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ORIGINAL PAPER

Impact of incorporating olive leaves during the industrial extraction of *cv.* Arbequina oils on the physicochemical–sensory quality and health claim fulfillment

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Abstract
The effect of olive leaves addition (1%, w/w, *cvs.* Arbequina or Santulhana), during the industrial extraction of Arbequina oils, on their physicochemical, color, phenolic profile, and sensory characteristics, was studied. Leaves' incorporation reduced the primary oxidation (peroxide value by 33% and K_{232} by 17%) and increased the oxidative stability (19%), with the impact being more pronounced for Arbequina leaves. For these latter oils, leaves incorporation increased the total phenolic content (293 ± 9 mg GAE/kg), which became richer in secoiridoid derivatives (143.7 ± 3.0 mg/kg). Also, only Arbequina oils extracted with their own leaves supported the health claim regarding the protection of blood lipids against oxidative stress (hydroxytyrosol and tyrosol derivatives content greater than 5 mg per 20 g of olive oil). On the other hand, the incorporation of leaves from *cvs.* Arbequina and Santulhana during extraction enhanced the bitterness (55–59%) and decreased the pungency (25–33%). Santulhana leaves promoted an increase of the green-fruitiness (5.3 ± 0.5), while Arbequina leaves enhanced the oils' sweetness (7.0 ± 0.4). Moreover, a potentiometric laboratory-made electronic tongue was applied, as a taste sensor device, being capable of successfully discriminating Arbequina oils extracted without or with addition of leaves, allowing the identification of (un)deliberated leaves incorporation during oils' extraction. Lastly, it was found that the quality and composition of Arbequina oils industrially extracted were leaf cultivar dependent, with the low level of phenolics of control oils promoted by the incorporation of Arbequina leaves.

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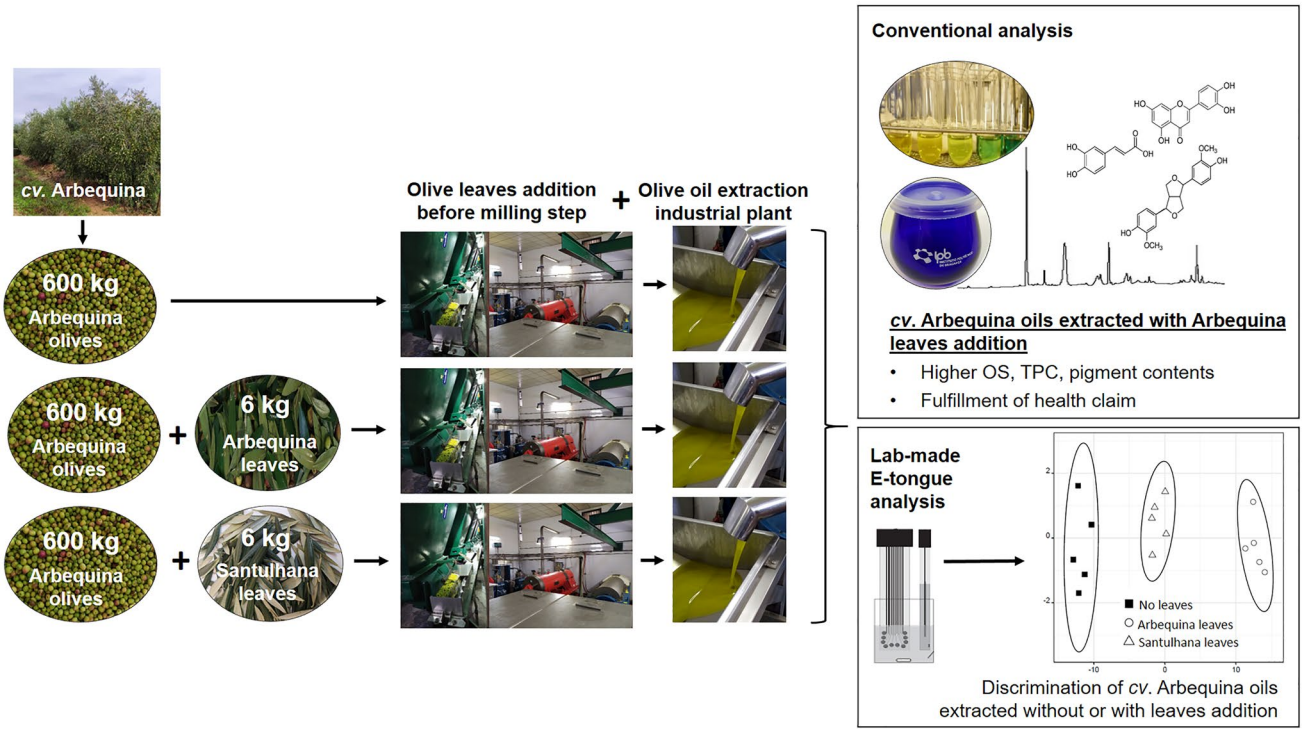
Graphic abstract



Keywords Olive leaves · Olive oil · Phenolic compounds · Health claim · Electronic tongue

Introduction

Olive oil plays a key role in the Mediterranean diet, being one of the most valued fats worldwide, with appreciated sensory characteristics and recognized health positive effects related to its phenolic composition [1]. This fact has been recognized by the European Food Safety Authority [2] leading to the health claim stating that *olive oil polyphenols contribute to the protection of blood lipids from oxidative stress.* This claim can only be applied to oils containing a minimum of 5 mg of hydroxytyrosol and derivatives per 20 g of olive oil [3]. Generally, oleuropein is the most abundant phenolic compound in olives, which is easily extracted as part of the phenolic fraction of olive fruits, leaves, and seeds [4] possessing high antioxidant activity and exhibiting strong preventive effects against oxidation, besides other pharmacological effects [5]. However, the chemical and sensory quality of olive oils can decrease during storage because of their natural oxidation, due to their high level of unsaturated fatty acids [6]. Thus, increasing their natural antioxidant contents could contribute to improve chemical stability, while enhancing their nutritional and nutraceutical properties [7].

Several strategies have been proposed to enhance the phenolics contents of olive oils, namely the optimization of the extraction conditions (e.g., malaxation/extraction time–temperature conditions during malaxation [8–10]). Another trend focused on the use of natural sources such as olive oil by-products, namely olive leaves [11]. Several studies have investigated the presence of a high number of phenolic compounds in olive leaves such as hydroxytyrosol, rutin, verbascoside, luteolin-7-*O*-glucoside, oleuropein (glycosidic form), as well as oleuropein and ligstroside aglycone [12].

