

# Key Odorant Compounds of Turkish Virgin Olive Oils: A Review

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DOI: <https://doi.org/10.5725/jrfoods.2025.9>

## Abstract

Virgin olive oil (VOO), a premium culinary oil, is highly prized for its delicious taste and aroma components in the world. It is particularly important in the Mediterranean diet. Aroma is one of the most critical quality criteria influencing consumer acceptance of the olive oils. More than 300 aroma compounds have been identified in olive oil; however, only a small fraction of these compounds are responsible for its characteristic aroma and are referred to as aroma-active compounds (AACs). Gas chromatography coupled with olfactometry (GC-O) technique is commonly used to identify the AACs. In addition, methods such as dilution analysis, frequency detection, and time–intensity techniques are employed to determine these compounds. Using the odor activity values (OAVs) obtained through these methods, the individual contribution of each aroma compound to the characteristic aroma of the olive oil can be calculated and evaluated. The number and concentration of the AACs in olive oils vary depending on numerous factors including the olive cultivar, ripening stage, climate, production process, storage conditions, etc. Türkiye is among the leading countries in olive oil production worldwide with the most prominent cultivars of Ayvalik, Beylik, Gemlik, Memecik, Halhali, Domat and Sari Ulak. This review aims to examine the AACs found in Turkish olive oils. The most important groups of volatile aroma compounds responsible for the characteristic aroma of olive oil are five- and six-carbon alcohols, aldehydes, and their corresponding esters. These are followed by terpenes, ketones, and carboxylic acids. Aroma and aroma-active compounds in olive oil are mainly formed through the lipoxygenase (LOX) pathway during oil extraction from olives. In this pathway, aldehydes are formed from fatty acids, which are then reduced to alcohols by alcohol dehydrogenase (ADH) and further converted into esters by alcohol acyltransferase (AAT). Among the AACs, hexanal (cut grass odor), octanal (citrus-like, lemon odor), and 1-penten-3-ol (oily odor) stand out due to their high OAV values.

**Keywords:** Turkish olive oils, GC-MS, olfactometry, aroma-active compounds

## 1. INTRODUCTION

Olive oil, one of the oldest known vegetable oils, is derived from the fruits of the olive tree (*Olea europaea* L.). Unlike most of the other vegetable oils, it possesses unique characteristics suitable for direct consumption without refining or further processing (Kesen et al., 2013<sup>a</sup>). Olive oil is a chemically complex substance composed primarily of two major categories of compounds. The first group, known as saponifiable components, accounts for approximately 98% of

the total composition and includes triglycerides, partial glycerides, and esters of fatty acids, as well as free (non-esterified) fatty acids (Cavalli et al., 2004). The second group, the unsaponifiable fraction, constitutes the remaining 2% and comprises a diverse array of bioactive compounds such as sterols, hydrocarbons, pigments, phenolic compounds, flavonoids, and volatile constituents (Escuderos, 2011).

Due to its bioactive components, olive oil consumption is suggested to provide protective effects against cardiovascular diseases and

cancer (Amanpour et al., 2016; Selli et al., 2018). The cultivation of olive trees is extensively practiced throughout the Mediterranean region and constitutes a vital component of the economic structure in olive-producing regions (Kesen et al., 2013<sup>a</sup>). According to the International Olive Council data of 2024, Türkiye ranks fifth in the world in olive oil production with 240,100 tons (International Olive Council, 2024). Türkiye is home to a diverse range of olive cultivars, including Ayvalik, Beylik, Gemlik, Memecik, Halhali, Domat and Sari Ulak. Olive oil quality is influenced by dynamic market demands and is primarily assessed based on consumer perception of organoleptic characteristics, namely aroma, flavor, and color, which may vary temporally and geographically (Kalua et al., 2007; Güçlü et al., 2016). Aroma is a critical quality parameter for virgin olive oils (VOO) and the identification of aroma-contributing compounds (aroma-active compounds or key odorants) is essential for quality assessment and authentication (Arslan and Ok, 2020). Volatile constituents, in particular, are of significant interest due to their strong association with the sensory quality and their utility in detecting adulteration (Escuderos, 2011).

Extra virgin olive oil (EVOO) is one of the most preferred vegetable oils in terms of its sensory properties due to the contributions of its volatile compounds to its aroma and flavor profile (Çevik et al., 2015). Non-volatile components affect the purity of olive oil while the volatile compounds contribute to its organoleptic properties, which play a significant role in human nutrition and consumer acceptability (Güçlü et al., 2016). For olive oil to be classified as "extra virgin" it must be free of any sensory defects (Kalua et al., 2007).

