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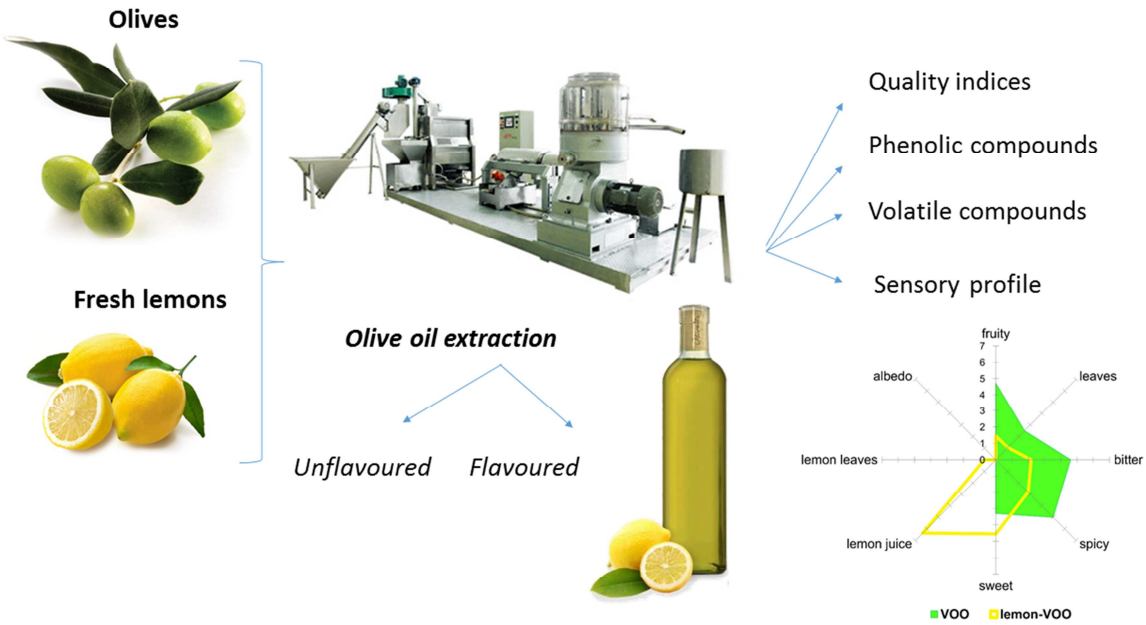

Characterisation of lemon-flavoured olive oils

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ABSTRACT

Fresh lemons (*Citrus limon* L.) from two locations in Italy were added to fresh olives before milling them to produce flavoured olive oils (FOO). FOOs were characterised for their quality parameters, fatty acid composition, biophenol content, volatile compound composition and sensory profile. Sensory results showed that lemon volatiles would mask negative (rancid) notes of olive oil obtained from olives with slight off-flavours by adding strong notes of lemon leaf, albedo and lemon juice, while they decreased the positive notes of olive fruit, green, leaf and bitter-pungent from good quality olives. Flavouring OO with lemon significantly affected quality indices, with a significant increase of the acidity value and UV indices. OO flavouring with fresh lemon also affected the phenolic compounds, particularly the simplest forms, hydroxytyrosol and tyrosol. A dramatic decrease of the concentration of aldehydic and dialdehydic forms of oleuropein was also observed in FOO. As expected, volatile profile of virgin OO dramatically changed due to lemons addition, which caused the presence of several terpene compounds, namely limonene, α-pinene, β-pinene, sabinene, β-mirceneand γ-terpinene. In contrast, positive notes of VOO decreased with lemon addition. In conclusion, addition of lemon to VOO should be carried out by considering these results, as well as the legislation for this kind of product.

Keywords: Aromatised olive oil; Lemon; Volatile compounds; Phenolic compounds.

1. Introduction

Olive oil is one of the most appreciated fat worldwide. Among “olive oil family”, the top commercial category is represented by extra virgin olive oil (EVOO), which is extracted from fresh olive fruits by only physical processing, with no addition of any chemical or any further refining process. VOO is one of the most appreciated products of the Mediterranean diet, and many positive nutritional properties have been associated to its consumption (Servili *et al*., 2009). Its popularity is linked both to its pleasant flavour, due to its volatile compounds and healthy properties, attributed to the phenolic compounds also responsible for bitterness and pungency (Angerosa *et al*., 2004; Vitaglione *et al*., 2015). The notable positive properties of olive oil on human health has been mainly attributed to its fatty acid composition, particularly its high content in oleic acid, and to its phenolic compounds, which have been proved to exert protective roles on humans (Servili *et al*., 2009; Frankel, 2011). There has been increasing interest on olive oil in the past few years as an ingredient to flavour gourmet food and as a healthier alternative to other forms of fats and oils (Vossen, 2007).

