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ORIGINAL ARTICLE

Unmasking Sensory Defects of Olive Oils Flavored with Basil and Oregano Using an Electronic Tongue-Chemometric Tool

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Abstract Olive oil price and consumers' preference depend on the commercial grade classification that can decrease if any sensory defect is perceived leading to an economic loss. Enriched oils, obtained by incorporating dried aromatic herbs, spices, or essential oils, which is a common practice in the Mediterranean region, are commercially available. This practice may conceal the fraudulent purpose of masking the perception of sensory defects. The detection of this type of fraud is a difficult task, requiring sensory analysis. Thus, in this study, extra-virgin and lampante olive oils, the latter classification being due to the perception of an intense winey-vinegary defect, were deliberately enriched with different amounts of basil-dried herbs and oregano-dried herbs. Sensory analysis showed that, depending on the aromatic herb and on the added amount (0.011–0.110 g herb per kg oil), the defect intensity could be masked leading to an erroneous classification of flavored lampante oils as flavored virgin oils. In contrast, the electronic tongue-chemometric approach could unmask the defect in flavored oils (predictive sensitivities: 70–78%) and semiquantitatively discriminate flavored oils according to the added levels of basil or oregano (predictive sensitivities: 93–100%). The electronic tongue approach showed satisfactory unmasking performance when compared with the sensory panel, and so, its future application as a quality control taste-sensor device for disclosing olive oil sensory defects masked by the incorporation of flavoring agents may be forseen.

Keywords Olive oil · Physicochemical analysis · Sensory analysis · Aromatic herbs flavoring · Electronic tongue · Sensory defect detection

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Introduction

Olive oil plays a key role in the Mediterranean diet, providing the intake of several bioactive compounds with recognized nutritional and healthy effects. These effects may even be enhanced by the consumption of enriched olive oils (En-VOO) (Rubió et al., 2012). The production of En-VOO is aimed to increase the oxidative stability (OS), and thus the oil shelf-life as well as to enhance oil taste and aroma, contributing to mask the intensity of some attributes, like bitterness, which although being considered a positive sensation from a sensory classification point of view, is not very appreciated by the consumers of some geographical regions (Moldão-Martins et al., 2004). Different nature-based matrices have been used to obtain En-VOO, including, aromatic herbs and spices (e.g., basil, caraway, chili peppers and red pepper, garlic, lemon and lemon peels, laurel, lavender, menthe, orange peels, oregano, rosemary, sage, and thyme), either through incorporation techniques (Ayadi et al., 2009; Gambacorta et al., 2007; Sousa et al., 2015) or by the addition of purified extracts or essential oils (Assami et al., 2016;

Baiano et al., 2016; Caponio et al., 2016; Clodoveo et al., 2016; Khemakhem et al., 2015; Sacchi et al., 2017).

Olive oil enrichment could also comprise a fraudulent practice. Indeed, the addition of aromatic herbs and spices may contribute to mask the sensory perception of organoleptic defects that when perceived leads to a great decrease of the olive oil commercial value, and so reducing the economic revenue of the olive oil producers. Indeed, even if from a physicochemical point of view an olive oil may be classified as extra-virgin olive oil (EVOO), its grade may decrease to virgin olive oil (VOO) if an organoleptic defect is perceived with an intensity up to 3.5 in a 0–10 scale intensity range or to a lampante olive oil (LOO, which cannot be commercialized) if the perceived defect intensity is greater than 3.5 (Commission Delegated Regulation [EU] 2015/1830, 2015). The implementation of this fraudulent practice may allow the commercial disposal of lower-quality olive oils.

Thus, the development of practical, fast, and accurate strategies that could be used to discriminate flavored from unflavored olive oils, of different quality grades, is of utmost relevance and could allow unmasking possible frauds related to the deliberated enrichment of sensory defected olive oils aiming its commercialization as high quality and more expensive olive oils. Officially, olive oils sensory profile must be established by a trained taste panel (Commission Delegated Regulation [EU] 2015/1830, 2015), which poses several drawbacks, namely due to the scarcity of official panels, lack of reference standards, and a low number of analyses per day (Sinelli et al., 2010). Alternatively, potentiometric and voltammetric electronic tongues (E-tongues) have been successfully used to evaluate positive sensory attributes (e.g., bitter, green, and/or fruity sensations) and organoleptic defects (e.g., rancid, winey-vinegary, musty, fusty, zapateria, butyric, and putrid sensations) of olive oils and table olives as well as to unmask the deliberated addition of rancid or winey-vinegary olive oil to EVOO (Harzalli et al., 2018; Rodrigues et al., 2019; Veloso et al., 2016, 2018). This work aims, for the first time, to evaluate the possibility of using a potentiometric E-tongue, comprising lipid-polymeric sensor membranes, in combination with chemometric tools to discriminate EVOO or LOO, flavored or not, with basil or oregano. The main goal is to use the E-tongue to unmask sensory defected olive oils after their flavor enrichment by the addition of natural aromatic herbs, which is a typical practice in the Mediterranean countries.