The main aroma compounds responsible for the olive oil aroma are aldehydes, alcohols, ketones, esters, terpenes, lactones, and carboxylic acids (Kılıç and Koyuncu, 2024). These compounds are used as important analytical parameters to assess the overall quality of olive oils and to detect adulteration and undesirable aromatic defects (Toker and Yavuz, 2015). A large portion of aroma compounds are formed as

a result of enzymatic reactions and auto-oxidation processes in olive oils (Arslan and Ok, 2020). The lipoxygenase (LOX) pathway, which acts on polyunsaturated fatty acids, stands out as one of the primary enzymatic mechanisms in the formation of these compounds (Amanpour et al., 2019). The genetic variety of the olive fruit is one of the most fundamental factors determining olive oil quality, influencing both the formation of volatile compounds and the sensory qualities of the olive oils (Caratti et al., 2025). The aroma profile of olive oil depends on various factors such as fruit variety, climate conditions, soil characteristics, harvest time, processing methods, and storage conditions (Kara and Bayrak, 2023).

Approximately 200 aroma compounds have been identified in olive oils to date but only a small fraction of these compounds are responsible for olive oil's characteristic aroma profile (Genovese et al., 2021). These compounds are called "aroma-active compounds (AACs)" or "key odorants" (Neugebauer et al., 2020). Gas chromatography-olfactometry (GC-O) is the most commonly used method for the detection of the AACs (Khursheed et al., 2024). Some aroma compounds, despite being present in relatively low amounts, can have high odor intensities, making them difficult to detect by using instrumental devices alone (Brattoli et al., 2011). Furthermore, some aroma compounds are unstable and can transform into other compounds (Weerawatanakorn et al., 2015). In such cases, the GC-O method allows the detection of the AACs even at very low concentrations (Kılıç-Büyükkurt, 2024). The purpose of this article was to examine the types and variations of the AACs available in olive oils produced in Türkiye.

## 2.FORMATION PATHWAYS OF AROMA AND AROMA-ACTIVE COMPOUNDS

Approximately 200 aroma compounds, generally grouped as alcohols, aldehydes, ketones, esters, terpenes, lactones, and carboxylic acids, have been identified in VOOs (Genovese et al., 2021). The three primary biosynthetic pathways, which have been identified for the

formation of these compounds are elucidated below:

The first and most well-known pathway is the "oxidative process" (Velasco and Dobarganes, 2002). It involves the oxidation of polyunsaturated fatty acids (PUFA) (especially linoleic and linolenic acids) by the enzyme lipoxygenase (LOX) and subsequent cleavage by the enzyme hydroperoxide lyase to form aldehydes (Figure 1). The resulting aldehydes are reduced to alcohols by the enzyme alcohol dehydrogenase and then esterified by alcohol acyltransferase to form ester compounds. The C6 and C5 volatile compounds synthesized through the LOX pathway are considered determinants of positive sensory attributes such as fruitiness, sweetness, greenness, or ripeness (Liu et al., 2022). A green and fruity odor indicates early harvest, fresh, high-quality olive fruit, and good processing and storage conditions. Aldehydes such as hexenal, *(E)*-(2)-hexenal, *(Z)*-(3)-hexenal, and *(E)*-2-pentenal and the alcohols such as *(E)*-2-hexen-1-ol, 1-hexanol, and 1-penten-3-ol are the AACs that contribute significantly to the characteristic "green" aroma of the olive oils. Alcohols, aldehydes, esters, and hydrocarbons are enzymatically produced from PUFAs (such as linoleic acid: LA and linolenic acid: LnA) via the LOX pathway. Other fatty acids (both saturated and unsaturated) and nitrogen-containing compounds can produce negative notes such as waxy, oily, winey, vinegary, moldy, fermented, creamy, soapy, fried, cheesy, ethereal, or mushroom-like, due to catabolism, autoxidation, thermal oxidation, and microbial fermentation (Aparicio and Harwood, 2013). The majority of the aroma compounds found in EVOOs are formed through the LOX pathway (Angerosa et al., 2000).