Spices and aromatic herbs are frequently added to VOOs to produce "flavoured oil", also called "aromatised oil" or "gourmet oil", in order to improve its health properties and/or sensory characteristics (Antoun & Tsimidou, 1997). The aroma composition of flavoured oil depends on the food ingredients used, as they are commonly added for the production of new aromas and sensory notes. For example, some papers reported the aromatisation of olive oil by using basil (Veillet, Tomao & Chemat, 2010), chilli pepper (Caporaso, Nicoletti, Paduano & Sacchi, 2013; Baiano, Terracone, Gambacorta & La Notte, 2009), essential oils from *Laminaceae* family (Tsimidou & Boskou, 1994), and many other ingredients such as vegetables (garlic, onion, pepper, chilli, sun dried tomatoes), herbs (rosemary, oregano, basil, sage, thyme, fennel, juniper, estragon), spices (clove, nutmeg, ginger), mushrooms, fruits (lemon, orange, mandarin, apple, banana) and nuts (almond, hazelnut, pine nuts) (Baiano *et al*., 2009; Sousa *et al*., 2015; Gambacorta *et al*., 2007).

Spices are also used for their antioxidant and antimicrobial properties (Yanishlieva, Marinova, & Pokorný, 2006). They are considered beneficial in the prevention of many human diseases such as breast, colon, and lung cancer (Kaefer & Milner, 2008). Moreover, the main reason of using flavoured oils is to confer new and more appreciated characteristics to the product and to have a ready to use dressing. For example, a flavoured olive oil obtained with fruity notes like citrus ones, gives a complex mixtures of substances, usually terpenes, sesquiterpenes and oxygenated derivatives, with general floral notes (Lucchesi, Chemat, & Smadja, 2004).

The characterisation of lemon volatile compounds has been previously reported in the literature, also discriminating between the peel and leaf of the lemon (Ayedoun, Sossou, Mardarowicz, & Leclercq, 1996; Sawamura *et al*., 1999), as well as the essential oils extracted from the fruit (Vekiari *et al*., 2002; Huang & Pu, 2000). The most abundant aroma compound in lemon is limonene, while other constituents have been reported at high concentrations in olive leaf oil, *i.e.* β-pinene, myrcene, neral, geranial, neryl acetate, geranyl acetate and β-caryophyllene. The essential oil of lemon peel mainly contains limonene, with also the presence of γ-terpinene, β-pinene, myrcene, neral, and geranial in the peel (Vekiari *et al*., 2002).

The flavouring of VOO causes significant changes in the quality indices, phenolic composition (Baiano *et al*., 2009; Gambacorta *et al*., 2007) and in volatile profiles. In the case of some spices, it has been reported that flavoured olive oil has higher antioxidant activity, *e.g.* olive oil flavoured with dried chili pepper (Baiano *et al*., 2009; Caporaso *et al*., 2013). Also, the free acidity of flavoured oils is affected by the presence of flavouring agents, as acidity value strongly dependent upon the food material used for flavouring and aromatisation process adopted (Paduano, Caporaso, Sacchi, & Santini, 2014), as well as the infusion time. The effect of storage time on the chemical composition of flavoured oils was previously studied by Baiano *et al*. (2009), where a noticeable decrease in phenolic content was observed in oils flavoured with garlic, lemon, oregano, hot pepper and rosemary compared with unflavoured oils. A contemporary increase of simple phenolics forms, namely tyrosol and hydroxytyrosol, was reported for all flavoured oils. Lemon-flavoured oils had

an intermediate antioxidant activity in comparison to the other flavoured oils (Baiano *et al*., 2009). Spices and herbs, after the infusion in VOO, commonly cause an increase of VOO biophenolic concentration, due to the release of some new phenolic compounds. Higher levels of phenolic compounds and tocopherols, as well as the presence of new flavonoids, have been reported for olive oils flavoured with oregano and rosemary (Damechki, Sotiropoulou, & Tsimidou 2001). Flavoured oil can be obtained in several forms: as whole spices, ground spices, essential oils or as oleoresins, or as prepared and filtered oil/vinegar infusions (Peter, 2001). In this latter case, there is a risk of physical instability of the product as it is an oil-in-water (W/O) dispersion. This aspect was investigated by researches focusing on the stability of dressings made by O/W emulsions formulated with olive oil and lemon juice (Paraskevopoulou, Boskou,& Paraskevopoulou, 2007). An alternative practice to infusion, in the Mediterranean area, is the addition of flavouring ingredients directly in the olive mill to immediately obtain a flavoured product, in particular using fresh lemons.

