

Article

Characterization of the Key Odorants of Mastic Gum (*Pistacia lentiscus* var. Chia) from Two Different Countries

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Abstract: Mastic gum, a plant-based resin from mastic trees, has become very popular in recent years and has been used in various food products due to its strong and positive aroma properties. In the present study, key odorant compounds of the mastic gum (MG) samples obtained from mastic gum trees (*Pistacia lentiscus* var. Chia) from two different countries, Türkiye (MGT) and Greece (MGG), were investigated and compared. The aroma-active compounds (AACs) were determined by aroma extract dilution analysis (AEDA) and by using gas chromatography-mass spectrometry-olfactometry (GC-MS-O). The two mastic gum samples exhibited similar aroma profiles but significant differences were observed in their concentrations. Among the aroma groups identified in both samples, monoterpenes were the most abundant group with α -pinene as the main compound followed by β -myrcene and β -pinene. On the other hand, the most dominant AAC in both samples was determined to be α -pinene (resinous, forest-like odor), followed by β -pinene (resinous, terpene-like odor), β -myrcene (pine-like, greenish odor), and linalool (floral, fruity odor), all of which had high flavor dilution (FD) values. The findings of the AEDA and sensory analysis revealed that the MGT sample contained more floral and fruity odors while the MGG sample had more resinous and pine-woody odors.



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Keywords: *Pistacia lentiscus*; mastic tree; mastic gum; resin; aroma; aroma-active; olfactometry

1. Introduction

The mastic plant (*Pistacia lentiscus*) is an evergreen shrub, belonging to the Anacardiaceae family [1]. Currently, it has two subspecies: *Pistacia lentiscus* L., which grows naturally as a shrub, and *Pistacia lentiscus* var. Chia, which is a cultivated subspecies [2]. It is commonly known as the mastic tree because it naturally produces a plant-based resin called mastic gum with a characteristic aroma from its trunk and branches [3]. The mastic gum produced by the *Pistacia lentiscus* var. Chia subspecies has a considerable economic importance and is recognized for its distinctive and unique quality as a spice [4]. Mastic gum has been used in traditional medicine since Ancient Greece for the treatment of various ailments, including cough, sore throat, eczema, and gastrointestinal diseases [5]. Additionally, due to its strong characteristic aroma, it is used as a flavoring agent in the food industry, particularly in chewing gums, sweets, and alcoholic beverages. The mastic tree is grown in various Mediterranean countries, including Türkiye, Greece, Tunisia, Morocco, Spain, and Italy [3,6]. Mastic gum is exclusively produced on the Greek island

of Chios, and is an important source of economic income. Although the mastic tree is widely distributed along the Aegean and Mediterranean coasts of Türkiye, it does not contribute to the economy. These trees are at risk of extinction due to some factors such as declining agricultural activities, urbanization, tourism, lack of maintenance (pruning, etc.), destruction, and aging of the trees [7,8].

An aroma compound is a volatile substance detected in the human nasal cavity, contributing to the sense of smell. The aroma of a food item is formed by the interaction of hundreds of different volatile compounds, present in the food in concentrations ranging from $\mu\text{g}/\text{kg}$ to ng/kg . It is one of the most important quality parameters affecting consumer preference. The aroma compounds that are naturally present in various parts of plants are categorized into chemical classes, including terpenes, aldehydes, alcohols, esters, and ketones [9]. These constituents are usually determined by gas chromatography-mass spectrometry instruments (GC-MS). Mastic gum contains numerous volatile compounds, predominantly terpenes and terpenoids. Researchers in various countries have identified major volatiles in mastic gum such as α -pinene, β -pinene, β -myrcene, limonene, and β -caryophyllene [10–12]. Mastic gum has a distinct strong aroma and a woody, bitter taste due to these terpenes [13]. The terpenes present in mastic gum are influenced by various factors, including the geographical region where the tree is grown, the collection time, the duration between exudation from the stem and collection, climate conditions, and the extraction methods applied. These factors also affect the general quality of mastic gum [2,13]. Some of these volatiles, known as the aroma-active compounds (AACs) or key odorants, are responsible for the characteristic aroma of the mastic gum. Despite being present in very low concentrations, these constituents significantly contribute to its distinctive odor and are detectable by the human nose. To identify these AACs, highly sensitive techniques such as GC-Olfactometry (GC-O) and/or GC-MS-O, which utilize the human nose as a detector, are commonly employed [14,15].

Most of the previous studies have focused on the volatile constituents, phenolic compounds, and antioxidant activity of both mastic gum resin and its essential oils [2,16–19]. However, only a single study has explored the AACs in mastic gums [11]. To the best of our knowledge, this is the first study to comparatively evaluate the AACs of mastic gum samples from two different geographical origins (Türkiye and Greece) by combining AEDA and sensory analysis. In this context, the present study aimed to investigate the effect of geographical location on the aroma and AACs of mastic gum samples from two different origins and the findings of this work may contribute to future research on the standardization, authentication, and origin-based utilization of mastic gum in food applications.

