

# Composition of fatty acids and volatile substances in the leaves of *Olea europaea* L.

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**Abstract.** The fatty-acid composition and some volatile components of essential oil extracted from the leaves of European olive (*Olea europaea*), growing in the conditions of a dry subtropical climate of the Mediterranean type of the Southern Coast of the Crimea, were determined. 11 fatty acids were found, including monounsaturated and polyunsaturated ones, the composition and ratio of which are similar to the lipid profile of oil from freshly harvested olive fruits. Among the fatty acids, the leading position is occupied by cis-9-oleic (35.07%), palmitic (32.88%) and stearic (10.21%) ones, a significant content of polyunsaturated (cis-9.12-linoleic (9.2%) and cis-12.15-linolenic (6.7%) acids, which determine the nutritional value of the leaves, was also revealed. Among the volatile components of the essential oil of the leaves, 4 aldehydes (pentanal, isobutanal, isopentanal, hexanal) and n-octane, which belongs to the class of alkanes, were determined. The major components of the essential oil are pentanal (valeric aldehyde) and isobutanal (isobutyraldehyde), the mass fraction of which is 60.5% and 22.1%, respectively, which determines the pharmacological value of the leaves.

## 1 Introduction

*Olea europaea* L. (European olive) is an evergreen tree of the Oleaceae family with a natural habitat in the Mediterranean.

Olive fruits are a rich source of various nutrients and beneficial compounds. They contain fatty oils, essential amino acids, vitamins, ascorbic acid, macro- and microelements, and dietary fiber. Additionally, they are packed with phytonutrients, including polyphenols, phytosterols, squalene, and triterpenes.

One of the notable compounds in olives is oleuropein, a phenolic compound that, along with its metabolite hydroxytyrosol, contributes to the fruit's antioxidant, antiatherogenic, anti-inflammatory, antiviral, and anticancer properties. However, these compounds also impart a bitter taste to the olives, making them unsuitable for fresh consumption.

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In terms of nutritional value, processed olive products are particularly valued for their content of monounsaturated fatty acids (MUFA), primarily oleic acid. They also contain polyunsaturated fatty acids (PUFA), such as linoleic acid (omega-6) and linolenic acid (omega-3), which are essential and therefore beneficial for health. Their biological importance stems from their role in the synthesis of eicosanoids, which are the precursors of prostaglandins and leukotrienes. These compounds are vital for several bodily functions, including the prevention of atherosclerosis, providing cardioprotective and antiarrhythmic effects, modulating inflammatory responses, and lowering cholesterol levels. [1].

By now, there is information about the content of significant amounts of biologically active substances, in particular phenols and fatty acids, also in olive leaves [2, 3, 4, 5, 6, 7, 8]. Thus, in the work of Brahmi et al. (2012) it was shown that the main components of the leaves were (E)-3-hexenol, 3-ethenylpyridine, (E)- $\beta$ -damascenone and phenylethyl alcohol, but their percentage varied depending on the processing of the leaves [9]. Studies have identified that the principal phenolic compounds contributing to the antioxidant properties of olive leaves are hydroxytyrosol, oleuropein, and tyrosol. Research conducted by Boss et al. (2016) highlights that olive leaves are significantly richer in oleuropein and other polyphenolic compounds compared to olive oil. Specifically, oleuropein content in the leaves ranges from 1% to 14%, whereas in olive oil it is only 0.005% to 0.12% [10].

Additionally, olive leaves are known to contain key secondary metabolites such as oleacein and oleuropein, which are associated with hypotensive effects [7, 11] and hypoglycemic properties [7, 12]. The presence of these biologically active compounds in olive leaves underpins their antioxidant, hypoglycemic, hypocholesterolemic, and cardioprotective effects, as well as their antiviral and antibacterial activities [4, 13, 14, 15, 16]. Moreover, extracts from olive leaves exhibit antiproliferative properties and hold promise for the treatment of malignant tumors, aiding in the maintenance of normal DNA repair mechanisms. [13, 14, 17, 18].

The variation of volatile substances of different *Olea europaea* cultivars grown in the regions of Algeria was 51 compounds in the leaves [19], 39 compounds were identified in Portugal ones, and 41 - in Italian ones, mainly volatile substances were represented by esters and alcohols, with a small percentage of aldehydes, ketones and terpene compounds, including sesquiterpenes [20, 21]. The article of Dursun et al (2017) evaluated the content of volatiles in the leaves collected from the olive cultivars 'Gemlik', 'Halhalı' and 'Sarı Hasebi' grown in Hatay province, Turkey. 97 of the 127 volatile compounds identified were found in all samples. Terpenes, aldehydes, alcohols and ketones were identified in olive leaves as the main groups of volatile substances, which accounted for about 36-60%, 20-28%, 6-14% and 4-8% of the total number of compounds detected in the leaves, respectively [22].