Therefore, the aim of the present work was to produce and characterise FOOs obtained by the direct addition of fresh lemons directly in the olive mill together with fresh olives for their extraction. The specific aim was to characterise FOOs obtained using fresh lemons, in comparison to unflavoured ones, through the analysis of their physico-chemical properties, and to verify whether the mixture of olive and lemon paste had significant effects on parameters such as free acidity, peroxide value and phenolic composition, beyond the merely addition of aroma compounds, which were also measured both through the analysis of volatile compounds and sensory analysis.

2. Materials and methods

2.1 Chemicals and olive oil samples

All reagents were of pure analytical grade methanol (>99.9% purity), acetonitrile (>99.9%), hexane (>95%), trifluoroacetic acid, fatty acids methyl esters mix, potassium hydroxide, Folin-Ciocalteu

reagent, sodium carbonate anhydrous (>99.5%). Authentic reference chemical compounds were obtained from Sigma-Aldrich (Steinheim, Germany) and Fluka (Buchs, Switzerland).

Samples of flavoured and unflavoured virgin olive oil were produced in two olive oil mills located in Roscigno (SA, Italy) (sample A) and Corigliano Calabro (CS, Italy) (sample B). Two olive batch used were mixture of local varieties (sample A: mix of 'Carpellese'/'Rotondella'; sample B: mix of 'Dolce di Rossano' and 'Tondina') and characterized by different ripening degree (sample A was composed by ripe black olives with a Jaen index of 5.1; sample B was composed by green unripe olives with a Jaen index of 2.6). After washing the olives, fresh lemons ("Feminello Siracusano" and "Comune di Rocca" varieties, for Sample A and B, respectively) were added at a concentration of 0.35 kg/kg olives. Crushing was made in both cases by using a stone mill, malaxation was carried out at 32-34 °C for 30-40 minutes in open malaxers, and oils were separated by centrifugal three-phase decanters in both mills, using low volumes of process water (0.1 kg/kg processed olives). The concentration of lemons added to the olives is very high as we aimed to reproduce the current way to make such a product. In fact, olive oil millers use a high ratio of lemons and small batch of olives, to produce a concentrated batch of FOO to be further diluted at the desired concentration.

2.2 Sensory analysis

VOO and FOOs were characterized by sensory analysis performed using quantitative descriptive analysis according to the EC Reg. 2568/91. The panel was composed by twelve assessors (age: 21-42), trained in the sensory assessment of virgin olive oils at the Department of Agricultural Sciences of the University of Naples Federico II (Portici, NA, Italy). In the case of FOOs, new sensory descriptors were added, obtained from preliminary tests during the panel training ('lemon juice', 'albedo', 'lemon leaf').

2.3 Legal quality parameters

Olive oil acidity (g oleic acid per 100 g olive oil), peroxide value (meq O_2/kg oil) and UV indices (K_{232}, K_{270} and ΔK) were measured according to the EC Reg. 2568/1991 standard method. Spectrophotometric parameters (K_{232}, K_{232} and ΔK) were obtained by using a Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan).

2.4 Fatty acid composition

The analysis of fatty acids methyl esters (FAMEs) was performed as per Sacchi, Caporaso, Paduano and Genovese (2015), using a Shimadzu GC-17A gas chromatograph (Shimadzu, Kyoto, Japan). The analyses were performed in triplicate.

2.5 Phenolic compounds

Phenolic composition of flavoured and unflavoured OOs was determined according to Sacchi *et al*., (2015). The quantification of individual phenolic compounds was carried out by HPLC-UV analysis of the hydro-alcoholic extracts while the total phenolic content was measured by using the Folin-Ciocalteau method. The analyses were performed in triplicate.

2.6 Volatile compounds

Volatile compounds were analysed by Dynamic Headspace (DHS) technique, using a Purge and Trap system (Tekmar Instruments, Manchester, UK). Three mL sample was submitted to a pre-purge step for 2 min, sample pre-heat for 3 min (at 40 °C), and purge last for 20 min, using Helium as stripping gas with a flow of 48 mL/min, and therefore sampled on a Tenax TA trap (Chrompack, Middleburg, The Netherlands), held at 33 °C. Dry-purge was carried out for 5 min, and Tenax trap was heated at 220 °C for the release of trapped volatile compounds, which were subsequently carried by a flow of Helium at 18 mL/min. Condensation was carried out at a temperature of -110 °C by using liquid Nitrogen. The cold trap was brought to 190 °C for 1 min and volatile compounds are finally injected in the GC column. A Shimadzu mod. GC-17A (Shimadzu, Milan, Italy)

