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Monitoring the debittering of traditional stoned green table olives during the aqueous washing process using an electronic tongue

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ABSTRACT

Stoned green olives are traditional table olives produced in the Northeast of Portugal. They are highly appreciated due to their sensory characteristics and levels of complex phenols with strong antioxidant activity, having a significant agro-economic relevance. During debittering, stoned olives are immersed in water, which is changed each 2 d during 20 d, leading to the reduction of initial bitterness turning them edible olives. So, monitoring the basic taste, total phenols contents and the bitterness index during washing is very important being a time-consuming and expensive task. Thus, the possibility of using an electronic tongue, coupled with chemometrics, for monitoring debittering of stoned green olives (*cvs.* Cobrançosa and Negrinha de Freixo), was evaluated for the first time. The electronic tongue allowed discriminating the different debittering time-periods (sensitivity ≥ 62%, internal-validation), monitoring the decrease of bitter and pungent intensities and the increase of the sweet intensity (0.793 ≤ *R*-Pearson ≤ 0.971; *RMSE* ≤ 1.388), assessing the decrease of the total phenols (*R*-Pearson ≥ 0.934; *RMSE* ≤ 19.1 g GAE/kg) and of the bitterness index (*R*-Pearson ≥ 0.892; *RMSE* ≤ 0.864 g Oleuropein/kg). Therefore, the device could be a practical tool to monitor debittering of stoned green olives during washing.

1. Introduction

Table olives, processed by drupes from the olive tree (*Olea europaea* L.), are a common component of the Mediterranean diet, which consumption is increasing worldwide due to their recognized nutritional and pleasant sensory characteristics (Ramírez, Brenes, Castro, Romero, & Medina, 2017). Moreover, table olives may be a source of monounsaturated fatty acids and phenolic compounds (Cicerale, Coulan, & Sinclair, 2008). Contrary to other fermented foods, table olives have low sugar levels, high fat content and a bitter taste caused by high phenolic content (Sakouhi, Harrabi, Absalon, Sbei, & Boukhchina Kallel, 2008). The natural presence of phenolic compounds in the raw fruit, particularly the bitter glucoside oleuropein, requires a pre-treatment to reduce their levels, by transforming them into other non-bitter compounds, allowing obtaining an edible fruit (Ramírez et al., 2017). Different styles of olive curing result in a different composition of hydrolysis products, which have a huge impact on the overall flavor and on the health-promoting properties of the cured table olives (Marsilio et al., 2005). The most common producing methods are the Spanish-style green olives, California-style black-olives, and Greek-style natural olives in brine (Papadaki & Mantzouridou, 2016), being the former the most popular one (Ramírez et al., 2017).

Other regional methods are also used to produce table olives, with a smaller representativeness but usually with a great agro-economic local relevance. In Trás-os-Montes, the Northeast region of Portugal, a regional type is commonly produced, the traditional stoned green olives, locally known as "*alcaparras*" (Sousa et al., 2006). The production of this type of table olives greatly differs from the most common production methods. While in the Spanish, Greek, and Californian styles the olives are subjected to lye treatments and/or fermentations in brine, traditional stoned green olives are debittered only using water. These traditional stoned green olives are processed from green or yellow-green healthy olive fruits, which are broken using a wood hammer, being the pulp and stone separated (Sousa et al., 2006). The pulp is then

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sliced into two approximately equal parts, perpendicularly to the major axis of the fruit, and immersed in water, which is changed every 1–2 d, during 1–2 wk, allowing reducing the natural bitterness of the raw olives, and so to obtain edible olives. This first stage (1–2 wk) consists in the debittering process, where washing is carried out with only water, after which NaCl, aromatic herbs or other condiments are added, turning the final product more palatable (Sousa et al., 2011). It is important to note that during debittering process no alkaline solutions are used, turning the process greener. Also, the olive overall taste after a NaOH-free debittering process is completely different from that of NaOH-treated fruits, mainly due to the highest residual bitterness that is retained by the olives subjected to the former procedure, even after a long period of storage in brine solutions (Montaño, Sánchez, López-López, De Castro, & Rejano, 2010). In the traditional stoned green olives, the epidermis of the fruit remains intact and acts as a barrier limiting the mass transfer phenomenon and so, the turning slower the diffusion of the phenolic compounds into the water, which are responsible for the natural bitterness of olives (Montaño et al., 2010).

Several researchers studied the composition of table olives produced according to the Spanish-style, Greek-style and Californian-style, namely total phenols and individual phenolic compounds contents, of the end-product (Johnson, Melliou, Zweigenbaum, & Mitchell, 2018) or during the debittering process (Ambra et al., 2017; Habibi, Golmakani, Farahnaky, Mesbahi, & Majzoobi, 2016; Kiai & Hafidi, 2014). Usually, conventional analytical techniques are used, such as high-performance liquid chromatography, ultra-high-pressure liquid chromatography, gas chromatography (GC) and GC coupled to a mass spectrometry detector (GC-MS), or the Folin–Ciocalteu spectrophotometric method. Some work also focused on the evaluation of sensory attributes, chemical composition (namely at the phenolic level), nutritional value and antioxidant activity of edible stoned green olives (Malheiro et al., 2012; Sousa et al., 2011, 2006), using conventional techniques or consumer panels.

Taking into account the regional agro-economic relevance of traditional stoned green olives, the capability of monitoring their production as well as the end-product, *in-situ* and at real-time, is of major interest for local producers. However, conventional techniques (spectrophotometry, liquid and gas chromatography, sensory panels) require skilled trained technicians/panelists, expensive non-green solvents and/or high purity standard compounds. Furthermore, the analysis cost is usually far beyond the economic possibilities of local producers. Therefore, the development of novel, fast and cost-effective analytical techniques for this purpose is a practical and challenging task for researchers with an unequivocally industrial interest not only for local producers of this type of traditional tables olives but also for larger producers of Spanish-style, Greek-style or California-style tables olives. In this framework, electrochemical based-sensor devices have emerged in the last years as versatile taste sensors for assessing positive and negative sensory attributes of table olives (Marx et al., 2017a,b,c).

Thus, the present work aims to evaluate, for the first time, the feasibility of using a potentiometric electronic tongue, coupled with chemometric tools, for monitoring the natural debittering process of traditional stoned green olives of two Portuguese olive cultivars (*cvs.* Cobrançosa and Negrinha de Freixo).

2. Materials and methods

2.1. Stoned green olives production: debittering process

Olives from *cvs.* Cobrançosa and Negrinha de Freixo were collected at the end of September 2017 from an olive grove located in Trás-os-Montes region (Mirandela, Portugal). Olives were collected from different trees of both cultivars and immediately transported to the laboratory. Then, olives were manually broken to separate the pulp from the stone. Afterwards, for each cultivar, 50 g of olive pulp were inserted into each of 100 transparent coded plastic pots, immersed with 50 mL of distilled water, closed and stored at ambient temperature (~16–20 °C) and exposed to usual lighting conditions, in order to promote the debittering of the stoned olives (10 pots for each debittering time-period evaluated, per olive cultivar: day_02 to day_20). Each 2 d, 10 pots from each olive cultivar were taken, being the washing water removed, the olive pulp drained and, for half of the pots (5 for each sampling date), the pulp was immediately frozen and later used for chemical and electrochemical analysis, being the pulps from the other 5 pots immediately subjected to the sensory analysis. For the remaining pots, the 50 mL of water were renewed and stored, under the previous mentioned conditions, until the next sampling day (each 2 d during 20 d of debittering). Besides, and to be used as control, for each olive cultivar, 10 plastic pots containing each 50 g of stoned green olives were also coded but not immersed in water (raw pulp not debittered: day_00), being half of them immediately frozen (−18 °C) for the chemical and electrochemical analysis and the other pots immediately subjected to the sensory analysis. Thus, for both olive cultivars (*cvs.* Cobrançosa and Negrinha de Freixo), a total of 220 pots were used.

