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Evaluation of extra-virgin olive oils shelf life using an electronic tongue—chemometric approach

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Abstract Physicochemical quality parameters, olfactory and gustatory–retronasal positive sensations of extra-virgin olive oils vary during storage leading to a decrease in the overall quality. Olive oil quality decline may prevent the compliance of olive oil quality with labeling and significantly reduce shelf life, resulting in important economic losses and negatively condition the consumer confidence. The feasibility of applying an electronic tongue to assess olive oils' usual commercial light storage conditions and storage time was evaluated and compared with the discrimination potential of physicochemical or positive olfactory/gustatory sensorial parameters. Linear discriminant models, based on subsets of 5–8 electronic tongue sensor signals, selected by the meta-heuristic simulated annealing variable selection algorithm, allowed the correct classification of olive oils according to the light exposition conditions and/or storage time (sensitivities and specificities for leave-one-out cross-validation: 82–96 %). The predictive performance of the E-tongue approach was further evaluated using an external independent dataset selected using the Kennard–Stone algorithm and, in general, better classification rates (sensitivities and specificities for external dataset: 67–100 %) were obtained compared to those achieved using physicochemical or sensorial data. So, the work carried out is a proof-of-principle that the proposed electrochemical device could be a practical and versatile tool for, in a single and fast electrochemical assay, successfully discriminate olive oils with different storage times and/or exposed to different light conditions.

Keywords Extra-virgin olive oil · Sensory attributes intensity perception · Electronic tongue · Linear discriminant analysis · Simulated annealing algorithm

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Introduction

Extra-virgin olive oils (EVOOs) are quite appreciated by consumers due to their quality, sensory attributes and potential health benefits. So, the compliance of olive oil quality with labeling is of great importance during storage and commercialization time. However, since olive oils contain high levels of polyphenolic compounds, they are quite prone to deterioration during storage due to oxidation caused namely by exposition to light, which may lead to significant changes on olive oils sensory attributes with

loss of positive sensations [1, 2]. Indeed olive oil quality is significantly influenced by storage conditions like time, temperature, type of packing material, exposition to air and/or light [1–6]. In fact, during storage the levels of some physicochemical parameters (e.g., free acidity, FA; peroxide values, PV; K_{232} and K_{270} extinction coefficients) may undesirably increase [1, 2, 5, 7–9], oxidative stability (OS) may decrease, positive olfactory and/or gustatory sensorial attributes may suffer dramatic changes [10, 11], resulting in an overall quality decrease that may even lead to the appearance of organoleptic defects [2, 12]. These issues may even arise in EVOO stored in dark glass bottles, which several authors pointed out as the most appropriate packing material for olive oils [6, 8, 13]. Olive oils physicochemical and organoleptic quality losses during storage are inevitable due to the lipid oxidation reactions that start immediately after the olive oil extraction. Since these reactions are catalyzed by light and heat, olive oils deterioration may be less pronounced for oils with higher antioxidant levels (e.g., phenolic compounds and tocopherols) or if an appropriate packaging material that minimized light exposition is used [2]. However, several authors pointed out that glass containers, which are commonly used, may be unsuitable for olive oil storage on supermarket shelves [7, 14–16]. Also, recently, Sinesio et al. [11] verified that a higher content of phenolic compounds in an olive oil did not result in greater stability of the sensory properties during olive oil storage. Moreover, it has been reported that some taste-active olive oil phenols may affect negatively bitterness and pungency positive sensory attributes of EVOO during storage [10]. So, the development of fast, simple and low-cost analytical devices to evaluate olive oils storage conditions (storage time and light/dark exposition) would be of great commercial interest for producers and consumers. Cosio et al. [17] demonstrated that the use of an electronic nose (E-nose) or an electronic tongue (E-tongue), individually or combined, together with chemometric tools could be successfully applied to differentiate olive oil samples stored under different light conditions (light exposition at shelf and darkness) and storage time (1 or 2 years). In that work, a commercial E-nose with 22 sensors (10 metal oxide semiconductor field effect transistors and 12 metal oxide semiconductors) and an E-tongue that comprised a flow injection analysis apparatus with amperometric detection were used. The quite satisfactory results reported were based on internal cross-validation procedures that may be an over-optimistic procedure since overfitting may occur. Also, the potential of the proposed methodology was not checked for the initial storage period (3–6 months), which is of major relevance since the main changes in olive oil sensorial sensations occur during the first months of storage and, furthermore, the shelf life of some olive oils stored in glass bottles may not exceed 6 months [6, 8]. So, in the present work, the possibility of using a potentiometric E-tongue, with nonspecific cross-sensitivity lipid membranes and linear discriminant analysis (LDA) coupled with the simulated annealing (SA) variable selection algorithm, to assess lightning storage conditions (light versus dark) and storage time (0, 3, 6, 9 and 12 months), was evaluated using external data for validation purposes. Electrochemical multisensors (potentiometric and/or voltammetric devices), including E-noses, E-tongues or fused approaches, were recently applied with success to evaluate olive oils according to quality grade, geographic origin or olive cultivar [18–29] as well as to assess olive oils sensory attributes [22, 30–32]. Furthermore, the profiles of positive sensorial attributes during storage were assessed by a sensorial panel and the observed time evolution trends were discussed and related with the olive oils physicochemical quality parameters previous reported [33]. Finally, the performance of the electrochemical approach for discriminating olive oils different storage conditions was further compared to those based on the physicochemical or organoleptic data.