equipped with a Flame Ionization Detector (FID) was used with a SupelcoWAX 10 fused silica gel column (60 m x 0.32 mm i.d., 0.50 μm film thickness of polyethylene glycol) (Supelco, Bellefonte, USA). Helium was used as carrier gas at a flow of 1.4 mL/min. Oven temperature was set as follows: isotherm at 40 °C for 4 min, then an increase at a rate of 3.5 K/min up to 240 °C, hold for 3 min. Injector temperature was 190 °C and FID temperature was 250 °C. Data were acquired by using a Class-VP Chromatography Data System vers. 4.6 (Shimadzu, Milan, Italy).The compound identification was confirmed using pure standards and comparing their retention indices and mass spectra. When reference compounds were not available, a tentative identification was given using the NIST database and comparing the data with those obtained by a Static Headspace (SHS) Butterfly mod. HT200H (Alfatech, Genova, Italy), coupled with a GC/MS-QP5000 (Shimadzu Corporation, Kyoto, Japan). Source temperature was set at 190 °C, and interface temperature was held at 240 °C. Scanning ratio ranged between 30 and 400 amu and scansion time was 0.2 sec. Results were expressed as a percentage of total peak area.

2.7 Statistical analysis

All the analytical determinations were carried out at least in triplicate. Statistical analysis was performed by using XLStat 2006 Version 6.6 software (Addinsoft, Paris, France). Differences were considered significant at $p<0.05$.

3. Results and discussion

A full characterisation of the lemon flavoured and unflavoured OO samples was carried out to understand the possible changes in several parameters, including the rate of secoiridoid aglycons hydrolysis, triacylglycerols hydrolysis, oxidation rate and the changes in the main volatile compounds.

3.1 Sensory profiles, quality indices and fatty acid composition

Sensory profiles of both FOOs obtained with fresh lemon are reported in **Fig. 1**. The results indicated a lowering of the "olive fruity" attribute in FOOs with a contemporary appearance of new notes, namely "lemon juice", "lemon leaf" and "albedo", with a slight but significant increase in the "bitter" and "pungent" notes, for samples A. In the case of sample B the intensity of the "sweet" note increased, while the "bitter" and "pungent" notes decreased. It has been reported that the release of terpene compounds from lemon stimulate taste receptors for the bitter and pungent sensory notes (Cometto-Muñiz, Cain, Abraham, & Kumarsingh, 1998). In sample A, which was characterised by negative organoleptic descriptors, namely 'fusty/muddy sediment', 'musty/humid' and 'rancid', the addition of lemons did not allow the sensory discrimination or recognition of these attributes in the resulting flavoured sample. On the contrary, in sample B, which had higher intensities of olive fruity, green, bitter and pungent, lemon flavouring caused a lowering of all these positive attributes, probably due to a masking effect of the new attribute "lemon". Interestingly, also the attribute "sweet" significantly increased compared to unflavoured sample, in case of sample B, while it was lower for sample A. This difference could be due to the initial intensity of bitter, pungent and sweet attributes. This finding also indicates the presence of a masking effect of lemon volatiles on olives with slight off-flavours, when the aromatisation is performed with fresh lemons directly milled with the olives. In fact, overripe olives were used in this case and they are known to be naturally prone to develop slight 'rancid' and 'fusty' sensory defects. The acidity of the lemon juice, in addition, is able to modify olive enzyme activity (lipoxygenase, β-glycosidase, lipase, esterase, etc.) and the partitioning equilibrium of biophenolic and volatile compounds between the oil and water/pomace phases. The sensory profiles of lemon-aromatised oils are not the simple sum of lemon flavour notes to that of VOO but the effect of complex biochemical and physicochemical interactions between lemons and olives during milling, malaxation and centrifugation.

Table 1 shows the main quality indices according to current legislation for olive oil. In both OOs considered, the acidity level increased in FOO compared to unflavoured VOO. The higher level of acidity might be due to the presence of organic acids in lemons, particularly citric acid which

caused a more acidic environment during malaxation with possible consequent increase of hydrolysis of triglycerides and therefore higher free acidity values.

Peroxide value (PV) was lower in flavoured samples with respect to unflavoured ones only in sample B. This decrease in PV could be related to the acidification during the malaxation phase caused by the very low pH of the lemons. Spectrophotometric indices K_{232} and K_{270}, resulted higher in FOO, while ΔK did not change significantly. A possible contribution to the higher level of K_{232} value might be due to the presence of terpenes from lemons which, due to their chemical structure, could influence the absorbance at 232 nm of hydroperoxydienes (Walker & Hawkins, 1952). In fact, some terpenes absorb at 232nm, e.g. citral and β-mircene, whose concentration in lemon oil has been reported to be up to 4.4 and 1.9%, respectively (Lota *et al*., 2002). Our results indicate that when the initial indices of the virgin olive oil are relatively high, the obtained flavoured oil would probably be above the legal limit for VOOs (Gambacorta *et al*., 2007).

