

Comparison between the chemical composition of commercial products containing orange essential oil

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Abstract

Introduction: Natural ingredients have grown in popularity in recent years, surpassing synthetic alternatives. Among them, orange essential oil (EO) stands out as one of the most widely used essential oils (EOs) globally, primarily due to its significant health benefits. Traditionally incorporated into the human diet, orange EO is now extensively utilized in the perfumery, cosmetic, pharmaceutical, and food industries for its antioxidant, antimicrobial, anti-inflammatory, and immunostimulatory properties. Orange EO is mainly extracted from the peels of *Citrus sinensis* L. through cold pressing.

Aim: This study aimed to evaluate the phytochemical profile of various commercial products containing sweet orange essential oil and to assess their quality in comparison to standardization documents.

Materials and methods: Gas chromatography with mass spectrometry (GC-MS) was used to carry out the analysis of the essential oils. The separation of the volatile compounds was achieved with a custom temperature program, using a Zebtron ZB-5MSplus capillary column (30 m×0.25 mm i.d. and 0.25 µm film thickness), and helium was used as a carrier gas.

Results: The analysis revealed that D-limonene was the predominant compound across all tested samples, consistent with established standards for sweet orange oil. Other identified components included α-pinene, sabinene, β-pinene, *n*-octanal, *n*-nonanal, *n*-decanal, linalool, neral, valencene, and geranial.

Conclusions: The analysis revealed that most of the samples meet the requirements for D-limonene content, but the overall quality of the samples did not comply with international standards requirements.

Keywords

essential oils, *Citrus sinensis* L., D-Limonene, natural products, orange oil

Introduction

Sweet orange essential oil (EO) has recently become one of the most popular essential oils (EOs) across a wide range of industries. Historically, it has been utilized for a myriad of purposes, including the masking of odors, the preservation of various foods and beverages, the enhancement of human interactions, and a multitude of other objectives.^[1] Today, the citrus aroma of *Citrus sinensis L.* is widely employed across multiple sectors such as beverage production, cosmetics, and perfumery. This broad utilization is largely due to its rich content of volatile compounds.^[2] Oranges contain a wide range of bioactive compounds, including essential oils, soluble sugars, phenolic compounds, flavonoids, vitamins, and dietary fibers like pectin, cellulose, and hemicellulose. The EOs of the orange are primarily concentrated in the fruit's peel, with only trace amounts present in the leaves.^[3]

The orange EO can be extracted by various methods, such as hydrodistillation and the cold pressing method. In hydrodistillation, the citrus fruit's peels or leaves undergo steam distillation, which separates the volatile compounds from the plant material. After the condensation process, the EO is collected from the water-oil mixture. In contrast, cold pressing involves mechanically pressing the orange peels, typically at room temperature, without the use of heat. This process yields a mixture of emulsion and EO, which is then separated to obtain the final product.^[2]

Sweet orange EO is associated with a great variety of biological activities, including antioxidant activity, antidiabetic activity (it inhibits α -amylase and α -glucosidase enzymes)^[4], significant antibacterial activity against various bacterial strains, including *Staphylococcus aureus*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Salmonella typhimurium*, *Escherichia coli*, and *Pseudomonas aeruginosa*^[5,6], and antifungal activity^[5]. In recent years, the number of investigations on sweet orange EO has increased.^[7,8] It was reported that the EO exhibits anticarcinogenic potential by inducing apoptosis in human leukemia (HL-60) cells and

human colon cancer cells, as well as by inhibiting angiogenesis and metastasis.^[6] In rodent models, sweet orange EO suppressed pre-neoplastic hepatic lesions and reduced tumor incidence following carcinogen exposure, likely due to its ability to restore normal cell phenotype and upregulate junctional complexes.^[6] Inhalation of sweet orange EO benefits physiological and psychological relaxation.^[6] The main biological activities of sweet orange EO, which are due to its main component in the composition—D-limonene, are shown in **Fig. 1**.