In Chinese tea made from olive leaves, a total of 105 volatile compounds have been identified. Among these, the predominant aromatic compounds include hexanal, benzaldehyde, and nonanal [13, 23].

Research into the volatile compound profile of olive leaf essential oils from Croatia has revealed significant diversity, both among different cultivars and across various harvest periods. A total of 45 distinct compounds have been identified. The primary components include aldehydes such as (E,Z)-2,4-heptadienal, (E,E)-2,4-heptadienal, and decanal; ketones like (E)- $\beta$ -damascone and dihydrodehydro- $\beta$ -ionone; sesquiterpenes such as cyclosativene,  $\alpha$ -copaene, and  $\alpha$ -muurolene; and saturated hydrocarbons including tetradecane and hexadecane [24]. In cultivars cultivated in Tunisia, 32 compounds were identified that make up 99.44% of the oil, where the main components were  $\alpha$ -pinene (52.70%), 2,6-dimethyloctane (16.57%) and 2-methoxy-3-isopropylpyrazine (6.01%) [25]. In essential oils of fresh and dried leaves of *Olea europaea* 25 and 47 components of cv.'Chemlali' were identified, respectively. The essential oils of fresh leaves were

dominated by  $\alpha$ -farnesene (17.85%) and  $\beta$ -maalien (17.16%), whereas n-hexadecanoic acid (7.44%) was the main component of essential oils of leaves dried in the shade [26]. In the study conducted by Vural and Akay (2021), the chemical composition of essential oil derived from *Olea europaea* leaves collected in Ayvalik, Turkey, was analyzed. The research identified 42 distinct compounds, which together account for  $99.59 \pm 1.15\%$  of the essential oil's total composition. As a result of detailed analysis, the predominant components of the essential oil and their relative concentrations were identified. The main components were alpha-pinene ( $9.82 \pm 0.33\%$ ), benzyl alcohol ( $8.83 \pm 0.27\%$ ), phenethyl alcohol ( $8.52 \pm 0.25\%$ ), 2-monopalmitin ( $8.13 \pm 0.28\%$ ), palmitic acid ( $5.53 \pm 0.41\%$ ), octadecanoic acid. 2.3-dihydroxypropyl acid ester ( $5.84 \pm 0.42\%$ ), phytol ( $4.22 \pm 0.17\%$ ) and benzaldehyde ( $4.21 \pm 0.38\%$ ) [27].

In the leaves, fruits and oil of *Olea europaea* cv. 'Olivastra Seggianese' from Italy has identified 80 volatile substances. The main components were aldehydes, in particular (E)-2-hexenal (9.8-48.0%); however, many terpenoids were also identified, mainly (E, E)- $\alpha$ -farnesene (0.2-27.0%), linalool (0-3.6%),  $\beta$ -caryophyllene (0-8.1%) and valentine (0-2.5%) [28].

All this increases scientific interest in the identification of the physico-chemical composition of olive leaves and determines the relevance of research.

The Southern Coast of Crimea, characterized by a dry subtropical Mediterranean climate [29], represents the northern limit of the olive-growing cultural region. The Nikitsky Botanical Gardens – National Scientific Center RAS (NBG-NSC RAS) holds the most extensive collection of olive cultivars in Russia, comprising over 260 different cultivars and forms. [17].

Previously, we studied the component composition of the phenolic complex (polyphenols, anthocyanins) and photosynthetic pigments in the leaves of the olives of the cv. 'Nikitskaya' bred in the NBG-NSC, and revealed a significant content of polyphenols (averaged 538.94 mg/100 g dry weight), flavonoids (flavanones (197.1 mg/100 g), flavanols (299.7 mg/100 g) and anthocyanidins (mainly represented by pelargonidins (184.0 mg/100 g), which possess anti-carcinogenic activity and cyanidins (160.1 mg/100 g)) [30].

Taking into account all the above, for a more complete assessment of the properties of olive leaves, we set a goal to study the content and composition of fatty and essential oils in the leaves of the olive cv. 'Nikitskaya'.