2. Materials and Methods

2.1. Mastic Gum Samples

The mastic gum samples from the shrub form of the mastic tree (*Pistacia lentiscus* var. Chia) were collected from Karaburun district ($38^{\circ} 38' 17.956''$ N, $26^{\circ} 30' 35.412''$ E) in Izmir province, Türkiye in 2020. In the same year, the mastic gum samples of Greek origin were obtained from a local producer in Chios Island, Greece ($38^{\circ} 23' 34''$ N $26^{\circ} 06' 46''$ E). Both regions are characterized by the hot, dry summers and mild, wet winters typical of the Mediterranean climate. About 80 g of each mastic gum sample was stored in a cool and dry place until analysis.

2.2. Chemicals

Standard aroma compounds (4-nonanol), dichloromethane, and anhydrous sodium sulfate were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). All chemi-

cals used in the present study were of chromatography grade. Water purification equipment (Millipore-Q, Millipore Corp., Saint-Quentin, France) was utilized to obtain distilled water.

2.3. Color Measurement

Color parameters of the mastic gum samples were quantified by using a colorimeter (HunterLab ColorQuest XE, Reston, VA, USA) based on the CIE color system (L^* , a^* , b^*) following the method described by Kelebek et al. [20]. The measurements were performed by placing the samples in a 5-cm-deep optical cuvette. In addition, based on the L^* , a^* , and b^* values, the Chroma (C , color intensity) and Hue angle (H° : 0° for red, 90° for yellow, 180° for green, 270° for blue) were calculated using Equations (1) and (2), respectively [21]. All measurements were conducted in triplicate at room temperature.

$$C = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$H^\circ = \arctan (b^*/a^*) \quad (2)$$

2.4. Aroma Compound Analysis

2.4.1. Aroma Extraction

The aroma compounds were extracted by using the purge and trap method which involved attaching aroma compounds to a trap containing 200 mg of LiChrolut EN resin, facilitated by purge nitrogen gas. The mastic gum samples (6 g each) were ground using a mortar and placed in 20 mL glass vials. An internal standard (5 μ L of 4-nonanol) was added and the vials were sealed with two holes made in the lids, one for the trap and one for the purge gas. Before placement in the vials, the traps were conditioned by passing 6 mL of dichloromethane through them. The samples were pre-incubated in a 60°C water bath for 10 min before initiating the gas flow. Nitrogen gas was then passed through each vial at a rate of 500 mL/min, allowing the aroma compounds to adsorb onto the LiChrolut EN resin in the traps. After 90 min of nitrogen gas flow with the samples maintained in the water bath at 60°C , the traps containing the absorbed aroma compounds were removed. The traps were washed with 12 mL of dichloromethane solvent to extract the aroma constituents. The solvent containing the aroma compounds was dehydrated using anhydrous sodium sulfate and then concentrated to 0.5 mL using a Vigreux distillation column in a 50°C water bath. The extracts were directly injected into the GC-MS, GC-FID, and GC-MS-O systems to assess the aroma and the AACs. All extractions were carried out in triplicate [22].

2.4.2. Identification of the Aroma Compounds

The aroma and aroma-active compounds were determined by using a GC system (Shimadzu Nexis GC-2030, Kyoto, Japan), an MS system (Shimadzu GC-MS-QP2020 NX, Kyoto, Japan), and an olfactometer integrated into the system. The column outlet was divided into three equal parts using a special separator: the first part was directed to the flame ionization detector (FID), the second to the mass spectrometer (MS), and the third to the olfactometer (O) for odor analysis. The GC-FID system was utilized to determine the aroma compounds that were separated by using a DB-WAX capillary column (60 m $\times$ 0.25 mm $\times$ 0.4 μ m). The injector and detector temperatures were set to 220°C and 250°C , respectively. The column temperature program started at 60°C (held for 3 min), followed by an increase of $2^\circ\text{C}/\text{min}$ up to 220°C , then by $3^\circ\text{C}/\text{min}$ up to 245°C , where it was maintained for 20 min. A 3 μ L sample was injected into the device in a split ratio of 1:10 and helium was used as the carrier gas at a flow rate of 1.5 mL/min [22].

The aroma compounds were identified by using the MS, which was connected to the same GC. The injector type and temperature program matched those used in the GC

analysis, with helium as the carrier gas at a flow rate of 1.5 mL/min. The ionization energy of the MS was set to 70 eV, the ion source temperature to 250 °C, and the quadrupole temperature to 120 °C. Scans were performed at one-second intervals across a mass-to-charge (m/z) range of 29–350. Peak identification was achieved by injecting standard solutions for compounds with available standards and comparing the mass spectra of the compounds without standards to those in flavor libraries (Wiley 10.0, NIST-11, and Flavor 2L). After the peak identification, the concentrations of the flavor constituents were calculated by using the internal standard method. The retention indices of the aroma compounds were calculated using the n-alkane series (C_5 – C_{30}) [22].

2.4.3. Determination of the Aroma-Active Compounds (AACs)

The aroma extract dilution analysis (AEDA) method was applied to determine the AACs. In this method, the concentrated aromatic extract was sequentially diluted with dichloromethane in a 1:1 ratio at each step (1:1, 1:2, 1:4, 1:8, ... 1:1024) and injected into the GC-MS-O system. The dilution process was terminated once the odor was no longer detectable, marking the completion of the analysis [9]. The flavor dilution (FD) value of an odorant is the highest dilution where it is still detectable at the sniffing port [23].

