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Does water addition during the industrial milling phase affect the chemical-sensory quality of olive oils? The case of cv. Arbequina oils

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ABSTRACT

At olive oil industrial extraction, water addition is a practice overcoming the formation of thick pastes. The effect of water addition (0 to 6.2%, $kg_{added\ water}/kg_{olives}$), during the industrial milling of *cv*. Arbequina olives, on the oils' chemical-sensory quality, was evaluated. Despite the extra virgin olive oil classification, compared with the water incorporation (1.2–6.2%), extraction without water addition resulted into oils that showed less primary oxidation (lower peroxide values and K_{232}), greater total phenolic content (+12–22%) and higher oxidative stability (+22–31%). No water addition increased the oils secoiridoids content (+5–13%), mainly oleacein (+27–79%). Oils extracted without water addition had a more intense ripe fruity sensation (≥ +11%) but lower fruit intensities (at least −4%). Thus, the quality and stability of the *cv*. Arbequina oils can be favoured if extracted without adding water during the olives industrial milling.

1. Introduction

Virgin olive oil (VOO) has a key role in the Mediterranean diet, contributing to its well-known health-related benefits (Jimenez-Lopez et al., 2020), particularly the oil's polyphenols, whose antioxidant activity plays a protective role against several diseases (Moral & Escrich, 2022). The quality and composition of VOO are influenced by several factors, including the composition of the olive fruits at harvest and the extraction conditions. Several approaches have been studied aiming to enhance the health-related composition and the physicochemical-sensory characteristics of VOO, by optimizing the extraction conditions, like milling settings, malaxation time–temperature, or ultrasound application (Manganiello et al., 2021; Marx et al., 2021a; Miho, Moral, López-González, Díez, & Priego-Capote, 2020; Polari, Garcí-Aguirre, Olmo-García, Carrasco-Pancorbo, & Wang, 2018; Taticchi et al., 2019, 2021). Water addition to the olive paste during malaxation has also been evaluated to increase the oil yield and extractability index (Kiritsakis, Rodríguez-Pérez, Gerasopoulos, & Segura- Carretero, 2017; Novoselić et al., 2021), since water acts as a phase separation coadjuvant, promoting the breakdown of the water–oil emulsions, facilitating oil extraction and thus, influencing the quality of the extracted olive oils (Ben-David et al., 2010; Clodoveo, 2012). Therefore, in the industry, the addition of water during the milling phase is a common but empirical procedure. This addition, besides standardizing the moisture content of the olive pastes, aims to control the temperature of the paste during the milling process, avoiding the overheating (Carrapiso et al., 2013; Clodoveo, 2012).

Several studies reported the effects of adding different amounts of water (varying from 5 to 43%, $kg_{added\ water}/kg_{olives}$) during the malaxation process on the oil yield, extractability index, physicochemical-sensory characteristics, and phenolic profiles (Ben-David et al., 2010; Carrapiso et al., 2013; Kiritsakis et al., 2017; Novoselić et al., 2021). The previous studies discussed the impact of adding different water levels (5 to 43%, w/w), during the oils extraction, from olives of different cultivars, on their characteristics. Although the addition of water is more common when olives had a low moisture content, the referred studies evaluated the effect of adding low to high water levels during the oil extraction from olives with medium to high moisture contents (varying

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from 49 to 68%) (Ben-David et al., 2010; Carrapiso et al., 2013; Novoselić et al., 2021). Globally, the results showed that the effects of water addition on the quality and phenolic composition of the extracted oils depended on the water levels used, the olive cultivar and the olives' moisture, being both positive and negative effects observed (Ben-David et al., 2010; Carrapiso et al., 2013; Kiritsakis et al., 2017; Novoselić et al., 2021), as can be inferred from Table S1.

The impact of the addition of water during the centrifugation phase on the quality and composition of the extracted olive oils has also been evaluated (Beltrán, Bejaoui, Sánchez-Ortiz, & Jiménez, 2021). Globally, the studies carried out reported contradictory results, which could be due to the fact that the effects of water addition during oils' extraction depend on the olive cultivar (Ben-David et al., 2010). However, none of these studies was performed at industrial scale, being carried out at laboratory or pilot scales. Additionally, it is important to highlight that extractions at laboratory scale can hardly mimic those performed at industrial scale. Only one study was conducted at industrial scale, evaluating the impact of water addition during malaxation on the contents of pigments, tocopherols, and squalene of Greek olive oils (more than 450) from 12 different olive cultivars (Martakos, Kostakis, Dasenaki, Pentogennis, & Thomaidis, 2020). It was found that the addition of water decreased the tocopherols content but increased the squalene content. However, the study only involved one *cv*. Arbequina oil.

To the authors' best knowledge, the effects of adding or not water during the industrial milling/malaxation process on the physicochemical, phenolic, and sensory characteristics of *cv*. Arbequina oils, have never been evaluated. Thus, it was studied the impact of adding different but low levels of water (0, 1.2, 3.5 and 6.2%, $kg_{added\ water}/kg_{olives}$) during the industrial extraction of *cv*. Arbequina oils. Arbequina cultivar was used since it is one of the most spread cultivars around the world. The amounts of water added were established since according to the olive oil producer, 5% ($kg_{added\ water}/kg_{olives}$) of water are usually added during the oil industrial extraction. Thus, it was decided to use percentages below and slightly above the referred set point, aiming to provide *cv*. Arbequina olive oil producers with a feasible decision tool regarding the addition or not of water during the extraction.

Additionally, a potentiometric electronic tongue (E-tongue) was used for assessing the overall impact of the water addition during the extraction of *cv*. Arbequina oils, considering the known relationships between the potentiometric signals and the phenolic-sensory characteristics of the oils (Rodrigues et al., 2019; Marx et al., 2021a; Marx et al., 2022).

2. Materials and methods

2.1. Olives and olive oil samples

Olives from *cv*. Arbequina were collected in mid-November 2019 from an orchard located in Trás-os-Montes region, Portugal. Olives were harvested with a maturity index between two and three (Uceda & Frias, 1975) and had an average moisture content of 61.2 ± 1.1% (according to the olive oil producer). Olive oils were extracted in an industrial olive oil mill (OLIMONTES, Macedo de Cavaleiros, Portugal), using a two-phase oil extraction method. In each of four independent batches, 600 kg of olives were used. In one batch, the extraction was performed without water addition during the milling phase (0% of water addition). In the other three batches, tap water (~18 °C) was added during the milling process of the olives, by supplying water at a flow rate of 0.7, 2.1 or 3.7 L/min, during 10 min, which corresponded to water levels of 1.2, 3.5 and 6.2% ($kg_{added\ water}/kg_{olives}$) (i.e., addition of ~ 7, 21 and 37 kg of water to 600 kg of olives). All the other processes (malaxation at 22 °C and 12 rpm, during 45 min; decantation; and, centrifugation) were the same for all independent extraction batches. Taking into account the temperature of the water added during the milling step (~18 °C), the relative low amounts of water added (1.2 to 6.2%), as well as the extraction temperature used (22 °C), it is not expected that the water addition had a great impact on the final temperature of the olive paste. Fig. 1 ilustrates the olive oil extraction process.

In total, four independent lots of *cv*. Arbequina oils were obtained. From each lot three oil bottles (amber glass bottles) were collected, closed, and immediately transported to the laboratory (Bragança, Portugal). Water traces in the oils were removed using anhydrous sodium sulfate (1 g for 100 mL of olive oil) and the oils were filtered using a cellulose filter. All oils were stored in the dark (18–22 °C) in amber glass bottles, being analysed after six months of storage.