The second formation pathway is the synthesis of the volatile compounds derived from branched-chain amino acid derivatives such as leucine and valine (Maoz et al., 2022). This pathway produces methyl-branched alkyl and acyl esters and methyl-branched alcohols, which can have significant impact on sensory properties.

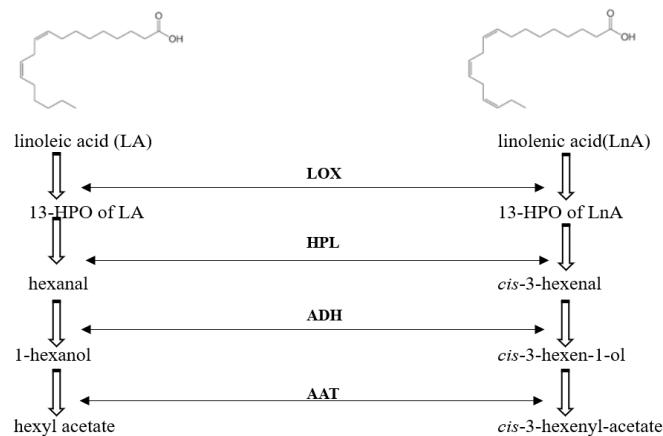


Figure 1. Lipoxygenase pathway (LOX) involved in the formation of virgin olive oil (VOO) aroma compounds (AAT: alcohol acyltransferase, ADH: alcohol dehydrogenase, 13-HPL: 13-hydroperoxide lyase, ISO: isomerase)

The third pathway is associated with the microbial activities and chemical oxidation processes. These processes produce compounds such as ethanol, C6–C10 dienals, C5 branched aldehydes, C8 ketones, various alcohols, and C7–C11 monounsaturated aldehydes (Yıldırım et al., 2023). These volatile compounds are associated with the negative flavor attributes such as spoiled, musty-damp, wine-vinegar, and/or rancid (Amanpour et al., 2019). Pentanal, hexanal, octanal, and nonanal are the primary compounds formed in the oxidatively degraded olive oils. The 2-pentenal and 2-heptenal are generally responsible for off-flavors. The presence of nonanal, in particular, is considered a significant indicator pointing to the beginning of the oxidation process. The vinegar-like off-flavor is often associated with acetic resonance. Various biochemical mechanisms, such as sugar fermentation, metabolism of branched-chain amino acids such as leucine, isoleucine, and valine, and oxidative reactions, also contribute to the formation of volatile compounds. However, excessive accumulation of these compounds leads to the development of undesirable off-flavors in olive oils. The development of the sensory defects is influenced by numerous factors including the quality and ripeness of the olives, harvest time, post-harvest practices, storage conditions, extraction methods, and filtration processes (Amanpour et al., 2016). In fact, each step in the production chain significantly impacts the volatile compound profile and consequently

the sensory quality. Molds such as *Penicillium* and *Aspergillus* oxidize free fatty acids leading to the formation of methylketone compounds such as 2-heptanone and 2-nonenone. Similarly, yeasts such as *Candida* and *Saccharomyces* reduce carbonyl compounds through esterification leading to the development of a musty-dank aroma. The AACs of 1-octen-3-one, 1-octen-3-ol, and 2-heptanol mostly contribute to this defect. The "wine-vinegar" characteristic is a typical sensory defect generally observed in oils obtained from olives that are not fresh and have undergone alcoholic and acetic fermentation. The primary bacteria involved in this process are lactic acid bacteria (especially *Lactobacillus* species) and acetic acid bacteria. In the absence of oxygen, ethanol is formed, followed by volatile compounds such as acetic acid and ethyl acetate. These compounds, along with 3-methylbutanol, are among the primary causes of this defect. Another sensory defect identified in olive oils caused by microorganisms is the "muddy sediment" note, which is the characteristic aroma of the oils that have been exposed to their own muddy sediment for extended periods of time and have undergone anaerobic, predominantly butyric, fermentation. Ethyl butanoate, ethyl propionate, and butyl acetate are generally found in higher levels in moldy VOOs. The high levels of 6-methyl-5-hepten-2-one are associated with the presence of *Pseudomonas* species, which play a role in the degradation of terpene alcohols. Conversely, the high concentrations of the butanoic and propanoic acids are likely due to the metabolic activities of *Clostridium* spp. and propionic acid bacteria. Rancidity is a typical off-flavor defect that develops as a result of oxidative deterioration of olive oils (Genovese et al., 2021). This deterioration is accelerated by environmental factors such as prolonged exposure to air, light, and storage at relatively high temperatures. The oxidation produces aldehydes, particularly 2-heptenal, 2-octenal, 2-decenal, hexanal, nonanal, octenal, pentanal, and heptanal, which are considered the primary chemical indicators of rancidity. In addition, the presence of short and medium chain fatty acids such as butanoic, hexanoic and heptanoic acids indicates that the

oxidation occurs at an advanced level, as these acids are formed by the oxidative conversion of the aldehydes (Karagöz et al., 2017; Üçüncüoğlu and Sivri-Özay, 2020; Genovese et al., 2021).