Materials and methods

Olive oil samples

The full design consisted of 36 samples of blend EVOOs (from the same lot and stored in dark amber glass bottles of 250 mL), produced in the Mirandela region, located at North of Portugal. The selected EVOO sample was an olive oil with a Protected Designation of Origin (PDO), having the designation of "Azeite de Trás-os-Montes," since it was obtained from olives (mainly from cultivars Cobrançosa and Verdeal Transmontana, with 10 % of olives of cultivar Madural, according to the producer information) collected at the initial maturation indexes (1–3) and extracted at low temperatures (near 22 °C). Samples of 4 fresh olive oils bottles were analyzed after packing, with respect to sensory attributes and electrochemical signal profiles (coded as "T0"). The other 32 samples were kept in the laboratory at ambient temperature (varying from 17 to 25 °C during different time periods till 1 year) under two storage lighting conditions that tried to mimic real-storage conditions of supermarkets: 16 samples were stored in the dark, protected from any exposition to daylight or any artificial light; other 16 samples were stored in laboratory open shelves exposed to natural daylight (entered through 3 windows but without direct exposition to sun) and artificial light (from 8 fluorescent lamps, Philips TL-D36 W/840) that remained connected for 14 h a day. Similarly to the storage conditions at supermarket facilities, each lamp provided a luminous flux of 3250 lm (according to the manufacturer information) that

illuminated a 6 × 9 m^2 laboratory area, corresponding to approximately 482 lx). For each lighting condition evaluated (coded as "dark" and "light"), a group of 4 samples were also analyzed regarding their sensory attributes and electrochemical signal profiles at 4 storage periods ("T3", "T6", "T9" and "T12" for olive oils stored during 3, 6, 9 and 12 months, respectively), resulting in a (2 × 4 × 4) experimental factorial design. It should be remarked that the number of independent olive oil bottles analyzed per treatment is in accordance with those reported by several authors regarding the study of the possible effects of different storage conditions in the quality and physicochemical contents of olive oils [1, 2, 5, 10, 16, 33–35].

Olive oils physicochemical quality parameters and oxidative stability data

The olive oil's quality parameters [free acidity (FA), peroxide value (PV) and the specific coefficients of extinction at 232 and 270 nm (K_{232}, K_{270}, and ΔK)] were determined according to the European Union standard methods [36] and the oxidative stability (OS) using the Rancimat 743 apparatus (Metrohm CH, Switzerland). The abovementioned parameters time evolution during the 1 year of storage and under different light exposition conditions were previously reported [33].

Olive oil sensory analysis

Olive oil samples were subjected to sensory assessment following the methods, grading scales and standards adopted by the International Olive Council (COI), namely COI/T.20/Doc. No 15/Rev. 6 [37] and COI/T.30/Doc. No 17 [38], as previously described [20, 32]. Each sample was subjected to the judgment of 4 trained panel members (instead of the 8 recommended by COI) that classified the samples according to olfactory sensations, gustatory–retronasal sensations and final olfactory–gustatory sensations, similarly to the procedure previously applied by the research team [20, 32]. Also, it should be pointed out that the expertise and skills of the 4 panelists, which integrated the sensory panel used in this work, are well recognized by that pairs, being often invited to be part of judging panels in national and international olive oil competitions (e.g., "Mario Solinas Prize"—Spain, "Terra Olivo"—Spain, "Concurso Nacional de Azeites"—Portugal), where a full discriminative analysis of each organoleptic attribute is required. For olfactory sensations, the following attributes were measured: olive fruitiness (0–7 scale); other fruits (0–3 scale); green (grass/leaves, 0–2 scale); other positive sensations (0–3 scale) and harmony (0–20 scale). Concerning gustatory–retronasal sensations were evaluated for the olive fruitiness (0–10 scale); sweet (0–4 scale); bitter (0–3 scale); pungent (0–3 scale); green (grass/leaves, 0–2 scale); other positive sensations (0–3 scale) and harmony (0–20 scale). A final olfactory–gustatory sensation for each sample was also pointed that conjugating all the organoleptic sensations pointed out the complexity (0–10 scale) and persistence (0–10 scale).

E-tongue device

The E-tongue multi-sensor device included two print-screen potentiometric arrays containing each one 20 sensors (3.6 mm of diameter and 0.3 mm of thickness) [19, 33]. The sensor membranes contained a lipid additive (octadecylamine, oleyl alcohol, methyltrioctylammonium chloride or oleic acid; ≈3 %); a plasticizer (bis(1-butylpentyl) adipate, dibutyl sebacate, 2-nitrophenyl-octylether, tris(2-ethylhexyl)phosphate or dioctyl phenylphosphonate; ≈65 %) and high molecular weight polyvinyl chloride (PVC; ≈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 chosen considering previous works of the research team [39], which showed the satisfactory signal stability over time (%RSD < 5 %) and repeatability (0.5 % < %RSD < 15 %) toward the basic standard taste compounds (sweet, acid, bitter, salty and umami). Also, the lipid polymeric membranes were selected since they enable interactions with taste substances via electrostatic or hydrophobic interactions [40]. As in previous works [19], each sensor was coded 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).