The addition of olive leaves during olive pressing can promote the oils' phenolic and chlorophyll content and nutraceutical properties as well as appreciated sensory attributes [13]. Some studies evaluated the impact of adding olive leaves (1–10%, *w*/*w*), dried or fresh, during laboratory or pilot-scale extraction, on the nutritional and sensory properties of olive oils [11, 14–17]. Contrarily, and to the authors' best knowledge, only one work was performed at an industrial scale to evaluate the effect on the oils' sensory sensations of mixing leaves and olives from the same Italian cultivars (*cvs*. Castiglionese, Dritta and Leccino) [18]. The addition of different leaves during the oil extraction process could enhance the olive oil

chemical and sensory quality. *Cv.* Arbequina olive oils generally present low concentrations of total phenolics when compared with other cultivars, such as *cvs.* Hojiblanca, Arbosana and Koroneiki [19, 20]. In this sense, olive leaves' incorporation during oil extraction may promote the phenolics' enhancement in *cv.* Arbequina oils, as well as improve their sensory quality. Arbequina is a Spanish olive cultivar, worldwide widespread, known by its moderate vigor, early bearing, and highly adaptation to high-density farming [21]. On the other hand, Santulhana is a typical Portuguese olive cultivar mainly cultivated in traditional olive groves located in Trás-os-Montes' region (northeast Portugal), which has recently emerged as being interesting due to its exceptional chemical and organoleptic characteristics [22]. However, it should be kept in mind that, independently of the leaves incorporation known benefits, only oils exclusively extracted from healthy olives can be commercialized as extra virgin olive oil (EVOO), according to the European Union (EU) regulations [23], and so, oils extracted after the incorporation of olives leaves should not be commercialized as EVOO.

Thus, the present study aimed to assess the effects, at physicochemical and sensory levels, of incorporating fresh olive leaves (1%, *w*/*w*) from two cultivars, one produced in a high-density (*cv.* Arbequina) and the other in a traditional system (*cv.* Santulhana), both in Trás-os-Montes' region, during the industrial extraction of *cv.* Arbequina olive oils. Lastly, the application of a potentiometric electronic tongue (E-tongue), together with chemometric tools, was envisioned as a single-run taste sensor device for discriminating the three types of extracted olive oils and, thus, to detect the possible (un)deliberated leaves' incorporation during the oils' extraction.

Materials and methods

Sampling

Olives from *cv.* Arbequina, maturity index between two and three [24], were collected in mid-November 2019 from an orchard located in Trás-os-Montes' region (northeast Portugal). Olive leaves from *cvs.* Arbequina and Santulhana were hand-picked in the olive mill, separated from branches, carefully washed and the excess of water removed and weighed. For each leaf cultivar, 6 kg of cleaned leaves were added to 600 kg of *cv*. Arbequina olives (1%, *w*/*w*) before the milling process. Then, oils were extracted (malaxation conditions: 22 °C, 45 min, 12 revolutions per minute) in an industrial olive oil mill (OLIMONTES, Macedo de Cavaleiros, Portugal) as previously described [8], totaling three independent batches, corresponding to 1800 kg of olives (3 × 600 kg). It is important to note that for an industrial-scale study, each independent extraction batch requires a huge amount of olives, limiting the number of independent extractions that can be performed for each type of olive oil, due to the related cost. However, although laboratory- or pilot-scale extractions would allow to increase the number of independent batches, it is known that the composition of olive oils extracted at laboratory or pilot scales can hardly mimic that obtained at industrial level [25].

In total, three different types of *cv.* Arbequina oils were obtained, in three independent industrial extraction batches:

(i) olive oil extracted from *cv.* Arbequina olives (600 kg) without addition of leaves (control);
(ii) olive oil extracted from *cv.* Arbequina olives (600 kg) with the addition of *cv.* Arbequina leaves (1%, *w*/*w*; 6 kg); and,
(iii) olive oil extracted from *cv.* Arbequina olives (600 kg) with the addition of *cv.* Santulhana leaves (1%, *w*/*w*; 6 kg).

From each batch, five amber glass bottles were filled and closed, within a 10 min time period before the oil entered the storage tank and immediately transported to the laboratory (Bragança, Portugal). At the laboratory, water traces were removed using anhydrous sodium sulfate (1 g for 100 mL of olive oil) and filtered through a cellulose filter. The 15 collected bottles (samples) for the three different extracted oils ($n = 3$ types of *cv.* Arbequina olive oils × 5 bottles) were stored in the dark, at 18–22 °C, in amber glass bottles (~ 100 mL) and analyzed after a 6-month storage period to enable the full development of the oils' sensory attributes [8].

Olive oil physicochemical analysis and color determination

Free acidity (FA, in % oleic acid), peroxide value (PV, in mEq O_2/kg) and specific coefficients of extinction at 232 nm and 268 nm (K_{232}, and K_{268}) were evaluated according to the EU Regulation (Annexes II and IX in Commission Regulation (EEC) No. 2568/91 from 11th July and amendments). Oxidative stability (OS, in h) was determined under accelerated oxidation conditions (120 °C) using the Rancimat method (Rancimat 743, Metrohm CH, Switzerland), as previously described [26].

Total phenolic contents (TPC) were assessed following the methodology proposed by Capannesi et al. [27] with some modifications, as previously described [8].

Total chlorophyll contents and total carotenoids contents (TCC) were determined by UV–Vis spectrophotometry, as previously described [28].

Color was determined according to the CIELAB color scale, using a colorimeter (model CR-400, Konica Minolta) and the monochromatic coordinates L*, a*, and b* were calculated after full immersion of the colorimeter lens in the oils in five different areas of the glass plate [29].