Materials and Methods

Olive Oil Samples and Flavoring Procedure

Cv. Arbequina olive oils from two different production lots (lot #1 and lot #2) were obtained from a local olive mill (Olimontes, Macedo de Cavaleiros, Portugal). An initial quality evaluation (*data not shown*) was performed showing that, from a physicochemical point of view, the oils from both lots were EVOO, fulfilling the legal thresholds established by the European Commission (EC) regulations (Commission Delegated Regulation [EU] 2015/1830, 2015; Sacchi et al., 2017). From a sensory point of view, and according to the sensory analysis performed by eight trained panelists of the Agricultural School of the Polytechnic Institute of Bragança, the oils from lot #1 and lot #2 had a fruity intensity sensation greater than 0 fulfilling the requirement for EVOO classification. However, although no organoleptic defect could be perceived by the panelists for oils from lot #1, confirming the EVOO classification; oils from lot #2 had a winey-vinegary gustatory-retronasal negative sensation, which could be perceived with an average intensity greater than 3.5, leading to a LOO quality classification, according to the EC recommendations (Commission Delegated Regulation [EU] 2015/1830, 2015).

The oils were split into 80 amber glass bottles (125 mL). Ten bottles from each quality grade (EVOO or LOO for lot #1 and #2, respectively) were used as control, no flavoring agent being added (2 oil grades × 10 bottle replicas = 20 bottles). The other 60 olive oil bottles were flavored using two different flavoring agents (oregano-dried herbs and basil aromatic-dried herbs from a commercial brand, purchased from a local supermarket). For each flavoring agent (oregano or basil) and oil quality grade (EVOO or LOO, for lot #1 and #2, respectively), three concentration levels (0.011, 0.055, and 0.110 g dried flavoring agent per kg olive oil) were studied, in quintuplicate resulting in a total of 60 bottles (3 levels × 2 flavoring agents × 2 oil grades × 5 bottle replicas = 60 bottles). All bottles were stored for 2 weeks in a dark environment, at room temperature (18–25°C), before being analyzed.

Evaluation of Olive Oils Quality Chemical Indices, OS, and Sensory Analysis

The values of the free acidity (FA), extinction coefficients at 232 and 270 nm (K_{232} and K_{270}), and peroxide value (PV) of the samples withdrawn from each of the 80 olive oil bottles, unflavored (20 bottles), or flavored (60 bottles) by incorporating different concentrations of oregano or basil dehydrated/dried leaves were determined according to the EC regulation (Commission Delegated Regulation [EU] 2015/1830, 2015). The oils OS was also assessed using the Rancimat method (Rodrigues et al., 2019). The oils sensory analysis was performed by the abovementioned panel, following the EC regulation (Commission Delegated Regulation [EU] 2015/1830, 2015), being specifically asked the assessment of the olfactory and gustatory-retronasal fruity positive attribute and of possible organoleptic defects, using an intensity scale ranging from 0 (absence of attribute) to 10 (maximum attribute intensity).

E-tongue Analysis

E-Tongue Device and Setup

A lab-made potentiometric multisensor E-tongue, comprising two cylindrical arrays, was used (Rodrigues et al., 2019). Each array contained 20 lipid polymeric cross-sensitive sensor membranes (40 sensors in total), with a composition (lipid additive, 3%; plasticizer, 32%; and, polyvinyl chloride, 65%) similar to that reported for the screen-printed E-tongues developed by the research team (Rodrigues et al., 2019; Veloso et al., 2016), although with greater diameter and thickness (greater contact surface), which allowed achieving more repeatable signal profiles and to minimize signal drifts (intraday coefficient of variation lower than 5%) (Rodrigues et al., 2019). The sensor membranes were connected to a multiplexer Agilent Data Acquisition Switch Unit (model 34970A) controlled by the Agilent BenchLink Data Logger software installed on a PC. Each potentiometric assay took 5 min and allowed recording the potentiometric signals of the 40 sensor membranes generated through the establishment of electrostatic or hydrophobic interactions between the sensor membranes and polar compounds (Kobayashi et al., 2010). A reference Ag/AgCl double-junction glass electrode (Crison, 5241) was used. The two sensor arrays were stored in a HCl solution (0.01 M). The same sensor coding used in previous works was adopted: each sensor was identified with a letter S (for sensor) followed by the number of the array (1: or 2:) and the number of the membrane (1–20, corresponding to different combinations of plasticizers and additives).

E-Tongue Analysis: Olive Oil Sample Preparation and Potentiometric Assays

To overcome the difficulty of performing electrochemical assays in nonconductive and highly viscous liquids (Apetrei et al., 2010), olive oils (10 g) were extracted with 100 mL of hydroethanolic solution (80:20, water: ethanol, v/v). Also, these extracts are rich in polar compounds, including phenolic compounds, esters, alcohols, and aldehydes, which are responsible for several sensory positive and negative attributes (Veloso et al., 2016, 2018) and with which the lipid sensor membranes can interact through the establishment of electrostatic or hydrophobic interactions (Kobayashi et al., 2010). The mixture (olive oil plus hydroethanolic solution) was agitated for 1–2 min using a vortex stirrer (LBX V05 series, LBX Instruments, LABBOX LABWARE S.L., Barcelona, Spain) at 500 rpm. Then, it was left at ambient temperature for 60 min, after which, 60 mL of the supernatant solution was carefully withdrawn and immediately analyzed with the E-tongue, for a 5-min period, which allowed reaching a pseudo-equilibrium between the lipid polymeric membranes of E-tongue' and the chemical compounds of the extract. Electrochemical assays were performed in duplicate for each sample, with a third assay carried out if the potentiometric signal of any of the 40 sensors showed a coefficient of variation greater than 20% (value set according to EC regulations for sensory analysis). The E-tongue signals were normalized previously to any further statistical use.