2.2. Olive oil physicochemical analysis

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 European Union Regulation (European commission delegated regulation (EU) 2015/1830, 2015). The oxidative stability (OS, in h) was determined under accelerated oxidation conditions (120 °C) using the Rancimat method (Rancimat 743, Metrohm CH, Switzerland) (Rodrigues et al., 2019).

Total phenolic contents (TPC) were assessed following the traditional method (Capannesi, Palchetti, Mascini, & Parenti, 2000), with minor modifications described by Marx et al. (2021a). The TPC quantification (in mg of gallic acid equivalents (GAE) per kg of oil) was carried out using a calibration curve ($R^2 \geq 0.999$). The aqueous methanolic extract (methanol:water, 80:20 (v/v)) obtained in this step was also used for the potentiometric E-tongue analysis.

2.3. HPLC-DAD phenolic compounds determination

The extraction and phenolics determinations were made according to the methodology proposed by the International Olive Council (2017), with minor modifications as described by Marx et al. (2022). Methanolic extracts (80% v/v), from duplicate extractions of each sample, were injected in a high-performance liquid chromatography system with a diode-array detector (HPLC-DAD) (Jasco, Japan). The apparatus included 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.

The phenolic compounds were identified by comparing with the pure standards (apigenin, hydroxytyrosol, luteolin and verbascoside, from ExtraSynthese; caffeic acid, cinnamic acid, gallic acid, oleocanthal (p-HPEA-EDA), oleuropein, p-coumaric acid, pinoresinol, vanillic acid and vanillin, from Sigma-Aldrich; tyrosol (2–4- hydroxyphenyl ethanol), from Fluka; oleacein (3,4-DHPEA-EDA), from Toronto Research Chemicals) and taking into account the peak characteristics, namely the UV–Vis spectra (200–600 nm) of each compound. Some peaks were also tentatively identified based on the study of Klen, Wondra, Vrhovšek, and Vodopivec (2015).

The concentration of hydroxytyrosol, tyrosol, vanillic acid, oleacein, oleocanthal, luteolin, and apigenin in the olive oil extracts was determined using individual calibration curves (Table 1), established following the internal standard calibration method, using the area of each identified compound (considering the UV–Vis absorption maxima, λ_{max}) and the area of the internal standard (syringic acid; Sigma-Aldrich). Table 1 presents data for calibration curves, concentration ranges (mg/mL), limit of detection (LOD), and limit of quantification (LOQ) for phenolic compounds. The LOD and LOQ were calculated based on the standard deviation of y-intercept of the regression line (Sy) and the slope (S), using the equations LOD = 3.3 × Sy/S and LOQ = 10 × Sy/S. The remaining phenolic compounds, for which no commercial standards were available, were quantified using the calibration curves of the phenolic compound with the closest chemical structure. Oleuropein

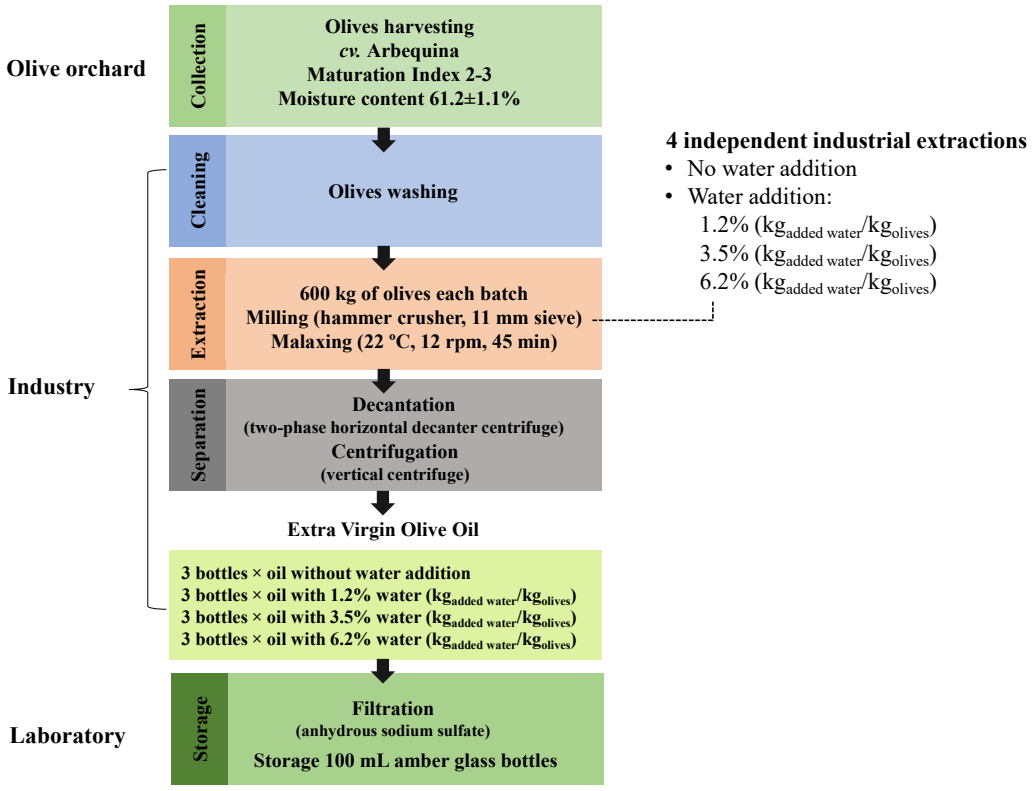

Fig. 1. Technological process of olive oil extraction on an industrial scale.

Table 1
Calibration curves established for the quantification of phenolic compounds (λ_{max}, coefficient of determination, slope, intercept values, limit of detection (LOD), limit of quantification (LOQ) and concentration range.

Compound	λ_{max} (nm)	Determination coefficient	Slope mL/mg	Intercept	LOD mg/mL	LOQ mg/mL	Concentration range mg/mL
Phenolic acids							
Vanillic acid	280	0.999	195.8 ± 1.3	−0.0005 ± 0.0057	0.0001	0.0003	0.0004–0.0083
Caffeic acid	325	0.997	392.8 ± 14.8	−0.045 ± 0.020	0.0001	0.0004	0.0002–0.0184
p-coumaric acid	325	0.990	310.4 ± 13.8	0.288 ± 0.272	0.0029	0.0090	0.0006–0.0457
Phenolic alcohols							
Hydroxytyrosol	280	0.999	84.2 ± 0.8	−0.032 ± 0.035	0.0014	0.0042	0.0006–0.2250
Tyrosol	280	0.996	66.2 ± 1.6	0.004 ± 0.064	0.0028	0.0086	0.0005–0.1029
Flavonoids							
Luteolin	350	0.995	149.6 ± 4.1	0.002 ± 0.062	0.0136	0.0410	0.0004–0.0364
Apigenin	325	0.996	786.2 ± 23.1	0.001 ± 0.097	0.0004	0.0012	0.0001–0.0097
Lignans							
Pinoresinol	280	0.993	43.1 ± 1.6	0.030 ± 0.040	0.0030	0.0092	0.0008–0.0562
Secoiridoids							
Oleuropein	280	0.991	34.7 ± 1.2	−0.053 ± 0.246	0.0230	0.0710	0.0016–0.5813
Oleacein (3,4-DHPEA-EDA)	280	0.990	39.0 ± 1.9	0.0007 ± 0.0167	0.0014	0.0040	0.0005–0.0187
Oleocanthal (p-HPEA-EDA)	280	0.994	17.2 ± 0.6	−0.130 ± 0.050	0.0010	0.0300	0.0025–0.1875

and ligstroside derivatives, which include oleuropein aglycone (3,4-DHPEA-EA), ligstroside aglycone (p-HPEA-EA), and their isomers (secoiridoids), were quantified using the calibration curve of oleuropein (Loubiri et al., 2017).