Phenolic compounds profile

The phenolic profile of the olive oil samples was established following the guidelines of the International Olive Council (IOC) [30], with minor modifications as previously described [9]. Methanolic extracts (80% *v*/*v*), from duplicate extractions of each sample, were injected in a HPLC–DAD system (Jasco, Japan) comprising a data transmitter (LC–NetII/ADC), two integrated pumps (PU–4180), an auto-sampler (AS–4050), a column oven (ECOM Eco2000, Czech Republic), and the DAD (MD–4010). Separation was accomplished on a pentafluorophenyl column (Kinetex 2,6 µm PFP 100 Å; LC length 100 mm; internal diameter: 4.60 mm) from Phenomenex (Spain), at 35 °C, using an eluent gradient with water and acetonitrile, both with 0.1% of formic acid, at 1.0 mL/min. Peaks were identified based on the retention times (RT) and UV/Vis spectra (200–600 nm), by comparison with pure standards (apigenin, apigenin-7-*O*-glucoside, hydroxytyrosol, luteolin, luteolin-7-*O*-glucoside and verbascoside, from ExtraSynthese; 4-hydroxybenzoic acid, caffeic acid, cinnamic acid, gallic acid, oleocanthal, oleuropein, p-coumaric acid, pinoresinol, vanillic acid and vanillin, from Sigma-Aldrich; tyrosol (2–(4-hydroxyphenyl)ethanol), from Fluka and oleacein, from Toronto Research Chemicals). Results were expressed as mg of tyrosol equivalents per kg of olive oil, using the experimental data recorded at 280 nm, following the IOC guidelines [30]. Some peaks were identified based on the literature [31].

Hydroxytyrosol and tyrosol derivatives contents after hydrolysis

The total content of hydroxytyrosol and tyrosol derivatives of the olive oils were chromatographically determined after acid hydrolysis of secoiridoids according to the method proposed by Romero and Brenes [32] with some modifications [9]. After hydrolysis, only the tyrosol and hydroxytyrosol moieties were quantified, thus the original bound forms were estimated using the correction factors (CF) proposed in the literature for hydroxytyrosol derivatives (CF = 2.2) and tyrosol derivatives (CF = 2.5) [33, 34].

Olive oil sensory gustatory analysis

The gustatory sensations of the olive oils were assessed by a trained sensory panel following the methodologies described by the EU standard methods (Annexes II and IX in the Commission Regulation (EEC) Nº 2568/91 from 11th July and amendments). The analysis was performed by eight trained panelists of the olive oil sensory panel of the School of Agriculture of the Polytechnic Institute of Bragança, Portugal [35]. The descriptive profile was assessed using a test sheet, with some modifications, as recommended by the International Olive Council [36] and as previously reported by [35].

E-tongue

A laboratory-made potentiometric E-tongue multisensor device, comprising 40 lipid polymeric cross-sensitive sensor membranes was used. The construction details, as well as the composition of the membranes were previously reported [8]. For the olive oil analysis with the E-tongue, the polar extract obtained for the TPC assays was used after a 1:5 (*v*/*v*) dilution in deionized water.

Statistical analysis

The composition of the three different *cv.* Arbequina olive oils (industrially extracted without or with the addition of *cvs.* Arbequina or Santulhana leaves) were analyzed using the one-way ANOVA followed by the Tukey's post hoc multicomparison test. Principal component analysis (PCA) was applied as an unsupervised multivariate pattern recognition technique for inferring about the impact of the leaves' addition during the oil extraction on the physicochemical, color, phenolic and sensory profiles. Linear discriminant analysis (LDA) was applied to discriminate the studied oils 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. The class membership regions were established using the posterior probabilities, computed using the Bayes' theorem. All statistical analyses were performed using the Sub-select and MASS packages of the open-source statistical program R (version 3.6.2), at a 5% significance level [8].

Results and discussion

Effect of addition of leaves during oil extraction on physicochemical and color parameters

The physicochemical quality data (FA, PV, K_{232} and K_{268}), the OS, TPC, TCC and total chlorophyll content, as well

Table 1 Physicochemical quality and color-scale parameters (mean ± standard deviation, for each oil $n = 5$ olive oil bottles × 2 analysis) of the studied *cv*. Arbequina olive oils

Parameters	Industrially extracted *cv.* Arbequina oils			*p*-value[a]
	Without addition of leaves (control)	With *cv.* Arbequina leaves	With *cv.* Santulhana leaves	
Quality parameters				
FA (g oleic acid/100 g)	0.31 ± 0.00^{C}	0.34 ± 0.01^{A}	0.32 ± 0.01^{B}	< 0.0001
PV (mEq O_2/kg oil)	2.48 ± 0.01^{A}	1.65 ± 0.01^{B}	1.65 ± 0.02^{B}	< 0.0001
K_{232}	1.84 ± 0.07^{A}	1.53 ± 0.05^{C}	1.60 ± 0.08^{B}	< 0.0001
K_{268}	0.07 ± 0.01^{A}	0.06 ± 0.00^{B}	0.05 ± 0.01^{C}	< 0.0001
Chemical parameters				
OS (h)	9.8 ± 0.3^{C}	12.1 ± 0.2^{A}	10.8 ± 0.6^{B}	< 0.0001
TPC (mg GAE/kg oil)	252 ± 7^{B}	293 ± 9^{A}	218 ± 4^{C}	< 0.0001
TCC (mg lutein eq/kg oil)	2.51 ± 0.02^{C}	2.85 ± 0.04^{A}	2.57 ± 0.02^{B}	< 0.0001
Chlorophylls (mg pheophytin eq. /kg oil)	3.68 ± 0.08^{B}	3.88 ± 0.14^{A}	3.01 ± 0.05^{C}	< 0.0001
CIELAB color space				
L*	71.4 ± 1.6^{B}	75.8 ± 1.4^{A}	75.2 ± 1.8^{A}	0.0021
a*	-11.0 ± 0.9^{A}	-12.5 ± 0.4^{B}	-14.1 ± 0.5^{C}	< 0.0001
b*	76.3 ± 1.1^{A}	73.1 ± 3.5^{A}	75.7 ± 2.0^{A}	0.1287

FA free acidity, *PV* peroxide value, K_{232} *and* K_{268} UV–Vis extinction coefficients at 232 and 268 nm, respectively, *OS* oxidative stability, *TPC* total phenols content, *TCC* total carotenoids content

[a]*p*-values for the one-way ANOVA. Different letters in the same row show statistically significant differences from the given mean (*p*-value < 0.05)

CIELAB color coordinates (L*, a* and b* parameters) of the *cv.* Arbequina olive oils extracted without (control) and after the addition of leaves from *cvs.* Arbequina or Santulhana are shown in Table 1. The results pointed out that, with the exception of b* values, all parameters were significantly influenced by the addition of olive leaves during the oil industrial extraction (*p*-value < 0.0001, for one-way ANOVA).