As reported in **Table 2**, fatty acid composition showed little changes due to the addition of fresh lemons. Significant differences between flavoured and unflavoured VOOs were found for some fatty acids, specifically palmitic, stearic, oleic and (E)-octadec-11-enoic acid. Stearic and palmitic acids always resulted in increased concentrations in flavoured VOOs, while oleic acid had a limited increase. Differences in fatty acid composition between the two samples could be attributed to the different olive batch used, with different varietal and geographical origins of the olives, as the genetic origin of the olives is known to be critical for olive oil fatty acid composition (Lanza, Russo & Tommaselli, 1998). Also, further studies are needed to understand whether this effect is due to the changes in the lipase activity due to the additional water added by the lemons and the consequent influence on the pH.

3.2. Phenolic compounds

As reported in **Table 3**, the phenolic composition of olive oil samples was strongly influenced by lemon addition. The dialdehydic form of the ligstroside aglycon (p-HPEA-EDA) was generally the

most abundant compound, followed by the dialdehydic form of the oleuropein aglycon (3,4-DHPEA-EDA) and oleuropein aglycon (3,4-DHPEA-EA). The concentration of total phenolic compounds was significantly lower in FOOs. These results could be explained both by the inhibition of β-glycosidase activity due to the acidity of lemon juice and by the hydrolysis/partition phenomena toward lipid and water phases of biophenols during the malaxation and centrifugation steps. The addition of lemons, in fact, represents about 0.25-0.30 kg/kg total olive paste during malaxation, and therefore there is a consequent partition of phenolic compounds in a relatively higher volume of water. The more acidic environment caused by the acids released from fresh lemons could also promote hydrolysis of secoiridoid aglycons with a consequent production of simple phenyl alcohols, such as tyrosol and hydroxytyrosol, which have higher affinity for water and are therefore more likely lost in the olive mill wastewater (Sacchi *et al*, 2002; Balasundram, Sundram, & Samman, 2006).

3.3. Volatile compounds

Table 4 shows the volatile compounds identified in flavoured and unflavoured virgin olive oil. The volatile profile of FOOs headspace was strongly affected by lemon addition, which resulted in the appearance of terpene compounds (α-pinene, β-pinene, sabinene, β-myrcene, limonene and γ-terpinene). This result was expected, as the main reason of producing lemon-FOO is to give new aromatic notes to VOO. The most abundant terpene compound was limonene, followed by β-pinene in both flavoured oils. Limonene and β-pinene represent more than 50% and 20%, respectively, of the total terpene compounds measured in FOOs. The main volatile compound in VOO samples was *trans*-2-hexenal, arising from the lipoxygenase (LOX) pathway and known to be quantitatively the most abundant one in EVOOs (Angerosa *et al*., 2004). In both samples, the following abundant volatile compound was hexanal. This compound is linked to lipid oxidation and to 'rancid' off-flavours in vegetable oils at high concentrations (Morales, Luna, & Aparicio, 2005) but it is also found at very low concentrations in good EVOOs, being synthetized in the LOX pathway

depending on ripening and olive variety (Angerosa *et al*., 2004). Other volatile compounds related to 'rancid' and 'fusty' defect were found in higher amount in sample A (**Table 4**), particularly pentanal and 3-methyl-1-butanol (Morales *et al*., 2005).

Volatile compounds released from lemons in the oil samples showed differences between the two samples analysed, with a greater relative concentration of terpene compounds in sample B (**Table 4**). This finding was attributed to the different lemons used in two experiments, as it was previously reported that volatile composition of lemon can undergo dramatic changes depending on the lemon varieties (Allegrone, Belliardo, & Cabella, 2006). A general decrease was observed in FOOs for other volatile compounds produced from the LOX pathway, with higher concentrations in sample B, due to the higher relative abundance of terpene compounds. The presence of lemon caused strong changes in the volatile pattern of olive oils, not only with the addition of terpene compounds but also through the influence of lemon juice on the LOX pathway, which is also likely to be influenced by the very low pH of the lemon juice, while the pH value of lemon paste is circa 5.5. Indeed, it was reported that some terpene compounds such as limonene and γ-terpinene have strong LOX inhibition activity (Baylag and Racine, 2003).