E-tongue analysis: sample preparation and potentiometric assays

Olive oils were extracted using water–ethanol solutions (80:20 v/v) and electrochemically analyzed as previously described [19]. Ethanol (analytical grade, Panreac, Barcelona) and deionized water (type II) were 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 was 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 [20]. The mixture was left at ambient temperature during 60 min, after which, 40.0 mL (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 be belong 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 1×10^{-3} mol/L of gallic acid (purchased from Sigma with a minimum purity ≥99 %) was analyzed before and after each olive oil measurement series. Possible signal drifts were overcome 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.

Statistical analysis

The possible effects of storage conditions (light/dark conditions or storage time) on EVOO's physicochemical parameters and sensorial sensations were evaluated by means of a Student's *t* test (for comparing light versus dark stored conditions for each storage time) and by means of a one-way ANOVA followed, when appropriate, by the Tukey's post hoc multi-comparison test, for assessing the effect of the storage time 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. Linear discriminant analysis (LDA) was used as a supervised pattern recognition method to infer about the capability of the E-tongue to correctly classify the EVOO according to the storage time (i.e., "T0", "T3", "T6", "T9" and "T12" for 0, 3, 6, 9 and 12 months, respectively) or storage conditions ("fresh," "dark" and "light"). Similarly, LDA was also applied for evaluating the qualitative classification capability of physicochemical and sensorial data. Detailed information regarding multivariate statistical tools can be found in the literature [41, 42]. 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 [43–45]. 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 subset of sensors under evaluation (possible combinations of 2–39 sensors), the set of sensors chosen was the one that maximized tau2 value [45]. To evaluate the LDA classification models, a leave-one-out cross-validation (LOO-CV) procedure was applied. This process may lead to over-optimistic results, although it has proven to be an adequate procedure when the number of samples is low [19, 46]. To minimize the risk of over-fitting, the initial set of olive oil samples (i.e., 36 bottles of olive oil) was also split into two datasets, one for training (for which the LOO-CV was applied), comprising two-thirds of the initial samples (i.e., data recorded for 24 different bottles of the EVOO) and the other for testing with the remaining one-third of the samples (i.e., data regarding 12 bottles of the EVOO) using the Kennard–Stone algorithm. For each splitting procedure, it was always ensured that representative olive oil samples belonging to a particular level of the main factors under evaluation (i.e., storage time and/or storage conditions) were simultaneously chosen for training and testing datasets. The Kennard–Stone sample selection algorithm is a sequential method that covers the experimental region uniformly [47]. The procedure consists on the selection of the next sample (candidate object) as the one that is most distant, based on the Euclidean or Mahalanobis distance, from those object already selected (calibration objects). At the initial step, the two objects chosen are those that are most distant from each other, or preferably, the one closest to the mean. From all the candidate points, the next selected point is the one furthest distant from those already selected and added to the set of calibration points. To do this, the distance from each candidate point i_0 to each point i that had been already selected is evaluated and that corresponding to the smallest distance is identified. Among these, that whose distance is maximal is chosen. In the absence of strong irregularities in the factor space, the procedure starts first selecting a set of points close to those selected by the D-optimality method, i.e., on the borderline of the dataset (plus the center point, if this is chosen as the starting point). It then proceeds to fill up the calibration space. Kennard and Stone [47] called their procedure a uniform mapping algorithm that yields a flat distribution of the data which is more suitable for a regression model. All statistical analysis was performed using the Subselect [45, 48] and MASS [49] packages of the open-source statistical program R (version 2.15.1) at a significance level of 5 %.