Statistical Analysis

The influences of the type of flavoring agent used (basil or oregano) and of the concentration (0, 0.011, 0.055, and 0.110 g dried flavoring agent per kg olive oil) on the physicochemical parameters and sensory attributes of the unflavored or flavored olive oils were evaluated through the *t*-Student test or the one-way ANOVA, the significance of the interaction of "flavoring agent × concentration" being assessed by a two-way ANOVA. For the one-way ANOVA, the post-hoc multicomparison Tukey's test was further applied if a significant statistical effect was found (P-value < 0.050). Besides, the *R*-Pearson correlation coefficients were also calculated to evaluate the existence of a linear trend between the OS or the intensity of sensory attributes and the added concentrations of each flavoring agent.

Linear discriminant analysis (LDA) coupled with the metaheuristic simulated annealing (SA) variable selection algorithm (Cadima et al., 2004) was used to evaluate the capability of the potentiometric E-tongue to simultaneously discriminate olive oils according to the oil quality grade (EVOO and LOO) and the deliberated oils flavoring/enrichment, or not, with aromatic herbs (oregano or basil). E-tongue-LDA-SA models were established based on the best subsets of the 40 normalized signal profiles generated during the electrochemical analysis, which were selected by the SA algorithm, aiming to minimize noise effects due to the inclusion of redundant potentiometric signals. The LDA predictive performance was assessed using the leave-one-out cross-validation (LOO-CV) technique taking into account the dimension of the independent dataset. The classification performance of each LDA model was also graphically evaluated using 2D or 3D plots of the main discriminant functions, the class membership boundary ellipses being determined based on the posterior probabilities computed using the Bayes' theorem (which enables controlling over-fitting issues) (Bishop, 2006). Finally, for each LDA model, the overall performance established was also assessed based on the sensitivity values, i.e., based on the percentage of correct classifications. All statistical analyses were performed using the open source statistical program R (version 2.15.1), at a 5% significance level.

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

Results and Discussion

Effect of Olive Oil Flavoring on the Physicochemical Quality Attributes, OS, and Fruity and Sensory Defect Intensities

Unflavored and flavored olive oils (EVOO: lot #1; and, LOO: lot #2) were evaluated after 2 weeks of flavoring agent incorporation (or not) at three pre-established concentrations of the two dried aromatic herbs (basil or oregano). The values of the evaluated parameters (FA, PV, K_{232}, K_{270}, ΔK, and OS) are given in Tables 1 and 2. The perceived intensities of fruity positive sensation and winey-vinegary negative attribute, assessed by the trained panelists, are also shown in Tables 1 and 2. A two-way ANOVA (*data not shown*) pointed out that, for both types of olive oils, and the evaluated physicochemical and sensory parameters, a significant interaction effect ("flavoring agent × concentration") was found (P-value ≤ 0.01 for the two-way ANOVA), with the exception of FA (EVOO: P-value = 0.1842), K_{232} (EVOO: P-value = 0.1520), and K_{270} (EVOO and LOO: P-value ≥ 0.4213). Nevertheless, the significance of each individual effect ("flavoring agent" or "concentration") was further evaluated by the *t*-Student test or one-way ANOVA, respectively (Tables 1 and 2). The data showed that the physicochemical quality parameters of the EVOO and LOO studied were, in general, significantly influenced by the presence and concentration of each aromatic herb (P-value < 0.05 for the one-way ANOVA). However, no clear trend could be established, an increase–decrease tendency being observed, depending on the olive oil quality, aromatic herb, and/or added concentration level. For the studied olive oils, the quality parameters changed and the extent of changes greatly depended on the starting olive oil grade and to a less extent on the flavoring agent and concentration used (Baiano et al., 2016). Gambacorta et al. (2007) reported that PV and K_{232} values decreased with the addition of the flavoring agents (rosemary, hot pepper, oregano, and garlic) although no significant effect was observed for FA and K_{270}. Baiano et al. (2009) compared unflavored and flavored olive oils (with garlic, lemon, hot pepper, oregano, or rosemary) and found lower FA but higher values of PV, K_{232}, and K_{270}. However, Khemakhem et al. (2015) observed no changes between FA of flavored (sweet lime and sweet orange) and unflavored olive oils, although a significant increase of the PV values occurred for some flavored oils. Caponio et al. (2016) found that olive oils aromatized with spices had significantly higher levels of PV and K_{232} compared to the unflavored oils. Other researchers reported an increase of FA values but not of the other quality indices (Ayadi et al., 2009; Sousa et al., 2015), while Caporaso et al. (2013) and Assami et al. (2016) observed a slight increase of all olive oil quality parameters with the addition of condiments. For example, Sacchi et al. (2017) verified a significant increase of FA, K_{232}, and K_{270} values on olive oils flavored with lemon. Nevertheless, Clodoveo et al. (2016) did not found any differences using thyme or oregano. Benmoussa et al. (2016) found that flavoring olive oils with rosemary leaves leads to an increase of the FA, a decrease of PV and K_{232}, while no significant change was observed for K_{270}. The different effects reported in the literature (increase or decrease trends) or the absence of a significant influence on olive oils quality parameter levels and their oxidation stability, after the addition of natural flavoring agents, may be tentatively explained considering: the differences in chemical composition and bioactive contents of the aromatic plants/herbs or species used, the use of fresh or dried natural flavoring agents, and consequently the incorporation of water in the system, the different flavoring techniques as well as the specific olive oil characteristics (Clodoveo et al., 2016). It should be noticed that, in this work, the final levels of the physicochemical quality parameters of the unflavored and flavored olive oils remained below the legal thresholds established for EVOO classification (Commission Delegated Regulation [EU] 2015/1830, 2015).