2.4. Olive oil sensory analysis

The olive oils' sensory analysis was performed by a trained sensory panel, from the School of Agriculture of the Polytechnic Institute of Bragança, Portugal The trained panel followed the guidelines of the European Union standard methods (European commission delegated regulation (EU) 2015/1830, 2015). The intensities of the sensory attributes were scored using an unstructured scale ranging from 0 (no sensory sensation perceived) to 10 (maximum intensity perceived). The descriptive profile was evaluated using a test sheet, according to the International Olive Council (2005).

2.5. E-tongue device

The potentiometric E-tongue was designed and built in the laboratory facilities of the School of Agriculture of the Polytechnic Institute of Bragança, Portugal. The device included two cylindrical arrays, containing each one 20 different lipid polymeric cross-sensitive sensor membranes. The composition of each sensor membranes, construction details and potentiometric signals acquisition have been previously described (Rodrigues et al., 2019). The potentiometric analysis was performed on the polar extracts previously obtained for the TPC assessment. Each extract was diluted (1:5, v/v) with deionized water

and vortexed for 1–2 min before being analysed (Rodrigues et al., 2019). Each assay took 5 min, allowing achieving a pseudo-equilibrium between the E-tongue membranes and the chemical compounds present in each polar extract solution (Marx et al., 2021a).

2.6. Statistical analysis

One-way ANOVA followed, when appropriate, by the Tukey's post-hoc multi-comparison test, or the Student's *t*-test, was used to evaluate the existence of statistical significant differences among the chemical and sensory profiles of the 4 different *cv*. Arbequina olive oils (industrially extracted without or with the addition of water during the milling phase). The Pearson correlation coefficient (*R*-Pearson) was used to evaluate the existence of linear correlations between the chemical or sensory data and the water addition level.

Linear discriminant analysis (LDA) was also applied to verify the possibility of using the potentiometric E-tongue to discriminate the extracted oils according to the addition or not of different amounts of water. The best subsets of E-tongue sensors were identified using the meta-heuristic simulated annealing (SA) variable selection algorithm. The classification performance of each selected LDA-SA model was evaluated based on the sensitivity values for the leave-one-out (LOO) cross-validation (CV) procedure. A 2D-plot of the two first discriminant functions (DF) for the original grouped data (training) was also used for evaluating the classification performance, being the confidence ellipses determined based on the posterior probabilities computed using the Bayes' theorem for each class considered.

All the referred statistical analyses were performed using statistical program R (version 3.6.2), at a 5% significance level.

3. Results and discussion

3.1. Effect of the water addition during the olives milling on the oils' physicochemical characteristics

The physicochemical data of the *cv*. Arbequina oils extracted without or with the addition of water (1.2, 3.5 and 6.2%, $kg_{added\ water}/kg_{olives}$) during the milling/malaxation phase are shown in Table 2. Independently of the amount of water added, based on the FA, PV, K_{232} and K_{268}, values, all extracted oils could be classified as extra virgin olive oils (EVOO) (FA $\leq$ 0.8 g oleic acid/100 g, PV $\leq$ 20 mEq O2/kg, $K_{232} \leq 2.50$, $K_{268} \leq 0.22$) (European commission delegated regulation (EU) 2015/1830, 2015). It can also be inferred that water addition had a significant negative effect on the quality parameters of the studied olive oils, similarly to the findings of Ben-David et al. (2010). The observed negative effect was more evident for PV and K_{232}, which suffered an increase up to 34% compared to the values of the oils extracted without water addition, showing that water addition enhanced undesirable primary oxidation reactions. The observed trend for PV is in agreement with the results of Kiritsakis et al. (2017) and Carrapiso et al. (2013). Regarding the FA and the K_{268}, the significant negative differences observed (Table 2) were of a smaller order of magnitude compared with the previous parameters. On the other hand, Ben-David et al. (2010) reported that the oils extracted from *cvs.* Picual or Barnea olives with a water addition of 14% ($kg_{added\ water}/kg_{olives}$) had higher FA than oils extracted with 32 and 43% ($kg_{added\ water}/kg_{olives}$). Contrary, Kiritsakis et al. (2017) observed that *cv*. Koroneiki oils extracted with 25% ($kg_{added\ water}/kg_{olives}$) of water had greater FA than those extracted without added water, in agreement with the findings reported by Carrapiso et al. (2013). The different trends reported may be attributed to the different olive cultivars and to the different levels of water added, studied in each research. In which concerns the K_{268}, the values for oils extracted without or with water addition were of the same order of magnitude, contrary to what would be expected based on the findings of Carrapiso et al. (2013) and Kiritsakis et al. (2017).

Also, water addition had a significant and clear negative impact on the TPC and OS of the extracted *cv*. Arbequina oils, with reductions of 11–18% and 18–24%, respectively, compared with the values found for oils extracted without water addition (Table 2). Thus, extraction without water addition had a positive effect on the olive oil quality. On the other hand, increasing the level of added water from 1.2 to 6.2% did not have a marked impact on the TPC or OS. Even so, the overall decreasing trends of OS or TPC when the level of added water increased are in agreement with the literature data, although much higher levels of added water were previously evaluated (14–43%, $kg_{added\ water}/kg_{olives}$) (Ben-David et al., 2010; Carrapiso et al., 2013; Kiritsakis et al., 2017). In fact, although the use of water facilitates the oil separation, usually it is responsible for a decrease of the total phenolic content, leading to hence a shorter shelf life (Velasco & Dobarganes, 2002).

Finally, the overall results pointed out that, for *cv*. Arbequina olives with a mean moisture content of 61.2 ± 1.1%, the quality of the oils industrially extracted would be promoted if no water is added during the milling phase. The water addition had a negative impact on the FA, PV, K_{232}, K_{268}, OS and TPC, and so, the extraction without water addition during the milling phase would allow obtaining EVOOs with superior chemical quality and higher resistance to the oxidation degradation.

Table 2
Physicochemical quality parameters (mean ± standard deviation, for each oil: n = 3 olive oil bottles × 2 analysis) of the studied *cv*. Arbequina olive oils analyzed 6 months after extraction.

Parameters[1]	Without water addition	Water addition ($kg_{added\ water}/kg_{olives}$)[2]			
		1.2%	3.5%	6.2%	*P*-value[3]
Quality parameters					
FA (g oleic acid/100 g)	0.34 ± 0.00^{b}	0.39 ± 0.00^{a}	0.34 ± 0.00^{b}	0.34 ± 0.00^{b}	<0.0001
PV (mEq O_2/kg oil)	2.48 ± 0.01^{c}	3.32 ± 0.02^{a}	3.32 ± 0.06^{a}	2.90 ± 0.03^{b}	<0.0001
K_{232}	1.84 ± 0.03^{d}	2.35 ± 0.04^{b}	2.46 ± 0.02^{a}	2.27 ± 0.01^{c}	<0.0001
K_{268}	0.10 ± 0.00^{b}	0.11 ± 0.01^{a}	0.11 ± 0.00^{a}	0.11 ± 0.01^{a}	<0.0001
Other parameters					
OS (h)	8.4 ± 0.4^{a}	6.4 ± 0.1^{b}	6.4 ± 0.3^{b}	6.9 ± 0.2^{b}	<0.0001
TPC (mg GAE/kg oil)	230.4 ± 2.4^{a}	189.2 ± 0.6^{d}	197.2 ± 2.9^{c}	205.2 ± 2.5^{b}	<0.0001

[1] 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 phenolic content.
[2] Mass percentage of added water during the milling phase: 1.2% corresponding to ~ 7 $kg_{added\ water}$ in 600 kg_{olives}; 3.5% corresponding to ~ 22 $kg_{added\ water}$ in 600 kg_{olives}; and, 6.2% corresponding to ~ 37 $kg_{added\ water}$ in 600 kg_{olives}.
[3] *P*-values for the one-way ANOVA. Different letters in the same row show statistically differences from the given mean ($p < 0.05$).

