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Evaluation of the Effect of Extracted Time Conditions on the Phenolic Content of Olive Pastes from *cv.* Arbequina and Discrimination Using a Lab-Made Potentiometric Electronic Tongue †

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Abstract: The present study investigated the effect of malaxation times (Mt) (0, 15, 30, 45 and 60 min), during the industrial extraction of *cv.* Arbequina oils at 25 °C on total phenolic content of olive pastes. Additionally, the possibility of applying a lab-made potentiometric electronic tongue (E-tongue), comprising 40 lipid/polymer sensor membranes with cross sensitivity, to discriminate the olive pastes according to the Mt, was evaluated. The results pointed out that the olive pastes' total phenolic contents significantly decreased (p-value < 0.001, one-way ANOVA) with the increase of the Mt (from 2.21 ± 0.02 to 1.99 ± 0.03 g gallic acid equivalents/kg olive paste), there being observed a linear decreasing trend (R-Pearson = −0.910). These findings may be tentatively attributed to the migration of the phenolic compounds from the olive pastes to the extracted oil and water phases, during the malaxation process. Finally, the E-tongue signals, acquired during the analysis of the olive pastes' methanolic extracts (methanol:water, 80:20 v/v), together with a linear discriminant analysis (LDA), coupled with a simulated annealing (SA) algorithm, allowed us to establish a successful classification model. The E-tongue-LDA-SA model, based on 11 selected non-redundant sensors, allowed us to correctly discriminate all the studied olive pastes according to the Mt (sensitivities of 100% for training and leave-one-out cross-validation). The satisfactory performance of the E-tongue could be tentatively explained by the known capability of lipid/polymeric sensor membranes to interact with phenolic compounds, through electrostatic interactions and/or hydrogen bonds, which total content depended on the Mt.

Keywords: electronic tongue; lipid sensor membranes; chemometrics; olive pastes; total phenolic content

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1. Introduction

The worldwide consumption of virgin olive oil (VOO) is associated with its appreciated sensory attributes as well as with the recognized health benefits, namely, the reduced risk of chronic diseases and increased longevity, mainly related to the unsaturated fatty acids and minor components like polyphenols [1]. One strategy to ensure the natural enrichment of olive oils in phenolic compounds is based on the optimization of the extraction conditions, namely, using different malaxation times and/or temperatures [2–4].

Several destructive and nondestructive analytical techniques (e.g., chromatography, electrochemical sensor devices and spectroscopy) have been applied to evaluate the olive oil physicochemical and quality characteristics, including the assessment of total and

individual compositions in phenolics [5,6]. Nevertheless, most of the studies are focused on the olive oil evaluation after being extracted or during the storage period. In those studies, the proposed methodologies were not used as prognostic tools of olive oil quality, i.e., to predict the quality of the olive oil to be processed from measurements on the olive pastes collected during the olive oil extraction process. Actually, a small number of works have been published on the potential prediction of olive oil composition and quality before or during olive oil production [4,7,8]. In this context, this study aimed to evaluate the effect of malaxation time (Mt), during the industrial extraction of oils, on the total phenolic content (TPC) of *cv.* Arbequina pastes. Additionally, the use of a potentiometric lab-made electronic tongue (E-tongue) to estimate the TPC in olive pastes collected at different Mt, was also evaluated. This capability could allow establishing indirect correlations between the composition of olive pastes and the TPC of the *cv.* Arbequina oils industrially extracted. It is important to emphasize that E-tongues comprising lipid sensor membranes have been extensively used to determine the phenolic profile and the sensory sensations of olive oils [3,7], which versatility has been related to the low selectivity and cross-sensitivity of the sensors that mimic the behavior of the human biological gustatory receptors [9].

2. Materials and Methods

2.1. Olives and Olive Pastes Samples

Olives from the *cv.* Arbequina were harvested in mid-November 2017 from an orchard located in Trás-os-Montes region (northeast Portugal). Olive pastes were collected at 5 time-periods (0, 15, 30, 45 and 60 min) during the oil extraction at 25 °C, in an industrial olive mill (OLIMONTES, Macedo de Cavaleiros, Portugal. Five samples of olive pastes (~100 g) were collected from the malaxers during the extraction, totalizing 25 olive paste sub-samples (5 replicas × 5 time-periods). The TPC and the potentiometric profiles of the olive pastes were determined.

2.2. Olive Pastes

2.2.1. Analytical Extraction for TPC and Potentiometric Analysis

The methodology applied was previously described by Marx et al. [6]. The polar extract containing the phenolic compounds was collected to assess the TPC and to establish the potentiometric profiles.