This study also showed that OS significantly decreased with the increase of the flavoring agent concentration, being more pronounced for basil compared to oregano and for EVOO compared to LOO. However, other researchers reported an OS increase due to the addition of aromatic herbs or natural condiments (Assami et al., 2016; Gambacorta et al., 2007; Sousa et al., 2015), although this could depend on the flavoring agent (Ayadi et al., 2009; Issaoui et al., 2011). For example, a significant decrease of the OS was observed when dried chili pepper, hot pepper, lemon, rosemary, or sweet lime/orange were added to oils (Baiano et al., 2009; Caporaso et al., 2013; Khemakhem et al., 2015).

Finally, the major impact of the aromatizing process was at the olive oil sensory level. The fruity intensity of EVOO linearly increased with the increase of the flavoring concentration (R-Pearson = +0.90 and + 0.94 for basil and oregano, respectively). A similar behavior was found for LOO (basil and oregano: R-Pearson = +0.89 and + 0.92, respectively). On the other hand, for EVOO, the addition of basil or oregano did not lead to the appearance of any sensory defect. Regarding LOO, the addition of basil or oregano

Table 1 Physicochemical and sensory parameters of extra-virgin olive oils (lot #1), 15 days after being flavored or not with dried aromatic herbs (basil or oregano at 0, 0.011, 0.055, and 0.110 g per kg olive oil)

EVOO attribute (lot #1)	Flavoring agent	Concentration (g dried oregano per kg olive oil)				
		0 g kg^{-1} (10 bottles)	0.011 g kg^{-1} (5 bottles)	0.055 g kg^{-1} (5 bottles)	0.110 g kg^{-1} (5 bottles)	*P*-value[a]
Physicochemical parameters						
FA (%)	Basil	0.17 ± 0.01	0.18 ± 0.01	0.17 ± 0.00	0.17 ± 0.01	0.6007
	Oregano	0.17 ± 0.01[a]	0.15 ± 0.01[b]	0.16 ± 0.01[a,b]	0.16 ± 0.01[a,b]	***0.0083***
	P-value[b]	—	***0.0013***	***0.0246***	0.3352	
PV (mEq O_2 kg^{-1})	Basil	4.8 ± 1.2	4.4 ± 0.2	4.6 ± 0.4	5.2 ± 0.5	0.1458
	Oregano	4.8 ± 1.2[a]	3.3 ± 0.3[b]	4.4 ± 0.9[a,b]	5.6 ± 1.4[a]	***<0.0001***
	P-value[b]	—	***<0.0001***	0.5773	0.4019	
K_{232}	Basil	1.507 ± 0.043[b]	2.073 ± 0.044[a]	2.042 ± 0.055[a]	2.024 ± 0.077[a]	***<0.0001***
	Oregano	1.507 ± 0.043[b]	2.053 ± 0.080[a]	2.107 ± 0.074[a]	2.034 ± 0.085[a]	***<0.0001***
	P-value[b]	—	0.4896	***0.0357***	0.7882	
K_{270}	Basil	0.119 ± 0.012[b]	0.138 ± 0.009[a]	0.136 ± 0.003[a]	0.139 ± 0.004[a]	***<0.0001***
	Oregano	0.119 ± 0.012	0.124 ± 0.007	0.119 ± 0.015	0.130 ± 0.007	0.0540
	P-value[b]	—	***0.0010***	***0.0019***	***0.0024***	
OS (h)	Basil	8.8 ± 0.2[a]	7.6 ± 0.3[b]	7.6 ± 0.2[b]	7.6 ± 0.2[b]	***<0.0001***
	Oregano	8.8 ± 0.2[a]	8.4 ± 0.2[b]	8.4 ± 0.2[b]	8.3 ± 0.2[b]	***<0.0001***
	P-value[b]	—	***<0.0001***	***<0.0001***	***<0.0001***	
Sensory analysis (intensity, 0–10 scale, 0: sensation not perceived to 10: maximum perceived intensity)						
Fruity sensation	Basil	3.9 ± 0.4[d]	5.3 ± 0.3[c]	6.0 ± 0.3[b]	6.7 ± 0.3[a]	***<0.0001***
	Oregano	3.9 ± 0.4[d]	5.3 ± 0.2[c]	6.4 ± 0.2[b]	7.4 ± 0.3[a]	***<0.0001***
	P-value	—	0.7974	***0.0064***	***<0.0001***	
Defect sensation	Basil	N.D.	N.D.	N.D.	N.D.	
	Oregano	N.D.	N.D.	N.D.	N.D.	