The addition of leaves slightly increased FA in comparison to control with statistical differences. On the other hand, primary and secondary oxidations were reduced up to 33.5%, 16.8% and 14.3% for PV, K_{232} and K_{268}, respectively, with *cv.* Arbequina leaves incorporation. A similar PV decreasing trend was reported by Tarchoune et al. [11] during laboratory-extracted oils from olives and leaves of *cv.* Oueslati. On the contrary, Di Giovacchino et al. [18] and Malheiro et al. [14] reported that the addition of leaves of the same cultivar as the olives during the industrial oil extraction induced peroxidation, resulting in oils with higher PV. Regarding the extinction coefficients, the reduction trend observed in this work with the addition of leaves is in line with the behavior described by Di Giovacchino et al. [18] for *cv.* Dritta oils, but opposite to that found by the same authors for *cvs.* Leccino and Castiglionese oils or by Malheiro et al. [14] for *cv.* Cobrançosa oils.

Table 1 shows that OS was significantly increased (up to 19%, *p*-value < 0.0001) after addition of leaves when compared to the control. Di Giovacchino et al. [18] found that the addition of leaves during industrial extraction of *cvs.* Leccino and Castiglionese oils slightly increased the OS (~ 2%), but an opposite trend was observed for *cv.* Dritta oils (a 7% reduction). On the other hand, Malheiro et al. [14] found that the addition of leaves enhanced the OS of *cv.* Cobrançosa over mature oils up to 20%. These findings may support the hypothesis that the impact of the addition of olive leaves during the oils' extraction may depend on the extraction scale and parameters as well as on the leaf olive cultivar. OS is an indispensable parameter in assessing the quality of oils and fats, and it is significantly influenced by their fatty acid composition and minor components such as tocopherols, phytosterols, and phenolic compounds [37]. This is known as induction time, and it results in a rapid increment in the lipid oxidation rate. In this present work it was possible to establish correlation with OS and PV (*R*-Pearson = −0.830). On the other hand, it was not possible to establish correlations between OS and TPC, as well as total individual phenolic content. These results point out that both OS and PV are not only associated with the phenolic content of oils, but also with other compounds that generally promote antioxidant activity such as tocopherols, squalenes, pigments, and sterols [38]. This hypothesis was confirmed by Tarchoune et al. [11] who showed that following 3% leaf addition, the PV halved, and the antioxidant capacity was increased by 87% in *cv.* Oueslati oils. The authors pointed

out that the wide change was probably due to the increases in chlorophyll, carotenoid, and total phenolic and flavonoid concentrations.

Regarding TPC, the current literature shows a large variation of the phenolics content in EVOO, varying from 50 to 940 mg/kg, depending on the cultivar, olives' maturity index, extractive technology, and environmental variables [39]. The present study showed that, compared with the control oils, the effect of addition of leaves was clearly leaf cultivar dependent as far as TPC and TCC are concerned (Table 1). Compared to control oils, lower TPC (reduction of 13%) and chlorophyll contents (reduction of 18%) were found for *cv.* Santulhana leaves added oils, while the addition of *cv.* Arbequina leaves increased TPC, TCC and chlorophyll content (14, 12 and 5%, respectively). Di Giovacchino et al. [18] observed a TPC decrease of 4% (also assessed following Folin–Ciocalteu method) for *cvs*. Leccino and Castiglionese oils, while Sevim and Tuncay [17], in a pilot-scale extraction system, observed a 2–25% increase (depending on the cultivar) as well as Sanmartin et al. [16] and Tarchoune et al. [11], for laboratory-extracted oils (increase of 3%). For chlorophyll contents (evaluated by the same methodology), similar increasing trends were reported by Di Giovacchino et al. [18] for industrial-scale extraction (increase of 38%), by Sevim and Tuncay [17] for pilot-scale extraction (rise of 32–45%), by Tarchoune et al. [11] (increase of 44–68%) and to a less extent (~ 1%) by Sanmartin et al. [16]. Regarding TCC, Tarchoune et al. [11] also reported an increase of 43–63%, but Sanmartin et al. [16] observed a slight decrease (~ 1%) in oils extracted after addition of leaves. Chlorophyll and carotenoids play key roles in olive oils' stability, acting as antioxidants in the dark or as pro-oxidants with light exposure [40]. It should be remarked that the literature focused on the leaves' addition effect on the composition of olive oils when both leaves and olives were from the same cultivar, and so, contrary to the present study, no inference could be established regarding the possible leaf cultivar effect, which has been found to be significant in this study.

Color evaluation (Table 1) showed that significantly higher a* values, toward a greener coloration, were found after the addition of *cvs.* Arbequina or Santulhana leaves (increase of 12 and 22%, respectively). The addition of olive leaves somewhat increased (up to 6%) the oils' luminosity, L*, resulting in brighter oils.

Effect of addition of leaves during oil extraction on the phenolic profile

Positive health effects related to olive oil consumption have been attributed, to some extent, to their phenolic composition [39]. Similarly, olive leaves have been traditionally used as a folk remedy, due to the recognized anti-inflammatory, hypoglycemic, antimicrobial, and hypocholesterolemic effects [1], partially attributed to low-molecular-weight polyphenols like oleuropein [41]. Talhaoui et al. [42] reported that, besides oleuropein, *cv.* Arbequina leaves have high amounts of verbascoside and flavonoids (e.g., luteolin glucoside isomers and rutin). On the other hand, Meirinhos et al. [43] found that *cv.* Santulhana leaves contained high concentrations of flavonoids, significantly luteolin-7-*O*-glucoside and luteolin-4´-*O*-glucoside.

Though olive leaves are rich in phenolic compounds, it does not mean that they can easily migrate to the oil during extraction. In fact, phenolic compounds are better extracted from dried leaves using organic solvents [44]. However, in this study, the extraction occurred from crushed fresh leaves (1%, *w*/*w*) mixed with olive paste, simulating in a more realistic way a non-intentional incorporation of leaves during olive oil industrial extraction. The small amount of added leaves and the mixing procedure may result in a low migration of phenolics from the leaves to the oily fraction (olive oil), not promoting an increase of the final content of oils' phenolics, namely with flavonoids that are present in *cvs.* Arbequina or Santulhana leaves. Indeed, in the present study, it was not possible to establish a direct relationship between the profile reported in the literature for the referred leaves [42, 43] and the phenolic compounds detected on the oils extracted after leaves' incorporation.