2.2. Sensory analysis of stoned green olives: preparation of samples and evaluation

For the sensory evaluation, the stoned green olive samples were analyzed immediately after being prepared, following the guidelines of the International Olive Council (IOC) official regulation, *i.e.*, COI/OT/MO No 1/Rev.2 November 2011 (IOC, 2011), for sensory table olives analysis. The sensory analysis was performed by 8 trained panelists (4 men and 4 women) of the School of Agriculture of the Polytechnic Institute of Bragança (Portugal), including teachers and staff, aging from 24 to 58 years-old. Sensory analysis included the perception of bitter, pungent, salty and sweet attributes, and the assessment of the respective intensities, using a profile sheet with a continuous unstructured scale ranging from 1 (absence of attribute perception) to 11 (strongest intensity perceived) following the IOC official regulations with slight adaptations (IOC, 2011).

2.3. Stoned green olives analysis: sample preparation, bitterness index, total phenols and pH

2.3.1. Sample preparation

For the chemical analysis, the stoned green olives were first defrozen overnight at ambient temperature, and after, reduced to a paste. First, 4 g of the drained pulps were weighed, placed into a coded polypropylene conical tube of 50 mL, and re-hydrated with 12 mL of distilled water. The mixture was crushed (Ultra-Turraz® T25 basic IKA®-werke) and an aqueous paste solution was obtained. After rest, a suspension with two-phases was formed: the solid phase (olive paste) and the liquid phase. The aqueous extract was removed by filtration under vacuum (vacuum pump GM-1.00 A-Laboratory Diaphragm®, equipment; paper filter Whatman nº4). The permeate was stored in 15 mL polypropylene conical tube, properly coded, and frozen until analysis.

2.3.2. Bitterness index determination

The bitterness index (or bitter index) was determined following the protocol described by Inarejos-García, Androulaki, Salvador, Fregapane, and Tsimidou (2009). From each defrozen aqueous extract, 1.0 g was withdrawn and diluted into 5 mL of n-hexane and re-extracted with 5 mL MeOH/H_2O (60:40, v/v). The solution was mixed during 1 min using a vortex (VWR®Vortex Mixers, 230 V) and then centrifuged at 3500 rpm for 10 min (Hettich® Centrifuge Universal 1200). After the removal of the hexane layer, the polar fraction (aqueous-ethanolic phase), was transferred to a volumetric flask and a final volume of 10 mL was obtained using a solution of MeOH/H_2O (60:40, v/v) (stock solution). Finally, an aliquot of 1.25 mL was withdrawn and further diluted to 5 mL with MeOH/H_2O (60:40, v/v). The absorbance of this final extract was recorded at 225 nm using a UV/Vis spectrophotometer

(SPECORD®200 from Analytik Jena®). A calibration curve was established using oleuropein (purity ≥90%, from ExtraSynthese) at different concentrations (dynamic concentration ranges: 0.084–4.6 mol/L, $R^2 = 0.9997$ for *cv.* Cobrançosa and *cv.* Negrinha de Freixo). The bitterness indexes were expressed as g of oleuropein per kg of olives (g Oleuropein/kg olives).

2.3.3. Total phenols content

The total phenols contents of the debittered stoned green olives were determined following the methodology proposed by Singleton and Rossi (1965). Briefly, 1 mL of the defrozen aqueous extract was added to 1 mL of Folin-Ciocalteau solution and agitated in a vortex (VWR® Vortex Mixers, 230 V) during 1 min. Then 1 mL of saturate sodium carbonate (Na_2CO_3) solution plus 7 mL of distilled water were added to the former mixture. The final mixture was exposed to light for 90 min, and then the absorbance was recorded at 725 nm with an UV–Vis spectrophotometer (GENESYS™ 10, from Thermo Scientific™). Distilled water was used as the blank solution. Gallic acid (purity ≥ 99%, from ExtraSynthese) was used as the standard compound to establish the calibration curve (dynamic concentration range: 0.001–1 mol/L, $R^2 = 0.9997$), being the results expressed as g of Gallic acid (GAE) per kg of olive (g GAE/kg olives).

2.3.4. pH

pH of stoned green olives was measured along the 20 d of the debittering process. For that, the pulp was crushed using a shredder knives Moulinex® equipment. A fine-grained paste was obtained, which was diluted with deionized water (20 g of olive paste diluted with 60 mL of deionized water). Each diluted paste was placed into transparent plastic pot properly coded and the pH recorded at ambient temperature (~16–20 °C) using a pH meter (HI 8417, from Hanna Instrument®).

2.4. E-tongue analysis

2.4.1. E-tongue device

A new lab-made potentiometric E-tongue, with two cylindrical sensor arrays was built (Fig. 1), each with 20 lipid polymeric cross-sensitive membranes (lipid additive, 3%; plasticizer, 32%; and, polyvinyl chloride, 65%, similarly to Marx et al. (2017a)). The sensor membranes were connected to a multiplexer Agilent Data Acquisition Switch Unit (model 34970 A) controlled by an Agilent BenchLink Data Logger software installed on a PC. Each potentiometric assay took 5 min, being recorded 40 sensor signals generated due to electrostatic or hydrophobic interactions between the membranes and the olive polar compounds (Kobayashi et al., 2010). A reference Ag/AgCl double-junction glass electrode (Crison, 5241) was also used. The two sensor arrays were stored at ambient temperature in a HCl solution (0.01 mol/L). The sensor codes were those of previous works: letter S for identifying the sensor followed by the number of the array (1: or 2:) and the number of the membrane (1–20) (Marx et al., 2017a).

2.4.2. Sample preparation and potentiometric analysis

The stoned green olives (110 independent samples: 10 raw and 100 debittered stoned olive samples) of *cvs.* Cobrançosa and Negrinha de Freixo were analyzed using the E-tongue, allowing obtaining representative potentiometric signal profiles, for the 11 time-periods evaluated. Before analysis, the stoned green olives were reduced to a paste by crushing the pulps using a shredder knives Moulinex® equipment. The process allowed obtaining a fine-grained paste, which was diluted using deionized water (20 g of olive paste diluted with 60 mL of deionized water). Each diluted paste was placed into a transparent plastic pot, properly coded and analyzed with the E-tongue. Each assay took 5 min, enabling achieving a pseudo-equilibrium between the non-specific lipid polymeric membranes and the chemical compounds present on the aqueous olive paste solution. The potentiometric assays were carried out in duplicate for each sample, with a third assay performed if a coefficient of variation greater than 20% was observed for any of the 40 sensors. Finally, to overcome/minimize the occurrence of signal drift, all the E-tongue assays were performed in the same day, which guaranteed a negligible signal variation (variation coefficients lower than 5%) (Prata et al., 2018; Veloso, Dias, Rodrigues, Pereira, & Peres, 2016).