The odor threshold of each volatile compound represents the lowest detectable concentration of that compound (Santos et al., 2010). Therefore, the overall aroma profile of olive oil is formed by the combination of the individual contributions of each volatile compound based on their odor threshold and unique aromatic properties (Kalua et al., 2007). Aroma extract dilution analysis (AEDA) is used in conjunction with the GC-O system to determine the odor threshold of each AAC (Amanpour et al., 2016).

The list of the AACs identified in various studies conducted on olive oils is provided in Table 1. C6 aldehydes (hexanal, 3-hexenal, (E)-2-hexenal and 2,4-hexadienal), C6 alcohols (1-hexanol, (Z)-3-hexen-1-ol and (E)-2-hexen-1-ol), C5 aldehydes (pentanal, (E)-2-pentenal) and C5 alcohols (1-penten-3-ol, 1-pentanol and (Z)-2-penten-1-ol) formed via the LOX pathway have been reported in previous studies as the most prevalent AACs in VOOs and are mainly responsible for the formation of the characteristic aroma of the olive oils studied (Kesen et al., 2013<sup>a</sup>; Keser et al., 2013<sup>b</sup>; Keser et al., 2013<sup>c</sup>; Korkmaz, 2023; Sevim et al., 2023).

### 3. EXTRACTION METHODS FOR THE AROMA-ACTIVE COMPOUNDS

An effective extraction of aroma compounds from the food matrix is one of the most crucial steps in aroma analysis before carrying out the GC-MS-O analysis. The selection of the appropriate extraction method is critical for the accuracy and reliability of the baseline data used in subsequent stages (Kılıç-Büyükkurt et al., 2024). For this purpose, various extraction techniques including steam distillation, simultaneous distillation-extraction (SDE), purge and trap extraction, solid-phase microextraction (SPME), rod-type adsorption extraction (SBSE), liquid-liquid extraction (LLE), and solvent-assisted aroma evaporation

(SAFE) are widely-employed techniques. These practices are briefly described below:

Steam distillation is utilized to separate the volatile compounds in plant materials. This method is effective in separating volatile substances such as essential oils and organic acids from the matrix but it can lead to aroma losses due to the high applied temperature (Perovic et al., 2024).

Simultaneous distillation-extraction (SDE), also known as the Lickens-Nickerson technique, allows distillation and extraction to be

Table 1. The aroma-active compounds reported in Turkish olive oil

Aroma-active compounds	LRI*	Odo odor r description	Reference
<b>Ethyl propanoate</b>	950	Fruity, sweet	Selli et al., 2018
<b>Methyl 2-methylbutyrate</b>	1057	Tropical, sweet	Selli et al., 2018
<b>Hexanal</b>	1074	Green, grassy, cut grass	Kesen et al., 2013 <sup>a</sup> ; Güçlü et al., 2016; Amanpour et al., 2019; Sevim et al., 2023
<b>Isobutanol</b>	1103	Pleasant	Selli et al., 2018
<b>(E)-2-pentenal</b>	1121	Green plant, grassy, fresh-plant	Kesen et al., 2013 <sup>a</sup> ; Sevim et al., 2023
<b>Isoamyl acetate</b>	1157	Fruity, pleasant	Selli et al., 2018
<b>(Z)-3-Hexanal</b>	1136	Green-olive paste, Fresh-cut grass	Güçlü et al., 2016; Amanpour et al., 2019; Sevim et al., 2023
<b>3-Hexanol</b>	1142	Woody, green	Kesen et al., 2013 <sup>a</sup>
<b>1-Penten-3-ol</b>	1157	Green-leafy, grassy, herbal	Kesen et al., 2013 <sup>a</sup> ; Amanpour et al., 2019; Sevim et al., 2023
<b>2-Ethyl-(E)-2-butenal</b>	1171	Grassy, floral	Kesen et al., 2013 <sup>a</sup>
<b>Heptanal</b>	1180	Green plant, oily	Kesen et al., 2013 <sup>a</sup> ; Amanpour et al., 2019; Sevim et al., 2023

carried out simultaneously in a specialized device. Water vapor helps separate aroma compounds from the matrix while the compounds condensed with the organic phase form a pure aroma extract (Selli and Cayhan, 2009).