Herein, the phenolic profile was similar for all studied olive oils regardless of whether leaves were added or not during their extractions. Six different phenolic compounds (i.e., hydroxytyrosol, tyrosol, vanillic acid, oleuropein (glycosidic form), oleacein and oleocanthal) were identified in the three types of *cv.* Arbequina oils, belonging to three phenolic groups, mainly secoiridoid derivatives followed by alcohols and acids (Table 2). The phenolic profile of control oils is in line with those previously reported for *cv.* Arbequina oils, although the individual contents may differ [20, 45–47]. In addition, several peaks related to compounds derived from oleuropein and ligstroside could be identified based on Klen et al. [31].

Although no flavonoids were detected in the studied oils, the literature reports that *cv*. Arbequina oils may contain luteolin (0.09–12.6 mg/kg) and apigenin (0.015–3.3 mg/kg) [20, 45–48]. Regarding lignans, the literature reported that *cv.* Arbequina oils generally presents significant amounts of acetoxypinoresinol (9.77–22.0 mg/kg) and lower amounts of pinoresinol (2.94–4.10 mg/kg) [47, 48]. In this study, although these phenolic compounds were not identified, it should be noticed that the chromatographic profile showed a peak with a retention time very close to the that recorded for the standard pinoresinol, which could hypothetically correspond to acetoxypinoresinol, although the lack of the related standard did not allow confirmation.

Table 2 Phenolic compounds content (mean ± standard deviation, mg of tyrosol equivalents/kg of olive oil; for each oil $n = 5$ olive oil bottles × 2 analytical extractions × 1 chromatographic analysis) of the studied *cv.* Arbequina olive oils

Phenolic compounds	Industrially extracted *cv.* Arbequina oils			*p*-value[a]
	Without addition of leaves (control)	With *cv.* Arbequina leaves	With *cv.* Santulhana leaves	
Hydroxytyrosol	1.3 ± 0.2^{A}	1.1 ± 0.1^{B}	0.2 ± 0.0^{C}	< 0.0001
Tyrosol	1.0 ± 0.1^{A}	0.6 ± 0.1^{B}	0.4 ± 0.1^{C}	< 0.0001
Vanillic acid	1.1 ± 0.2^{A}	0.6 ± 0.5^{C}	0.9 ± 0.1^{B}	< 0.0001
Oleuropein[b]	1.7 ± 0.3^{A}	1.4 ± 0.3^{A}	1.0 ± 0.2^{B}	< 0.0001
Oleacein	38.5 ± 3.5^{B}	54.9 ± 1.5^{A}	15.1 ± 0.8^{C}	< 0.0001
Oleocanthal	12.8 ± 1.0^{A}	12.4 ± 0.9^{A}	8.7 ± 0.5^{B}	< 0.0001
ΣOleuropein derivatives	53.9 ± 2.3^{B}	56.9 ± 1.4^{A}	52.8 ± 2.3^{B}	< 0.0001
ΣLigstroside derivatives	18.4 ± 1.0^{B}	18.0 ± 0.7^{B}	28.8 ± 1.6^{A}	< 0.0001
$\Sigma_{\text{phenolic acids}}$	1.1 ± 0.2^{A}	0.6 ± 0.5^{C}	0.9 ± 0.1^{B}	< 0.0001
$\Sigma_{\text{phenolic alcohols}}$	2.3 ± 0.3^{A}	1.7 ± 0.1^{B}	0.6 ± 0.1^{C}	< 0.0001
$\Sigma_{\text{secoiridoid derivatives}}$	125.3 ± 5.6^{B}	143.7 ± 3.0^{A}	106.4 ± 4.9^{C}	< 0.0001
$\Sigma_{\text{identified phenols}}$	128.7 ± 6.0^{B}	145.9 ± 3.0^{A}	107.9 ± 4.9^{C}	< 0.0001
$\Sigma_{\text{non-identified phenols}}$	36.2 ± 3.1^{A}	35.4 ± 3.7^{A}	34.7 ± 3.8^{A}	0.6605
$\Sigma_{\text{phenolics content}}$[c]	164.9 ± 6.2^{B}	181.3 ± 4.7^{A}	142.6 ± 5.8^{C}	< 0.0001
Identified phenols (rel. %)	78.0 ± 1.8^{B}	80.5 ± 1.7^{A}	75.7 ± 2.3^{C}	< 0.0001

[a] *p*-values for the one-way ANOVA. Different letters in the same row show statistically significant differences from the given mean (*p*-value < 0.05)

[b] Oleuropein in glycosidic form

[c] Sum of $\Sigma_{\text{identified phenols}}$ and $\Sigma_{\text{non-identified phenols}}$

Other phenolic compounds have also been detected in *cv*. Arbequina oils, although in rather small concentrations (lower than 0.7 mg/kg) such as vanillin, cinnamic, ferulic and p-coumaric acids, but they were not identified in the present study. The differences observed may be tentatively attributed to different geographical origins, agro-climatic conditions, oils' extraction scale, and enzymatic reactions that occur during the mechanical extraction of oils, which highly depend on the malaxation conditions [45, 48, 49], as well as on the analytical methodology applied for their identification.

Table 2 also shows that all the phenolic families were significantly affected (*p*-value < 0.0001) by the addition of olive leaves during the *cv.* Arbequina oil extraction, as already pointed out by other studies [11, 16].

In general, the incorporation of *cv.* Arbequina leaves significantly increased the content of the secoiridoid derivatives (rise of 13%), as described in the literature [11, 15, 16]. Also, these leaves significantly increased the total content of the identified phenols (146 ± 3.0 mg/kg oil; increase of 12%), which is in accordance with the results of Ammar et al. [15]. However, the addition of *cv.* Santulhana leaves had an opposite impact, resulting in a significant decrease of the total content (107.9 ± 4.9 mg/kg oil; reduction of 16%).

The incorporation of *cv.* Arbequina leaves promoted an increase of oleacein and oleuropein derivative contents (30 and 5%, respectively) compared to the control oils, but no significant changes were observed for oleuropein, oleocanthal and ligstroside derivatives. On the contrary, the addition of *cv*. Santulhana leaves resulted in a decrease of the oleuropein, oleacein and oleocanthal contents (41, 40 and 32%, respectively), without variation in the oleuropein derivatives but with a significant increase in the ligstroside derivative contents by 36%. A similar trend was reported by Ammar et al. [15] who extracted oils from different cultivars, adding 3% of leaves from the same studied cultivars, during the extraction process. The authors noticed, for *cv.* Chemlali and *cv*. Chétoui oils extracted with the addition of their leaves, an increase in the concentrations of hydroxytyrosol acetate and oleacein. On the other hand, oils extracted from *cv*. Zalmati leaves showed a significant reduction of these amounts. These findings demonstrate that the results obtained are clearly dependent on the cultivar studied.