The 2023 global citrus oil market is valued at 8.70 billion USD and is expected to expand and grow by 8.0% annually from 2024 to 2030.^[9] This positive trend also includes the sweet orange EO. The increasing need for natural ingredients in the food and beverage sector, cosmetics, and healthcare is driving this rapid growth. Moreover, the wide availability of citrus oil, its expanding range of applications, and increasing consumer awareness of its health and functional benefits are anticipated to further accelerate market demand.

Aim

The aim of the present study is to determine whether orange EOs available in Bulgarian markets meet the required standards.

Materials and methods

Sample preparation

Five commercial products labeled as containing pure sweet orange EO were purchased at random from Bulgarian pharmacies and stored according to instructions prior to the analyses. The tested samples were labeled S1, S2, S3, S4, and S5. The samples were diluted in hexane before being

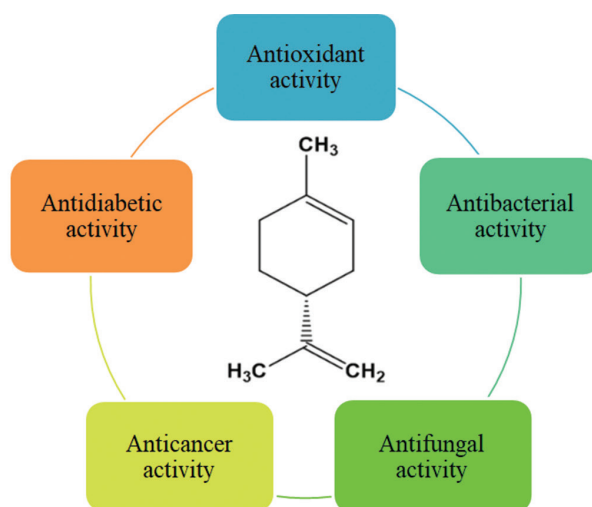


Figure 1. Biological activities of sweet orange essential oil.

analyzed using gas chromatography-mass spectrometry.

Chemicals and reagents

Retention indices (RI) were determined using the following hydrocarbons: nonane ($\geq 99\%$), decane ($\geq 99\%$), undecane ($\geq 99\%$), dodecane (99%), tridecane ($\geq 99\%$), tetradecane ($\geq 99\%$), and hexadecane ($\geq 99\%$), purchased from Merck KGaA (Darmstadt, Germany). Hexane (Thermo Fisher Scientific GmbH, Bremen, Germany) was used to dilute the essential oil.

Physicochemical analyses

Density measurements

The density was determined using a pycnometer. The pycnometer, which had been carefully cleaned previously, was rinsed with ethanol and dried. The density measuring bottle was thermally equilibrated for 20 minutes before weighing. The bottle was then filled with distilled water (to the measuring mark), tempered for 20 minutes, and weighed again. The pycnometer was emptied, rinsed with ethanol, then dried. The procedure described for distilled water was also performed for the essential oils (EOs). Each sample was given an average value based on three independent measurements.

Refractive index measurements

The refractive indices of all essential oil samples were determined using an Abbe refractometer (Carl Zeiss, model ORT 1RS, Jena, Switzerland), with a measurement error of $n_D \pm 0.0003$ units. The instrument was calibrated using distilled water ($n=1.333$). The samples were introduced into the refractometer's prism assembly with a syringe. The average value of three independent measurements was recorded for each sample.

Chromatographic conditions

Gas chromatography coupled with mass spectrometry (GC-MS) was used to analyze the samples. The separation of the volatile compounds was achieved with a Bruker Sci-on 436-GC SQ MS (Bremen, Germany), equipped with a Zebron ZB-5MSplus capillary column (30 m \times 0.25 mm i.d. and 0.25 μ m film thickness). Helium served as a carrier gas at a constant flow rate of 1.0 mL/min. The injection volume was 1 μ L. The injector temperature was set to 250°C and a split ratio of 1:10. The temperature program of the oven was as follows: initial temperature at 60°C, ramped to 110°C at 5°C/min, then further increased to 240°C at 15°C/min. The detector (ion source) temperature was maintained at 300°C. Mass spectra were recorded in full scan mode over a mass range of 50–350 m/z. Identification of EO constituents was based on comparison of their mass spectra and retention indices (RI) with those in the Wiley NIST11 Mass Spectral Library (NIST11/2011/EPA/NIH) and with literature data. Retention indices were calculated using the retention times

of a standard n-alkane series (C8–C20), analyzed under identical chromatographic conditions. Quantitative results were expressed as the percentage of the relative peak area, calculated as the average of three replicate measurements. The standard error of the mean was omitted, as it did not exceed 2%.