FA, free acidity; K_{232} and K_{270}, extinction coefficients at 232 and 270 nm, respectively; N.D., not detected (no sensations perceived by the trained panelists); OS, oxidative stability; PV, peroxide values.

[a] For each line, a *P*-value < 0.05 (bold and italic) means that for each flavoring agent (basil or oregano), the mean value of the evaluated parameter of at least one aromatizing concentration differs from the others, according to the one-way ANOVA. In each line, different small letters mean significant statistical differences of the parameter under evaluation, at a 5% significance level, according to multiple comparison Tukey's HSD test.

[b] For each column and for aromatizing concentration level, a *P*-value < 0.05 (bold and italic) means that the mean value of the evaluated parameter varied significantly with the type of flavoring agent, according to *t*-Student test.

contributed to mask the sensory defect (winey-vinegary negative attribute) intensity, which linearly decreased with the increase of the flavoring concentration (basil and oregano: *R*-Pearson = −0.95 and −0.99, respectively). It should be remarked that the olive oil aromatization, with the highest oregano concentration (0.110 g dried oregano per kg olive oil) could mask the intensity of the perceived defect to such an extent that the initial LOO classification could be changed to VOO. This fact is of major relevance, pointing out the possibility of using flavoring agents for masking olive oil sensory negative attributes allowing the fraudulent commercialization of LOO as aromatized VOO. Although the defect intensity decrease was not so sharp for LOO flavored with basil, the final value achieved (defect mean intensity: 3.8 ± 0.3) also opens the possibility of using this flavoring agent at a higher concentration level to mask the presence and intensity of negative organoleptic attributes. Therefore, the development of fast, user-friendly, and cost-effective analytical tools that could unmask low-quality olive oils flavored with aromatic herbs is a real need and can play an important role as a quality control tool. In this context, the use of a potentiometric E-tongue as a taste sensor quality device was evaluated for the first time to the authors' best knowledge.

E-Tongue Analysis of Flavored and Unflavored Olive Oils

Basil Flavoring Process

The results of the sensory analysis (Table 2) showed that flavoring LOO with basil allowed masking the intensity of

Table 2 Physicochemical and sensory parameters of lampante olive oils (lot #2), 15 days after being flavored or not with dried aromatic herbs (basil or oregano at 0, 0.011, 0.055, and 0.110 g kg^{-1} olive oil)

LOO attribute (lot #2)	Flavoring agent	Concentration (g dried oregano per kg olive oil)				
		0 g kg^{-1} (10 bottles)	0.011 g kg^{-1} (5 bottles)	0.055 g kg^{-1} (5 bottles)	0.110 g kg^{-1} (5 bottles)	*P*-value[a]
Physicochemical parameters						
FA (%)	Basil	0.25 ± 0.01[a,b]	0.26 ± 0.01[a]	0.26 ± 0.02[a]	0.24 ± 0.02[b]	***0.0104***
	Oregano	0.25 ± 0.01[b]	0.27 ± 0.02[a]	0.27 ± 0.02[a]	0.28 ± 0.02[a]	***<0.0001***
	P-value[b]	—	0.0538	0.1783	***0.0001***	
PV (mEq O_2 kg^{-1})	Basil	8.4 ± 1.0	8.5 ± 0.8	7.8 ± 0.4	8.6 ± 0.6	0.1529
	Oregano	8.4 ± 1.0[a]	8.9 ± 1.6[a]	7.9 ± 1.0[a,b]	7.1 ± 0.3[b]	***0.0025***
	P-value[b]	—	0.5299	0.7536	***<0.0001***	
K_{232}	Basil	2.127 ± 0.070[a]	2.161 ± 0.055[a]	1.925 ± 0.025[b]	2.156 ± 0.043[a]	***<0.0001***
	Oregano	2.127 ± 0.070[a]	2.056 ± 0.108[a,b]	2.024 ± 0.115[b]	2.026 ± 0.103[b]	***0.0132***
	P-value[b]	—	***0.0137***	***0.0160***	***0.0017***	
K_{270}	Basil	0.126 ± 0.008[b]	0.141 ± 0.008[a]	0.139 ± 0.006[a]	0.127 ± 0.004[b]	***<0.0001***
	Oregano	0.126 ± 0.008[b]	0.138 ± 0.013[a]	0.132 ± 0.014[a,b]	0.120 ± 0.013[b]	***0.0053***
	P-value[b]	—	0.5734	0.1406	0.1372	
OS (h)	Basil	6.8 ± 0.2[a]	6.0 ± 0.1[b]	6.0 ± 0.3[b]	6.0 ± 0.1[b]	***<0.0001***
	Oregano	6.8 ± 0.2[a]	6.3 ± 0.2[b]	6.4 ± 0.1[b]	6.3 ± 0.1[b]	***<0.0001***
	P-value[b]	—	***0.0004***	***0.0005***	***< 0.0001***	
Sensory analysis (intensity, 0–10 scale, 0: sensation not perceived to 10: maximum perceived intensity)						
Fruity sensation	Basil	1.7 ± 0.3[d]	2.7 ± 0.1[c]	3.1 ± 0.2[b]	3.6 ± 0.3[a]	***<0.0001***
	Oregano	1.7 ± 0.3[c]	2.2 ± 0.2[b]	3.6 ± 0.4[a]	3.8 ± 0.3[a]	***<0.0001***
	P-value[b]	—	***<0.0001***	***0.0090***	0.2205	
Defect sensation	Basil	5.9 ± 0.4[d]	5.1 ± 0.4[c]	4.5 ± 0.4[b]	3.8 ± 0.3[a]	***<0.0001***
	Oregano	5.9 ± 0.4[d]	5.1 ± 0.6[c]	3.8 ± 0.3[b]	2.2 ± 0.3[a]	***<0.0001***
	P-value[b]	—	0.9675	***0.0003***	***<0.0001***	