2.5. Statistical analysis

Boxplots were used to graphically evaluate the trend of the bitter, pungent, salty and sweet intensities perceived by trained panelists, as well as, the total phenols contents and the bitterness index of the stoned green olives along their processing time-periods (from 0 to 20 d of washing procedure, each 2 d). Besides, principal component analysis (PCA) was applied for evaluating the unsupervised split of the stoned

Plasticizer (~65%)	Additive (~3%)	Identification code
Bis(1-butylpentyl) adipate	Octadecylamine	S1:1 or S2:1
	Oleyl alcohol	S1:2 or S2:2
	Methyltrioctylammonium chloride	S1:3 or S2:3
	Oleic acid	S1:4 or S2:4
Dibutyl sebacate	Octadecylamine	S1:5 or S2:5
	Oleyl alcohol	S1:6 or S2:6
	Methyltrioctylammonium chloride	S1:7 or S2:7
	Oleic acid	S1:8 or S2:8
2-Nitrophenyl-octylether	Octadecylamine	S1:9 or S2:9
	Oleyl alcohol	S1:10 or S2:10
	Methyltrioctylammonium chloride	S1:11 or S2:11
	Oleic acid	S1:12 or S2:12
Tris(2-ethylhexyl)phosphate	Octadecylamine	S1:13 or S2:13
	Oleyl alcohol	S1:14 or S2:14
	Methyltrioctylammonium chloride	S1:15 or S2:15
	Oleic acid	S1:16 or S2:16
Dioctyl phenylphosphonate	Octadecylamine	S1:17 or S2:17
	Oleyl alcohol	S1:18 or S2:18
	Methyltrioctylammonium chloride	S1:19 or S2:19
	Oleic acid	S1:20 or S2:20

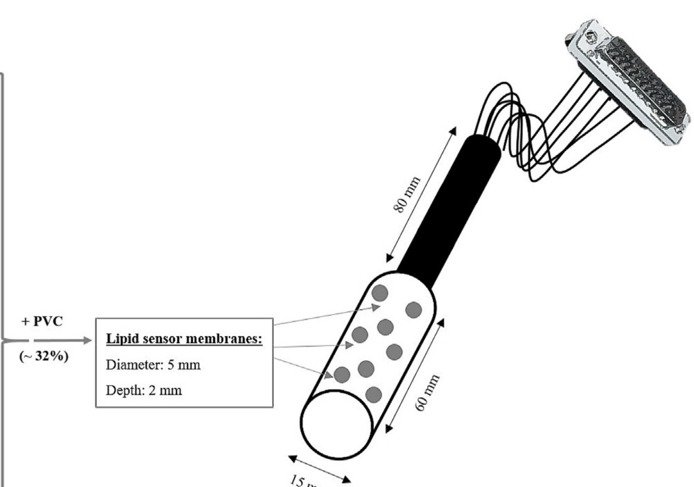

Fig. 1. E-tongue sensor array and lipid polymeric sensor membranes composition.

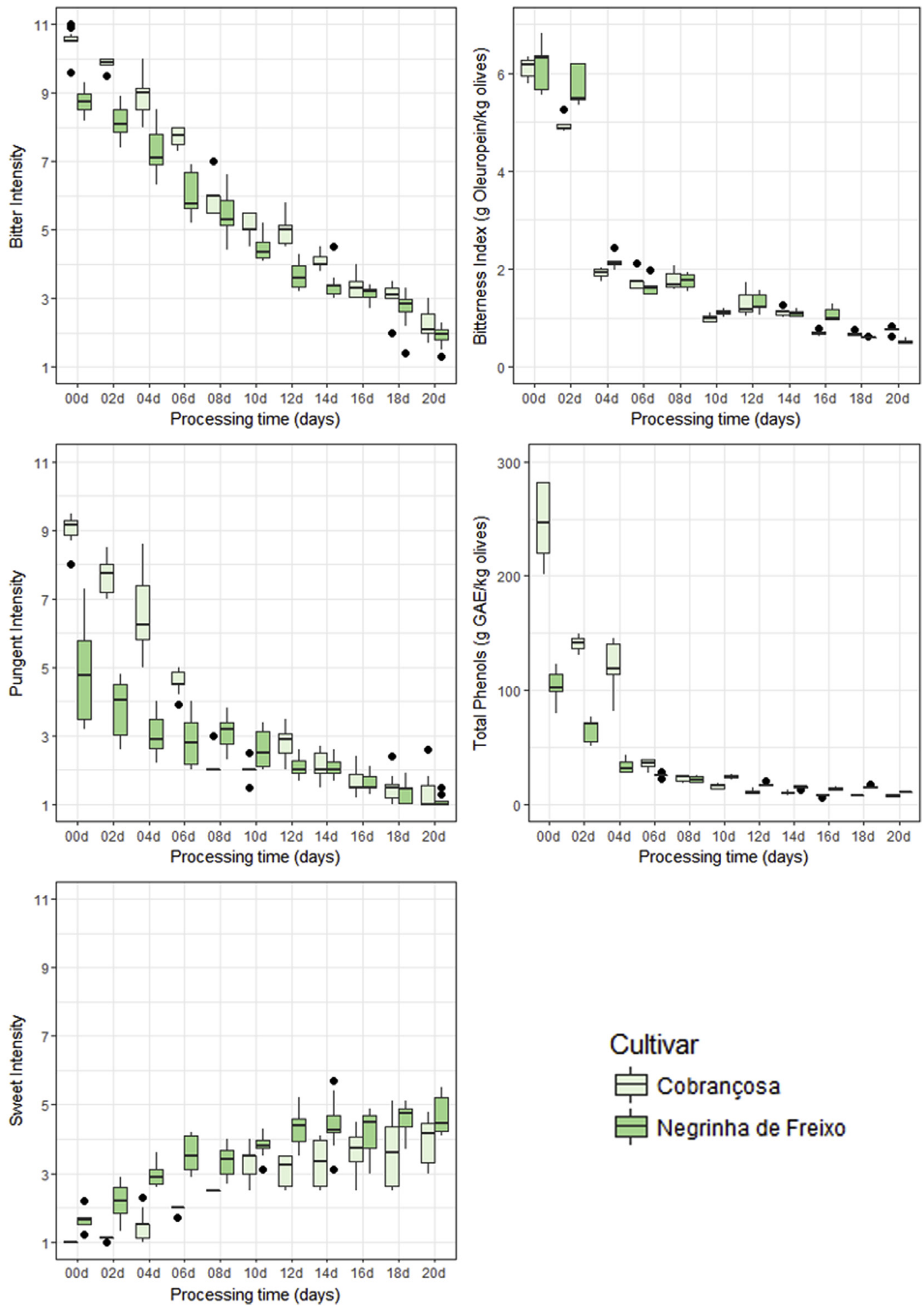

Fig. 2. Boxplots showing the time evolution trends of the sensory attributes (bitter, pungent and sweet intensities) and chemical parameters (bitterness index and total phenols contents) of the stoned green olives (*cvs.* Cobrançosa and Negrinha de Freixo) during the 20 d of debittering-washing process. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

green olives according to the processing time-periods, based on the sensory and chemical data. The results were evaluated by plotting the most significant principal component (PC) functions, using 3D plots.