2.5. Sensory Analysis

The sensory analysis of the mastic gum samples was conducted by seven trained panelists (five females and two males between 23 and 35 years old) from the Department of Food Engineering at Cukurova University, Adana, Türkiye. The panelists were familiar with resin aromas and had training in odor recognition and sensory evaluation techniques. The samples, randomly served as coded with three-digit numbers, were placed in 25 mL brown glass bottles with caps. Each panelist smelled the samples and assessed their characteristics using a 100 mm unmarked scale. The results were statistically analyzed and presented in a spider web diagram.

2.6. Statistical Data Analysis

The data obtained from the analyses were subjected to a *t*-test by using the SPSS software (v.20.0, SPSS Inc., Chicago, IL, USA). The results were presented as means $\pm$ standard deviations.

3. Results and Discussions

3.1. Color Properties of the Mastic Gum Samples

The color parameters (L^* , a^* , b^* , C^* , and H°) of the mastic gum samples from different origins (Türkiye: MGT, Greece: MGG) are presented in Table 1. The L^* , a^* , and b^* values of the MGT sample were determined as 61.92, -0.93 , and 6.74 while those of the MGG sample were 47.36, -1.79 , and 27.23, respectively. The MGT sample exhibited a higher L^* value, indicating greater brightness, whereas the MGG sample showed higher a^* and b^* values, suggesting a more matte and yellowish appearance. Statistical analysis revealed significant differences ($p < 0.01$) between the L^* , a^* , and b^* values of the gum samples from two distinct geographical origins.

The C^* and H° color parameters were 6.80 and -82.14 for the MGT sample and 27.29 and -86.23 for the MGG sample, respectively (Table 1). A comparison of the two samples revealed that the MGT sample exhibited brighter and greener tones, while the MGG sample displayed a more pronounced yellow color. Statistical analysis indicated significant differences ($p < 0.01$) in the C^* and H° values of the two samples. The higher C^* value in the MGG sample suggested greater color saturation and intensity, resulting in an opaquer appearance. These differences in color characteristics may be related to the variations in harvest time, cultivation, and storage conditions, which can also influence the

overall composition of the samples. This study marks the first reporting of color difference in mastic gum samples from two different geographical origins.

Table 1. The color properties of the mastic gum samples from Türkiye (MGT) and Greece (MGG).

Color Properties	MGT	MGG	<i>p</i> ¹
<i>L</i> *	61.92 ± 0.06	47.36 ± 0.01	**
<i>a</i> *	−0.93 ± 0.01	−1.79 ± 0.02	**
<i>b</i> *	6.74 ± 0.11	27.23 ± 0.01	**
<i>C</i> *	6.80 ± 0.11	27.29 ± 0.01	**
<i>H</i> °	−82.14 ± 0.21	−86.23 ± 0.03	**

Results are presented as the means ± standard deviations of three replicates. ¹ Statistical significances according to the *t*-test; **: significant at *p* < 0.01.

3.2. Volatile Profile of the Mastic Gum Samples

The aroma profiles of the mastic gum samples from Türkiye (MGT) and Greece (MGG) were determined using the purge and trap method. Mastic gum usually possesses a unique aroma characterized by woody, fresh-greenish, and resinous odors [13]. The identified aroma constituents in both samples in the present study are listed in Table 2. In the MGT sample, 31 compounds were detected and identified, including 11 monoterpenes, seven monoterpeneoids, three alcohols, two sesquiterpenes, two aldehydes, one sesquiterpene oxide, one diterpenoid, one volatile phenol, one volatile acid, one ketone, and one ester. The MGG sample on the other hand included 33 constituents, including 10 monoterpenes, eight monoterpeneoids, five alcohols, two volatile phenols, two aldehydes, two ketones, one volatile acid, one sesquiterpene, one sesquiterpene oxide, and one diterpenoid (Table 2). The total concentration values of the aroma compounds were calculated as 4021 µg/kg for the MGT sample and 6427 µg/kg for the MGG sample. The MGG sample possessed more aroma substances in terms of both number and concentration (Table 2). The aroma profiles of the gum samples from the two different geographical origins were similar; however, their concentrations showed significant differences. The *t*-test results indicated that the geographical origin significantly affected the quantitative properties of the aroma compounds (*p* < 0.01).

3.2.1. Monoterpenes

Monoterpenes were the most dominant volatile composition detected in both mastic gum samples in the current study. It has been previously reported that the prenyltransferase enzyme activity present in the structure of plants plays a crucial role in the formation of the monoterpene aroma compounds during the growth and maturation stages of many plants [24]. The analysis results in the current study revealed 11 and 10 aroma constituents, with total concentrations of 3862.1 and 5815.8 µg/kg in the MGT and MGG samples, respectively (Table 2). Comparison of the two samples showed that the MGG sample had a higher concentration of monoterpenes compared to the MGT sample. Monoterpenes accounted for approximately 96% and 90% of the total aroma concentration in the MGT and MGG samples, respectively. Similarly, Dhouibi et al. [25] reported that the aroma profile of mastic gum oil consisted of 96.2% monoterpenes. In parallel to our results, previous studies indicated that monoterpenes were dominant in mastic gum and essential oils [11,17,18].