FA, free acidity; K_{232} and K_{270}, extinction coefficients at 232 and 270 nm, respectively; N.D., not detected (no sensations perceived by the trained panelists); OS, oxidative stability; PV, peroxide values.

[a] For each line, a *P*-value < 0.05 (bold and italic) means that for each flavoring agent (basil or oregano), the mean value of the evaluated parameter of at least one aromatizing concentration differs from the others, according to the one-way ANOVA. In each line, different small letters mean significant statistical differences of the parameter under evaluation, at a 5% significance level, according to multiple comparison Tukey's HSD test.

[b] For each column and for aromatizing concentration level, a *P*-value < 0.05 (bold and italic) means that the mean value of the evaluated parameter varied significantly with the type of flavoring agent, according to *t*-Student test.

the winey-vinegary defect. Indeed, the panelists' perceived intensities significantly decreased with the increasing basil concentration (from an average intensity of 5.9 ± 0.4, for unflavored LOO, to 3.8 ± 0.3 for the LOO aromatized with the maximum basil concentration). In fact, the addition of basil to LOO allowed masking the sensory defect perception and for the highest concentration evaluated, the flavored oil almost reached a defect intensity that could allow its fraudulent classification as En-VOO (a classification of VOO requires that any sensory defect could only be perceived at a maximum intensity ≤3.5). In this context, the potentiometric signals of the 40 sensor E-tongue membranes recorded during the analysis of the flavored and unflavored olive oil samples (EVOO; LOO; EVOO enriched with basil: EVOO-basil; and, LOO enriched with basil: LOO-basil) were used, after normalization, to establish multivariate supervised classification models. The best subset of sensors was selected by applying the meta-heuristic SA algorithm, based on the predictive classification performance (LOO-CV procedure). An E-tongue-LDA-SA model was established (three linear discriminant [LD] functions explaining 74.5, 14.7, and 10.8% of the original data variability). The model used the normalized potentiometric data collected from 20 sensors (1st array: S1:3, S1:4, S1:6, S1:7, S1:8, S1:10, S1:12, and S1:14-S1:20; 2nd array: S2:5, S2:9, S2:12, S2:13, S2:16, and S2:19) during analysis of the hydroethanolic extracts of olive oils. The multivariate supervised classification model allowed correctly classifying 94% of the original grouped samples (Fig. 1) and correctly classify 70% of the samples (LOO-CV). It should be remarked that the misclassifications were observed for all groups with the exception of LOO samples, which were all correctly classified. The predicted classification performance was satisfactory taking into account the complexity of this challenging task. Actually, all unflavored oils evaluated (lots #1 and #2) could be classified as EVOO from a physicochemical point of view, fulfilling the legal thresholds required for this classification (Commission Delegated Regulation [EU] 2015/1830, 2015). Moreover, the basil aromatization was performed at three different concentration levels (ranging from 0.011 to 0.110 g dried basil per kg olive oil). The reduced olive oil aromatizing time and the low flavoring levels assessed may pose additional practical difficulties. Therefore, the capability of the E-tongue to identify LOO-basil samples and differentiate them from EVOO, LOO, and EVOO-basil samples is of utmost commercial importance.

Furthermore, the use of the E-tongue for differentiating the flavored EVOO or flavored LOO according to the three different added basil concentration levels was further investigated. For EVOO-basil and LOO-basil, it was possible to establish E-tongue-LDA-SA models with two discriminant functions (that explained 100% of the original data variability), based on the normalized signals of six sensors (1st array: S1:16, and S1:18; 2nd array: S2:2, S2:3, S2:11, and S2:20) and seven sensors (1st array: S1:5, S1:12, and S1:19; 2nd array: S2:3, S2:11, S2:18, and S2:20), respectively. The models allowed 100% of correct classifications for the original grouped samples (Fig. 1) and 93% for the LOO-CV procedure. The results showed that the E-tongue was able to detect and discriminate the different levels of basil flavored oils, confirming its potential as a taste sensor device for olive oil analysis.