Linear discriminant analysis (LDA) was used to evaluate the performance of the E-tongue for correctly classify the stoned green olives according to the processing time-periods. The best subsets of independent predictors (*i.e.*, sensors) were established from the 40 potentiometric sensors, applying a meta-heuristic simulated annealing (SA) variable selection algorithm (Bertsimas & Tsitsiklis, 1993; Cadima, Cerdeira, & Minhoto, 2004; Kirkpatrick, Gelatt, & Vecchi, 1983). The LDA performance was assessed using the leave-one-out cross-validation (LOO-CV) procedure, which although being an over-optimistic internal validation technique has been widely used when the number of samples is limited. To normalize the weight of each variable in the final linear

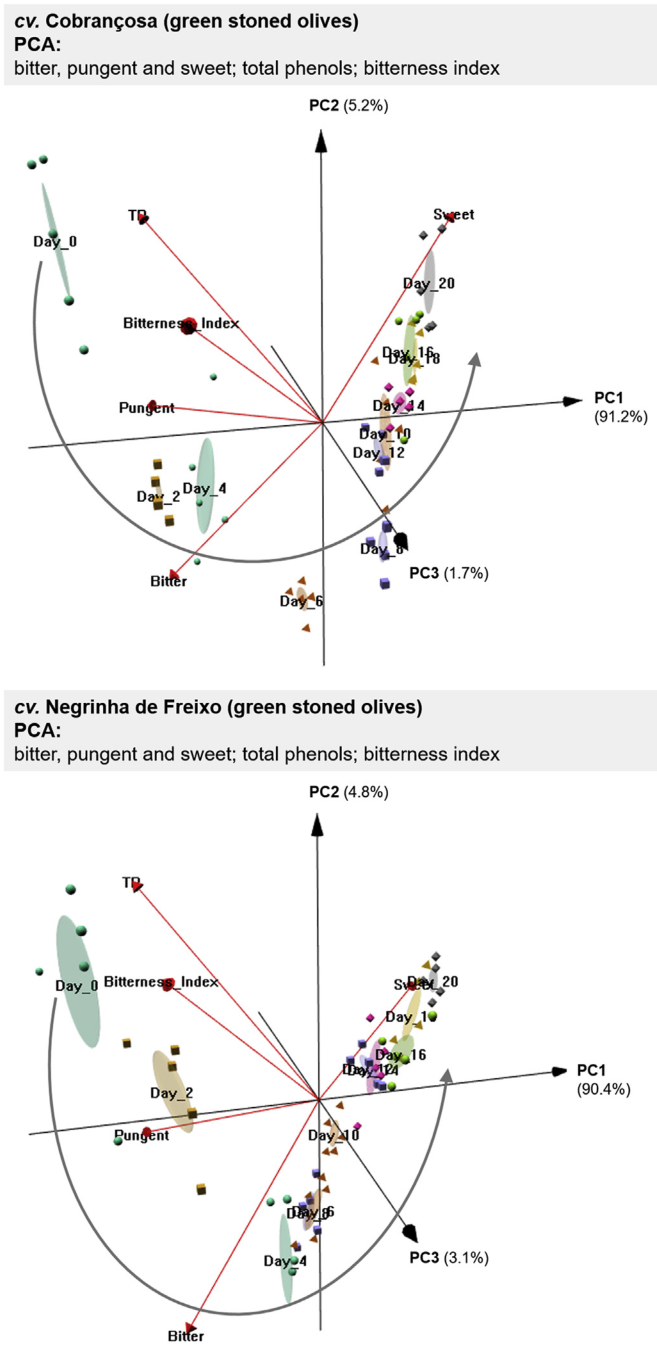

Fig. 3. Unsupervised classification of stoned green olives (*cvs.* Cobrançosa or Negrinha de Freixo) according to the debittering time-periods (day_00, day_02, day_04, day_06, day_08, day_10, day_12, day_14, day_16, day_18 and day_20): 3D-PCA plots of the first 3 PCs based on 3 descriptive gustatory attributes (bitter, pungent and sweet intensities sensations) and 2 chemical parameters (bitterness index and total phenols, TP). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

classification model, variable scaling and centering procedures were implemented. The classification capability of each LDA model was graphically evaluated using 3-D plots of the three most significant linear discriminant (LD) functions and by calculating the sensitivity values (*i.e.*, the percentage of correctly classified samples according to the pre-established data groups).

Multiple linear regression (MLR) models were used to estimate and/or predict the median intensities of the descriptive gustatory attributes perceived by the panelists. MLR models were used since, they can satisfactorily model data generated from a potentiometric E-tongue, and with better results than partial least squares (PLS) and principal component regression (PCR) (Rodrigues, Dias, Veloso, Pereira, & Peres, 2016). The meta-heuristic SA algorithm was used to select the subsets of different number of sensors used to establish the best E-tongue-MLR models, using the correlation coefficients (R) and the root mean square errors (*RMSE*) as the quality criteria (Cadima, Cerdeira, Silva, & Minhoto, 2012; Cadima et al., 2004). In this work, the E-tongue-MLR-SA models established were based on subsets of 2–39 signals, from a total set of 40 signal profile recorded for each table olive sample by the E-tongue (40 signals concerning the evaluation of olive pastes). The best model was selected by its quantitative prediction performance, being set a maximum adjusted R^2 value for the LOO-CV with the lowest number of sensors. All statistical analysis was performed using the Subselect (Cadima et al., 2004, 2012) and MASS (Venables & Ripley, 2002) packages of the open source statistical program R (version 2.15.1), at a 5% significance level.

3. Results and discussion

3.1. *Stoned green olives: sensory and chemical profiles during the debittering-washing process*

The stoned green olives were evaluated along natural debittering (during 20 d of the aqueous washing step). Regarding sensory analysis, the intensities of four descriptive gustatory attributes (bitter, pungent, salty and sweet sensations) were assessed by the trained panelists. The results showed that, for both olive cultivars (*cvs.* Cobrançosa and Negrinha de Freixo), the salty sensation was not perceived when tasting the stoned olives along the 20 d of washing (intensities ranging from 1.0 to 1.1 for a 1–11 continuous unstructured scale). This was expected since, during the aqueous washing stage of the debittering process no salt or other chemicals (e.g., NaOH) are added. On the contrary, and as expected, the bitter and pungent intensities linearly decreased with the increase of the washing time-periods (*cv.* Cobrançosa: 11.0 ≥ Bitter ≥ 1.1 and R-Pearson = −0.985, 9.5 ≥ Pungent ≥ 1.0 and R-Pearson = −0.898, P-value ≤ 0.0002; *cv.* Negrinha de Freixo: 9.3 ≥ Bitter ≥ 1.3 and R-Pearson = −0.985, 7.3 ≥ Pungent ≥ 0.5 and R-Pearson = −0.962, P-value < 0.0001). Furthermore, as can be inferred, the initial bitterness and pungency intensities of *cv.* Cobrançosa olives were higher than those from *cv.* Negrinha de Freixo. Nevertheless, after 16–20 d of debittering-washing steps, the bitter and pungent intensities perceived by the trained panelists, for both cultivars, were of the same order of magnitude, showing that the debittering procedure was successful, allowing obtaining edible olives. On the other hand, the sweet perceived sensation linearly increased with the debittering time (*cv.* Cobrançosa: 1.0 ≤ Sweet ≤ 5.1 and R-Pearson = +0.966, P-value < 0.0001; *cv.* Negrinha de Freixo: 1.2 ≤ Sweet ≤ 5.7 and R-Pearson = +0.938, P-value < 0.0001), being similar intensities found for both cultivars along the studied washing time-periods. The bitterness index, pH and total phenols contents of the stoned green olives were also determined along the debittering process. For both olive cultivars the pH was almost constant during the 20 d of analysis (5.00 ≤ pH ≤ 5.50 to *cv.* Cobrançosa and 4.86 ≤ pH ≤ 5.43 to *cv.* Negrinha de Freixo), being the aqueous olive pastes slightly acid. However, both bitterness index and total phenols contents significantly decreased with the time (*cv.* Cobrançosa: 282.1 ≥ Total phenols (g GAE/kg) ≥ 5.4, 6.33 ≥ Bitterness index (g Oleuropein/kg) ≥ 0.61; *cv.* Negrinha de Freixo: 122.5 ≥ Total phenols (g GAE/kg) ≥ 9.5, 6.82 ≥ Bitterness index (g Oleuropein/kg) ≥ 0.48). Once again, stoned olives of *cv.* Cobrançosa showed the greatest initial total phenols contents but, after 18–20 d of production, the remained level was similar to that found for *cv.* Negrinha de Freixo. In Fig. 2, the evolution trends observed for the sensory attributes and chemical parameters along debittering-washing are shown. From the boxplots it can be inferred that, in general, the greatest changes occurred during the first 4–6 d of olives' debittering process, with the exception of bitter and sweet sensations, for which the intensity evolution was clearly smoother. Too, the decrease of the bitterness index could be directly related to decrease of the bitter taste intensity (for both cultivars) and, the decrease of the pungent sensation could be related with the decrease of the total phenols contents.