Table 2. The aroma profiles of the mastic gum samples from Türkiye (MGT) and Greece (MGG).

No	LRI ¹	Aroma Compounds	Concentration (µg/kg) ²		<i>p</i> ³	Identification ⁴
			MGT	MGG		
Monoterpenes						
1	1011	<i>α</i> -Pinene	2179.05 ± 126.24	4417.56 ± 422.71	**	LRI, MS, std
2	1057	Camphene	3.43 ± 0.08	30.28 ± 2.68	**	LRI, MS, std
3	1096	<i>β</i> -Pinene	82.51 ± 7.85	134.98 ± 11.77	**	LRI, MS, std
4	1113	Sabinene	13.23 ± 0.38	20.74 ± 0.51	**	LRI, MS, std
5	1167	<i>β</i> -Myrcene	1448.49 ± 76.38	1056.05 ± 91.49	**	LRI, MS, std
6	1196	Limonene	72.6 ± 6.98	73.13 ± 5.96	ns	LRI, MS, std
7	1212	<i>β</i> -Phellandrene	4.25 ± 0.42	3.06 ± 0.22	**	LRI, MS, std
8	1265	<i>p</i> -Cymene	1.93 ± 0.18	6.1 ± 0.43	**	LRI, MS, std
9	1431	Perillene	44.51 ± 2.43	14.62 ± 0.6	**	LRI, MS, std
10	1666	Citral	9.34 ± 0.25	59.23 ± 3.83	**	LRI, MS, std
11	1679	(<i>Z</i>)-verbenone	2.75 ± 0.14	nd	**	LRI, MS, std
		Total	3862.1	5815.8		
Monoterpenoids						
12	1497	Linalool	4.65 ± 0.3	44.71 ± 3.44	**	LRI, MS, std
13	1648	Myrtenal	0.62 ± 0.03	11.69 ± 0.55	**	LRI, MS, tent.
14	1651	(<i>E</i>)-Pinocarveol	3.56 ± 0.31	33.76 ± 2.9	**	LRI, MS, std
15	1669	(<i>E</i>)-Verbenol	4.89 ± 0.39	85.06 ± 1.02	**	LRI, MS, std
16	1763	Myrtenol	0.47 ± 0.02	16.66 ± 0.27	**	LRI, MS, tent.
17	1830	(<i>E</i>)-Anethole	5.66 ± 0.09	7.51 ± 0.04	**	LRI, MS, std
18	1836	(<i>E</i>)-Carveol	nd	13.01 ± 1.3	**	LRI, MS, std
19	1985	Perilla alcohol	2.49 ± 0.04	3.84 ± 0.18	**	LRI, MS, std
		Total	22.34	216.24		
Sesquiterpenes						
20	1585	<i>β</i> -Caryophyllene	66.42 ± 3.66	25.07 ± 0.47	**	LRI, MS, std
21	1667	<i>α</i> -Humulene	4.59 ± 0.45	nd	**	LRI, MS, std
		Total	71.01	25.07		
Sesquiterpene oxide						
22	1953	Caryophyllene oxide	8.74 ± 0.41	12.08 ± 1.2	*	LRI, MS, std
		Total	8.74	12.08		
Diterpenoid						
23	2551	Geranyl linalool	2.95 ± 0.16	12.57 ± 0.18	**	LRI, MS, std
		Total	2.95	12.57		
Alcohols						
24	1036	2-Methyl-3-buten-2-ol	10.74 ± 0.63	36.06 ± 0.95	**	LRI, MS, std
25	1152	3-Penten-2-ol	2.48 ± 0.23	69.34 ± 4.42	**	LRI, MS, std
26	1226	2-Hexanol	1.42 ± 0.01	21.33 ± 1.96	**	LRI, MS, std
27	1322	2-Methyl-2-buten-1-ol	nd	5.2 ± 0.12	**	LRI, MS, std
28	1480	2-Ethylhexanol	nd	6.46 ± 0.34	**	LRI, MS, std
		Total	14.64	138.39		
Volatile phenols						
29	1390	2-Methylanisole	25.69 ± 1.48	54.98 ± 4.04	**	LRI, MS, std
30	2126	Methyl isoeugenol	nd	2.33 ± 0.09	**	LRI, MS, tent.
		Total	25.69	57.31		
Volatile acid						
31	1452	Acetic acid	4.53 ± 0.26	60.3 ± 0.67	**	LRI, MS, std
		Total	4.53	60.3		
Aldehydes						
32	1447	<i>α</i> -Campolenal	1.63 ± 0.15	69.95 ± 1.23	**	LRI, MS, tent.
33	1514	Isoneral	3.63 ± 0.36	10.64 ± 0.65	**	LRI, MS, std
		Total	5.26	80.59		
Ketones						
34	1341	Sulcatone	1.98 ± 0.18	3.66 ± 0.19	**	LRI, MS, tent.
35	1610	2-Undecanone	nd	5.07 ± 0.1	**	LRI, MS, tent.
		Total	1.98	8.73		
Ester						
36	1499	Linalyl acetate	1.55 ± 0.15	nd	**	LRI, MS, std
		Total	1.55	0.00		
General total			4021	6427	**	

¹ Linear retention index (LRI) was calculated on DB-WAX capillary column. ² Concentrations are expressed in µg/kg $\pm$ standard deviation, based on the average of three replicates. nd: not detected. ³ Statistical significances according to the *t*-test; ns: not significant, *: significant, *p* < 0.05, **: significant, *p* < 0.01. ⁴ Identification methods; LRI: linear retention index; tent.: tentative identification by MS; std: identified using standard chemical substance.