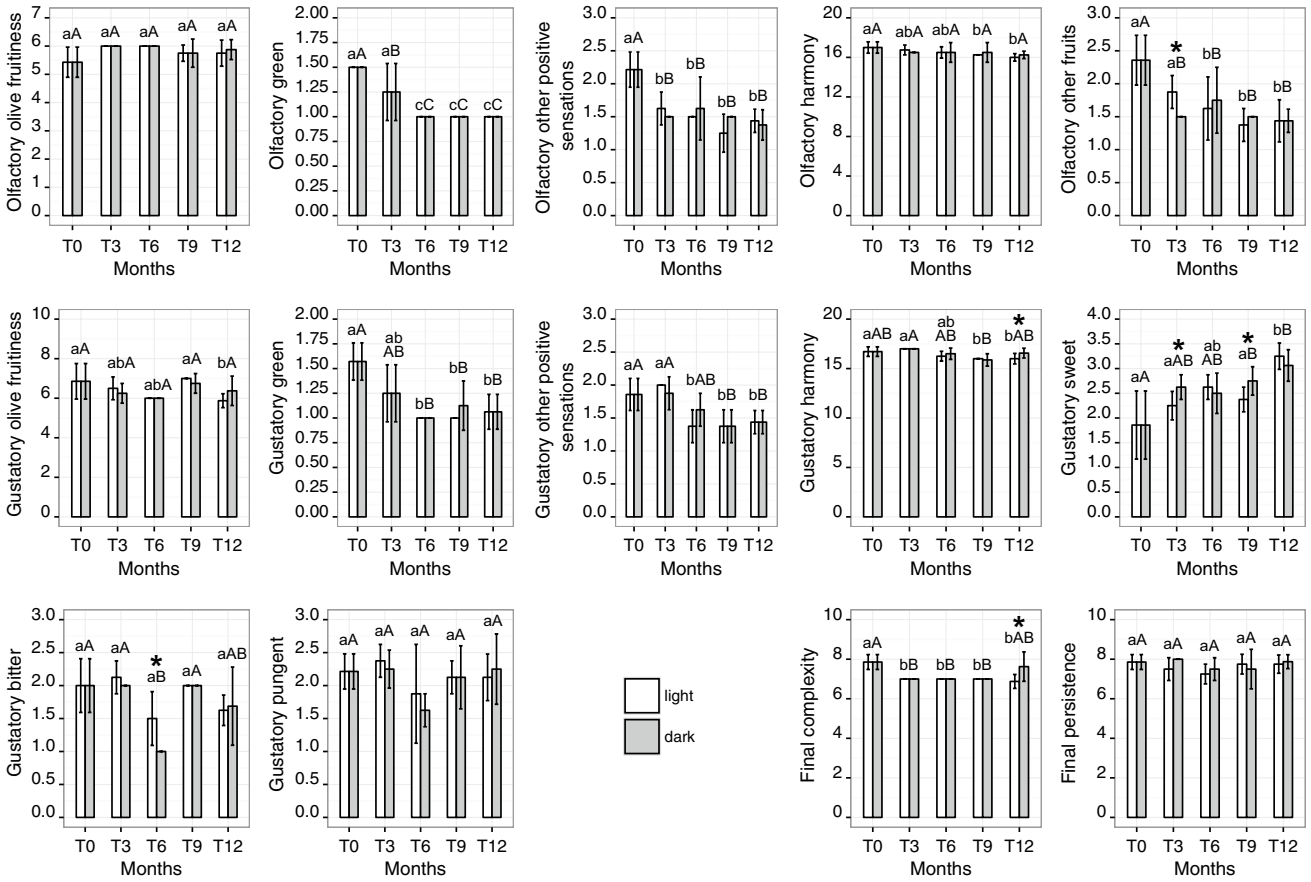

Fig. 1 Time evolution of EVOO's sensorial attributes intensity perception along 1 year of storage (0 months, T0; 3 months, T3; 6 months, T6; 9 months, T9; and 12 months, T12) and under different light/dark storage conditions (light and dark). Each attribute was assessed following the guidelines and grade scales established by the International Olive Council [24, 25] (The statistical significance effect of the storage time on the sensorial attributes, for each light exposition storage condition studied, was evaluated by one-way ANOVA followed by Tukey's post hoc multiple comparison test: *different lowercase* or *uppercase letters* at the *top* of the *bars* indicate a significant statistical difference, at a 5 % significance level, for olive oils stored at light or dark, respectively. Significance of light storage conditions for each storage period time was assessed by Student's *t* test: *asterisk* means the existence of a significant statistical difference, at a 5 % significance level)

Results and discussion

Time evolution trend of EVOO's overall quality during storage

The levels of the main physicochemical quality parameters and positive sensorial attributes of EVOO change during storage toward a decrease in olive oil global quality [1, 2, 10, 11, 34, 35, 50]. These changes that occur during storage and depending on the light/dark conditions may lead to the appearance of olive oil organoleptic defects (e.g., rancidity) simultaneously with the increase in some physicochemical parameter levels (e.g., FA, PV, K_{232} and/or K_{270}) to values greater than the legal thresholds [36], which make the commercialization of olive oils as EVOO impossible, with the related loss of profit. Indeed, recently the research team [33] showed that the physicochemical quality parameters and the OS of bottled blend EVOO stored during 1 year at dark or exposed to natural/artificial light (aiming to mimic the usual storage commercial conditions) were quite influenced by the storage time and light conditions, although the first effect was more significant. The less significant effect of the light conditions on the changes observed for physicochemical quality parameters could be due to the fact that olive oils were stored in dark brown glass bottles, which prevent olive oil degradation due to the exposition to light. Nevertheless, a previous study [33] showed that the EVOO in dark brown glass bottles only kept their physicochemical quality during the first 9 months of storage, regardless the storage light conditions, after which the mean K_{232} and K_{270} levels exceeded the legally limits required for keeping possible to use the designation of "extra-virgin" in the olive oil label. Indeed, Garrido-Delgado et al. [16] reported that traditional glass bottles were unsuitable for EVOO storage on

supermarket shelves. The present work tries to complement the study of Rodrigues et al. [33] by including the evaluation of the positive sensorial attributes of olive oils during the 1-year storage period and their time evolution profiles.