## 2 Objects and methods of research

For the study, the leaves of the olives of cv. 'Nikitskaya', bred by the NBG-NSC, isolated during the study of the oil plantations of the SCC in 1950, which is frost-resistant and productive, with an oil yield of 36% (on the raw mass), were taken. The leaves of this cultivar are relatively small, 4.9 cm long and 1.1 cm wide, pointed-lanceolate, the greatest width is closer to the top, dark green from above, leathery, with an opposite arrangement [3]. The primary lipid biochemical profile of freshly harvested fruits of this cultivar was characterized by a high proportion of unsaturated and a low proportion of saturated fatty acids, which distinguished this cultivar from others in terms of the content of the most important biological substances for human health [1].

Sampling for research was carried out during the harvest of olive fruits (the second decade of October 2022). Plants are grown at the production sites of the NBG-NSC (Nikita village, Republic of the Crimea, Russia). The samples were collected manually. The leaves were dried at room temperature.

Laboratory analyses were performed on the basis of the Department of Plant Physiology and Biochemistry of the Federal Research Center "Subtropical Scientific center" RAS

(Sochi, Russia). The composition of volatile substances of the essential oil of the leaf apparatus of the studied plants was studied by the method of headspace analysis (HSA) [31, 32, 33]. In a penicillin bottle with a volume of 15 ml, a 2 g suspension was placed from a medium sample of the leaves of the plant crushed by slicing, 5 ml of distilled water was added. Next, laying with a polyethylene film, it was hermetically sealed with a rubber stopper, which was fixed with a special clamp. After that, the vial with the contents was placed in the socket of the DRP-10 equilibrium steam dispenser (manufactured by RPC “Meta-Chrome”, Russia, Yoshkar-Ola), which is a water bath (thermostat) with additional vibration effect. The sample preparation time is 5 minutes, the temperature is 80°C. At the end of the procedure, 1 ml of a vapor-gas mixture was taken from the vial by puncturing a rubber stopper with a syringe and injected into the injector of a gas chromatograph with a Focus SSL/DSQ II (Thermo Scientific) mass spectrometric detector. The mode of operation of the analytical complex was as follows:

- the temperature of the source is 200°C
- detection mode 30-600 Da (positive ion)
- 70 eV ionization mode
- capillary separation column TR-5MS 30 m – 0.25 mm ID – 0.25 microns
- programmed chromatography temperature mode: 70°C – 1 min, then heating at a speed of 30°C/min to 280°C.
- the temperature of the injector is 230°C.
- interface temperature (transfer-line) 260°C
- carrier gas helium, velocity 1.2 ml/min, discharge 12 ml/min
- flow division 12.

Identification was carried out by individual mass spectra based on the electronic spectral database NIST-2011. Chemical compounds were taken into account, the mass spectra of which coincided with the standard ones by more than 40%. The relative content of the essential oil components was determined by the internal normalization method [4, 34].

The fatty-acid composition of olive leaf extract obtained by extraction freezing under the influence of a centrifugal force field (EFC) [35] was determined after methylation of carboxylic acids according to [36]. The high efficiency of extraction of carboxylic acids using EFC was previously confirmed by a series of experimental studies [37, 38]. This method also allows us to significantly reduce the sample preparation time.

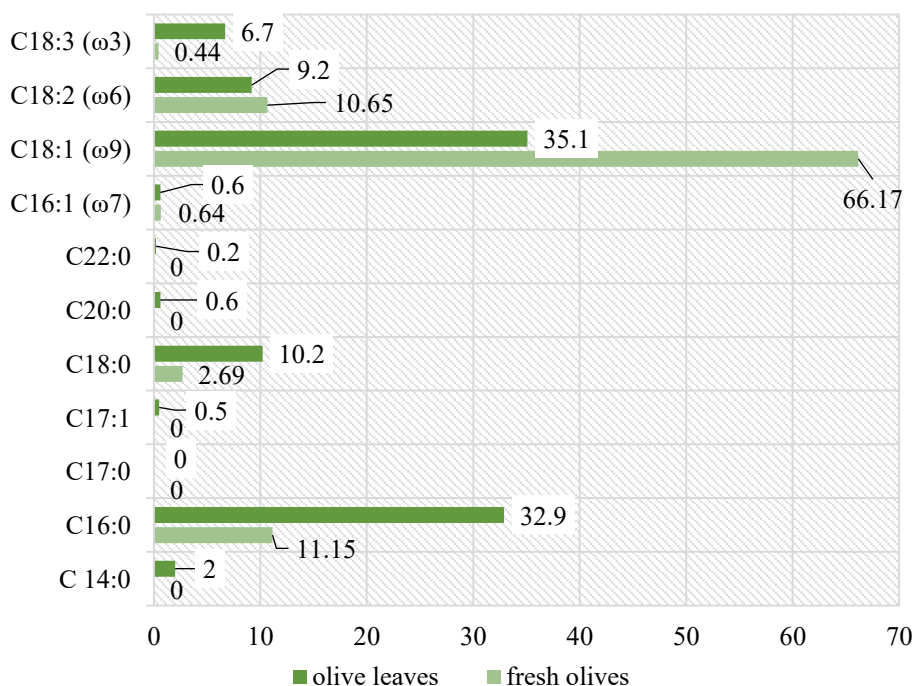
The fatty-acid composition of fatty oils was determined by the procedure for obtaining methyl esters of fatty acids according to GOST 31665 and their gas chromatographic separation according to GOST 30623 on a “Crystal 2000M” chromatograph. This method makes it possible to determine the ratios of individual carboxylic acids in fat mixtures [39]. Fatty acids were identified by comparing their retention time with the retention time of standard reference compounds. In our studies, the identification of the fatty-acid composition of the oil was carried out using the standard Food Industry FAME Mix 35077.

