} values using E-tongue-MLR-SA models: (A) leave-one-out cross-validation ($R^2_{LOO-CV} = 0.98$; 26 sensor signal profiles); (B) repeated K-folds cross-validation (K = 7 folds with 10 repeats; $R^2_{repeated\ K-folds} = 0.96 \pm 0.07$; 28 sensor signal profiles). Dashed lines: EVOOs' correct classifications or misclassifications according to legal thresholds (Commission regulation (ECC) nº 2568/91).
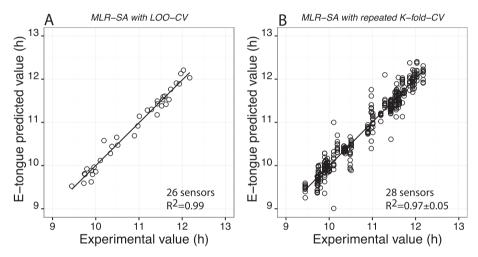

Fig. 6. Quantification of olive oils' *OS* values (Rancimat assays) using E-tongue-MLR-SA models: (A) leave-one-out cross-validation ($R^2_{LOO-CV} = 0.99$; 26 sensor signal profiles); (B) repeated K-folds cross-validation (K = 7 folds with 10 repeats; $R^2_{repeated\ K-folds} = 0.97 \pm 0.05$; 28 sensor signal profiles).

that at least 15% of independent data was used for internal validation during each one of the 10 repetition cycles, increasing the accuracy of the estimates and minimizing any bias effect. This quantification capability demonstrated by the E-tongue device could be tentatively attributed to the changes of nature and amount of the polar compounds extracted from the olive oils at each storage date, due to the natural occurrence of oxidation processes, affecting the intensity of olive oil sensorial characteristics (e.g., pungency, astringency and bitterness), which may be detected by the E-tongue.

Also, the capability of quantifying three physicochemical quality parameters (PV, K_{232} and K_{270}), which levels must be evaluated to verify the fulfillment of the legal requirements for classifying an olive oil as EVOO, represents a proof-of-principle that the electrochemical device may be applied as a complementary tool for olive oil analysis. Indeed, it can be easily verified (Fig. 3) that for all the olive oils analyzed during the one-year storage time, the estimated PV levels with the E-tongue were quite lower than the maximum legal value of 20 mEq O_2/kg, and so all olive oils would be classified as EVOO according to this physicochemical parameters likewise to the classification that would be made based on the classical titration technique. Similarly, based on the K_{232} values calculated from

the E-tongue data (Fig. 4), olive oils would be correctly classified as EVOO or not (K_{232} values ≤ 2.5 or > 2.5, respectively) in total accordance with the conclusions that could be drawn based on the spectophotometric classical analysis. For both cases, the E-tongue/MLR-SA quantification method did not reveal any false negative or false positive olive oil misclassification. On the contrary, the quantification of the K_{270} levels using the E-tongue device (Fig. 5) led to some false negative olive oils classifications (i.e., EVOO misclassified: (K_{270})$_{estimated\ E-tongue}$ > 0.22) and some false positive olive oils classifications (i.e., olive oils misclassified as EVOO: (K_{270})$_{estimated\ E-tongue}$ < 0.22). However, the number of olive oils that could be misclassified based on the electrochemical analysis represented less than 15% of the 36 olive oil bottles analyzed during the one-year storage time (~4 olive oils), which is quite satisfactory considering the reduction of the time and cost of the assay compared to the classical standard technique. Finally, the capability of estimating olive oils' OS from the E-tongue analysis (Fig. 6) may envisage a significant reduction of the analysis time reduction, since instead of carrying out a rancimat assay during a long period of time (hours or even days) an accurate estimate of the OS value could be electrochemically performed in few minutes.

The overall satisfactory performance was further demonstrated

Table 2
Linear regression lines obtained for the representation of predicted peroxide values (PV, mEq O$_2$/kg), extinction coefficients (K_{232} and K_{270}) and oxidative stability (OS, h) of olive oils stored during one year under different lighting conditions (using MLR-SA models plus E-tongue data) versus experimental data, for LOO-CV and repeated K-fold-CV procedures: slope, intercept values and respective 95% confidence intervals (CI).