Globally (Fig. 1), olive oils stored at different light conditions showed similar time evolution trend profiles were observed for the olfactory and gustatory–retronasal sensations, including olfactory and gustatory harmony notes ($0.80 \leq R$-Pearson ≤ 0.999). Depending on the storage light conditions, slight different time evolution trends (R-Pearson ≤ 0.66) were detected for the final olfactory–gustatory sensations like complexity and persistence.

Also, the results pointed out that not all olive oil's olfactory positive sensations were affected by the storage conditions (time and light/dark conditions) evaluated, as shown in Fig. 1. Although a small increase in the intensity perception of olfactory olive fruitiness sensation could be observed during the first 3 months of storage, as expected since aromas need some time to develop, this olfactory attribute was not significantly influenced by storage time (one-way ANOVA: P value ≥ 0.1040). The intensity perception of the other three olfactory attributes evaluated (other fruits, green and other positive olfactory sensations) significantly decreased with storage time (one-way ANOVA: P value ≤ 0.0003), more drastically during the first 3–6 months of storage remaining then almost constant (P value ≤ 0.0235 and P value ≥ 0.1559 for Tukey's test, respectively). The intensity decrease in some olfactory sensations with storage time was expected due to the possible occurrence of oxidation/hydrolysis of secoiridoids during olive oil storage [11]. Olfactory harmony notes of olive oils were not significantly affected by the storage time remaining almost constant during the one year of storage, for both light/dark storage conditions assessed (one-way ANOVA: P value ≥ 0.2922). On the contrary, light storage conditions did not showed a significant effect on any of the olfactory attributes evaluated, for olive oils with the same time of storage (P value ≥ 0.0577, for Student's t test). Furthermore, globally, for both light/dark storage conditions studied, the evolution trends of the intensity perception of the olive oils olfactory sensations were significantly correlated (R-Pearson ≥ 0.80) with the change of some of the physicochemical quality parameters previous reported [33]. Olfactory olive fruitiness evolution was positively correlated with the observed change of PV (R-Pearson $\geq +0.85$, respectively). Olfactory other fruits sensations, green sensations and other positive sensations were, in general, negatively correlated with PV and K_{270} values ($-0.94 \leq R$-Pearson ≤ -0.80), and positively correlated with the OS ($0.84 \leq R$-Pearson ≤ 0.93). Also, high olfactory harmony notes corresponded to olive oils with lower FA, PV and K_{270} levels ($-0.95 \leq R$-Pearson ≤ -0.80) and with higher OS ($+0.82 \leq R$-Pearson $\leq +0.99$).

Concerning the six gustatory–retronasal positive attributes evaluated, the results (Fig. 1) demonstrated that the storage time significantly influenced the intensity perception of sweet, bitter, green and other positive attributes of the EVOO stored at light or dark conditions (one-way ANOVA: P value ≤ 0.0164). Overall it can be stated that the intensity perception of the olive oils' sweet sensation remained almost constant during the first 9 months of storage and then significantly increases till the 12 months of storage (Tukey's test: P value ≥ 0.0600 and P value ≤ 0.0342, respectively). Opposite trend was found for the intensity perception of green attribute, for which there was a significant decrease during the first 6 months of storage, remaining then almost constant until 1 year of storage (Tukey's test: P value ≤ 0.0130 and P value ≥ 0.1095, respectively). The intensity perception of gustatory other positive sensation did not changed significantly during the first 3 months of storage (Tukey's test: P value ≥ 0.4965), then significantly decreased till the 6 months of storage (Tukey's test: P value ≤ 0.0099) and remained after that almost constant between until the 12 months of storage (Tukey's test: P value ≥ 0.5403). For the bitterness intensity perception, it was not possible to established a clear time evolution trend during the storage period studied, although it would be expected a decrease in the bitter intensity with the storage time [10, 11]. Olive fruitiness intensity perception and gustatory harmony notes were only significantly affected for olive oils stored at light conditions, being no significant effect detected on olive oils stored at dark (one-way ANOVA: P value ≤ 0.0034 or P value ≥ 0.0500, respectively). Olive fruitiness intensity did not changed during the first 9 months of storage (P value ≥ 0.1141 for Tukey's test) and then significantly decreased till 12 months (P value $= 0.0233$ for Tukey's test). The effect detected on the gustatory harmony notes could be mainly attributed to the significant lower harmony level of olive oils stored during 12 months compared with the harmony notes of fresh olive oils or stored up to 3 months (P value ≤ 0.0326, Tukey's test). On the other hand, olive oils' pungency sensation was not significantly affected by the storage time (one-way ANOVA: P value ≥ 0.1422, for both light/dark conditions studied) although it was expected a decrease in the pungency note during the storage [10, 11]. Contrary to the storage time, the light/dark storage conditions evaluated did not significantly influence the intensity perception of any gustatory–retronasal positive attribute (Student's t test: P value ≥ 0.0972, for light versus dark olive oils storage conditions at each storage time), being only detected a slight effect on the gustatory harmony notes, which decreased from 9 to 12 months of storage (Student's t test: P value $= 0.0233$). The inexistence of a significant effect of the light/dark storage conditions on the gustatory positive quality attributes is in disagreement with

the findings of other researchers [34], which reported that extended artificial illumination largely affects the organoleptic sensations of olive oils during storage. Finally, no statistical significant correlations were identified between olive fruitiness, bitter and pungency intensity perceptions and the olive oils' physicochemical quality parameters previously reported [33]. On the other hand, sweeter olive oils had greater K_{232} levels (R-Pearson = 0.85) when stored at light, and higher PV (R-Pearson = 0.86) when stored at dark. Contrary, olive oils with greater green intensities had lower PV (R-Pearson ≤ -0.88). In general, olive oils with greater OS showed higher green intensities, other positive sensations and global harmony notes ($0.80 \leq R$-Pearson ≤ 0.92). Olive oils with greater other positive sensations had lower FA (R-Pearson = −0.87).