Among the terpenes, α -pinene was present in the highest concentration in both gum samples (Table 2). Likewise, D'Auria and Racioppi [12] identified α -pinene as the major compound in mastic gum and its oil originating from Chios Island, Greece. A literature review revealed a limited number of studies that directly examined the aroma profile of the mastic gum samples [11,12,26,27]. In a study conducted by Zachariadis and Langioli [27], 26 and 34 compounds were determined in mastic gum and mastic oil, respectively, and α -pinene was the main compound in both samples, followed by β -myrcene, β -pinene, limonene, and caryophyllene. Similarly, Rigling et al. [11] analyzed the aroma and the AACS of mastic gums using GC-MS-O and found that α -pinene was the dominant compound, with minor components such as β -myrcene, limonene, β -linalool, and perillene. Koutsoudaki et al. [26] also reported that α -pinene was the major constituent in mastic gum while β -pinene, β -myrcene, limonene, and β -caryophyllene were present in smaller amounts. Some studies, however, focused on the aroma compounds of the volatile oil obtained through steam distillation from the mastic gum. Tabanca et al. [18] carried out a study on mastic gum oil originating from the Cesme district of Izmir province, Türkiye, and reported α -pinene as the most abundant monoterpene. Similarly, α -pinene was identified as the main compound in several studies on mastic gum oil [2,17,28].

Following α -pinene, β -myrcene was the second most dominant monoterpene, with concentrations of 1448 and 1056 $\mu\text{g}/\text{kg}$ in the MGT and MGG samples, respectively (Table 2). In several previous studies on the volatile oil of *P. lentiscus* var. Chia mastic gum, similar to our findings, α -pinene was the main compound, followed by β -myrcene and β -pinene [12,27,29,30]. In another study on the aroma substances in various mastic gum volatile oils [2], the ratios of α -pinene and β -myrcene were reported to range from 33.7–72.8% and 3.8–63.5%, respectively. The wide variation in these ratios was attributed to differences in the timing between the gum seeping from the tree trunk and its collection. Additionally, verbenone, α -humulene, sabinene, and (*E*)-anethole were detected in low amounts, similar to previous studies [2,31]. In the literature, β -pinene, camphene, limonene, β -phellandrene, *p*-cymene, perillene, and verbenone were reported in mastic gum oil studies, similar to the findings of the present study [28,31–33]. Finally, the citral compound was detected in the MGT sample but not in the MGG sample, marking the first identification of this constituent in *P. lentiscus* var. Chia mastic gum.

Some qualitative and quantitative differences determined in mastic gum and mastic oil have been attributed to factors such as geographical location, tree age, storage period, environmental conditions, soil physicochemical properties, and extraction method [7,17,18].

3.2.2. Monoterpenoids

Seven and eight monoterpenoids were detected in the MGT and MGG samples, with total concentrations of 22 and 216 $\mu\text{g}/\text{kg}$, respectively (Table 2). Linalool, (*E*)-pinocarveol, (*E*)-verbenol, (*E*)-anethole, myrtenal, myrtenol, and perilla alcohol were present in both mastic gum samples, whereas (*E*)-carveol was found only in the MGG sample. All monoterpenoids, except for perilla alcohol, were also reported in mastic gum and mastic oil in previous studies [26,27]. Among the monoterpenoids, (*E*)-anethole and (*E*)-verbenol were the most abundant in the MGT and MGG samples, respectively (Table 2). Similarly, linalool, myrtenol, and (*E*)-anethole have been reported in the *P. lentiscus* var. Chia mastic gum essential oil before [2,16,32]. Previous studies have also identified myrtenol, carveol, and pinocarveol in *P. lentiscus* L. mastic gum oil [31]. In addition, linalool, myrtenal, verbenol, and (*E*)-carveol were detected in the essential oil of mastic gum originating from Japan [28].

3.2.3. Sesquiterpenes

Sesquiterpenes were detected at the concentrations of 71 and 25 µg/kg in the MGT and MGG samples, respectively, in the present study (Table 2). Both β -caryophyllene and α -humulene were identified in the MGT sample while only β -caryophyllene was present in the MGG sample. Similarly, β -caryophyllene and α -humulene were previously reported in *P. lentiscus* var. Chia mastic gum and its essential oil from Chios Island, Greece [12,26,27,30,33].

3.2.4. Alcohols

Three and five alcohol compounds were identified in the MGT and MGG samples, respectively, in the current study (Table 2). Both samples contained 2-methyl-3-buten-2-ol, 3-penten-2-ol, and 2-hexanol. However, the MGG sample also included two additional alcohols, 2-methyl-2-buten-1-ol and 2-ethylhexanol, which were absent in the MGT sample. The most abundant alcohol was 2-methyl-3-buten-2-ol (11 µg/kg) in the MGT sample whereas 3-penten-2-ol (69 µg/kg) was the most dominant alcohol in the MGG sample. These alcohols detected in both samples were identified for the first time in the mastic gum of *P. lentiscus* var. Chia in the present study.