In the purge and trap method, also called dynamic headspace extraction, volatile compounds in the sample are collected in an adsorbent trap using an inert gas (helium or nitrogen) and then desorbed by heating or using a solvent for further analysis (Sönmezdağ et al., 2017).

<b>3-Penten-2-ol</b>	1181	Woody, winey	Güçlü et al., 2016; Selli et al., 2018
<b>dl-Limonene</b>	1186	Floral-citrusy	Güçlü et al., 2016; Sevim et al., 2023
<b>(E)-2-Hexenal</b>	1190	Green-cut grass, apple-like	Kesen et al., 2013 <sup>a</sup> ; Güçlü et al., 2016; Amanpour et al., 2019; Yıldırım et al., 2023
<b>Isoamyl alcohol</b>	1194	Alcohol	Selli et al., 2018
<b>2-Methyl-1-penten-3-ol</b>	1220	Plastic-chemical	Amanpour et al., 2019
<b>3-Hydroxybutanone</b>	1273	Buttery	Selli et al., 2018
<b>Hexyl acetate</b>	1285	Fruity, green	Kesen et al., 2013 <sup>a</sup> ; Sevim et al., 2023
<b>Octanal</b>	1292	Green, citrusy, lemon, oily-floral	Kesen et al., 2013 <sup>a</sup> ; Amanpour et al., 2019; Sevim et al., 2023
<b>1-Octen-3-one</b>	1298	Chemical	Kesen et al., 2013 <sup>a</sup>
<b>(Z)-3-Hexenyl acetate</b>	1300	Fruity, green	Kesen et al., 2013 <sup>a</sup> ; Yıldırım et al., 2023; Sevim et al., 2023
<b>Isoamyl isovalerate</b>	1307	Fruity	Kesen et al., 2013 <sup>a</sup>
<b>(E)-2-Pentenol</b>	1319	Waxy-fatty	Amanpour et al., 2019
<b>(E)-2-heptenal</b>	1320	Chemical, fatty	Kesen et al., 2013 <sup>a</sup>
<b>6-Methyl-5-hepten-2-one</b>	1332	Fruity	Kesen et al., 2013 <sup>a</sup>
<b>Hexanol</b>	1350	Flowery-spicy, fruity, green, floral, grassy, floral-herbal	Kesen et al., 2013 <sup>a</sup> ; Selli et al., 2018; Amanpour et al., 2019; Yıldırım et al., 2023; Sevim et al., 2023
<b>(Z)-3-Hexenol</b>	1378	Green-herbal, cut grass, flowery	Kesen et al., 2013 <sup>a</sup> ; Selli et al., 2018; Amanpour et al., 2019; Sevim et al., 2023