Oregano Flavoring Process

Multivariate supervised classification models were established for the evaluation of oregano flavored and unflavored olive oils (EVOO; LOO; EVOO enriched with oregano: EVOO-oregano; and LOO enriched with basil: LOO-oregano). In this case, a subset of 17 sensors (1st array: S1:7, S1:9, S1:11-S1:13, S1:15, and S1:16; 2nd array: S2:4–S2:6, S2:9, S2:11, S2:12, S2:14-S2:16, and S2:19) was selected using the SA algorithm and further used to establish the E-tongue-LDA-SA model

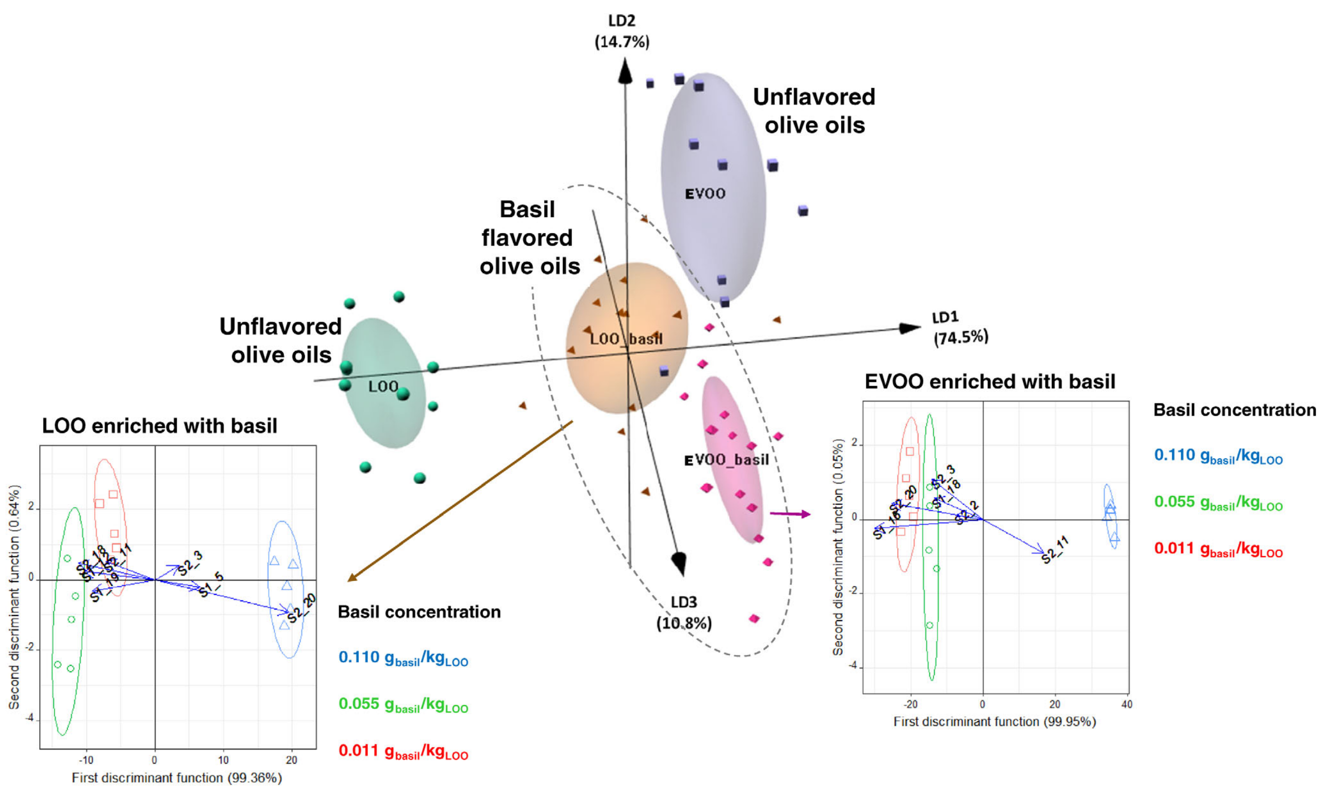

Fig. 1 Discrimination of unflavored and flavored olive oils according to the grade quality (EVOO and LOO) and the level of added flavoring agent (basil-dried herb): E-tongue-LDA-SA models established based on the E-tongue potentiometric signals gathered by the lipid polymeric sensor membranes during the analysis of hydroethanolic extracts of olive oils