3.2. Effect of water addition during the olives milling on the oils' phenolic profiles

The HPLC-DAD method implemented allowed identifying and quantifying seven phenolic compounds (apigenin, luteolin, hydroxytyrosol, oleacein, oleocanthal, tyrosol, and vanillic acid) in the four types of *cv*. Arbequina oils, extracted without or with the addition of water at the milling process, belonging to 4 phenolic classes: acids, alcohols, flavonoids, and secoiridoids. The sum of identified phenolic compounds represented 64–66% of the total area of the chromatogram for *cv*. Arbequina oils extracted with or without water (Table 3). New methodologies, like liquid chromatography coupled to tandem mass spectrometry have been successfully applied allowing the identification of a large number of phenolic compounds in olive oils, thus providing

Table 3
Phenolic compounds content (mean ± standard deviation, mg/kg of olive oil; for each oil: n = 3 olive oil bottles × 2 analytical extractions × 1 chromatographic analysis) of the studied *cv.* Arbequina olive oils.

Phenolic compounds	Without water addition	Water addition ($kg_{added\ water}/kg_{olives}$)[1] 1.2%	3.5%	6.2%	*P*-value[2]
Acids					
Vanillic acid	0.21 ± 0.02^{b}	0.27 ± 0.01^{a}	0.28 ± 0.03^{a}	0.27 ± 0.04^{a}	0.0003
Alcohols					
Hydroxytyrosol	0.69 ± 0.08^{b}	0.86 ± 0.12^{a}	0.79 ± 0.07^{ab}	0.86 ± 0.06^{a}	0.0059
Tyrosol	0.58 ± 0.09^{b}	0.86 ± 0.10^{a}	0.86 ± 0.09^{a}	0.91 ± 0.07^{a}	<0.0001
Flavonoids					
Luteolin	4.40 ± 0.64^{a}	4.45 ± 0.45^{a}	3.74 ± 0.67^{a}	3.73 ± 0.49^{a}	0.0610
Apigenin	0.48 ± 0.02^{a}	0.47 ± 0.02^{a}	0.53 ± 0.16^{a}	0.45 ± 0.03^{a}	0.4190
Secoiridoids					
Oleacein (3,4-DHPEA-EDA)	23.08 ± 1.52^{a}	12.90 ± 0.83^{c}	15.48 ± 0.99^{c}	18.20 ± 0.82^{b}	<0.0001
Oleocanthal (p-HPEA-EDA)	8.20 ± 0.56^{a}	6.72 ± 0.19^{b}	8.30 ± 0.64^{a}	8.53 ± 0.73^{a}	<0.0001
ΣOleuropein derivatives	65.52 ± 2.07^{a}	65.38 ± 2.34^{a}	64.57 ± 1.98^{a}	64.26 ± 2.18^{a}	0.8701
ΣLigstroside derivatives	14.88 ± 0.70^{ab}	14.13 ± 1.58^{b}	14.75 ± 0.81^{ab}	15.44 ± 0.84^{a}	0.0604
$\Sigma_{phenolic\ acids}$	0.21 ± 0.02^{b}	0.27 ± 0.01^{a}	0.28 ± 0.03^{a}	0.27 ± 0.04^{a}	<0.0001
$\Sigma_{phenolic\ alcohols}$	1.27 ± 0.16^{b}	1.72 ± 0.21^{a}	1.65 ± 0.14^{a}	1.77 ± 0.09^{a}	0.0003
$\Sigma_{flavonoids}$	4.88 ± 0.66^{a}	4.92 ± 0.47^{a}	4.27 ± 0.56^{a}	4.18 ± 0.51^{a}	0.0570
$\Sigma_{secoiridoids\ derivatives}$	111.68 ± 1.86^{a}	99.13 ± 2.04^{d}	103.10 ± 2.04^{b}	106.43 ± 2.98^{b}	<0.0001
$\Sigma_{identified\ phenols}$	118.04 ± 2.09^{a}	106.04 ± 2.34^{c}	109.30 ± 2.28^{bc}	112.66 ± 2.96^{d}	<0.0001

[1] Mass percentage of added water during the milling phase: 1.2% corresponding to ~ 7 $kg_{added\ water}$ in 600 kg_{olives}; 3.5% corresponding to ~ 22 $kg_{added\ water}$ in 600 kg_{olives}; and, 6.2% corresponding to ~ 37 $kg_{added\ water}$ in 600 kg_{olives}.
[2] *P*-values for the one-way ANOVA. Different letters in the same row show statistically differences from the given mean ($p < 0.05$).

new and more detailed insights about the phenolic profiles of olive oils (López-Yerena et al., 2021). However, in this study, a HPLC-DAD technique was adopted, based on the methodology recommended by the IOC (International Olive Council, 2017).

The phenolic compounds identified in the studied *cv.* Arbequina oils are in-line with the profiles reported for *cv.* Arbequina oils (Loubiri et al., 2017; Marx et al., 2022), although different contents were found, which can be due to different maturation indices of the olives at harvest, the different agro-climatic and extraction conditions (Clodoveo, 2012).

According to the results (Table 3), the most abundant class was the secoiridoids (99 to 112 mg/kg), followed by flavonoids (4 to 5 mg/kg). Phenolic alcohols and phenolic acids were the less abundant classes (lower than 1.8 mg/kg). The low amounts of phenolic acids and alcohols agree with the results reported by Loubiri et al. (2017) for *cv.* Arbequina oils. Morevover, the results also pointed out that, in general, water addition during the extraction of the oils had a significant effect ($p < 0.05$, for the one-way ANOVA) on the contents of the identified individual phenolics, as well as on three of the four phenolic classes (with the exception of the flavonoids). Different increasing/decreasing trends were found depending on the phenolic compound and the phenolic class (Table 3).

Globally, the addition of water during the oils extraction increased the contents of the less abundant classes, namely the phenolic acids (an increase of ~ 30%) and the phenolic alcohols (an increase of 30–40%). The results for the phenolic acids are in accordance with those previously reported in the literature (Kiritsakis et al., 2017; Novoselić et al., 2021). The increasing content of phenolic alcohols is in agreement with the slight increase of tyrosol content reported by Kiritsakis et al. (2017), but contrary to the significant decrease reported by Novoselić et al. (2021). Hydroxytyrosol content was less affected by the water addition during the oil extraction, as also pointed out by Novoselić et al. (2021). This could be explained since it is soluble in both oily and aqueous media (Bouaziz, Grayer, Simmonds, Damak, & Sayadi, 2005). The increasing trend of the phenolic alcohols contents could be related with the decrease of secoiridoids contents, since hydroxytyrosol and tyrosol can be formed from the hydrolysis of oleuropein or ligstroside (Johnson, Melliou, Zweigenbaum, & Mitchell, 2018). In fact, for the studied oils, a negative correlation (*R*-Pearson = −0.763) was established between the phenolic alcohols and the secoiridoid contents. On the other hand, for the studied oils, the flavonoids contents were not affected by the addition of water, in opposition to the findings of Novoselić et al. (2021) that reported that the addition of water (5%, $kg_{added\ water}/kg_{olives}$) resulted in oils richer in flavonoids. On the other hand, Kiritsakis et al. (2017) verified that the addition of 25% ($kg_{added\ water}/kg_{olives}$) of water increased luteolin content, but decreased apigenin concentration.