Finally, PCA was used to infer about the possibility of using the sensory-chemical data for the unsupervised splitting of the stoned green olives (*cvs.* Cobrançosa or Negrinha de Freixo) according to the debittering-washing time-periods (Fig. 3). The results pointed out that the sensory data, the bitterness index and the total phenols contents allowed correctly grouping the stoned olive samples of each cultivar (*cvs.* Cobrançosa or Negrinha de Freixo) by time-periods (based on the first 3 PCs, which explained 98% of the data variability). Fig. 3 shows that it is possible to easily differentiate the initial time-period (day_00, *i.e.*, not debittered raw olives) from the following time-periods till the 6th-8th d of processing (day_02 to day_08, corresponding to 4 changes of the washing water solution), which is related to the deepest changes observed at sensory and chemical levels (decrease trends over time). Moreover, as can be inferred from the 3D-PCA biplot, the bitter, pungent, bitterness index and total phenols contents were the most influent differentiation parameters. Also, lower changes were observed for the remaining debittering-washing time-periods (day_10 to day_20), leading to a partial overlapping of those groups that are located in the same 3D region, mainly influenced by the increase of the sweet sensation.

3.2. E-tongue analysis

The intensities of the basic tastes perceived by the trained panelists together with the values of the bitterness index and of the total phenols contents allowed an unsupervised identification of the 11 different time-periods studied (day_00 to day_20, with 2 d of sampling interval). However, the availability of these data poses some practical difficulties. First, the scarcity of trained sensory panels makes the sensory assessment a hard and expensive task, which is far beyond the economical possibilities of the majority of local stoned green olive producers. Moreover, the sensory analysis implies a human factor, being a time-consuming methodology since only a limited number of samples can be evaluated per day (Panagou, Sahgal, Magan, & Nychas, 2008; Sinelli, Cerretani, Egidio, Bendini, & Casiraghi, 2010). On the other hand, although the bitterness index and the total phenols contents can be determined by spectrophotometry, the use of non-green solvents and of high-cost standards together with the multiple sample pre-treatment steps required, turn out a time-consuming and expensive procedure. Thus, the feasibility of applying a fast, simple and cost-effective E-tongue, coupled with chemometric tools, for monitoring the sensory and chemical trends during the natural debittering of the stoned green olives, would be of utmost practical and economical relevance.

3.2.1. Discrimination of stoned green olives according to the debittering time-periods

The capability of the E-tongue to discriminate the different debittering time-periods of stoned green olives from *cvs.* Cobrançosa or Negrinha de Freixo, was evaluated using the LDA (supervised technique) and the meta-heuristic SA variable selection algorithm. For each olive cultivar, the LDA-SA approach identified the best sub-set of E-tongue sensors, with the minimum number of sensors (between 2 and 39 sensors, of an overall of 40 possible sensors), which enabled achieving the greatest correct classification rates (sensitivity value) for the LOO-CV. Globally, the potentiometric signals recorded during the stoned green olive aqueous pastes analysis ranged from −24 to +288 mV for *cv.* Cobrançosa and −12 to +313 mV for *cv.* Negrinha de Freixo (for day_00 till day_20). Multivariate classification models, with

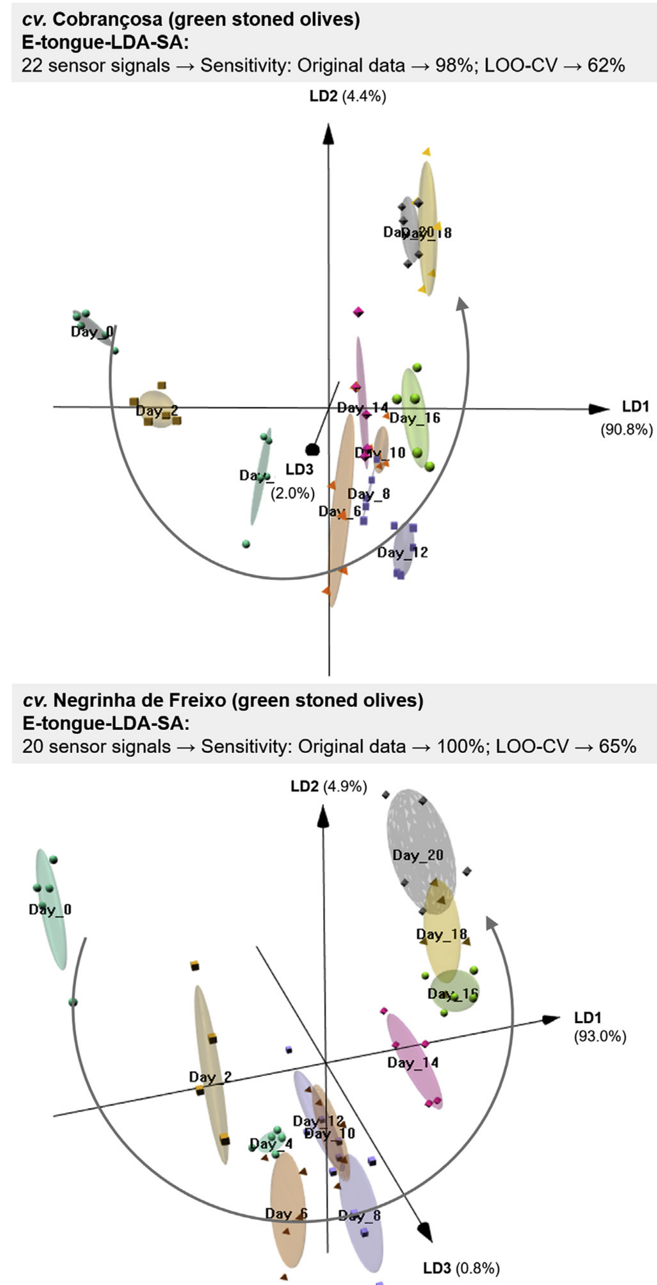

Fig. 4. Classification of stoned green olives (*cvs.* Cobrançosa or Negrinha de Freixo) according to the debittering time-periods (day_00, day_02, day_04, day_06, day_08, day_10, day_12, day_14, day_16, day_18 and day_20): 3D-E-tongue-LDA-SA plots of the first 3 PCs based on E-tongue signals profiles recorded by 22 or 20 sensors (*cvs.* Cobrançosa or Negrinha de Freixo, respectively). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3 significant functions (*P*-value < 0.0001; explaining 97.2% and 98.7% of the original data variability for *cvs.* Cobrançosa and Negrinha de Freixo, respectively), based on the signal profiles of 22 sensors for *cv.* Cobrançosa (S1:1, S1:2, S1:5, S1:7-S1:9, S1:11-S1:13, S1:16, S1:18, S2:1, S2:5, S2:7-S2:10, S2:12, S2:13, S2:16, S2:17 and S2:20) and 20 sensors for *cv.* Negrinha de Freixo (S1:1, S1:5-S1:7, S1:10, S1:14, S1:16, S1:17, S2:1, S2:2, S2:5-S2:7, S2:9-S2:11 and S2:17-S2:20) were established. For *cv.* Cobrançosa, the best E-tongue-LDA-SA model correctly classified 98% of the original grouped data (Fig. 4) and 62% for LOO-CV. Similarly, for *cv.* Negrinha de Freixo, the best E-tongue-LDA-SA model showed sensitivities of 100% for the original grouped data

Table 1
Predictive capability of the E-tongue-MLR-SA models established to quantify the mean intensities of 3 descriptive gustatory attributes (bitter, pungent and sweet) evaluated by trained panelists (using a 1–11 continuous intensity scale), as well as of total phenols contents and bitterness index values, assessed by UV–Vis spectrophotometry, of *cv.* Cobrançosa and *cv.* Negrinha de Freixo stoned green olives, during their production, along a 20 d aqueous washing step time-period.