Oleuropein and ligstroside derivatives, which are precursors of smaller molecules (as oleuropein, oleacein and oleocanthal), were present in all analyzed oils, but their relative proportions were different. The phenolic fraction is often affected by the hydrolytic enzymes' activity (e.g., *β*-glucosidase), which catalyzes the release of aglycone secoiridoids from their respective glucoside forms, as well as by the oxidative degradation catalyzed by the polyphenol oxidases (PPO) and peroxidases (POD) [49]. Thus, differences at an enzymatic level can influence the phenolics' composition, thus partially explaining the differences observed for the studied oils.

Finally, it could be stated that the addition of *cvs*. Arbequina or Santulhana leaves resulted in a decrease of the phenolic acids and phenolic alcohol contents, though some studies did not observe a direct effect on their amounts due to the leaves' incorporation [11, 16].

Effect of addition of leaves during oil extraction on the health claim evaluation

The EFSA [2] has recognized the protective effect of polyphenols from olive oils against the oxidative stress of blood lipids. This has led to a health claim related to olive oil consumption, which establishes a minimum content of 5 mg of hydroxytyrosol and derivatives per 20 g of olive oil [3]. Therefore, the effect of addition of olive leaves on the fulfillment of this health claim was determined.

Table 3 shows the hydrolyzed secoiridoidic fraction of the oils that comprises hydroxytyrosol and tyrosol contents as their sum (in mg/20 g of oil). For *cv.* Arbequina oils (control), the hydroxytyrosol derivatives contents almost double those of tyrosol, which is in accordance with the literature data [9, 34]. The addition of *cv.* Arbequina leaves during the extraction process led to a significant increase (p-value < 0.0001) of the hydroxytyrosol derivatives, but an opposite finding was observed for the addition of *cv.* Santulhana leaves, which led to a significant reduction. This trend is in line with the results previously discussed regarding the individual phenolic profile of olive oils extracted with or without olive leaves (Table 2).

The results pointed out that although the *cv.* Arbequina control oils were unable to attain the health claim, as well as those extracted after addition of *cv.* Santulhana leaves, the oils extracted after the addition of *cv.* Arbequina leaves could fulfill the health claim by surpassing the phenolic threshold (hydroxytyrosol–tyrosol derivatives greater than 5 mg/20 g oil). Though these oils should not be commercialized as EVOO, since the extraction was performed in the presence of olive leaves, which is not according to the EU regulations [23], they possess a commercial health claim advantage. Thus, the addition of *cv.* Arbequina leaves during

Table 3 Estimated content of hydroxytyrosol and tyrosol derivatives (mean ± standard deviation, mg/20 g of olive oil; for each oil $n = 5$ olive oil bottles × 2 analytical extractions × 1 chromatographic analysis) in the hydrolyzed *cv.* Arbequina olive oils

Phenolic derivatives	Industrially extracted *cv.* Arbequina oils			p-value[a]
	Without addition of leaves (control)	With *cv.* Arbequina leaves	With *cv.* Santulhana leaves	
Hydroxytyrosol	2.54 ± 0.28^{B}	3.55 ± 0.28^{A}	1.31 ± 0.11^{C}	< 0.0001
Tyrosol	1.82 ± 0.34^{A}	1.72 ± 0.22^{A}	1.59 ± 0.21^{A}	0.1722
Total	4.36 ± 0.23^{B}	5.28 ± 0.18^{A}	2.90 ± 0.25^{C}	< 0.0001

[a]p-values for the one-way ANOVA. Different letters in the same row show statistically significant differences from the given mean (p-value < 0.05)

Table 4 Intensities of gustatory attributes (mean ± standard deviation, $n = 5$ olive oil bottles × 2 samples × 8 panelists) of the studied *cv.* Arbequina olive oils

Perceived gustatory attributes	Industrially extracted *cv.* Arbequina oils			p-value[a]
	Without addition of leaves (control)	With *cv.* Arbequina leaves	With *cv.* Santulhana leaves	
Green fruity	2.4 ± 0.3^{B}	2.6 ± 0.3^{B}	5.3 ± 0.5^{A}	< 0.0001
Sweet	6.1 ± 0.4^{B}	7.0 ± 0.4^{A}	6.2 ± 0.6^{B}	< 0.0001
Bitter	1.3 ± 0.3^{B}	2.9 ± 0.5^{A}	3.2 ± 0.2^{A}	< 0.0001
Pungent	2.4 ± 0.5^{A}	1.6 ± 0.4^{B}	1.8 ± 0.2^{B}	0.0001
Apple	6.0 ± 0.5^{A}	5.2 ± 0.4^{B}	5.7 ± 0.5^{A}	0.0052
Tomato	3.2 ± 0.6^{A}	3.1 ± 0.5^{A}	3.3 ± 0.5^{A}	0.7887
Dry fruits	1.9 ± 0.3^{A}	1.8 ± 0.7^{A}	1.9 ± 0.3^{A}	0.8050
Banana	5.1 ± 0.4^{A}	4.3 ± 0.4^{B}	5.1 ± 0.5^{A}	0.0011
Fresh herbs	2.2 ± 0.7^{A}	2.2 ± 0.3^{A}	2.1 ± 0.1^{A}	0.9670
Cabbage	3.6 ± 0.6^{B}	4.2 ± 0.4^{A}	3.4 ± 0.2^{B}	0.0017
Harmony	8.0 ± 0.5^{A}	7.9 ± 0.2^{A}	8.2 ± 0.3^{A}	0.2086

[a]p-values for the one-way ANOVA. Different letters in the same row show statistically significant differences from the given mean (p-value < 0.05)

the oil extraction could be foreseen as an important strategy for increasing the phenolic content of *cv.* Arbequina oils, well known by their usual low-level phenolic content, and potentially attaining similar levels of bioactive phenolics to those ensuring the health claim.