3.2.5. Other Compounds

Sesquiterpene oxide, diterpenoid, volatile phenol, volatile acid, ketone, and aldehyde compounds were detected in both MGT and MGG samples in addition to the monoterpenes, monoterpenoids, sesquiterpenes, and alcohols in the present study (Table 2). However, unlike the MGG sample, the MGT sample contained one ester compound (linalyl acetate). A previous study reported this constituent in lower-quality mastic oil, while high-quality oil had it only in trace amounts [16]. It was observed in the current study that caryophyllene oxide (a sesquiterpene oxide) and geranyl linalool (a diterpenoid) were present at higher concentrations in the MGG sample (Table 2). Caryophyllene oxide has also been identified in mastic gum and its essential oil in prior studies [10,12,28]. In addition, two volatile phenols, 2-methylanisole and methyl isoeugenol, were identified in the MGG sample, whereas only 2-methylanisole was detected in the MGT sample. Previous studies have similarly reported the presence of methyl isoeugenol in mastic essential oil [2,16,28,30]. Furthermore, methylanisole was found in mastic gum essential oil obtained through both hydrodistillation and supercritical extraction [10]. Earlier studies similarly reported this compound in mastic oils from Greece [19,27]. Acetic acid, a volatile acid, was present in both mastic gum samples in the present study, with a much higher concentration in the MGG sample (60.0 µg/kg) compared to the MGT sample (4.5 µg/kg). In a prior study, Rigling et al. [11] also reported acetic acid in mastic samples. Two ketones, sulcatone (6-methyl-5-hepten-2-one) and 2-undecanone, were identified in the MGG sample, while only sulcatone was detected in the MGT sample in the current study. Sulcatone was previously detected in trace amounts in the essential oils obtained from the *P. lentiscus* L. and *P. lentiscus* var. Chia grown in the Cesme district of Izmir province, Türkiye [18] while 2-undecanone was reported in mastic gum and its oil [27,29]. It was found that both mastic gum samples contained the aldehydes isoneral and α -campholenal in the present study. α -campholenal was also previously identified in the *P. lentiscus* var. Chia mastic oil [2,10,30]. Overall, these aroma groups, such as volatile phenols, volatile acids, ketones, and aldehydes, constituted a minor fraction of the mastic aroma. Geranyl linalool and isoneral were identified in mastic gum for the first time in this study.

Consequently, it was observed in the present study that the geographical region plays a significant role in determining the composition and aroma profile of mastic gums. Environmental factors such as temperature, humidity, light, and soil properties strongly influence

the aroma properties of the mastic gums from different origins [34,35]. In addition, after the harvest, physicochemical reactions such as oxidation, polymerization, and evaporation occur, altering the aroma profile of mastic gums. The environmental temperature, particularly during the formation of mastic gums on the tree trunk, can further accelerate these reactions, contributing to variations in its aroma composition [17,19,36].

3.3. Aroma-Active Compounds (AACs) of the Mastic Gum Samples

The AACs identified in the mastic gum samples, their associated odors, and flavor dilution (FD) values are provided in Table 3. The analysis revealed 10 and 17 AACs in the MGT and MGG samples, respectively, indicating a greater number of AACs in the MGG sample. The variation in the number of the AACs of the two samples is likely due to regional differences such as climate and soil composition, which highlights the significant influence of geographical factors on the AACs. The sensory analysis results obtained in the present study aligned with this observation, as the panelists described the odor of the MGG sample as more intense and resinous. To the best knowledge of the authors of the present article, only one study in the literature examined the characteristic odors of mastic resins using GC-MS-O [11]. As can be seen in Table 3, the MGT sample contained five monoterpenes, two monoterpenoids, one volatile phenol, one volatile acid, and one unknown compound (LRI: 1480), which could not be identified via GC-MS due to the peaks being too small and/or the mass/charge (m/z) ratios not being available in the MS library. On the other hand, the MGG sample included eight monoterpenes, four monoterpenoids, one sesquiterpene, one alcohol, one volatile phenol, one volatile acid, and one aldehyde.

The most active AAC in the MGT and MGG samples was identified as α -pinene (a monoterpene), with FD values of 256 and 512, respectively. This compound contributed resinous and forest-like odors to the samples. Similarly, in a previous study that identified the AACs in mastic gums obtained from Greece's Chios Island using two different aroma extraction techniques (Headspace Solid-Phase Microextraction (HS-SPME) and Headspace Sorptive Extraction (HS-SBSE)), α -pinene was found to be one of the most dominant AACs, imparting forest-like and resinous odors (with FD values of 256 and 128, respectively) [11]. Furthermore, α -pinene was also identified as an AAC in frankincense (*Boswellia sacra* Flueck.) in a prior study [37].