Regression line parameters	Physicochemical quality parameters of olive oils during storage (at dark and light conditions)							
	PV		K_{232}		K_{270}		OS	
	LOO-CV	*Repeated K-fold-CV*	*LOO-CV*	*Repeated K-fold-CV*	*LOO-CV*	*Repeated K-fold-CV*	*LOO-CV*	*Repeated K-fold-CV*
R^2	0.990	0.970	0.990	0.959	0.983	0.903	0.987	0.944
Slope	0.998	1.001	0.989	0.994	0.989	1.023	0.989	1.009
Slope CI[a]	[0.949, 1.047]	[0.982, 1.019]	[0.939, 1.039]	[0.976, 1.012]	[0.924, 1.053]	[0.988, 1.058]	[0.933, 1.046]	[0.983, 1.034]
Intercept	0.039	0.022	0.018	0.009	0.003	−0.005	0.106	−0.093
Intercept CI[b]	[−0.335, 0.413]	[−0.118, 0.161]	[−0.070, 0.106]	[−0.022, 0.040]	[−0.010, 0.016]	[−0.012, 0.002]	[−0.511, 0.722]	[−0.372, 0.185]

[a] 95% slope confidence interval.
[b] 95% intercept confidence interval.

for the two internal-validation procedures applied (LOO-CV and repeated K-fold-CV) since, as can be inferred from Figs. 3–6, plotting olive oil quality physicochemical levels estimated using the E-tongue signal profiles and the previously established MLR-SA models versus the experimental data generated by the classical analytical techniques, linear straight lines were obtained (slope and intercept values and the 95% confidence intervals are gathered in Table 2). These results demonstrate that, at 5% of significance level, the respective slopes and intercept values (of each regression line) are statistically equal to the theoretical expected values (slope equal to one; intercept equal to zero). These findings confirmed the robustness of the proposed MLR-SA models and their possible practical application. Indeed, the overall satisfactory quantitative performance achieved is indicative that the proposed approach could be implemented for routine olive oils quality analysis, allowing an accurate estimative of the most relevant physicochemical quality parameters as well as the OS in a 5 min single electrochemical run that requires a small amount of olive oil sample (10 g, ~10 mL), which are major advantages compared to the time-consuming classical reference analytical techniques.

The quantitative potential of the E-tongue-MLR-SA method to simultaneous quantify PV, K_{232}, K_{270} and OS values of olive oils, constitutes the main contribution of the present work and may be seen as a proof-of-concept of a novel applicability field of electrochemical-based strategies within olive oil quality control during storage. Nevertheless, the capability of simultaneously evaluating the FA levels of olive oils must be evaluated in future works in order to establish the E.-tongue device as a complementary/alternative tool for physicochemical quality assessment of olive oils.

4. Conclusions

The study carried out showed that, in general, the EVOOs' physicochemical quality parameters (FA, PV, K_{232} and K_{270}) followed the expected time-evolution during storage, although the influence of lighting conditions on PV and K_{232} values was contrary to the reported in the literature. On the other hand, it was also observed that some of the premium EVOOs studied, although stored in recommended dark amber bottles, suffered a degradation of the K_{232} and K_{270} quality parameters in such a level that no longer could be classified as EVOOs after 9–12 months of storage. This fact, which has also been described in the literature, points out the real need of quantitatively monitoring the levels of these legal required parameters throughout the olive oils' storage time in order to ensure the correctness of the olive oils label (and indirectly their shelf-life) as well as to enhance the consumers' confidence when

purchasing this type of high-value food product.

In this context, the potentimetric E-tongue multi-sensor device coupled with MLR-SA models exhibited satisfactory predictive potential to assess PV, K_{232}, K_{270} and OS values of olive oils during one-year of storage. Globally, the olive oils' physicochemical levels assessed by the E-tongue procedure were in agreement with those determined using the time-consuming analytical standard techniques. Nevertheless, it should be remarked that K_{270} levels evaluated from the E-tongue may lead to some, although few, EVOO misclassifications (both false negative and positive cases). Finally, since a single electrochemical assay enable the simultaneously quantification of physicochemical olive oil parameters, reducing the analysis time and cost, the application of this kind of electrochemical device in the olive oil industry may be foreseen in a near future as a practical and accurate quality control tool. However, prior to the possible implementation of this proof-of-concept, a significant larger number of olive oil samples must be evaluated.

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