2.2.2. TPC of Olive Paste Extracts

The TPC was determined following the methodology proposed by Singleton and Rossi [10] and previous described by Marx et al. [7]. Gallic acid was used as the external standard compound to establish the calibration curve ($R^2 > 0.999$), being the results expressed as g of Gallic acid equivalents (GAE) per kg of olive paste (g GAE/kg olive paste).

2.2.3. E-Tongue Apparatus and Potentiometric Analysis of Olive Paste Extracts

A lab-made potentiometric E-tongue, comprising two cylindrical arrays, was used. Each array contained 20 lipid polymeric cross-sensitive sensor membranes (1st array: S1:1 to S1:20; 2nd array: S2:1 to 2:20). The construction details, as well as the composition of the membranes were previously reported by Marx et al. [3]. The device was connected to an Agilent Data Acquisition unit (model 34970A), which was controlled by an Agilent BenchLink Data Logger software. For the olive pastes analysis, the TPC polar extract was used after a 1:5 (*v*/*v*) dilution in deionized water [7]. The diluted solution was analyzed with the E-tongue during 5 min to allow reaching a pseudo-equilibrium between the non-specific lipid polymeric membranes and the dissolved chemical compounds [7].

2.3. Statistical Analysis

The TPC of olive pastes were analyzed using the one-way ANOVA followed by the Tukey's post-hoc multi-comparison test. Linear discriminant analysis (LDA) was applied to evaluate the correct discrimination of the studied pastes based on the best subsets of

E-tongue sensors selected using the simulated annealing (SA) algorithm. The leave-one-out cross-validation (LOO-CV) variant was used to evaluate the predictive performance of the classification model and the repeated K-fold-CV. The quality of the results was assessed considering the sensitivity (i.e., the percentage of corrected classified samples). The statistical analysis was performed using the Sub-select and MASS packages of the open-source statistical program R (RStudio version Version 1.2.5033), at a 5% significance level, as previous detailed by Marx et al. [3,7].

3. Results and Discussion

3.1. TPC of Olive Pastes

The TPC of the olive pastes, collected at five time-periods (0, 15, 30, 45 and 60 min) during the industrial extraction of *cv*. Arbequina oils, were determined following the Folin-Ciocalteau spectrophotometric method and are shown in Table 1. According to the results, the TPC of the pastes linearly decreases with the Mt (*R*-Pearson = −0.910). However, until 30 min of malaxation, the observed decrease is not significant, there being observed a reduction of 0.46% between 15 and 30 min. On the other hand, after 30 min of malaxation, the reduction on the TPC of the studied pastes was more pronounced. Similar trends (negative correlation between the TPC of the olive paste and the Mt) have already been reported in the literature [4]. According to Trapani et al. [4], the decreasing trend was attributed to the enzymatic oxidation of phenolic compounds, probably due to the fact that during the malaxation process the olive paste was exposed to air. The knowledge of the TPC of olive pastes during malaxation could pave the way towards a real-time control of the impact of the Mt on the olive oils being extracted in order to promote the increase of the total phenolics in olive oils.

Table 1. Statistical analysis of TPC of olive pastes collected at five different malaxation times, during the industrial extraction of olive oil (average, standard deviation, *p*-value and *R*-Pearson).

Folin–Ciocalteau Spectrophotometric Method	Malaxation Time					*p*-Value [1]	*R*-Pearson
	0 min	15 min	30 min	45 min	60 min		
TPC (g GAE/kg olive paste)	2.21 ± 0.02 [A]	2.18 ± 0.02 [A]	2.17 ± 0.04 [A]	2.04 ± 0.03 [B]	2.00 ± 0.03 [B]	<0.0001	−0.910

[1] *p*-values for the one-way ANOVA. Different letters in the same row show statistically differences from the given mean ($p < 0.05$). n = 5.

3.2. Estimating TPC of Olive Pastes Based on the Potentiometric E-Tongue Analysis of Olive Paste Extracts

Among the 40 lipid polymeric sensors, it was possible to establish linear correlations (positive or negative, i.e., signal-on or signal-off) between the potentiometric signals recorded by the E-tongue sensor membranes and the decimal logarithm of TPC, for 75% of the sensors ($0.836 \leq R^2 \leq 0.998$). The sensors mean sensitivities varied from +3.7 to +376 mV/decade, or between −185 to −42 mV/decade. The linear correlations were obtained for 30 E-tongue sensors (1st array: S1:1, S1:2, S1:3, S4, S1:7, S1:8, S1:10, S1:12, S1:13, S1:15, S1:16, S1:17, S1:18, S1:19 and S1:20; 2nd array: S2:2, S2:3, S2:4, S2:5, S2:7, S2:8, S2:9, S2:10, S2:12, S2:13, S2:15, S2:17, S2:18, S2:19 and S2:20), and the mean TPC calculated by applying the referred linear correlations are shown in Table 2.