The results (Table 3) also showed that the extraction without water addition significantly increased (5–13%) the total content of secoiridoids (depending on the level of added water), which is of major relevance taking into account the vast positive pharmacological effects attributed to this class of phenolic compounds (Jimenez-Lopez et al., 2020). The abovementioned rising trend could be attributed to the amphiphilic characteristics of these aglycons, which are partitioned between the oily layer and the vegetation water, due to their polar functional groups (Bendini et al., 2007) and so, the increase of the water amount due to its addition during the extraction would favour the migration of the secoiridoids to the water phase. A remarkable increase of the oleacein content was observed when the *cv.* Arbequina oils were extracted without water addition, compared to the contents found in oils extracted with water addition (an increase 27 to 79%). Additionally, the reduction of the content of the total identified phenolic compounds observed for the oils extracted with water addition could be partially associated with the hydrophilic characteristics of some phenolic compounds (Clodoveo, 2012), being less soluble in oil than in water. Thus, increasing the amount of water added during oil extraction would result in a higher loss of these phenolics to the aqueous phase, which is discarded (Kalogeropoulos, Kaliora, Artemiou, & Giogios, 2014). This could also partially explain the decreasing trend of the TPC of the oils extracted with water addition, based on the positive correlation (*R*-Pearson = +0.984) established between the total contents determined by both methods (HPLC and spectrophotometry).

These findings are in agreement with the results reported by Kiritsakis et al. (2017) that have found higher concentrations of oleacein in *cv.* Koroneiki oils extracted without water addition compared with those obtained after the addition of 25% ($kg_{added\ water}/kg_{olives}$) of water. An opposite trend was reported by Novoselić et al. (2021), when extracting *cv.* Leccino oils with 5% ($kg_{added\ water}/kg_{olives}$) of water. Lastly, secoiridoids have a higher antioxidant capacity than the other phenolic compounds classes (Bouaziz et al., 2005). Thus, it can be assumed that a higher amount of secoiridoids in oils extracted without water, could contribute to a higher stability of these oils. In fact, in this work, it was possible to establish a positive correlation between the OS values and the total content of secoridoids (*R*-Pearson = +0.928) for the studied *cv.* Arbequina oils.

Finally, the results pointed out that oils extracted from *cv.* Arbequina olives (average moisture of 61.2 ± 1.1%) without the addition of water during the milling step, were richer in phenolic compounds with greater contents of secoiridoids, like oleacein, compared to oils extracted with water addition. Considering the recognized beneficial health properties attributed to the phenolic compounds, namely to oleacein (Jimenez-Lopez et al., 2020), it can be assumed that *cv.* Arbequina oils extracted without water addition have a superior health-related composition. These findings are in-line with the findings reported in the literature. In

fact, although water added during the extraction can act as a coadjuvant for VOOs' extraction namely in low-moisture fruits (Clodoveo, 2012), for fruits with medium–high moisture contents, the water addition decreases the oil yield, the phenolic contents and/or the chemical quality of the oils (Ben-David et al., 2010; Carrapiso et al., 2013; Kiritsakis et al., 2017). Thus, Carrapiso et al. (2013) even suggested to avoid the addition of water to the olive pastes, for olives grown in irrigated orchards. In the present study the extraction yield for each type of olive oil was not determined. However, taking into account the low levels of water used (1.2–6.2 %, $kg_{added\ water}/kg_{olives}$), the moisture content of the *cv.* Arbequina olives (61.2 ± 1.1%), and the recent findings of Novoselić et al. (2021), similar extraction yields are expected for all the oils studied.

3.3. Effect of water addition during olives milling on the oils' sensory quality

During the sensory analysis of the *cv.* Arbequina oils, no defect sensation was perceived, confirming the previous classification of the oils as EVOO. In total, 9 positive gustatory and 6 positive olfactory attributes were detected and assessed, which intensities are listed in Table 4. The overall sensory profiles established by the sensory panel are in agreement with the literature data available for *cv.* Arbequina oils produced in Portugal (Marx et al., 2021b; Marx et al., 2022), although different intensities were observed, probably due to differences in the agro-climatic and extraction process conditions rather than to the addition of water during the olives milling process.

Regarding the gustatory attributes, it can be noticed that oils extracted without water addition during the milling phase, denoted a more intense ripe fruity sensation (an increase of 11–17%, compared to the oils extracted with water addition), which is in accordance with the findings of Kiritsakis et al. (2017). Linear correlations were established between the oils' ripe fruity intensity and the PV (*R*-Pearson = −0.934) or the OS (*R*-Pearson = +0.967), meaning that oils with higher ripe fruity intensities would be more resistant to oxidation and more stable. A similar, but less pronounced trend in magnitude, could be observed for the pungent sensation, whose intensity increased 20–25% when no water was added at the olives milling phase (from intensities of 0.8–1.0 to 1.2). Kiritsakis et al. (2017) also observed a decrease in oils' pungency with the addition of 25% of water. This sensation is characteristic of early harvest VOOs produced from unripe olives and can be related to some phenolic compounds like oleocanthal (des Gachons et al., 2011). However, in the present study it was not possible to establish a correlation between the oleocanthal content and the pungent intensities of the *cv.* Arbequina oils extracted with different levels of water.

Contrary, for the other perceived gustatory sensations (i.e., sweet, apple, banana, tomato, dry fruits, and dry hay grass), the addition of water during the milling phase, independently of the added amount, promoted a significant and clear increase of the respective intensities. This was even more relevant for the dry hay grass sensation, which was not perceived in the oils extracted without water addition and was detected in the other oils with medium intensities (ranging from 4 to 5). The absence of the perception of this sensation in the oils extracted when no water was added during the olives milling could be related to the high intensity of the ripe fruity sensation, which may have mask the other attribute. It should be remarked that for tomato and dry fruits sensations, positive correlations could be established between the perceived intensities and the amount of water added during the extraction (*R*-Pearson = +0.930 or +0.959, respectively). Finally, it could be stated that all oils had a similar and low bitterness (intensities varying from 1 to 1.3 for oils extracted without water addition or with 6.2% of water added, respectively), even if a significant statistical effect was observed. This fact could be partially explained taking into account that the bitter sensation is a characteristic of olive oils obtained from green olives, being related with the phenolic composition and contents in the oils (Cui et al., 2021). Indeed, oleacein can be responsible for the bitterness of the oils (Gutiérrez-Rosales, Ríos, & Gómez-Rey, 2003), being ligstroside and

Table 4
Intensities of sensory attributes (mean ± standard deviation, n = 3 olive oil bottles × 2 analysis × 8 panellists) of the studied *cv.* Arbequina olive oils.