<b>(E)-2-Hexenol</b>	1400	Green-fruity, grassy-cool	Kesen et al., 2013 <sup>a</sup> ; Amanpour et al., 2019; Sevim et al., 2023
<b>Nonanal</b>	1408	Waxy-fatty, pungent, grassy, citrusy	Kesen et al., 2013 <sup>a</sup> ; Güçlü et al., 2016; Amanpour et al., 2019; Sevim et al., 2023
<b>Acetic acid</b>	1414	Vinegar	Selli et al., 2018
<b>(E,E)-2,4-Heptadienal</b>	1481	Spicy-fatty, oily	Amanpour et al., 2019; Sevim et al., 2023; Sevim et al., 2023
<b>Heptanol</b>	1498	Fatty	Amanpour et al., 2019
<b>(E)-2-hepten-1-ol</b>	1529	Floral	Kesen et al., 2013 <sup>a</sup>
<b>(E)-2-nonenal</b>	1536	Fruity, grassy	Kesen et al., 2013 <sup>a</sup>
<b>Decanal</b>	1538	Fatty-wet-soapy	Kesen et al., 2013 <sup>a</sup> ; Amanpour et al., 2019
<b><math>\alpha</math>-Copaene</b>	1544	Sweet, fruity	Kesen et al., 2013 <sup>a</sup> ; Amanpour et al., 2019; Sevim et al., 2023
<b>Linalool</b>	1547	Lilac, lavender	Kesen et al., 2013 <sup>a</sup>
<b>Octanol</b>	1554	Flowery-herbal, fruity-green	Amanpour et al., 2019; Sevim et al., 2023
<b>4-OH-2-hexenoic acid lactone</b>	1580	Fruity, sweet	Kesen et al., 2013 <sup>a</sup>
<b>2,3-Butanediol</b>	1584	Oily	Selli et al., 2018
<b><math>\gamma</math>-Butyrolactone</b>	1597	Olive, pleasant	Selli et al., 2018
<b>Butanoic acid</b>	1624	Cheesy	Amanpour et al., 2019
<b>(E)-2-Decenal</b>	1644	Soapy, fatty , wet-boiled potato	Kesen et al., 2013 <sup>a</sup> ; Amanpour et al., 2019
<b>Nonanol</b>	1660	Fruity-citrusy	Amanpour et al., 2019
<b>(E,E)-2,4-Nonadienal</b>	1693	Fatty-deep fried	Kesen et al., 2013 <sup>a</sup> ; Amanpour et al., 2019
<b>Valencene</b>	1694	Mint, orange blossom	Kesen et al., 2013 <sup>a</sup>
<b>Zingiberene</b>	1702	Floral	Kesen et al., 2013 <sup>a</sup>

<b>2-Methyl-butanoic acid</b>	1711	Cheesy	Selli et al., 2018
<b>(E)-2-nonen-1-ol</b>	1712	Fruity, waxy	Kesen et al., 2013 <sup>a</sup>
<b>γ-Crotonolactone</b>	1713	Fruity	Kesen et al., 2013 <sup>a</sup>
<b>α-Farnesene</b>	1745	Floral, green plant	Kesen et al., 2013 <sup>a</sup> ; Sevim et al., 2023
<b>Methyl salicylate</b>	1747	Cooked-caramel	Amanpour et al., 2019
<b>(E,E)-α-farnesene</b>	1749	Floral, herb	Kesen et al., 2013 <sup>a</sup>
<b>(E,E)-2,4-hexadienal</b>	1759	Fatty, solvent	Kesen et al., 2013 <sup>a</sup>
<b>(E,E)-2,4-Decadienal</b>	1781	Cooked-fatty	Kesen et al., 2013 <sup>a</sup> ; Amanpour et al., 2019
<b>(E,E)-α-Farnesene</b>	1768	Flowery-herbal, grassy	Selli et al., 2018; Amanpour et al., 2019
<b>β-Sesquiphellandrene</b>	1784	Floral	Kesen et al., 2013 <sup>a</sup>
<b>Hexanoic acid</b>	1810	Oily	Kesen et al., 2013 <sup>a</sup>
<b>Guaiacol</b>	1824	Olive paste, soapy	Kesen et al., 2013 <sup>a</sup> ; Sevim et al., 2023
<b>(E)-2-undecenal</b>	1876	Olive, fatty	Kesen et al., 2013 <sup>a</sup>
<b>2-Phenyl ethanol</b>	1922	Rose	Kesen et al., 2013 <sup>a</sup>
<b>Tridecanol</b>	1934	Olive paste	Kesen et al., 2013 <sup>a</sup>
<b>γ-Decalactone</b>	2103	Fruity	Kesen et al., 2013 <sup>a</sup>
<b>Phenylethyl alcohol</b>	2130	Flowery, olive	Selli et al., 2018; Sevim et al., 2023
<b>δ-Decalactone</b>	2216	Fruity	Kesen et al., 2013 <sup>a</sup>
<b>Methyl palmitate</b>	2233	Fruity	Kesen et al., 2013 <sup>a</sup>
<b>Ethyl palmitate</b>	2270	Fruity	Kesen et al., 2013 <sup>a</sup>
<b>γ-Dodecalactone</b>	2384	Fruity	Kesen et al., 2013 <sup>a</sup>
<b>3-Penten-2-ol</b>		Woody, herbal-fruity	Güçlü et al., 2016; Sevim et al., 2023

\*LRI: Linear retention indices (LRI)

The solid-phase microextraction (SPME) technique is based on the adsorption of the

volatile compounds onto a coated fiber and is a rapid and environmentally-friendly method for

the GC analysis that does not require the use of solvents. However, this method should be used with caution due to the delicate nature of the fibers (Jalili et al., 2020).