In general, lemon addition results in an increase of limonene concentration higher than trans-2-hexanal, which is the most abundant VOO volatile compound. Our results are in agreement with literature on lemon volatile composition, being mainly represented by mono- and sesquiterpene hydrocarbons and oxygenated molecules (aldehydes, monoterpene alcohols, and monoterpene esters) (Allegrone *et al*., 2006). The fact that limonene was by far the most abundant compound in FOOs is in agreement with previous studies on lemon composition (Lota, de Rocca Serra, Tomi, Jacquemond, & Casanova, 2002; Allegrone *et al*., 2006). The chemical variability of lemon species has been scarcely reported in the literature, especially in terms of volatile profile. Lota *et al*. (2002) reported on the volatile composition of 9 species of lemon peel oil, and they attributed some of the observed difference to environmental factors, which suggests the difficulty of evaluating all possible conditions for the standardisation in terms of food industry needs, for lemon-aromatised

306 olive oils.

307 Our results confirmed the complexity of VOO direct aromatisation using fresh lemon in the olive
308 oil mill, as the final results in terms of volatile compounds is strongly linked to the variety and
309 ripening degree, as well as the environmental growing conditions of both lemons and olives. These
310 parameters should be carefully assessed to obtain the desired final concentration of lemon notes
311 without drowning out the typical aroma of VOO. Moreover, it is important to stress that this
312 product is not the final product sold to the consumer but it is generally diluted with unflavoured
313 olive oil at a desired concentration to avoid excessive lemon notes and to be able to standardise the
314 intensity of some sensory notes. In this way, it is possible to partially compensate the high
315 variability due to the combination of olive and lemon fruits, as their chemical composition and
316 aroma development strongly depends on their variety, several agronomical factors and other
317 environmental factors such as the maturity stage.
318 Further studies are in progress on typical productions in the area of Sorrento (Italy) combining the
319 use of IGP “Limone di Sorrento” lemons and PDO “Penisola sorrentina” EVOOs.
320

321 **4. Conclusions**

322 The present paper reported on the complete characterisation of VOOs processed with the addition of
323 fresh lemons directly in the olive mill to the olive fruits, to produce lemon-flavoured oils. Despite
324 the industrial interest toward flavoured olive oils with methods involving the direct use of the fresh
325 products in the mill, this is one of the first papers with the aim to specifically understand the effect
326 of directly adding fresh lemons in the olive batches on the physico-chemical and sensory attributes
327 of lemon flavoured olive oil. Our results indicated that the potential sensory defects of VOO
328 obtained from low quality olives would be masked by the addition of fresh lemons to produce
329 lemon-flavoured OO, where strong notes of lemon leaf, albedo and lemon juice when using fresh
330 lemons cover the typical olive notes. In the case of a good-quality VOO the positive notes of olive
331 fruit, green, leaf and bitter-pungent decrease, as they are “covered” by the lemon flavour, while they

still contribute to the final sensory profile of the aromatised oil.

It was highlighted that the addition of fresh lemons in the olive mill causes a dramatic influence on quality parameters, with an important loss of phenolic compounds. Due to their role as antioxidant, their influence on the shelf life of the product and their healthy positive effects, this factor should be carefully taken into consideration in the production of such a flavoured olive oil.

Further research can be carried out to check whether better quality is obtained when removing the lemon juice before processing the raw materials, thus adding just the lemon peel in the olive mill. Moreover, further studies are in progress to compare the flavoured oils obtained by using the technique here reported with respect those obtained by infusion/maceration techniques, also in combination to ultrasonic treatments, as recently reported for the production of chilli pepper-flavoured oils (Caporaso *et al*., 2013; Paduano *et al*., 2014).

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448 ***Figure caption.***

449

450 **Fig. 1**. Sensory profile of two flavoured olive oils (lemon-OO) produced using fresh lemons added
451 in olive mills in comparison to unflavoured ones (OO). Area within black line: unflavoured OO;
452 area within grey area: flavoured OO.
453 *All sensory descriptors were statistically different (p<0.05), except for 'sweet' intensity in sample
454 A.

455

456

Table 1. Quality indices of two samples of unflavoured and flavoured olive oil produced by fresh lemons added to olives in the olive mill.