Effect of addition of leaves during oil extraction on the gustatory sensations

During the sensory analysis of the olive oils, no defective sensation was perceived, with ten positive gustatory attributes being detected (Table 4). The mean intensities varied from 1.3 ± 0.3 (bitter sensation intensity for control) to 7.0 ± 0.4 (sweet sensation intensity for oils extracted with *cv*. Arbequina leaves). Table 4 also shows that the addition of olive leaves did not influence the intensity perception of tomato, dry fruit, and fresh herb sensations (p-value ≥ 0.7887). On the contrary, for the other seven positive gustatory attributes, the addition of olive leaves had a significant effect (p-value ≤ 0.0052) on the perceived intensities, depending on the sensory sensation and the leaf cultivar added. The addition of *cv.* Santulhana leaves significantly increased the positive attributes, namely green-fruity (by 55%) and bitter (by 59%) sensations. Instead, the addition of *cv.* Arbequina leaves significantly increased the sweet (13%), bitter (55%) and cabbage (14%) sensations, but decreased the perceived intensities of apple (15%) and banana (19%) sensations. On the other hand, the addition of any type of leaves resulted in a significant lower pungent sensation (25–33%, for *cvs.* Santulhana and *cv.* Arbequina, respectively. Di Giovacchino et al. [18] and Malheiro et al. [14] also reported that addition of olive leaves during the oil extraction could improve some sensory characteristics of the extracted oils. The observed changes of oils' bitterness and pungency after the leaves' incorporation may be partially related to the known influence of the phenolic fraction on those sensations [49]. Derivatives of oleuropein like oleacein and the oleuropein aglycone are mainly responsible for the oil's bitterness, while oleocanthal is mainly responsible for the pungent sensation [50]. For all the *cv*. Arbequina oils studied, it was possible to establish a strong correlation between the perceived bitter intensities and the contents of secoiridoid derivatives of oleuropein ($R^2 = 0.999$), confirming the literature hypothesis pointed out by Romero et al. [51]. Finally, addition of leaves did not have a significant effect (p-value $= 0.2086$) on the harmony note, which takes into account the overall sensory perception.

Unsupervised and supervised recognition of *cv.* Arbequina oils

From the oxidative quality evaluation (FA, PV, K_{232} and K_{268}) and the sensory analysis (Tables 1 and 4), all *cv.* Arbequina oils fulfilled the required legal thresholds for EVOO classification (FA $\leq 0.8\%$ of oleic acid, PV ≤ 20 mEq O_2/kg, $K_{232} \leq 2.50$ and $K_{268} \leq 0.22$; green-fruity intensity > 0 and intensity of defects equal to 0) (Commission Delegated Regulation (EU) 2015/1830 of 8th July) [52]. However, according to the EU Commission Regulation (CE 1989/2003) [23], not all the studied oils could be commercialized as EVOO, since some of them were extracted in the presence of olive leaves. Therefore, the possibility of using the chemical–sensory data as biomarkers to detect oils extracted without or with the addition of a low amount of leaves (1%, *w*/*w*) was tested.

In this sense, PCA was applied to infer the unsupervised differentiation of the studied oils. The 2D-PCA biplots showed that the physicochemical quality data together with the TPC, TCC, total chlorophyll content and OS (Fig. 1A) allowed a satisfactory recognition of the oil type, with 94% of data variability explained by the first two principal components (PCs). A similar satisfactory differentiation was achieved using the oils' phenolic profiles (Fig. 1C, explaining the first two PCs approximately 78% of the data variability). However, the differentiation requires the use of different analytical techniques (e.g., titration, spectrophotometry plus Rancimat, or chromatography, respectively) which are time-consuming. Besides, the in situ/online implementation of those analytical techniques is not straightforward. The color data (Fig. 1B) did not enable such a satisfactory oil recognition, with a partial overlap of control and oils extracted with *cv*. Arbequina leaves observed, even if the first two PCs explained more than 95% of the data variability. For the gustatory sensations (Fig. 1D), the first two PCs only explained 47% of the data variability, pointing out to their lower differentiation capability, with an overlap being observed for oils extracted after the addition of *cvs*. Arbequina or Santulhana leaves.

On the other hand, potentiometric E-tongues have been reported to be a powerful, fast, accurate and cost-effective taste device for olive oil analysis [8, 53]. Thus, the possibility of using a potentiometric laboratory-made E-tongue, as a single-run taste sensor analytical tool, was further evaluated to discriminate *cv*. Arbequina oils extracted without or with the addition of olive leaves. An E-tongue-LDA-SA model, with two linear discriminant functions (DFs) was established (explaining 100% of data variability) based on the potentiometric signals recorded by six non-redundant sensors (1st array: S1:10, S1:14; 2nd array: S2:5, S2:10, S2:13 and S2:17). The multivariate linear classification model correctly classified all samples (100% sensitivity and specificity) for both original grouped data (Fig. 2) and LOO-CV variant. The successful classification could be tentatively attributed to the capability of the lipid sensor membranes to interact, through electrostatic and hydrogen bonds, with oils' polar compounds responsible for the observed differences at the sensory and