In the MGT sample, the most abundant AACs after α -pinene were identified as β -pinene (FD: 128) and β -myrcene (FD: 256), both being monoterpene compounds, along with linalool (FD: 256), a monoterpenoid compound (Table 3). These constituents were found to impart resinous, terpene-like; pine-like, greenish; and floral, fruity odors, respectively. Similarly, in the MGG sample, the most dominant AACs after α -pinene were β -myrcene (FD: 128), β -pinene (FD: 128), and linalool (FD: 128), respectively. The β -pinene compound has been identified before in various products, including mastic gums [11] and the fresh and roasted fruits of another *Pistacia* species, *P. terebinthus* [38], contributing resinous, piney, and terpene-like notes. β -Myrcene was identified in frankincense as an AAC [37], while linalool was associated with floral and sweet notes in previous studies [39].

Limonene, a monoterpene compound, was determined as an AAC in the current study (Table 3) with FD values of 32 and 16 in the MGT and MGG samples, respectively, giving a citrusy, fruity odor to the samples. Similarly, it was detected in the mastic gum sample obtained from Chios Island of Greece by Rigling et al. [11] and in frankincense gum by Niebler and Buettner [37]. Limonene is responsible for the characteristic aroma of many citrus fruits such as orange, tangerine, and lemon and it was also detected as the AAC in lemon juice [40] and bergamot essential oil [41]. Another monoterpene, perillene, was determined with FD values of 16 and 64 in the MGT and MGG samples, respectively, providing greenish, pea-like

odors to the mastic gum samples. In the study conducted by Rigling et al. [11], perillene was determined as one of the most dominant AACs in mastic gums.

Table 3. The aroma-active compounds of the mastic gum samples from Türkiye (MGT) and Greece (MGG).

No	LRI ¹	Compounds	Odor Descriptions	Flavor Dilution, FD ²	
				MGT	MGG
1	1011	α -Pinene	Forest like, resinous	256	512
2	1057	Camphene	Pungent, spicy, pine-like	nd	2
3	1096	β -Pinene	Resinous, terpene-like	128	128
4	1167	β -Myrcene	Pine-like, greenish	256	128
5	1196	Limonene	Citrusy, fruity	32	16
6	1265	<i>p</i> -Cymene	Citrusy	nd	2
7	1226	2-Hexanol	Wine like	nd	4
8	1390	2-Methylanisole	Woody, fresh	2	4
9	1431	Perillene	Greenish, pea like	16	64
10	1452	Acetic acid	Vinegar like	4	4
11	1447	α -Campolenal	Grassy	nd	4
12	1497	Linalool	Floral	256	128
13	1480	Unknown	Earthy	2	nd
14	1648	Myrtenal	Spicy, pungent	nd	16
15	1669	(<i>E</i>)-Verbenol	Pine-like	2	4
16	1679	(<i>E</i>)-Verbenone	Camphorous	nd	4
17	1953	Caryophyllene oxide	Woody	nd	4
18	1985	Perilla alcohol	Greenish	nd	8

¹ LRI: Linear retention index calculated on DB-WAX column. ² Flavor dilution factor was determined by the aroma extract dilution analysis (AEDA); nd: not detected.

In both MGT and MGG samples, one volatile phenol (2-methylanisole), one volatile acid (acetic acid), and one monoterpenoid ((*Z*)-verbenol) were identified as AACs (Table 3). The 2-methylanisole compound was found to impart woody, fresh odors to the mastic gums, also reported by Rigling et al. [11], who noted a woody aroma contribution in mastic gums. Additionally, this constituent was identified as an AAC in frankincense by Niebler and Buettner [37]. Acetic acid was found to contribute a vinegary odor to the samples, while (*Z*)-verbenol was associated with a pine-like aroma in the present study. Similar findings were reported by Rigling et al. [11], where both acetic acid and (*E*)-verbenol were identified as AACs in mastic gums. Furthermore, acetic acid has been reported as an AAC contributing to the odor in certain Citrus species [42].

In the MGG sample, unlike the MGT sample, three monoterpenes (camphene, *p*-cymene, (*E*)-verbenone), two monoterpenoids (myrtenal and perilla alcohol), one sesquiterpene (caryophyllene oxide), one alcohol (2-hexanol), and one aldehyde (α -campolenal) were identified as AACs in the present study (Table 3). Among these, (*E*)-verbenone, 2-hexanol, caryophyllene oxide, and perilla alcohol were identified as AACs in mastic gum for the first time. In an earlier study, compounds such as camphene (pungent, spicy, buttery), myrtenal (sharp, sweet), and α -campolenal (herbaceous, fresh) were also found to be AACs in the mastic gum samples, which is consistent with our findings [11]. *p*-Cymene and (*E*)-verbenone were identified as AACs in frankincense in a prior study, imparting fruity and spicy odors, respectively [37].

Most of the compounds identified in the mastic gum samples have pine-like, resinous, and pungent notes in the present study (Table 3) and the combination of these constituents is responsible for the characteristic odor of the mastic gum samples. Papanicolaou et al. [36] reported that the overall organoleptic quality of mastic essential oils is not dependent on a single aroma compound but is influenced by minor components in addition to the main

constituents. However, they also noted that these components change with the harvesting of mastic gums, thereby affecting their characteristic odors.