Sensory attributes perceived by the sensory panel[1]	Without water addition	Water addition ($kg_{added\ water}/kg_{olives}$)[2]			*P*-value[3]	*R*-*Pearson*
		1.2%	3.5%	6.2%		
Gustatory attributes						
Ripe fruity	8.3 ± 0.3^{a}	7.4 ± 0.2^{bc}	7.1 ± 0.3^{c}	7.5 ± 0.3^{b}	<0.0001	−0.534
Bitter	1.0 ± 0.2^{b}	1.1 ± 0.3^{ab}	1.1 ± 0.2^{ab}	1.3 ± 0.3^{a}	0.0152	+0.805
Pungent	1.2 ± 0.2^{a}	0.9 ± 0.1^{b}	1.0 ± 0.4^{a}	0.8 ± 0.2^{b}	0.0002	−0.777
Sweet	7.5 ± 0.4^{c}	7.3 ± 0.2^{c}	8.7 ± 0.2^{a}	8.1 ± 0.4^{b}	<0.0001	+0.606
Apple	3.3 ± 0.4^{c}	4.2 ± 0.2^{b}	5.4 ± 0.3^{a}	4.4 ± 0.4^{b}	<0.0001	+0.590
Banana	3.6 ± 0.7^{c}	5.6 ± 0.2^{b}	5.4 ± 0.4^{b}	6.9 ± 0.4^{a}	<0.0001	+0.878
Dry fruits	4.5 ± 0.6^{b}	4.7 ± 0.2^{b}	5.0 ± 0.3^{b}	6.0 ± 0.2^{a}	<0.0001	+0.959
Dry hay grass	n.d.	4.0 ± 0.1^{c}	4.3 ± 0.1^{b}	4.6 ± 0.1^{a}	<0.0001	+0.743
Tomato	2.2 ± 0.3^{b}	2.4 ± 0.3^{b}	3.9 ± 0.2^{a}	4.0 ± 0.3^{a}	<0.0001	+0.930
Harmony	8.3 ± 0.5^{a}	8.6 ± 0.3^{a}	8.1 ± 0.3^{b}	8.0 ± 0.2^{ab}	0.0024	−0.733
Olfactory attributes						
Ripe fruity	8.6 ± 0.3^{a}	6.4 ± 0.3^{c}	5.2 ± 0.4^{b}	5.3 ± 0.3^{b}	<0.0001	−0.841
Apple	3.0 ± 0.3^{d}	4.2 ± 0.4^{c}	5.2 ± 0.5^{a}	4.8 ± 0.2^{b}	<0.0001	+0.796
Banana	n.d.	n.d.	2.3 ± 0.5	6.2 ± 0.3	<0.0001	+0.717
Dry fruits	2.0 ± 0.3^{b}	4.0 ± 0.2^{a}	4.2 ± 0.2^{a}	4.2 ± 0.2^{a}	<0.0001	+0.972
Dry hay grass	n.d.	3.8 ± 0.3^{b}	4.1 ± 0.4^{a}	3.7 ± 0.2^{b}	0.0003	+0.637
Tomato	n.d.	3.2 ± 0.3^{b}	4.3 ± 0.2^{a}	2.9 ± 0.2^{c}	<0.0001	+0.572
Harmony	8.3 ± 0.2^{a}	8.2 ± 0.2^{ab}	8.1 ± 0.3^{b}	7.8 ± 0.2^{c}	<0.0001	−0.985
Global						
Complexity	3.5 ± 0.5^{d}	5.3 ± 0.3^{c}	6.0 ± 0.3^{b}	6.9 ± 0.4^{a}	<0.0001	+0.941
Persistence	4.3 ± 0.5^{c}	5.9 ± 0.4^{b}	6.3 ± 0.5^{b}	7.4 ± 0.3^{a}	<0.0001	+0.934

[1] Intensity means (n = 6) in the same line with the same uppercase letter are not significantly different from a statistical point of view according to the Test of Tukey, at a significance level of 0.05, following the IOC regulations (International Olive Council, 2005). Intensity scale: from 0 (n.d.: attribute not perceived by the panellists) to 10 (maximum attribute intensity).

[2] Mass percentage of added water during milling phase: 1.2% corresponding to ~ 7 $kg_{added\ water}$ in 600 kg_{olives}; 3.5% corresponding to ~ 22 $kg_{added\ water}$ in 600 kg_{olives}; and, 6.2% corresponding to ~ 37 $kg_{added\ water}$ in 600 kg_{olives}.

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

oleuropein aglycones the most potent bitter tastants in olive oils (Cui et al., 2021). Since the concentrations of oleuropein and ligstroside derivatives were similar for all studied oils (Table 3), extracted without or with the addition of water, the similar bitterness could be expected.

Concerning the olfactory attributes and respective intensities (Table 4), similar trends could be established regarding the effect of adding or not water during the extraction of the oils. For example, if no water is added during the milling phase, the ripe fruity intensities significantly increase (an increase up to 10%), but the intensities of the fruits and herbaceous related sensations greatly decrease, being some attributes no more perceived by the sensory panel (e.g., banana, tomato and dry grass).

3.4. Olive oils discrimination using a lab-made potentiometric E-tongue

E-tongues have emerged as alternative/complementary analytical taste sensor devices for EVOO analysis, which applications have been recently reviewed (Bounegru & Apetrei, 2021). Thus, it was decided to evaluate the possibility of applying a lab-made potentiometric E-tongue to qualitatively evaluate the impact of adding or not water, during the milling of *cv.* Arbequina olives, on the chemical-sensory quality of the extracted oils. Despite some statistical differences between the oils extracted with (1.2, 3.5 and 6.2%, $kg_{added\ water}/kg_{olives}$) or without water addition during the milling of *cv.* Arbequina olives, in general, the physicochemical data (Table 2), phenolic profiles (Table 3) as well as the sensory profiles (Table 4) of the oils extracted with the two highest levels of added water (i.e., 3.5 and 6.2%, $kg_{added\ water}/kg_{olives}$), were similar. Contrary, the composition was quite different when compared to oils extracted without water addition or with the lowest level of water (1.2%, $kg_{added\ water}/kg_{olives}$). Thus, the E-tongue was used aiming to discriminate three groups of extracted oils, i.e., one corresponding to the oils extracted without water addition, another including the oils extracted with 1.2% of added water and, the last one, containing the oils extracted with 3.5 and 6.2% of added water.

The olive oils polar extracts were analysed with the E-tongue, and the potentiometric signals of the 40 sensor membranes comprised in the taste sensor device were recorded, which varied from 18 to 312 mV. The signal profiles generated corresponded to a potentiometric fingerprint of each type of *cv.* Arbequina olive oil under study, allowing to establish a LDA-SA classification model. A linear multivariate model with two discriminant functions (which explained 99 and 1% of the data variability) was developed based on the signals recorded by 8 selected non-redundant sensors (1st array: S1:7, S1:8; S1:16; S1:19; and 2nd array: S2:2, S2:15, S2:16, S2:18). The model correctly discriminated (sensitivities of 100% for training, Fig. 2; and, LOO-CV) the three groups of *cv.* Arbequina oils, probably due to the phenolic-sensory differences found on the extracted oils, result of the different levels of added water during the milling phase. Indeed, the successful classification performance (discrimination of oils extracted without, with 1.2% or 3.5–6.2% of added water), could be tentatively attributed to the known capability of the lipid sensor membranes to interact, through electrostatics and/or hydrogen bonds, with the polar compounds present in the oils' extracts (Wu et al., 2020), which are partially responsible for the differences at the sensory and phenolic composition levels. The results confirmed the initial hypothesis regarding the possibility of using a potentiometric E-tongue with lipid membranes as practical taste sensor device capable of discriminating *cv.* Arbequina oils with different chemical-sensory characteristics as a result of the no addition of water or a low or medium–high levels of water addition during the milling of olives with a mean moisture content of 61.2 ± 1.1%. Thus, the E-tongue could indirectly allow assessing the impact of the water addition during the extraction on the phenolic-sensory profiles of the studied oils.