Table 2. TPC (mean ± standard deviation) of olive pastes estimated using the correlations established between the E-tongue signals and the decimal logarithm of TPC, for the five different malaxation times studied (minimum and maximum contents in brackets).

E-Tongue Analysis	Malaxation Time				
	0 min	15 min	30 min	45 min	60 min
Estimated TPC (g GAE/kg olive paste)	2.21 ± 0.03 (2.06–2.25)	2.13 ± 0.04 (2.03–2.22)	2.15 ± 0.03 (2.08–2.23)	2.05 ± 0.02 (1.99–2.09)	1.99 ± 0.01 (1.96–2.02)

The agreement between the experimental TPC (Table 1) and those estimated by the device (Table 2), pointed out that the E-tongue could be applied as a real-time analytical tool to estimate the TPC in olive pastes collected during the oil extraction, allowing establishing the best Mt of the olive pastes that would ensure the extraction of an olive oil rich in phenolic compounds.

Finally, the E-tongue signals acquired during the analysis of the olive pastes' methanolic extracts (methanol: water, 80:20 v/v), allowed the establishing of a successful classification LDA-SA model. The E-tongue-LDA-SA model (Figure 1), based on 11 selected non-redundant sensors, correctly discriminated all the studied olive pastes according to the Mt (sensitivities of 100% for training and LOO-CV) and 91 $\pm$ 12% for repeated K-fold-CV. The satisfactory performance of E-tongue could be tentatively attributed by the known capability of the lipid sensor membranes to interact with phenolic compounds, through electrostatic interactions and/or hydrogen bonds, which total content depended on the Mt [7].

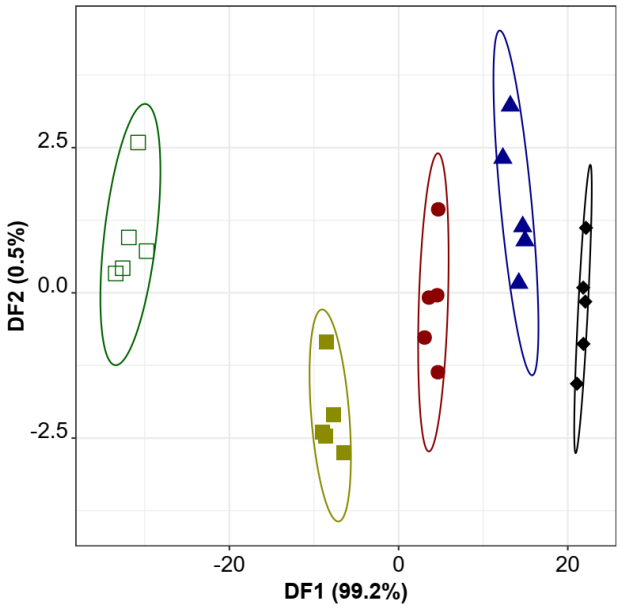

Figure 1. E-tongue-LDA-SA model performance regarding the supervising classification of *cv.* Arbequina olive pastes extracted at 0 min (□); 15 min (■); 30 min (●); 45 min (▲) and 60 min (♦) based on the potentiometric signals gathered by eleven lipid sensor membranes (1st array: S1:1, S1:8, S1:14, S1:17, S1:18, S1:20; 2nd array: S2:2, S2:3, S2:4, S2:5 and S2:18), selected using the SA algorithm from a set of 40 sensors.

4. Conclusions

The spectrophotometric evaluation of the olive pastes showed that until 30 min of malaxation, the TPC of the olive pastes were not significantly different. Oppositely, after 30 min of malaxation, the TPC of the pastes decreased, being the lowest contents determined for pastes after 60 min of malaxation. However, monitoring the TPC of olive pastes by spectrophotometry is a time-consuming task that requires several sample pre-treatments. Furthermore, this conventional spectrophotometric technique has some practical limitations, like the difficult regarding its implementation as an in-situ and *online* tool, besides being an invasive/destructive technique.

The present study showed that the potentiometric E-tongue analysis of extracts of olive pastes, collected during the industrial extraction of *cv.* Arbequina oils, coupled with chemometric tools, allowed estimating of the TPC. In addition, the E-tongue was capable to correctly discriminate all olive pastes studied according to the malaxation time.

Taking into account its portability, the lab-made E-tongue could be easily implemented in an industrial olive mill allowing estimating of the TPC of the olive pastes and, indirectly, establishing of the optimal malaxation time of the olive pastes to obtain a high-quality oil.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/CSAC2021-10556/s1.

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