3.4. Sensory Analysis of the Mastic Gum Samples

The sensory properties of the MGT and MGG samples were evaluated by a panel of seven panelists, who examined the aroma profiles based on 100 mm scales without marks. Both samples were evaluated based on eight criteria: resinous, fruity, floral, pine-like, woody/earthy, vinegary/sourish, citrus-like, and green. The result of the sensory analysis is shown in Figure 1 as a spider web diagram. No statistically significant difference was found between the two samples in terms of odor properties such as green, citrus-like, floral, and fruity ($p > 0.05$). However, resinous, vinegar/sourish, woody/earthy, and pine-like attributes showed statistically significant differences ($p < 0.05$) according to the *t*-test results. When the samples were compared, it was found that the MGG sample received higher scores in resinous, green, pine-like, and woody/earthy properties compared to the MGT sample. It is believed that these properties were due to the aroma compounds of α -pinene, β -pinene, and β -myrcene found in the samples. Moreover, the (*Z*)-verbenol and (*E*)-verbenone, which were detected as AACs only in the MGG sample, and the perillene, which had a higher FD value (64), were thought to contribute to a more resinous, pine-like, and green odor in the MGG sample. On the other hand, the MGT sample was evaluated to have higher scores in terms of fruity, floral, vinegary/sour, and citrus properties compared to the MGG sample. The higher FD values of the limonene (FD: 32) and linalool (FD: 256), which were determined in the MGT sample through the olfactometric analysis, supported this observation.

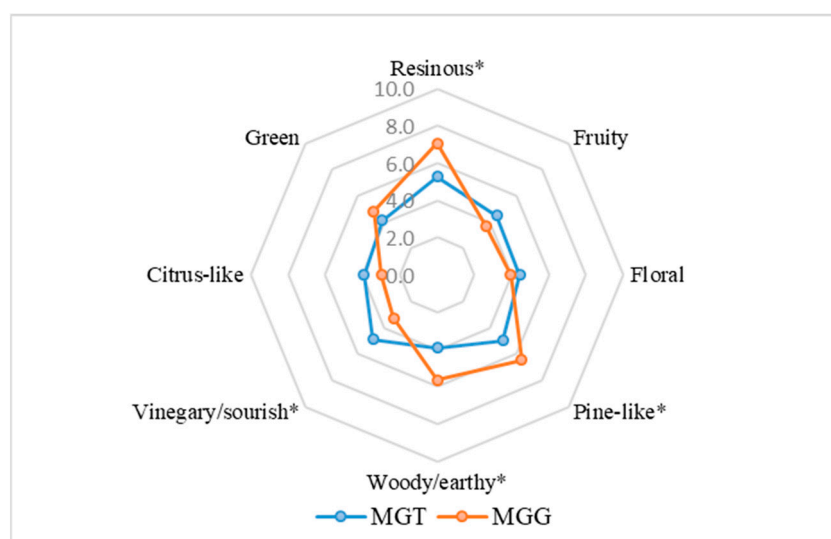


Figure 1. The sensory properties of the mastic gum samples from Türkiye (MGT) and Greece (MGG).
* Statistical significances ($p < 0.05$) according to the *t*-test.

4. Conclusions

This study investigated the influence of geographical region on the aroma and aroma-active compound (AAC) profiles of the mastic gum (MG) samples from Türkiye (MGT) and Greece (MGG). It was observed that the aroma profiles of the two samples were similar, but significant differences were observed in their concentrations. The identified aroma groups included monoterpenes, monoterpeneoids, sesquiterpenes, sesquiterpene oxides, diterpenoids, alcohols, volatile phenols, volatile acids, ketones, aldehydes, and esters. Among these, monoterpenes were the most dominant, with α -pinene as the major compound, followed by β -myrcene and β -pinene in both samples. The most abundant AAC in both samples was α -pinene, imparting resinous, forest-like odors. This was fol-

lowed by β -pinene (resinous, terpene-like), β -myrcene (pine-like, greenish), and linalool (floral, fruity), with high FD values. Sensory analysis results aligned with these findings, highlighting resinous, pine-like, vinegary-sour, and green odors as the most prominent sensory attributes. (*E*)-Verbenone, 2-hexanol, caryophyllene oxide, and perilla alcohol were identified as AACs in mastic gum samples for the first time. AEDA and sensory analysis results indicated that the MGT sample exhibited more pronounced floral and fruity notes, whereas the MGG sample was characterized by stronger resinous and pine-woody aromas. Depending on the desired aroma characteristics in different food applications, each sample may offer unique advantages. This study and a literature comparison elucidated that the aroma profiles and the AACs of the mastic gum samples were highly influenced by geographical origin. In conclusion, mastic gums, as plant-based natural resins, represent promising sources of volatile compounds that could be utilized to enhance the sensory quality and consumer acceptance of different food products.

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Abbreviations

The following abbreviations are used in this manuscript:

MG	Mastic gum
MGT	Mastic gum from Türkiye
MGG	Mastic gum from Greece
AEDA	Aroma Extract Dilution Analysis
GC	Gas Chromatography
MS	Mass Spectrometer
FID	Flame Ionization Detector
GC-MS-O	Gas Chromatography-Mass Spectrometry-Olfactometry
GC-O	Gas Chromatography-Olfactometry
AACs	Aroma-active compounds
FD	Flavor Dilution

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