The statistical analysis of the research data was conducted using STATGRAPHICS Centurion XV for statistical computations and MS Excel for mathematical processing. The analysis employed single-factor analysis of variance (ANOVA), with differences deemed statistically significant at a p-value of less than 0.05.

### 3 Results and Discussion

The value of olive oil is due to its lipid profile, namely the high content of mono- (55-83% is oleic acid – omega-9) and essential polyunsaturated fatty acids (2.5–21% is linoleic acid – omega-6 and 1% is linolenic acid – omega-3), which are the most biologically

significant, being essential nutrients of food products [1, 14, 40]. During the research, 11 fatty acids were identified (Fig. 1).



**Fig. 1.** Fatty-acid composition of fatty oil from the leaves and fruits of European olive, %

The main components are monounsaturated and polyunsaturated components C18:0-C18:3 (stearic, oleic, linoleic and linolenic acids), C20:0 (arachinic acid) and C22:0 (beogenic acid). Of the fatty acids, the leading ones are *cis*-9-oleic (35.07%), palmitic (32.88%) and stearic (10.21%), as well as a significant content of polyunsaturated (*cis*-9.12-linoleic (9.2%) and *cis*-12.15-linolenic (6.7%)) acids, which determine the nutritional value of the leaves.

A comparative analysis of the level of similar indicators of the lipid profile of fatty oil obtained from freshly harvested olive fruits of the studied cultivar showed that the fatty oil contained in olive leaves is characterized by a high content of *cis*-12.15-linolenic acid (omega-3) and exceeds the same indicator in fatty oil obtained from fruits by 6.26 percentage points. Also, leaf oil differs slightly from fruit oil in terms of the content of *cis*-9.12-linoleic acid (omega-6) – lower by 1.45 percentage points. All this confirms the high nutritional value of fatty oil from olive leaves.

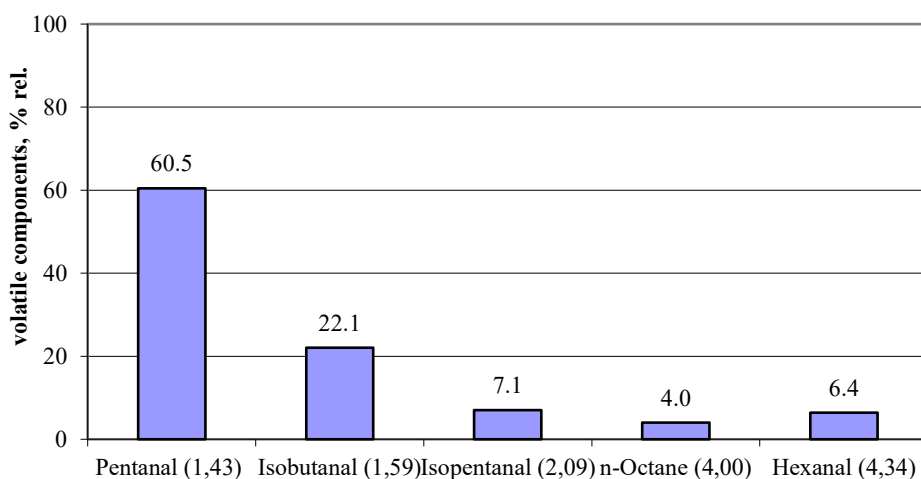
It is known that olive oil consists of approximately 95-98% lipid components. The remaining part of the oil, in particular extracted from the leaves, contains a very large number of aromatic compounds, including a number of volatile components that give the oil its smell and enhance the taste. These include aldehydes, ketones, thiols, alcohols and acids. Namely, aldehydes, as established by the headspace analysis, make up the bulk of the volatile organic substances of the studied olive leaves. Substances such as volatile aldehydes, which are biologically active compounds derived from unsaturated fatty acids, have been identified in the essential oil extracted from olive leaves grown in the Southern Coast of Crimea (SCC). Research indicates that these aldehydes are produced through the thermally accelerated oxidation of unsaturated fatty acids during the distillation process.