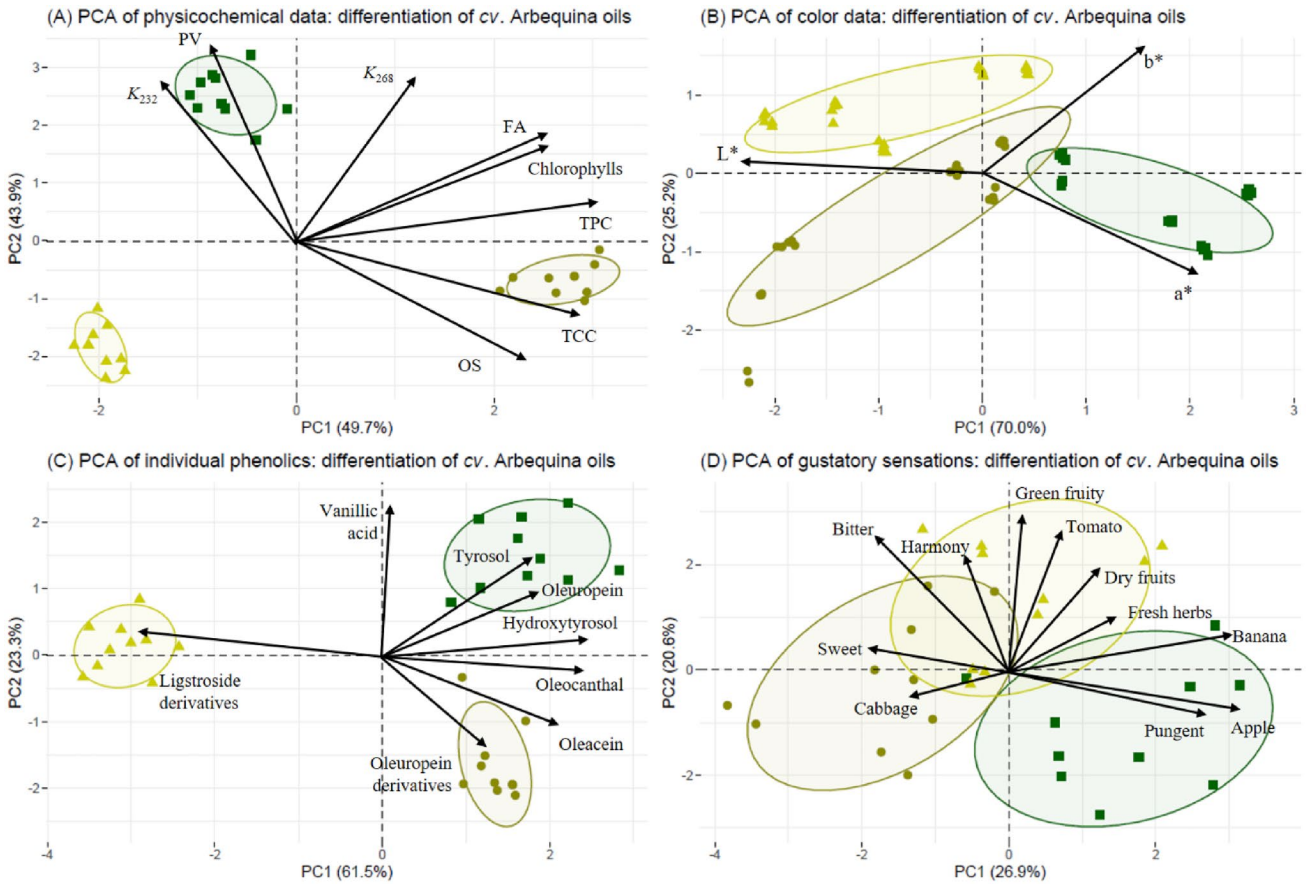


Fig. 1 PCA analysis (biplot) differentiation of *cv.* Arbequina olive oils extracted without (control, ■) or with the addition of olive leaves (1%, *w*/*w*) from *cv.* Arbequina (●) or *cv.* Santulhana (▲). **A** Physicochemical data. **B** CIELAB color data. **C** Phenolic compounds profile. **D** Gustatory sensations. Free acidity, FA; peroxide value, PV; extinction coefficient, K_{232} and K_{268}; oxidative stability, OS; total phenolic content, TPC; total carotenoids content, TCC; and chlorophyll contents

phenolic composition levels [54]. The satisfactory predictive performance demonstrated the potential of using the E-tongue as a single-run complementary/alternative tool to discriminate *cv.* Arbequina oils extracted without or with the addition of *cvs*. Arbequina or Santulhana leaves, foreseeing its use to detect the (un)deliberated incorporation (of at least 1%, *w*/*w*) of olive leaves during oil extraction.

Conclusions

The effects of incorporating olive leaves during the oil extraction, at industrial scale, are leaf cultivar dependent. In this study, it was verified that the addition of 1% (*w*/*w*) of *cv*. Arbequina leaves during the industrial extraction of *cv*. Arbequina oils had a global positive effect on the extracted oil quality. This can lead to lower lipid primary oxidation, greater oxidative stability, higher total phenols, and pigments contents as well as a greater content of secoiridoid phenolic derivatives. Indeed, the addition of *cv*. Arbequina leaves allowed obtaining greener and brighter oils that could support the polyphenols-related health claim, which is a clear commercial advantage. Furthermore, it also enhanced the perceived intensity of some appreciated gustatory sensations, such as green-fruity and bitter sensations. The results also pointed out that the use of leaves and olives of the same cultivar (*cv*. Arbequina) had a higher positive synergetic impact on overall oils' chemical–sensory quality compared to the use of *cv*. Santulhana leaves. Notwithstanding, the use of *cv.* Santulhana leaves promoted a significant increase of positive sensory attributes (green and bitter sensations). Furthermore, satisfactory correlation between bitter intensities and the oils' content on secoiridoid derivatives of oleuropein was found in this research. Finally, it was demonstrated that a laboratory-made potentiometric electronic tongue, with lipid polymeric membranes, could be satisfactorily applied, as a single-run sensor device, to discriminate the *cv*. Arbequina oils extracted without or with the addition of leaves and thus as a genuineness tool.

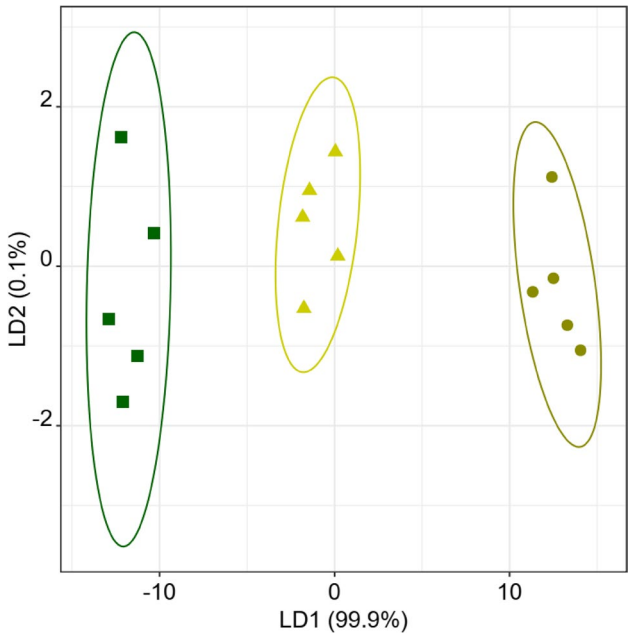


Fig. 2 LDA-SA model performance regarding the supervising classification of *cv.* Arbequina olive oils extracted without (control, ■) or with the addition of olive leaves (1%, *w/w*) from *cv.* Arbequina (●) or *cv.* Santulhana (▲) based on the potentiometric signals gathered by six E-tongue lipid sensor membranes (1st array: S1:10, S1:14; 2nd array: S2:5, S2:10, S2:13 and S2:17), selected using the SA algorithm from a set of 40 sensors. The ellipses represent the boundary lines based on the posterior probabilities computed using the Bayes' theorem for each class

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Availability of data and material Data will be available upon request.

Declarations

Conflict of interest The authors declare that they have no financial or non-financial conflict/competing interests.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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