The rod-type adsorption extraction (SBSE) is a technique that uses polydimethylsiloxane-coated magnetic rods to adsorb aroma compounds during mixing. This method offers an environmental advantage as it does not require a solvent (Nogueira, 2012).

The liquid-liquid extraction (LLE) procedure is based on the removal of target compounds with organic solvents of similar polarity. Benzene, dichloromethane, diethyl ether, and ethyl acetate are the commonly-preferred solvents used in the method (Sell et al., 2014).

Finally, the solvent-assisted flavor evaporation (SAFE) method is based on the evaporation and concentration of the aroma compounds without damage under low temperature and high vacuum condition. It minimizes the aroma losses and ensures the production of pure and rich aroma extracts (Amanpour et al., 2019).

#### 4. USE OF GC-O IN THE ANALYSIS OF AROMA-ACTIVE COMPOUNDS

The GC-O technique is an innovative method that holds great promise, particularly in the food aroma analyses. First introduced in 1964, this method has gained renewed interest by utilizing the human nose, which is a natural detector more sensitive than other detectors. The GC-O technique, derived from the Greek term "Osme (Olfactometric Sensory Method)", is based on the psychophysical assessment of individual aroma compounds' odors based on Stevens' Law (Biniecka and Caroli, 2011).

The GC-O method holds a significant place in the food industry due to its high sensitivity and selectivity in the detection of specific compounds. In this technique, the eluted substances are directed in two different directions through a quartz Y-junction at the end of the analytical capillary column: one to a detector such as a conventional flame ionization detector

(FID) or mass spectrometry (MS) and the other to the sniffing port (Drira et al., 2021). The system is surrounded by a heated sheath to prevent condensation. A glass funnel is located at the end of the sniffing port allowing the analyst to directly sniff the eluted compounds. The sample volume passing through this port is adjusted according to the diameter and length of the two capillary tubes at the column outlet to ensure optimal detection. Additionally, a nitrogen gas flow through a water-filled container cools and humidifies the carrier gas before it reaches the analyst's nose. When the analyst detects an odor, the system generates a signal via a potentiometer, which is then recorded by the chromatography software along with the data from the FID or MS detectors. At this stage, the analyst also defines the character and intensity of the odor.

The GC-O technique has been significantly developed, particularly through the studies by Terry Acree and Werner Grosch. In the aroma-extract dilution analysis (AEDA) technique introduced by Grosch, GC-O is used to obtain the initial olfactogram of a sample and then analyze successive 1:1 or 1:2 diluted solutions of the same sample (Biniecka and Caroli, 2011). This process is repeated until the analyst no longer detects any odor and the odor threshold values (OTV) of the eluted compounds are determined. While the detection devices can separate and identify the aroma compounds, their concentrations do not directly reflect their contribution to the overall aroma profile. Therefore, the odor activity value (OAV), defined as the ratio of the concentration to the odor threshold, has become an important indicator for the evaluation and selection of key aroma compounds (Neugebauer et al., 2020).

Only a portion of the volatile components are the compounds that contribute to the aroma profile (AACs) of the food sample; thus, the GC-O system, and particularly the AEDA technique, is of great importance for the analysis of food essential oils (Ruth, 2001; Biniecka and Caroli, 2011; Pu et al., 2025). One of the fundamental requirements of the GC technique is that the analyzed substances be volatile enough to be eluted and detected at the operating temperature. Furthermore, the stationary phase must be less

volatile and thermally stable to serve as the separation surface. The molecular mass range of the GC method ranges from 2–1500 atomic mass units (amu) (Biniecka and Caroli, 2011). This means that the compounds that can be separated by the GC system range from continuous gases (i.e., highly volatile substances) to volatile compounds up to 200 amu and semi-volatile compounds above 200 amu (Ranjan et al., 2023). Since most essential oils elute at relatively low temperatures, the use of the columns with high thermal stability may not always be necessary. The thermal stability of the column also ensures long-term reliability, which contributes to the increased reproducibility of the analyses and thus to the reliability of the analytical characterization process (Biniecka and Caroli, 2011).