For the final olfactory–gustatory notes (Fig. 1), different time evolution profiles were obtained for complexity or persistence levels of olive oils stored at light or at dark conditions. Indeed no significant correlation could be found (R-Pearson ≤ 0.66). The final complexity notes of the olive oils were significantly influenced by the storage time (P value ≤ 0.0092 for one-way ANOVA) for both light/dark storage conditions. However, no statistical significant differences could be identified for olive oils stored at dark (P value ≥ 0.0520, Tukey's test). For olive oils stored at light, it could be concluded that fresh olive oils (just bottled) had a significant greater complexity note than those stored for 3 months or more (P value ≤ 0.0006, Tukey's test), having these last similar complexity notes (P value ≥ 0.9478, Tukey's test). Contrary, the final persistence notes of olive oils stored at light or at dark were not significantly affected by the storage time (P value ≥ 0.3028 for one-way ANOVA). Light exposition conditions during storage did not significantly affect the persistence notes of the olive oils (Student's t test: P value ≥ 0.1340 for all the storage periods). The final complexity notes of olive oils stored at light or dark conditions were statistically similar but significantly higher for olive oils stored at dark for one year compared to those stored at light (Student's t test: P value = 0.0138). Finally, only a significant negative correlation could be detected between complexity notes of olive oils and their PV ($-0.90 \leq R$-Pearson ≤ -0.80). No significant correlations could be identified between persistence sensorial notes and the physicochemical quality parameters. The analysis carried out also pointed out that olive oils stored at light conditions were those that showed the highest number of significant correlations among the respective sensorial attributes evaluated (R-Pearson ≥ 0.80).

E-tongue signal profiles of olive oils during storage

In total, 36 different bottles of the selected EVOO were analyzed with the potentiometric E-tongue during 12 months (4 bottles at the initial time, corresponding to fresh olive oil, and then 8 bottles each 3 months, 4 of them stored at dark and the other 4 exposed to natural/artificial light, simulating the usual storage at supermarket shelves). Prior to the analysis, a sample of 10 g of olive oil, collected from each bottle, was extracted using 100 mL of water–ethanol solutions (80:20 v/v), and then, 50 mL of the ethanolic–aqueous phase was removed and analyzed, allowing to obtain an overall fingerprint of the matrix under analysis, richer in polar compounds that are known to influence the sensorial attributes of olive oils, namely their bitterness, pungency and astringency. Each analysis provided 40 signals (for the 20 sensors and the respective replicas) varying from −0.25 to +0.35 V, showing each pair of sensor/sensor-replica slight signal differences due to the slight membrane composition, transparency and porosity variations attributed to the drop-by-drop technique applied, which may lead to the formation of inhomogeneous membranes [19]. 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 12-month period), the sensor signals recorded for each olive oil extract and time period were corrected by subtracting the average signal (+0.04 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 to 0.19 V.

Discriminant potential of physicochemical parameters, sensorial attributes and E-tongue signals regarding olive oil's light/dark storage conditions and storage time

The potential of discriminating olive oils according to storage time (T0, T3, T6, T9 and T12), light exposition conditions (fresh, dark and light) and the two factors together (T0, T3_Dark; T3_Light; T6_Dark; T6_Light; T9_Dark; T9_Light; T12_Dark and T12_Light), which may simulate usual commercial storage conditions of olive oils, is of utmost interest. Indeed, it is known that, with storage, olive oils' physicochemical quality may decrease, sensorial positive attributes may change, organoleptic defects may arise, which can lead to economic losses as well as to the misleading of the consumers of EVOO, since olive oils, after storage, may not fulfill all requirements for being labeled as "extra-virgin." To assess the possibility of classifying olive oils according to storage time and/or storage light/dark conditions, the 36 olive oils bottles under study were split into two subsets using the Kennard–Stone selection algorithm [47], one for training purposes (training and internal validation set: 75 % of the independent samples) and the other (test set: 25 % of the independent samples) for external validation

Table 1 Potential of physicochemical parameters, sensory attributes or E-tongue potentiometric signals for discriminating olive oils' storage time, light conditions or light–time conditions, based on the LDA-SA models accuracy for internal (LOO-CV) and external validation procedures