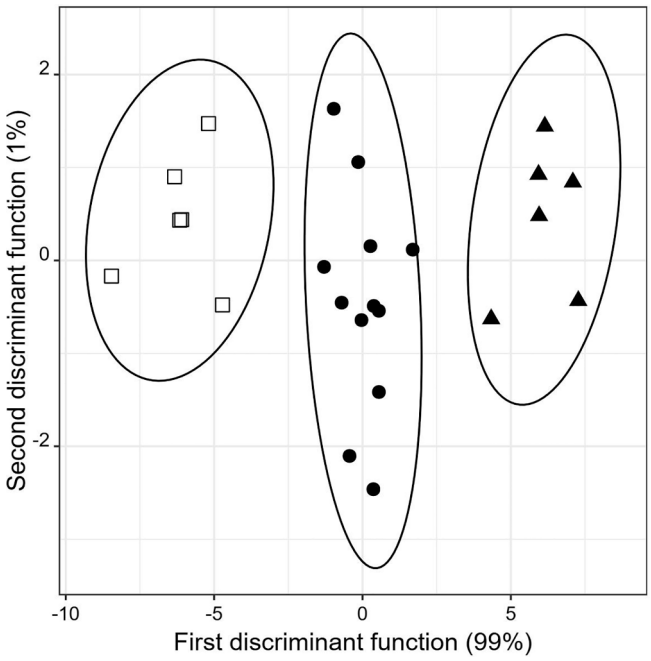

Fig. 2. E-tongue-LDA-SA model performance, based on the potentiometric signals gathered by eight lipid sensor membranes (1st array: S1:7, S1:8; S1:16; S1:19; 2nd array: S2:2, S2:15, S2:16, S2:18), regarding the supervising classification of *cv.* Arbequina oils extracted without or with water addition ($kg_{added\ water}/kg_{olives}$) during the milling phase: 0% (□); 1.2% (▲); and, 3.5–6.2% (●).

4. Conclusions

At the industrial level, water addition during the olive oil extraction, namely during the milling phase, is a common practice for olives with low moisture content, facilitating the extraction process and increasing the oil extraction yield. At the same time, this practice could have an undesirable impact on the overall chemical and sensory quality of the extracted oils, namely at the phenolic compounds' composition, mainly due to their hydrophilic nature. Thus, the effects of the addition of water during the oil extraction on its quality and phenolic composition were previously studied by other researchers. This work evaluated, for the first time, the impact of extracting *cv.* Arbequina oils at industrial scale, without or with the addition of low levels of water (0 and 1.2 to 6.2%, $kg_{added\ water}/kg_{olives}$) during the milling phase, on the physicochemical-sensory quality and phenolic profiles. The results obtained showed that, for *cv.* Arbequina fruits with an average moisture content of 61.2 ± 1.1%, the oil extraction without water addition during the milling phase, improved the quality of the oils, namely by decreasing the primary oxidation reactions (lower peroxide values and extinction coefficients at 232 nm), by increasing the secoiridoids concentrations, especially the oleacein content, and by increasing the oxidative stability of the oils and so, the expected shelf life. Thus, the oils' extraction without adding water during the milling phase seem to promote the content of some health-related phenolic compounds. At sensory level, oils extracted without water addition had higher ripe fruity intensities but showed a lower richness in fruit-herbaceous sensations. The differences observed at the chemical-sensory levels allowed the successful application of a potentiometric electronic tongue for assessing the impact of the added water level (no addition, 0%; low, 1.2%; or, medium–high, 3.5–6.2%) on the *cv.* Arbequina olive oils industrially extracted. The taste sensor device could be used as a complementary, fast, low-cost, and user-friendly tool for olive oil analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133570.

References

Beltrán, G., Bejaoui, M. A., Sánchez-Ortiz, A., & Jiménez, A. (2021). Water Addition During Oil Extraction Affects on Virgin Olive Oil Ethanol Content, Quality and Composition. *European Journal of Lipid Science and Technology, 123*(11), 2000400. https://doi.org/10.1002/ejlt.202000400

Ben-David, E., Kerem, Z., Zipori, I., Weissbein, S., Basheer, L., Bustan, A., & Dag, A. (2010). Optimization of the Abencor system to extract olive oil from irrigated orchards. *European Journal of Lipid Science and Technology, 112*(10), 1158–1165. https://doi.org/10.1002/ejlt.201000056

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A. M., Segura-Carretero, A., Fernández-Gutiérrez, A., & Lercker, G. (2007). Phenolic Molecules in Virgin Olive Oils: A Survey of Their Sensory Properties, Health Effects, Antioxidant Activity and Analytical Methods. An Overview of the Last Decade Alessandra. *Molecules, 12*(8), 1679–1719. https://doi.org/10.3390/12081679

Bouaziz, M., Grayer, R. J., Simmonds, M. S. J., Damak, M., & Sayadi, S. (2005). Identification and antioxidant potential of flavonoids and low molecular weight phenols in olive cultivar Chemlali growing in Tunisia. *Journal of Agricultural and Food Chemistry, 53*(2), 236–241. https://doi.org/10.1021/jf048859d

Bounegru, A. V., & Apetrei, C. (2021). Evaluation of olive oil quality with electrochemical sensors and biosensors: A review. *International Journal of Molecular Sciences, 22*(23), 12708. https://doi.org/10.3390/ijms222312708

Capannesi, C., Palchetti, I., Mascini, M., & Parenti, A. (2000). Electrochemical sensor and biosensor for polyphenols detection in olive oils. *Food Chemistry, 71*(4), 553–562. https://doi.org/10.1016/S0308-8146(00)00211-9

Carrapiso, A. I., García, A., Petrón, M. J., & Martín, L. (2013). Effect of talc and water addition on olive oil quality and antioxidants. *European Journal of Lipid Science and Technology, 115*(5), 583–588. https://doi.org/10.1002/ejlt.201200252

Clodoveo, M. L. (2012). Malaxation: Influence on virgin olive oil quality. Past, present and future - An overview. *Trends in Food Science and Technology, 25*(1), 13–23. https://doi.org/10.1016/j.tifs.2011.11.004

Cui, M., Chen, B., Xu, K., Rigakou, A., Diamantakos, P., Melliou, E., Logothetis, D. E., & Magiatis, P. (2021). Activation of specific bitter taste receptors by olive oil phenolics and secoiridoids. *Scientific Reports, 11*(1), 22340. https://doi.org/10.1038/s41598-021-01752-y

des Gachons, C. P., Uchida, K., Bryant, B., Shima, A., Sperry, J. B., & Breslin, P. A. S. (2011). Unusual pungency from extra-virgin olive oil is attributable to restricted spatial expression of the receptor of oleocanthal. *Journal of Neuroscience, 31*(3), 999–1009. https://doi.org/10.1523/JNEUROSCI.1374-10.2011

Gutiérrez-Rosales, F., Ríos, J. J., & Gómez-Rey, M. L. (2003). Main polyphenols in the bitter taste of virgin olive oil. Structural confirmation by on-line high-performance liquid chromatography electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry, 51*(20), 6021–6025. https://doi.org/10.1021/jf021199x

European commission delegated regulation (EU) 2015/1830 (2015). Amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis Official Journal of the European Union, L266, 9–13.

International Olive Council (2005). Method for the Organoleptic Assessment of Virgin Olive Oil applying to use a designation of origin, COI/T.20/Doc. no. 22 November, 29.

International Olive Council (2017). Determination of Biophenols in Olive Oils by HPLC, COI/T.20/Doc No 29/Rev.1. 29, 1–8.