Indices	**Sample A**				**Sample B**			
	Unflavoured		Flavoured		Unflavoured		Flavoured	
Acidity	0.80±0.33	b	1.50±0.16	a	0.50±0.03	b	1.00±0.14	a
PV	7.27±1.77	a	6.44±0.23	a	10.07±0.35	a	6.36±0.90	b
K_{232}	1.821±0.03	b	2.114±0.01	a	2.081±0.11	b	2.416±0.14	a
K_{270}	0.132±0.02	a	0.183±0.01	a	0.159±0.01	b	0.235±0.01	a
ΔK	0.002±0.01	a	0.003±0.01	a	-0.005±0.01	a	0.000±0.01	a

Values are the average of three replicates (n=3). Acidity was expressed as percentage (%) of oleic acid; PV (peroxide value) was expressed as meq O_2 per kg oil. Different letters on the same sample indicate significant different values (p<0.05).

Table 4. Volatile profile of two samples of unflavoured and flavoured olive oil produced by fresh lemons added to olives in the olive mill, analysed by Dynamic Headspace (DHS) and gas-chromatography/mass spectrometry (GC/MS).

Volatile compound	I.M.*	Sample A						Sample B					
		Unflavoured		SD	Flavoured		SD	Unflavoured		SD	Flavoured		SD
Octane	RC, MS	4.61	±	0.12a	2.19	±	0.20a	5.16	±	0.63b	0.64	±	0.07a
Ethanol	RC, MS	7.57	±	0.85a	3.24	±	0.30a	4.33	±	0.12a	0.35	±	0.03a
Pentanal	RC, MS	2.36	±	0.07a	1.09	±	0.22a	1.47	±	0.09b	0.29	±	0.02a
3-Pentanone	RC, MS	2.50	±	0.03a	0.93	±	0.06b	8.21	±	0.04a	0.68	±	0.08a
α-Pinene	MS		nd		4.11	±	1.67		nd		10.23	±	0.32
Hexanal	RC, MS	23.00	±	0.67a	10.76	±	0.53b	12.36	±	3.37b	1.64	±	0.14a
β-Pinene	RC, MS		nd		13.68	±	3.60		nd		19.94	±	0.13
Sabinene	MS		nd		2.28	±	0.65		nd		6.04	±	0.32
1-Penten-3-ol	RC, MS	2.28	±	0.05a	1.02	±	0.08b	5.11	±	0.29b	0.53	±	0.00a
β-Mircene	MS		nd		1.62	±	0.43		nd		4.59	±	0.17
3-Methyl-1-butanol	RC, MS	9.18	±	0.12a	4.20	±	0.28b	2.53	±	0.28a	0.23	±	0.01a
Limonene	RC, MS		nd		29.25	±	4.57		nd		38.77	±	0.02
t*rans*-2-Hexenal	RC, MS	41.60	±	0.86a	19.29	±	1.96b	56.21	±	13.10b	6.98	±	0.38a
γ-Terpinene	RC, MS	0.61	±	0.01b	3.17	±	0.66a		nd		8.20	±	0.45
cis-2-penten-1-ol	RC, MS	0.68	±	0.01a	0.33	±	0.02a	1.18	±	0.26b	0.15	±	0.01a
1-Hexanol	RC, MS	1.83	±	0.01a	0.93	±	0.01b	1.02	±	0.32b	0.19	±	0.02a
cis-3-Hexen-1-ol	RC, MS	0.42	±	0.01a	0.21	±	0.02a	0.76	±	0.25b	0.14	±	0.01a
trans-2-Hexen-1-ol	RC, MS	3.35	±	0.09a	1.71	±	0.19a	1.65	±	0.47b	0.41	±	0.05a

All values are shown as means ± standard deviation (n = 3) and are expressed as percentage of the total volatile composition. *I.M. = identification method: RC, pure reference compounds; MS, mass spectra and comparison with NIST libraries. nd = not detected. Different letters on the same sample indicate significant different values (p<0.05).

Table 2. Fatty acid composition of two samples of unflavoured and flavoured olive oil produced by fresh lemons added to olives in olive mills.