#### 4.1 Methods for Detecting the AACs

Various methods are utilized to identify the AACs in foods. These methods can be grouped into four main categories: dilution analysis, frequency of detection, time-intensity method, and subsequent intensity method (Grosch, 2001; Ruth, 2001; Amanpour et al., 2019; Kılıç-Büyükkurt, 2024). These methods are briefly explained below:

##### 4.1.1. Dilution analysis

Various quantitative methods have been developed to assess the odor thresholds of the aroma compounds in the GC-O analyses. These methods are based on the OAV, which is the ratio of an aroma compound's concentration to its odor threshold. Other terms such as "odor unit number," "odor intensity index," "flavor unit," and "threshold odor number" are also used for the OAV. Using the GC-O technique, the odor thresholds of the aroma compounds can be directly determined and these thresholds can be evaluated by comparing them with their concentrations in food products. Two dilution analysis-based methods, AEDA and Charm Analysis, are intended to determine the odor potential of the aroma compounds. In the AEDA method, the sample is continuously diluted at specific ratios (e.g., 1:2, 1:3) and the final perceived dilution level is defined as the flavor dilution (FD) value. These values are usually

presented in log (FD) format. Charm Analysis is similarly a dilution-based method, but in this, dilutions are presented in random order and the duration of the odors detected by the panelists is also taken into account. A chromatogram is generated from the obtained data and the peak areas are quantified as the "Charm value". This method combines the detection time with the degree of dilution to assess the odor potency of the compound. The key difference between the AEDA and Charm Analysis methods is that the latter also includes the duration of odor but the dilution coefficients obtained by both methods are generally equivalent (Ruth, 2001).

##### 4.1.2. Detection frequency analysis

This method was developed to eliminate the use of a limited number of panelists and the reliance on perception threshold values. It relies on the participation of a group of panelists rather than individual assessments. The number of panelists detecting a particular aroma compound in the odor port (detection frequency) is used as a measure reflecting the relative intensity of that compound. In this method, a "sniffing chromatogram" that shows the number of times the compounds are detected is produced. This method has also been tested in studies assessing different amounts of aroma compounds and despite varying sampling times, the number of detected aroma compounds remained the same, demonstrating the robustness of the method. A disadvantage of this method is that the perceived frequency is based solely on the number of panelists, not the actual (numerical) intensity, and therefore, it cannot directly reflect absolute aroma intensity (Ruth, 2001).

##### 4.1.3. Time-Intensity Method

The time-intensity method is based on the magnitude estimation of odor intensity. One of the most well-known of these approaches is the Osme technique (Grosch, 2001). In this method, trained panelists directly record the intensity and duration of each aroma compound detected in the GC output and provide odor-related descriptors. Evaluation is performed using a computerized feedback system and a 16-point scale (Kılıç-Büyükkurt, 2024).

#### 4.1.4. Posterior intensity method (PIM)

The PIM method, used in the GC-O analyses, is a technique for assessing the perceived concentrations of aroma compounds. In this method, after a peak emerges from the GC device, the evaluator scores the perceived odor intensity using a previously memorized five-step intensity scale. While it is similar to the OSME technique, the intensity assessment in this method is made after the peak. Therefore, it relies on the evaluator's memory and subjective perception. The PIM method has been relatively less studied in literature. Its relationship with the physical concentrations of the aroma compounds and the other GC-O methods has not yet been sufficiently validated (Grosch, 2001).

### CONCLUSION

The aroma profile of the EVOOs produced in Türkiye is rich and diverse in terms of sensory properties. The formation of the AACs, which determine the characteristic aroma of olive oil, varies depending on many factors, including olive variety, harvest time, environmental factors, processing, and storage conditions. The majority of the AACs are synthesized via lipoxygenase (LOX) while some of them are formed as a result of amino acid metabolism and microbial activities. C5 and C6 aldehydes, alcohols, and esters, in particular, contribute to the green, fruity, and fresh aromatic profile of olive oil as the compounds formed due to oxidative and microbial spoilage cause negative sensory defects. Therefore, the accurate extraction and analysis of the volatile compounds plays a critical role in determining the quality of olive oils. The use of advanced techniques such as GC-O contributes significantly to the identification of the AACs. In particular, analysis methods such as AEDA and OAV allow the true contribution of the aroma compounds to be assessed, enabling the development of more reliable and scientific approaches in food industry studies such as quality control, product standardization, and geographical indication. Consequently, Türkiye's olive diversity and production potential offer a rich array of AACs,

a significant asset that could enhance the competitiveness of Turkish olive oils in the international market.

### DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### FUNDING

None.

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