Parameters		E-tongue-MLR-SA models[a] (LOO-CV[b])							
		cv. Cobrançosa				*cv.* Negrinha de Freixo			
		Dynamic range	Nº of signals[c]	Correlation coefficient (*R*)	Root mean square error (*RMSE*)	Dynamic range	Nº of signals[c]	Correlation coefficient (*R*)	Root mean square error (*RMSE*)
Sensory gustatory attributes	Bitter	[1.1, 11.0]	13[d]	0.949	0.886	[1.3, 9.3]	15[i]	0.793	1.388
	Pungent	[1.0, 9.5]	11[e]	0.971	0.647	[0.5, 7.3]	11[j]	0.852	0.602
	Sweet	[1.0, 5.1]	9[f]	0.920	0.427	[1.2, 5.7]	5[k]	0.850	0.526
Chemical parameters	Bitterness index (g Oleuropein/kg)	[0.6, 6.3]	16[g]	0.973	0.407	[0.5, 6.8]	13[l]	0.892	0.864
	Total phenols (g GAE/kg)	[5.4, 282.1]	13[h]	0.969	19.1	[9.5, 122.5]	11[m]	0.934	10.0

[a] Multiple linear regression (MLR) models based on the sub-sets of potentiometric signals, established using the simulated annealing (SA) algorithm, selected among the 40 possible signal profiles obtained with the electronic tongue (E-tongue) during the analysis of stoned green olive aqueous pastes.
[b] LOO-CV: leave-one-out cross-validation procedure.
[c] Number of signals included in the E-tongue-MLR-SA model, selected from the 40 electrochemical signals recorded by E-tongue during analysis of each olive paste.
[d] E-tongue signals included in the E-tongue-MLR-SA model based on the potentiometric signals recorded by 13 E-tongue sensors (1st array: S1:2, S1:6, S1:15 and S1:17; 2nd array: S2:3, S2:4, S2:7, S2:10, S2:11, S2:15, S2:17, S2:18 and S2:20) during the analysis of the stoned olive aqueous pastes.
[e] E-tongue signals included in the E-tongue-MLR-SA model based on the potentiometric signals recorded by 11 E-tongue sensors (1st array: S1:1, S1:2 and S1:11; 2nd array: S2:1, S2:3, S2:7, S2:10, S2:12, S2:17, S2:18 and S2:20) during the analysis of the stoned olive aqueous pastes.
[f] E-tongue signals included in the E-tongue-MLR-SA model based on the potentiometric signals recorded by 9 E-tongue sensors (1st array: S1:1, S1:6 and S1:15; 2nd array: S2:3, S2:10, S2:11, S2:15, S2:17 and S2:20) during the analysis of the stoned olive aqueous pastes.
[g] E-tongue signals included in the E-tongue-MLR-SA model based on the potentiometric signals recorded by 16 E-tongue sensors (1st array: S1:1, S1:2, S1:3, S1:5, S1:10, S1:15, S1:17 and S1:18; 2nd array: S2:2, S2:5, S2:7, S2:8, S2:10, S2:12, S2:13 and S2:14) during the analysis of the stoned olive aqueous pastes.
[h] E-tongue signals included in the E-tongue-MLR-SA model based on the potentiometric signals recorded by 13 E-tongue sensors (1st array: S1:1, S1:2, S1:4, S1:6, S1:8, S1:11, S1:12 and S1:18; 2nd array: S2:3, S2:5, S2:7, S2:10 and S2:19) during the analysis of the stoned olive aqueous pastes.
[i] E-tongue signals included in the E-tongue-MLR-SA model based on the potentiometric signals recorded by 15 E-tongue sensors (1st array: S1:3, S1:5, S1:6, S1:9, S1:12, S1:15 and S1:18; 2nd array: S2:1, S2:4, S2:5, S2:7, S2:8, S2:10, S2:13 and S2:19) during the analysis of the stoned olive aqueous pastes.
[j] E-tongue signals included in the E-tongue-MLR-SA model based on the potentiometric signals recorded by 11 E-tongue sensors (1st array: S1:9, S1:11, S1:12, S1:15, S1:16, S1:18 and S1:20; 2nd array: S2:1, S2:8, S2:15 and S2:19) during the analysis of the stoned olive aqueous pastes.
[k] E-tongue signals included in the E-tongue-MLR-SA model based on the potentiometric signals recorded by 5 E-tongue sensors (1st array: S1:4 and S1:12; 2nd array: S2:1, S2:13 and S2:15) during the analysis of the stoned olive aqueous pastes.
[l] E-tongue signals included in the E-tongue-MLR-SA model based on the potentiometric signals recorded by 13 E-tongue sensors (1st array: S1:1, S1:2, S1:4, S1:8, S1:12, S1:14, S1:16 and S1:20; 2nd array: S2:1, S2:7, S2:14, S2:19 and S2:20) during the analysis of the stoned olive aqueous pastes.
[m] E-tongue signals included in the E-tongue-MLR-SA model based on the potentiometric signals recorded by 11 E-tongue sensors (1st array: S1:4, S1:12, S1:14, S1:18 and S1:20; 2nd array: S2:1, S2:9, S2:10, S2:16, S2:19 and S2:20) during the analysis of the stoned olive aqueous pastes.

(Fig. 4) and of 65% for the LOO-CV. It should be remarked that, for the first 3 debittering time-periods (*i.e.*, up to the 4th-6th d of washing steps: day_00, day_02 and day_04) the group sensitivities ranged from 80 to 100%, being the majority of the misclassifications observed between the 6th–8th till the 20th d, for *cvs.* Cobrançosa and Negrinha de Freixo. The better performance observed for the initial debittering days could be tentatively attributed to the known capability of the E-tongue lipid sensor membranes to generate different potentiometric responses in the presence of polar compounds that are responsible for table olives sensory positive or negative attributes (Marx et al., 2017a,b,c). Also, this type of polymeric membranes can generate different signal profiles depending on the levels of the bitterness index (related to changes of the oleuropein or similar compounds amounts) or due to changes of the contents of total phenols or individual phenolic compounds (Borges, Peres, Dias, Seiquer, & Pereira, 2018; Prata et al., 2018; Semenov et al., 2019). Since the changes of these attributes occurred in a greatest extent during the initial debittering days (Figs. 2 and 3), it can explain the satisfactory performance of the E-tongue-chemometric approach, for the initial debittering days. In fact, it could be tentatively concluded that the E-tongue would be a helpful monitoring tool mainly if 3 time-periods were considered within the debittering process: initial period (0–4 d), middle period (6–16 d) and end period (18-20 d).