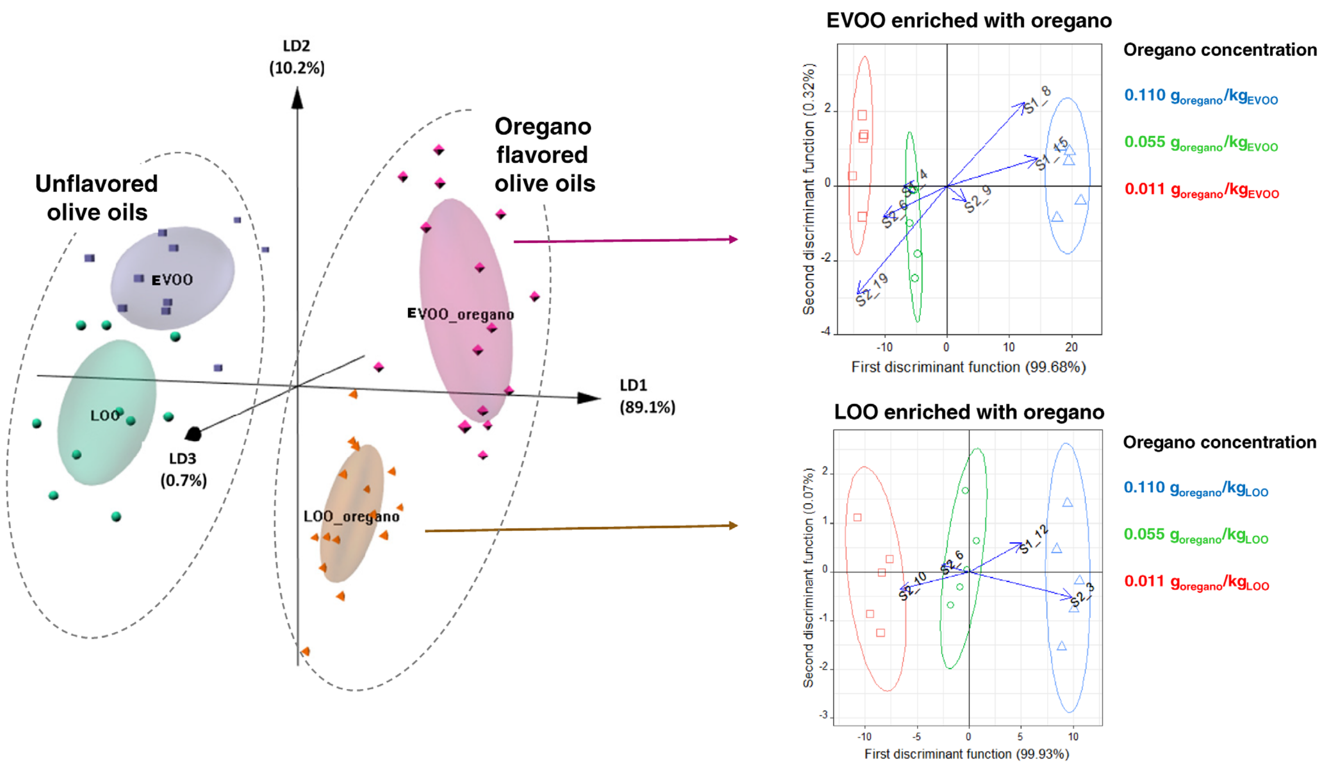

Fig. 2 Discrimination of unflavored and flavored olive oils according to the grade quality (EVOO and LOO) and the level of added flavoring agent (oregano-dried herb): E-tongue-LDA-SA models established based on the E-tongue potentiometric signals gathered by the lipid polymeric sensor membranes during the analysis of hydroethanolic extracts of olive oils

(LD explaining 89.1%, 10.2%, and 0.7% of the original data variability). The selected classification model showed sensitivities of 98% for the original grouped samples (Fig. 2) and 78% for the LOO-CV procedure. The slightly better predictive performance achieved (compared to that of the basil flavoring process) may be tentatively attributed to the higher influence of the oregano enrichment on the olive oils' physicochemical and sensory levels. It should be remarked that olive oils flavored with oregano showed lowest misclassification ratios, and so, at the studied flavoring levels, oregano seems to be a more potent/intense flavoring agent than basil. The increasing addition of oregano leads to a higher decrease of perception of the winey-vinegary defect (in comparison to the basil addition) leading to a minimum perceived average intensity of 2.2 ± 0.3 for the highest oregano concentration (Table 2). The defect masking potential of oregano would allow an erroneous classification, by the trained panelists, of the LOO with 0.1100 g kg^{-1} as an enriched VOO. However, the E-tongue enabled the correct classification of 12 of the15 LOO-oregano samples (predictive sensitivity of 80% for the LOO-CV procedure), demonstrating that it could be a practical complementary tool for the sensory analysis of flavored oils. The models allowed the correct classifications of 100% of the flavored oils for the original grouped samples (Fig. 2) as well as for the LOO-CV procedure. Once again, these results demonstrate the feasibility of using the E-tongue for olive oil analysis, specifically for their complex sensory evaluation.

Conclusions

The study carried out allowed concluding that olive oil flavoring with basil or oregano slightly influences the quality of physicochemical parameters but mainly their OS (which was reduced compared to the unflavored oils) and their sensory attributes (with an increase of the fruity positive sensation and a significant decrease of the winey-vinegary negative intensity), leading to a possible classification of a flavored lampante oil as a flavored virgin oil. This work also demonstrated that a potentiometric E-tongue, comprising lipid sensor membranes, can be efficiently used to unmask the presence of the winey-vinegary negative sensation in flavored olive oils, which have been enriched by the addition of different basil or oregano concentrations. The proposed electrochemical-chemometric approach showed to be a practical and accurate taste-sensor device that could be used as a complementary tool for olive oil sensory analysis. The satisfactory results pointed out that the E-tongue is a powerful screening procedure, implementation of which can be foreseen in a near future, if one can overcome the usual skepticism of the industrial partners regarding this novel sensor-based devices.

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

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