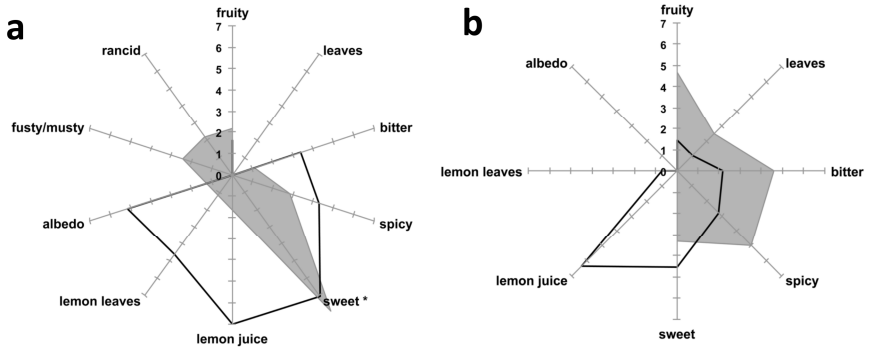
Fatty acids		**Sample A**				**Sample B**			
		Unflavoured		Flavoured		Unflavoured		Flavoured	
C 16:0	Palmitic acid	9.93±0.04	a	9.46±0.26	b	13.38±0.24	a	11.78±0.19	b
C 16:1w9	Palmitoleic acid	0.34±0.00	a	0.27±0.01	a	1.16±0.07	a	1.37±0.05	a
C 17:0	Heptadecanoic acid	0.04±0.00	a	0.04±0.00	a	0.11±0.01	a	0.12±0.03	a
C 17:1	Heptadecanoic acid	0.06±0.01	a	0.06±0.01	a	0.22±0.03	b	0.32±0.01	a
C 18:0	Stearic acid	1.95±0.04	b	2.26±0.04	a	2.24±0.09	a	2.18±0.00	b
C 18:1w9	Oleic acid	75.76±0.25	b	76.72±0.40	a	72.25±0.21	b	72.86±0.08	a
C 18:1w7	Vaccenic acid	1.41±0.02	a	1.14±0.07	b	3.00±0.14	b	3.21±0.05	a
C 18:2	Linoleic acid	8.24±0.06	a	8.00±0.00	b	6.03±0.07	a	6.04±0.02	a
C 18:3	Linolenic acid	0.34±0.00	a	0.36±0.04	a	0.38±0.04	a	0.37±0.00	a
C 20:0	Arachidic acid	0.66±0.01	a	0.60±0.09	a	0.65±0.00	a	0.56±0.01	a
C 20:1	Eicosenoic acid	0.35±0.03	a	0.36±0.01	a	0.22±0.03	a	0.25±0.02	a
C 22:0	Behenic acid	0.10±0.00	a	0.11±0.00	a	0.10±0.01	a	0.12±0.00	a
C 24:0	Lignoceric acid	0.55±0.13	a	0.47±0.01	a	0.05±0.00	a	0.06±0.01	a
C 30:6	Squalene	0.04±0.00	a	0.04±0.00	a	0.44±0.05	a	0.66±0.01	b

Values are the average of three replicates (n=3). Fatty acids were expressed as percentage expressed as % of total fatty acids.
Different letters on the same sample indicate significant different values (p<0.05).

Table 3.
Phenolic compounds of unflavoured and flavoured olive oils produced by fresh lemons added to olives in olive mills.

Phenolic compound	**Sample A**				**Sample B**			
	Unflavoured		Flavoured		Unflavoured		Flavoured	
Hydroxytyrosol	12.62±1.16	a	7.19±2.29	b	29.98±1.30	a	7.96±0.93	b
Tyrosol	22.66±0.99	a	17.66±3.10	b	8.34±1.18	a	2.50±0.71	b
3,4-DHPEA-EDA	26.68±1.71	a	15.11±4.10	b	102.71±1.40	a	7.20±0.99	b
p-HPEA-EDA	58.38±4.12	a	45.25±4.03	b	64.06±2.46	a	27.00±1.70	b
Lignans	30.62±2.60	a	20.94±1.68	b	16.81±1.54	a	7.94±0.62	b
3,4-DHPEA-EA	16.70±0.45	a	15.37±2.28	a	43.60±1.41	a	12.81±1.29	b
p-HPEA-EA	9.00±1.41	a	0.40±0.28	b	6.43±0.78	a	0.67±0.46	b
Total phenolics (Folin–Ciocalteau)	165.09±11.55	a	133.49±1.42	b	271.93±3.32	a	66.09±3.81	b

Phenolic compounds obtained by HPLC analysis were expressed as mg tyrosol/kg of oil; total phenolics obtained by Folin–Ciocalteau essay were expressed as mg caffeic acid/kg of oil. Values are the average of three replicates (n=3). Different letters on the same sample indicate significant different values (p<0.05). 3,4-DHPEA-EDA: dialdehydic form of elenoic acid linked to hydroxytyrosol; p-HPEA-EDA: dialdehydic form of elenoic acid linked to tyrosol; 3,4-DHPEA-EA: oleuropein aglyone; p-HPEA-EA: ligstroside aglyone; Lignans: sum of pinoresinol and acetoxypinoresinol.



Highlights

- Flavoured olive oil produced by direct addition of lemon in the mill was studied
- Changes in quality indices, phenolics, volatiles and sensory scores were found
- Addition of lemon caused a decrease in acidity and other quality parameters
- Olive oil biophenol concentration decreased significantly in flavoured oils
- Abundant lemon terpene levels covered the typical virgin olive oil sensory notes