Jimenez-Lopez, C., Carpena, M., Lourenço-Lopez, C. A. M. G.-G., Lorenzo, J. M., Barba, F. J., Prieto, M. J., & Simal-Gandara, J. (2020). Bioactive Compounds and Quality of Extra Virgin Olive Oil. *Foods, 9*(8), 1014. https://doi.org/10.3390/foods9081014

Johnson, R., Melliou, E., Zweigenbaum, J., & Mitchell, A. E. (2018). Quantitation of Oleuropein and Related Phenolics in Cured Spanish-Style Green, California-Style Black Ripe, and Greek-Style Natural Fermentation Olives. *Journal of Agricultural and Food Chemistry, 66*(9), 2121–2128. https://doi.org/10.1021/acs.jafc.7b06025

Kalogeropoulos, N., Kaliora, A. C., Artemiou, A., & Giogios, I. (2014). Composition, volatile profiles and functional properties of virgin olive oils produced by two-phase vs three-phase centrifugal decanters. *LWT - Food Science and Technology, 58*(1), 272–279. https://doi.org/10.1016/j.lwt.2014.02.052

Kiritsakis, K., Rodríguez-Pérez, C., Gerasopoulos, D., & Segura- Carretero, A. (2017). Olive oil enrichment in phenolic compounds during malaxation in the presence of olive leaves or olive mill wastewater extracts. *European Journal of Lipid Science and Technology, 119*(9), 1600425. https://doi.org/10.1002/ejlt.201600425

Klen, T., Wondra, A., Vrhovšek, U., & Vodopivec, B. (2015). Phenolic Profiling of Olives and Olive Oil Process-Derived Matrices Using UPLC-DAD-ESI-QTOF-HRMS Analysis. *Journal of Agricultural and Food Chemistry, 63*(15), 3859–3872. https://doi.org/10.1021/jf506345q

López-Yerena, A., Ninot, A., Jiménez-Ruiz, N., Lozano-Castellón, J., Pérez, M., Escribano-Ferrer, E., … Vallverdú-Queralt, A. (2021). Influence of the ripening stage and extraction conditions on the phenolic fingerprint of 'corbella' extra-virgin olive oil. *Antioxidants, 10*(6), 877. https://doi.org/10.3390/antiox10060877

Loubiri, A., Taamalli, A., Talhaoui, N., Mohamed, S. N., Carretero, A. S., & Zarrouk, M. (2017). Usefulness of phenolic profile in the classification of extra virgin olive oils from autochthonous and introduced cultivars in Tunisia. *European Food Research and Technology, 243*(3), 467–479. https://doi.org/10.1007/s00217-016-2760-7

Manganiello, R., Pagano, M., Nucciarelli, D., Ciccoritti, R., Tomasone, R., Di Serio, M. G., Giansante, L., Del Re, P., Servilli, M., & Veneziani, G. (2021). Effects of ultrasound technology on the qualitative properties of italian extra virgin olive oil. *Foods, 10*(11), 2884. https://doi.org/10.3390/foods10112884

Martakos, I., Kostakis, M., Dasenaki, M., Pentogennis, M., & Thomaidis, N. (2020). Simultaneous Determination of Pigments, Tocopherols, and Squalene in Greek Olive Oils: A Study of the Influence of Cultivation and Oil-Production Parameters. *Foods, 9*(1), 31. https://doi.org/10.3390/foods9010031

Marx, Í. M. G., Casal, S., Rodrigues, N., Cruz, R., Veloso, A. C. A., Pereira, J. A., & Peres, A. M. (2022). Impact of incorporating olive leaves during the industrial extraction of *cv*. Arbequina oils on the physicochemical–sensory quality and health claim fulfillment. *European Food Research and Technology, 248*, 171–183. https://doi.org/10.1007/s00217-021-03870-3

Marx, Í. M. G., Rodrigues, N., Veloso, A. C. A., Casal, S., Pereira, J. A., & Peres, A. M. (2021b). Volatile-olfactory profiles of *cv*. Arbequina olive oils extracted without/with olive leaves addition and their discrimination using an electronic nose. *Journal of Chemistry*. , Article 5058522. https://doi.org/10.1155/2021/5058522

Marx, Í. M. G., Rodrigues, N., Veloso, A. C. A., Casal, S., Pereira, J. A., & Peres, A. M. (2021a). Effect of malaxation temperature on the physicochemical and sensory quality of *cv*. Cobrançosa olive oil and its evaluation using an electronic tongue. *LWT - Food Science and Technology, 137*, Article 110426. https://doi.org/10.1016/j.lwt.2020.110426

Miho, H., Moral, J., López-González, M. A., Díez, C. M., & Priego-Capote, F. (2020). The phenolic profile of virgin olive oil is influenced by malaxation conditions and determines the oxidative stability. *Food Chemistry, 314*, Article 126183. https://doi.org/10.1016/j.foodchem.2020.126183

Moral, R., & Escrich, E. (2022). Influence of Olive Oil and Its Components on Breast Cancer : Molecular Mechanisms. *Molecules, 27*(2), 477. https://doi.org/10.3390/molecules27020477

Novoselić, A., Klisović, D., Lukić, M., Horvat, I., Lukić, I., & Brkić Bubola, K. (2021). Influence of small amount of water addition in the extraction process on the olive oil yield and phenolic compounds. *Agriculturae Conspectus Scientificus, 86*(3), 251–257.

Polari, J. J., Garcí-Aguirre, D., Olmo-García, L., Carrasco-Pancorbo, A., & Wang, S. C. (2018). Impact of industrial hammer mill rotor speed on extraction efficiency and quality of extra virgin olive oil. *Food Chemistry, 242*, 362–368. https://doi.org/10.1016/j.foodchem.2017.09.003

Rodrigues, N., Marx, Í. M. G., Casal, S., Dias, L. G., Veloso, A. C. A., Pereira, J. A., & Peres, A. M. (2019). Application of an electronic tongue as a single-run tool for olive oils' physicochemical and sensory simultaneous assessment. *Talanta, 197*, 363–373. https://doi.org/10.1016/j.talanta.2019.01.055

Taticchi, A., Esposto, S., Veneziani, G., Minnocci, A., Urbani, S., Selvaggini, R., Sordini, B., Daidone, L., Sebastiani, L., & Servili, M. (2021). High vacuum-assisted extraction affects virgin olive oil quality: Impact on phenolic and volatile compounds. *Food Chemistry, 342*, Article 128369. https://doi.org/10.1016/j.foodchem.2020.128369

Taticchi, A., Selvaggini, R., Esposto, S., Sordini, B., Veneziani, G., & Servili, M. (2019). Physicochemical characterization of virgin olive oil obtained using an ultrasound-assisted extraction at an industrial scale: Influence of olive maturity index and malaxation time. *Food Chemistry, 289*, 7–15. https://doi.org/10.1016/j.foodchem.2019.03.041

Uceda, M., & Frias, L. (1975). Harvest dates. Evolution of the fruit of content, oil composition and oil quality. In Proceedings of II Seminario Oleıcola Internacional; International Olive-oil Council: Cordoba, Spain, pp 125-130.

Velasco, J., & Dobarganes, C. (2002). Oxidative stability of virgin olive oil. *European Journal of Lipid Science and Technology, 104*, 661–676. https://doi.org/10.1002/1438-9312(200210)104:9/10<661::AID-EJLT661>3.0.CO;2-D

Wu, X., Miyake, K., Tahara, Y., Fujimoto, H., Iwai, K., Narita, Y., Hanzawa, T., Kobayashi, T., Kakiuchi, M., Ariki, S., Fukunaga, T., Ikezaki, H., & Toko, K. (2020). Quantification of bitterness of coffee in the presence of high-potency sweeteners using taste sensors. *Sensors and Actuators, B: Chemical, 309*, Article 127784. https://doi.org/10.1016/j.snb.2020.127784