3.2.2. Monitoring bitter, pungent, sweet, total phenols and bitterness during stoned olives processing

Besides classifying the stoned green olive samples according to the debittering time-period, the possibility of assessing the evolution of the basic tastes intensities as well as of the bitterness index and the total phenols contents along the debittering-washing process would be of major importance for producers. Indeed, a similar E-tongue, coupled with MLR-SA models, was successfully used by the research team for quantifying the intensities of positive (Marx et al., 2017b) and negative attributes (Marx et al., 2017c) of commercial table olives (natural fermentation, Spanish-style and Californian-style) of different olive cultivars, including *cvs.* Cobrançosa and Negrinha de Freixo, based on the analysis of olive aqueous pastes and the respective brine solutions. Furthermore, this type of potentiometric device also enabled quantifying the contents of total phenols as well as individual flavonoids, phenolic acids and phenol alcohols, found in olive oils (*cv.* Arbequina), using a similar potentiometric-chemometric strategy.

Thus, the performance of E-tongue-MLR-SA approach for monitoring the bitterness index, total phenols contents, bitter, pungent and sweet intensities during the debittering of traditional stoned green olives (*cvs.* Cobrançosa and Negrinha de Freixo) was evaluated for the first time. As can be inferred from the results shown in Table 1 and Fig. 5, the established quantitative multiple linear models (based on the signals profiles generated by 9–16 E-tongue sensors for *cv.* Cobrançosa,

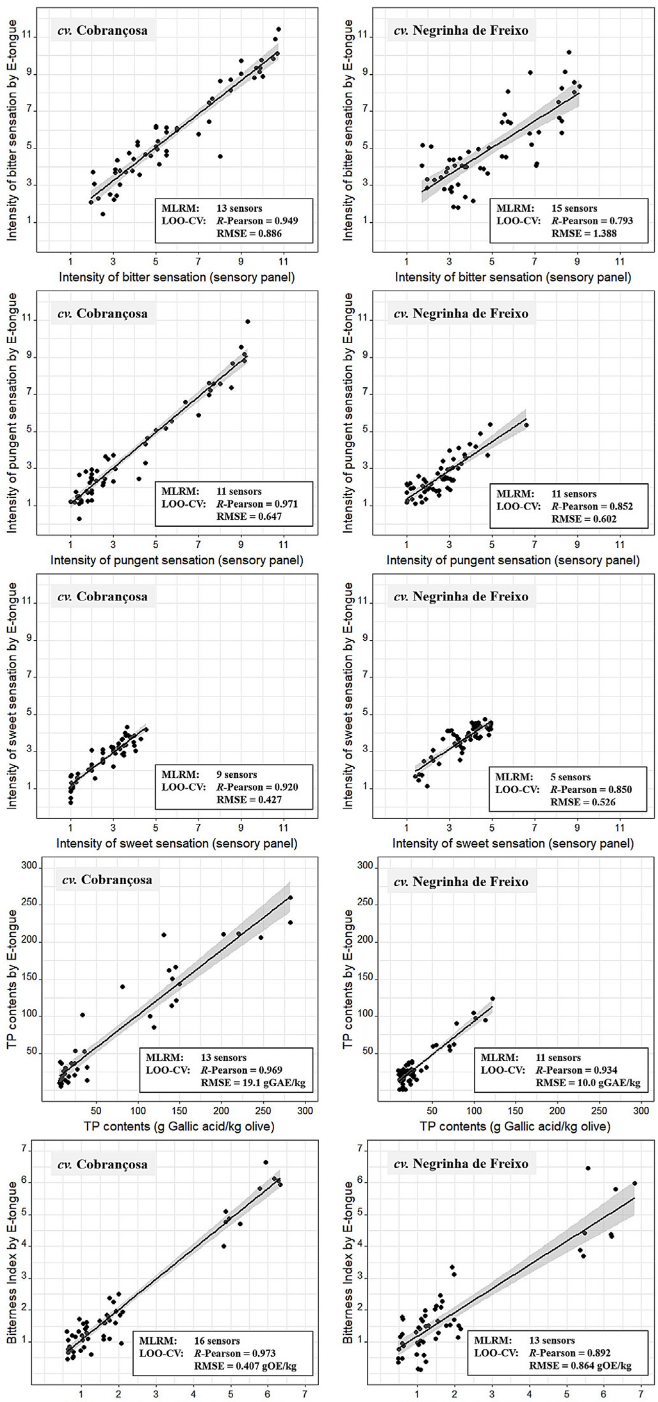

Fig. 5. Predictive performance of the E-tongue-MLR-SA models (LOO-CV): quantification of bitter, pungent and sweet intensities perceived, by trained panelists, during the sensory analysis of traditional stoned green olives (*cvs.* Cobrançosa and Negrinha de Freixo) along 20 d of debittering-washing process (being the water replaced each 2 d), as well as of the bitterness index and total phenols contents assessed by UV–Vis spectrophotometry.

and by 5–15 E-tongue sensors for *cv.* Negrinha de Freixo) allowed predicting (LOO-CV) the intensities of the 3 descriptive gustatory attributes (bitter, pungent and sweet sensations) as well as the levels of the two chemical parameters (bitterness index and total phenols). The satisfactory results achieved (Table 1 and Fig. 5) demonstrated the feasibility of using the E-tongue as a fast, cost-effective, *in-situ* and real-time device for monitoring the debittering process of these traditional stoned green olives, namely during the aqueous washing steps, which aimed reducing the natural initial high bitter-pungent sensations of green olives and increasing the sweet sensation, turning the olives into edible olives. However, it should be remarked that more accurate predictions (LOO-CV, Table 1 and Fig. 5) were achieved for *cv.* Cobrançosa, possibly due to the greater dynamic ranges observed for this olive cultivar, compared to those of *cv.* Negrinha de Freixo, which may indicate that this methodology would be more suitable for olives with higher natural bitterness. Finally, the proposed potentiometric-chemometric approach could be foreseen as a complementary analytical strategy to the time-consuming sensory analysis, allowing increasing the number of samples daily assessed. Moreover, since potentiometric assays only require turning the olive samples into an aqueous olive paste (minced olives diluted with water) the E-tongue analysis is a greener analytical technique, compared to the spectrophotometric analysis (bitterness index and total phenols evaluation), which needs multiple sample pre-treatments, non-green reagents/solvents and/or expensive standard chemical compounds, besides the longer required time needed by the latter, from sample pre-treatments till analysis. In conclusion, the E-tongue allows, in a single-run assay, to acquire a fingerprint of the debittering status of the stoned green olives, being possible to simultaneously monitor both sensory profiles, bitterness index and total phenols contents.

4. Conclusions

The study carried out demonstrated, for the first time, that a potentiometric electronic tongue, coupled with linear discriminant analysis or multiple linear regression models together with the simulated annealing algorithm, could be accurately used to monitor the changes of bitter, pungent and sweet intensities as well as of the bitterness index and the total phenols contents during the natural debittering of traditional stoned green table olives (*cvs.* Cobrançosa and Negrinha de Freixo). The proposed E-tongue-chemometric approach could be used as a fast, green, cost-effective, *in-situ* and real-time analytical tool, allowing assessing in a single-run the sensory and chemical key parameters related to the natural debittering process. The overall satisfactory qualitative and quantitative performance observed show that the device could be used as a taste sensor, enabling the sensory evaluation of the stoned green olives, and so, being a helpful complementary tool, reducing the number of samples that must be assessed by a trained sensory panel. However, the results also allowed verifying that this electrochemical based strategy is more accurate for bitter olive cultivars, thus, a future effort must be carried out to overcome this limitation.