Overall performance of the LDA-SA models proposed						
Dataset	Storage time: T0, T3, T6, T9 and T12 months					
	Physicochemical parameters[a]		Sensorial attributes[b]		E-tongue sensors[c]	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Training set (internal LOO-CV)*	67	60	68	70	96	95
Test set (external validation)**	89	80	89	90	89	90
Dataset	Light/dark storage conditions: fresh, dark and light					
	Physicochemical parameters[d]		Sensorial attributes[e]		E-tongue sensor[f]	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Training set (internal LOO-CV)*	96	97	74	79	82	86
Test set (external validation)**	100	100	67	75	67	75
Dataset	Light–time conditions: T0, T3_dark, T3_light, T6_dark, T6_light, T9_dark, T9_light, T12_dark and T12_light					
	Physicochemical parameters[g]		Sensorial attributes[h]		E-tongue sensors[i]	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Training set (internal LOO-CV)*	52	52	47	38	96	96
Test set (external validation)**	89	89	44	44	100	100

* Contains 75 % of olive oil bottles chosen using the Kennard–Stone sample selection algorithm for establishing the best LDA-SA model

** Contains 25 % of olive oil bottles chosen using the Kennard–Stone sample selection algorithm for external validation purposes

[a] Best LDA model: 3 physicochemical parameters (free acidity, K_{232} extinction coefficient and oxidative stability) selected by the SA algorithm

[b] Best LDA model: 11 sensorial attributes (olfactory attributes: olive fruitiness, green and other positive sensations; gustatory attributes: olive fruitiness, sweet, bitter, pungent, green, other positive sensations and harmony; final perception: complexity) selected by the SA algorithm

[c] Best LDA model: 5 E-tongue sensors (S1:14, S1:16, S2:3, S2:5 and S2:6) selected by the SA algorithm

[d] Best LDA model: 3 physicochemical parameters (free acidity, peroxide value and K_{270} extinction coefficient) selected by the SA algorithm

[e] Best LDA model: 4 sensorial attributes (olfactory attributes: other positive sensations; gustatory attributes: pungent and green; final perception: complexity) selected by the SA algorithm

[f] Best LDA model: 6 E-tongue sensors (S1:11, S2:3, S2:13, S2:17, S2:18 and S2:20) selected by the SA algorithm

[g] Best LDA model: 4 physicochemical parameters (free acidity, peroxide value, K_{270} extinction coefficient and oxidative stability) selected by the SA algorithm

[h] Best LDA model: 4 sensorial attributes (olfactory attributes: green; gustatory attributes: olive fruitiness and sweet; final perception: persistence) selected by the SA algorithm

[i] Best LDA model: 8 E-tongue sensors (S1:7, S1:13, S1:15, S1:16, S2:7, S2:16, S2:18 and S2:20) selected by the SA algorithm

purposes. Then, the best subset of independent variables to be included in each LDA model was chosen by applying the SA meta-heuristic variable selection algorithm among: (1) the 5 physicochemical parameters; (2) the 14 sensorial positive attributes; or (3) the 40 E-tongue sensors (sensor and the respective sensor-replica were assumed as independent variables), in order to study the influence of the different olive oils' storage conditions. The overall predictive performances of the best LDA-SA models established were assessed by calculating the sensitivity (i.e., probability to correctly classify the samples) and specificity (probability to incorrectly classify the samples) statistical measures for the training dataset (i.e., internal validation using LOO-CV procedure) and the test dataset (i.e., external validation using independent samples that were not used to establish the LDA model). The results are given in Table 1. It can be concluded that the