According to several studies [33, 41, 42], this thermal oxidation is a key factor in the formation of aldehydes in essential oils.

Many of the aldehydes perform not only protective functions (antifidants, allelopathic compounds, phytoalexins), but are also antibiotic components [43, 44]. Aldehydes exhibit anti-inflammatory, anticoagulant, antibacterial, anti-tuberculosis, antifungal, antiviral, neuroprotective and neurotoxic activities [45]. An immunostimulating effect associated with a certain allergenicity of some of these substances has also been established [43, 46]. The content of aldehydes in plants varies significantly depending on the species of plant and growing conditions.

Therefore, the aldehydes of essential oils are of interest to modern pharmacy and medicine.

In the studied samples, pentanal, isobutanal, isopentanal, hexanal were identified among aldehydes, and an insignificant content of alkane, represented as n-octane, was also found (Fig. 2).



**Fig. 2.** Volatile components of olive leaf essential oil

The largest share of the total of all detected components (Fig. 2) falls on pentanal (valerian aldehyde) – 60.5%, which plays the role of a plant metabolite and is a saturated fatty aldehyde. Pentanal is a compound known for its distinctive aroma, which can be described as fermented, bread-like, fruity, nutty, and berry-like. Its flavor profile is akin to almonds, berries, and bread, which contributes to its extensive use as a flavoring agent. This compound is present in a variety of vegetable products and natural sources, including olive oil and various essential oils. It can also be found in cardamom, rice, vanilla, sage, coriander leaves, apples, bananas, cherries, black currants, and in smaller amounts in corn and safflower [40]. It is also produced in human tissues and cells by lipid peroxidation and potentially inhibits tubulin polymerization. Its highest content forms the aroma of the essential oil of olive leaves.

The second in terms of content (22.1%) is isobutanal (methylpropal, isobutyric aldehyde), which, for example, belongs to the usual aroma-forming components of honey, isobutyric aldehyde also forms a specific taste and smell of banana [11].

Isopentanal (3-methylbutanal, isovalerian aldehyde) and hexanal (capron aldehyde) account for almost equal amounts – 6-7%. Capron aldehyde is a fairly common component of essential oils obtained from the fruits of many plants: olives, pears, avocados, it is also

determined in green peas, to which hexanal gives a characteristic dissonant herbaceous aroma [47].

The identified volatile component of olive leaf essential oil n-octane belongs to the organic compounds of the alkane class. The relative content of this component is insignificant compared to aldehydes and amounts to 4.0%. According to a number of authors, n-octanes form a solid wax layer on plant tissues (mainly leaves and fruits) [48, 49]. The function of alkanes, on the one hand, is to protect against dehydration, on the other – to protect against biotic stressors (bacteria, fungi, insects) [50, 51]. At the same time, the possibility of the participation of n-Octane in the formation of aroma is noted [52].

## 4 Conclusion

The composition of fatty acids of fatty oil and volatile substances of essential oil obtained from the leaves of olive (*Olea europaea*), growing in a dry subtropical climate of the Mediterranean type of the Southern Coast of the Crimea, has been studied. 11 fatty acids have been identified in the fatty oil, the composition and ratio of which are similar to the lipid profile of oil from freshly harvested olive fruits. Of the fatty acids, the leading ones are cis-9-oleic (35.07%), palmitic (32.88%) and stearic (10.21%), as well as a significant content of polyunsaturated (cis-9.12-linoleic (9.2%) and cis-12.15-linolenic (6.7%)) acids, which determine the nutritional value of the leaves. Among the volatile components of the essential oil of the leaves, 4 aldehydes (pentanal, isobutanal, isopentanal, hexanal) and n-octane, belonging to the class of alkanes, have been identified. The major components of the essential oil are pentanal (valeric aldehyde) and isobutanal (isobutyric aldehyde), the mass fraction of which is 60.5% and 22.1%, respectively, which determines the pharmacological value of the leaves.

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