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References

Ambra, R., Natella, F., Bello, C., Lucchetti, S., Forte, V., & Pastore, G. (2017). Phenolics fate in table olives (*Olea europaea* L. *cv*. Nocellara del Belice) debittered using the Spanish and Castelvetrano methods. *Food Research International, 100*, 369–376.
Bertsimas, D., & Tsitsiklis, J. (1993). Simulated annealing. *Statistical Science, 8*, 10–15.
Borges, T. H., Peres, A. M., Dias, L. G., Seiquer, I., & Pereira, J. A. (2018). Application of a potentiometric electronic tongue for assessing phenolic and volatile profiles of Arbequina extra virgin olive oils. *LWT – Food Science and Technology, 93*, 150–157.
Cadima, J., Cerdeira, J. O., & Minhoto, M. (2004). Computational aspects of algorithms for variable selection in the context of principal components. *Computational Statistics & Data Analysis, 47*, 225–236.

Cadima, J., Cerdeira, J. O., Silva, P. D., & Minhoto, M. (2012). *The subselect R package.* Accessed date http://cran.rproject.org/web/packages/subselect/vignettes/subselect.pdf/, Accessed date: 12 December 2018.

Cicerale, S., Conlan, X. A., & Sinclair, A. J. (2008). Chemistry and health of olive oil phenolics. *Critical Reviews in Food Science and Nutrition, 49*, 218–236.

Habibi, M., Golmakani, M., Farahnaky, A., Mesbahi, G., & Majzoobi, M. (2016). NaOH-free debittering of table olives using power ultrasound. *Food Chemistry, 192*, 775–781.

Inarejos-García, A. M., Androulaki, A., Salvador, M. D., Fregapane, G., & Tsimidou, M. (2009). Discussion on the objective evaluation of virgin olive oil bitterness. *Food Research International, 42*, 279–284.

IOC, International Olive Council (2011). *Method for the sensory analysis of table olives.* 2011 Accessed date http://www.internationaloliveoil.org/estaticos/view/224-testing-methods, Accessed date: 10 November 2018.

Johnson, R. L., Melliou, E., Zweigenbaum, J. A., & Mitchell, A. E. (2018). Quantitation of oleuropein and related phenolics in cured Spanish-style green, California-style black ripe and Greek-style natural fermentation olives. *Journal of Agricultural and Food Chemistry, 66*, 2121–2128.

Kiai, H., & Hafidi, A. (2014). Chemical composition changes in four green olive cultivars during spontaneous fermentation. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 57*, 663–670.

Kirkpatrick, S., Gelatt, C. D., & Vecchi, M. P. (1983). Optimization by simulated annealing. *Science, 220*, 671–680.

Kobayashi, Y., Habara, M., Ikezazki, H., Chen, R., Naito, Y., & Toko, K. (2010). Advanced taste sensors based on artificial lipids with global selectivity to basic taste qualities and high correlation to sensory scores. *Sensors, 10*, 3411–3443.

Malheiro, R., Casal, S., Sousa, A., Pinho, P. G., Peres, A. M., Dias, L. G., et al. (2012). Effect of cultivar on sensory characteristics, chemical composition, and nutritional value of stoned green table olives. *Food and Bioprocess Technology, 5*, 1733–1742.

Marsilio, V., Seghetti, L., Iannucci, E., Russi, F., Lanza, B., & Felicioni, M. (2005). Use of a lactic acid bacteria starter culture during green olive (*Olea europaea* L *cv.* Ascolana tenera) processing. *Journal of the Science of Food and Agriculture, 85*, 1084–1090.

Marx, Í. M. G., Rodrigues, N., Dias, L. G., Veloso, A. C. A., Pereira, J. A., Drunkler, D. A., et al. (2017a). Sensory classification of table olives using an electronic tongue: Analysis of aqueous pastes and brines. *Talanta, 162*, 98–106.

Marx, Í. M. G., Rodrigues, N., Dias, L. G., Veloso, A. C. A., Pereira, J. A., Drunkler, D. A., et al. (2017b). Quantification of table olives' acid, bitter and salty tastes using potentiometric electronic tongue fingerprints. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 79*, 394–401.

Marx, Í. M. G., Rodrigues, N., Dias, L. G., Veloso, A. C. A., Pereira, J. A., Drunkler, D. A., et al. (2017c). Assessment of table olives' organoleptic defects intensities based on the potentiometric fingerprint recorded by an electronic tongue. *Food and Bioprocess Technology, 10*, 1310–1323.

Montaño, A., Sánchez, A., López-López, A., De Castro, A., & Rejano, L. (2010). Chemical composition of fermented green olives: Acidity, salt, moisture, fat, protein, ash, fiber, sugar and polyphenol. In V. R. Preedy, & R. R. Watson (Eds.). *Olives and olive oil in health and disease prevention* (pp. 291–297). Academic Press.

Panagou, E. Z., Sahgal, N., Magan, N., & Nychas, G.-J. E. (2008). Table olives volatile fingerprints: Potential of an electronic nose for quality discrimination. *Sensors and Actuators B: Chemical, 134*, 902–907.

Papadaki, E., & Mantzouridou, F. (2016). Current status and future challenges of table olive processing wastewater valorization. *Biochemical Engineering Journal, 112*, 103–113.

Prata, R., Pereira, J. A., Rodrigues, N., Dias, L. G., Veloso, A. C. A., Casal, S., et al. (2018). Olive oil total phenolic contents and sensory sensations trends during oven and microwave heating processes and their discrimination using an electronic tongue. *Journal of Food Quality, 2018*, 7826428.

Ramírez, E., Brenes, M., Castro, A., Romero, C., & Medina, E. (2017). Oleuropein hydrolysis by lactic acid bacteria in natural green olives. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 78*, 165–171.

Rodrigues, N., Dias, L. G., Veloso, A. C. A., Pereira, J. A., & Peres, A. M. (2016). Monitoring olive oils quality and oxidative resistance during storage using an electronic tongue. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 73*, 683–692.

Sakouhi, F., Harrabi, S., Absalon, C., Sbei, K., & Boukhchina Kallel, H. (2008). α-Tocopherol and fatty acids contents of some Tunisian table olives (*Olea europaea* L.): Changes in their composition during ripening and processing. *Food Chemistry, 108*, 833–839.

Semenov, V., Volkov, S., Khaydukova, M., Fedorov, A., Lisitsynab, I., Kirsanov, D., et al. (2019). Determination of three quality parameters in vegetable oils using potentiometric e-tongue. *Journal of Food Composition and Analysis, 75*, 75–80.

Sinelli, N., Cerretani, L., Egidio, V. D., Bendini, A., & Casiraghi, E. (2010). Application of near (NIR) infrared and mid (MIR) infrared spectroscopy as a rapid tool to classify extra virgin olive oil on the basis of fruity attribute intensity. *Food Research International, 43*, 369–375.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Sousa, A., Casal, S., Bento, A., Malheiro, R., Oliveira, M. B. P. P., & Pereira, J. A. (2011). Chemical characterization of "Alcaparras" stoned table olives from northeast Portugal. *Molecules, 16*, 9025–9040.

Sousa, A., Ferreira, I. C. F. R., Calhelha, R. C., Andrade, P. B., Valentão, P., Seabra, R., et al. (2006). Phenolics and antimicrobial activity of traditional stoned table olives "alcaparra". *Bioorganic & Medicinal Chemistry, 14*, 8533–8538.

Veloso, A. C. A., Dias, L. G., Rodrigues, N., Pereira, J. A., & Peres, A. M. (2016). Sensory intensity assessment of olive oils using an electronic tongue. *Talanta, 146*, 585–593.

Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S (statistics and computing)* (4th ed.). New York: Springer.