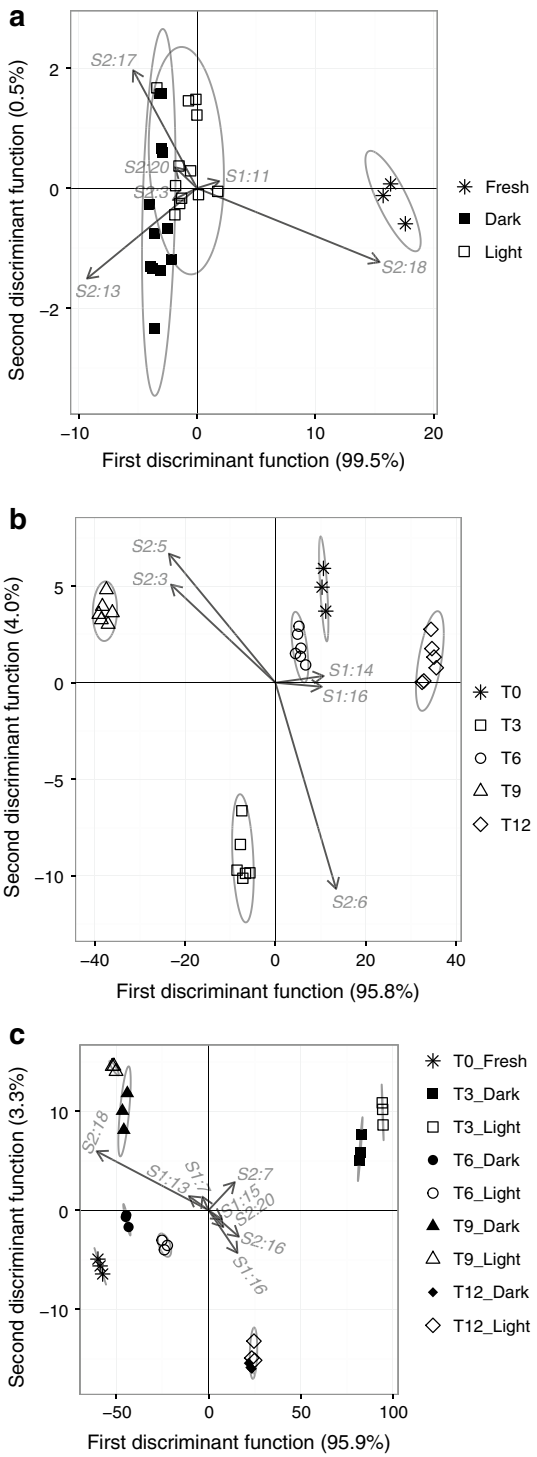

Fig. 2 E-tongue LDA-SA procedure: **a** olive oils light storage conditions ("Fresh," "Dark" and "Light") discrimination for the original training data for the best LDA model established with 6 sensor signals (S1:11, S2:3, S2:13, S2:17, S2:18 and S2:20); **b** olive oils storage time ("T0", "T3", "T6", "T9" and "T12") discrimination for the original training data for the best LDA model established with 5 sensor signals (S1:14, S1:16, S2:3, S2:5 and S2:6); and **c** olive oils time–light storage conditions ("T0", "T3_Dark", "T3_Light", "T6_Dark", "T6_Light", "T9_Dark", "T9_Light", "T12_Dark" and "T12_Light") discrimination for the original training data for the best LDA model established with 8 sensor signals (S1:7, S1:13, S1:15, S1:16, S2:7, S2:16, S2:18 and S2:20). For all the LDA models, the sensors subsets were selected using the SA meta-heuristic algorithm. The graphical outputs include the respective sensors loading *arrows*

sensorial attributes had the lowest potential for discriminating the different olive oils' storage conditions evaluated (time and/or light/dark conditions). Physicochemical parameters showed some potentiality for differentiating olive oil storage conditions, mainly, between light storage conditions. Nevertheless, globally it is clear that the E-tongue device enables an overall better predictive discrimination performance namely when storage time is considered, individually or in combination with the light/dark conditions. Indeed, the 3 best LDA-SA models established were based on a minimum number of E-tongue signal profiles (from 5 to 8 sensor/sensor-replicas used as independent variables; data shown in Table 1) allowing to exclude non-informative, redundant and highly collinear variables. Each LDA model had 2 significant discriminant functions (explaining from 98.2 to 100 % of the original data variability) with sensitivities and specificities greater than 82 and 86 % for the internal LOO-CV procedure (Table 1), respectively. The good performance of the E-tongue combined with the LDA-SA approach was further demonstrated by the satisfactory results achieved for the external validation dataset, for which sensitivities and specificities greater than 67 and 75 % were obtained, respectively (Table 1). Figure 2 exemplifies the discriminating capability of E-tongue/LDA-SA approach as a tool for discriminating storage time and/or light storage conditions, for the original grouped data. Finally, it should be remarked that the sensors and sensor-replicas selected (for the 3 models: S1:7, S1:11, S1:13 to S1:16, S2:3, S2:5 to S2:7, S2:13, S2:16 to S2:18 and S2:20) included all the plasticizer–additives combinations, being 3 plasticizers the most used (in the subsequent order: tris(2-ethylhexyl) phosphate > dioctyl phenylphosphonate > dibutyl sebacate) and all the 4 lipid additive compounds similarly present in all combinations (in the following order: methyltrioctylammonium chloride > oleic acid > octadecylamine = oleyl alcohol). The results obtained in this study as well as in previous works of the research team [18–20, 32, 33] point out the capability of the lipid-based sensor membranes to give a representative fingerprint of the polar compounds present in hydroethanolic olive oil's extracts analyzed by the E-tongue. However, although the interaction mechanisms between the lipid membranes and the polar compounds, namely phenolics, are still quite limited, it is known that natural lipid–phenolic interactions may occur in olive oils [51], and so, it would also be expected that similar interaction may be established between the E-tongue lipid membranes and the polar compounds extracted from olive oils by using hydroethanolic solutions.

Conclusions

The potentiometric E-tongue multi-sensor device coupled with a LDA-SA approach showed satisfactory predictive potential to classify EVOO stored under different storage conditions, namely light/dark exposition and time. Indeed, it was proven that the sensor device could successfully monitor the storage time of olive oils in glass bottles and so could be used to assess their freshness under the usual commercial light exposition conditions (either kept at dark or exposed to natural/artificial light), during the first year of storage. The quite satisfactory overall performance of the proposed procedure was further demonstrated using external data for validation purposes, showing to be quite superior compared to the use of physicochemical or sensory data. So, considering the reported predictive feature of the E-tongue for assessing olive oils storage conditions and taking into account the previous reported capability of this type of device to discriminate EVOO according to olive cultivar as well as to evaluate olive oil's sensory intensity levels, it seems fair to foreseen and hopefully expect, in a near future, the application of this kind of electrochemical device in the olive oil industry.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human participants or animals performed by any of the authors.

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