Results and discussion

The results obtained from the physicochemical analysis of the five samples (appearance, color, relative density, and refractive index) were compared with the International Organization for Standardization (ISO)^[10] and European Pharmacopoeia (Ph. Eur.)^[11] requirements, as shown in **Table 1**. The average value was calculated with a relative standard deviation (RSD) of less than 2% for each of the samples.

The comparative analyses performed and the results shown in the table led to the conclusion that all of the tested sam-

Table 1. Physicochemical requirements of sweet orange essential oil

Characteristics	S1	S2	S3	S4	S5
Appearance	✓	✓	✓	✓	✓
Color	✓	✓	✓	✓	✓
Relative density	✓	x	x	✓	✓
Refractive index	✓	✓	✓	✓	✓

ples met the European Pharmacopoeia^[11] and ISO^[10] requirements for EO appearance, color, and refractive index, while the S2 and S3 samples did not meet the relative density criteria.

Volatile constituents of orange essential oils

The chemical composition of commercial products containing orange essential oils (EOs) was evaluated using gas chromatography with mass spectrometry (GC-MS). A total of twenty-seven, twenty-nine, twenty-nine, twenty-two, and twenty-nine volatile compounds were identified in the EOs, representing 99.19%, 99.66%, 99.08%, 92.22%, and 97.07% of the total oil content for Sample 1, Sample 2, Sample 3, Sample 4, and Sample 5, respectively.

The chromatograms from the GC-MS analysis are presented in **Figs 2-6**. The chemical composition of the analyzed EOs is presented with retention indices, formulas, class of the compound, and % of the total EO in **Table 2**.

Monoterpene hydrocarbons (MH) are the dominant class of terpenes in orange EOs. The percentage of MH in the total oil content in all five samples was from 84.18% to 97.45%, with four of them being determined with concentrations above 90%, as shown in **Fig. 7**.

Compared to MH, the percentage content of the re-

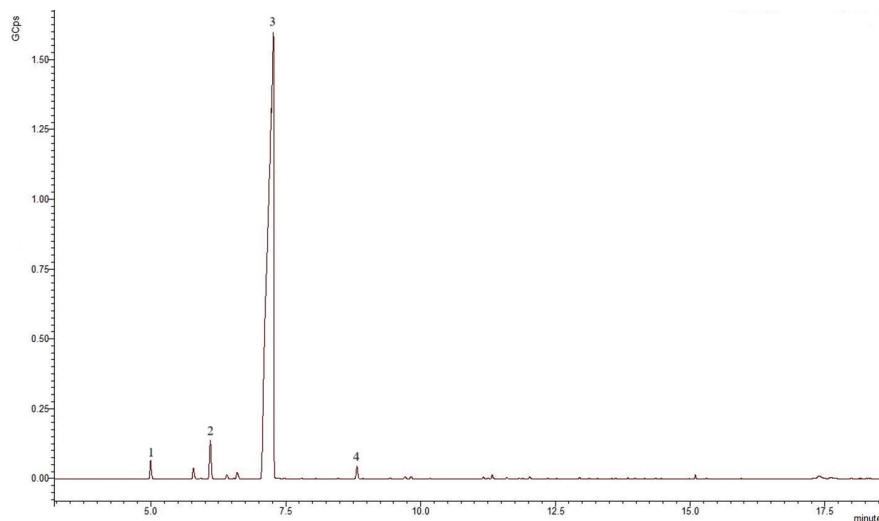


Figure 2. GC-MS chromatogram of Sample 1 (S1) EO, where MCps is Mega Counts per second, and the numbers refer to the following compounds: 1- α -pinene; 2- β -pinene; 3-D-limonene; 4-linalool.

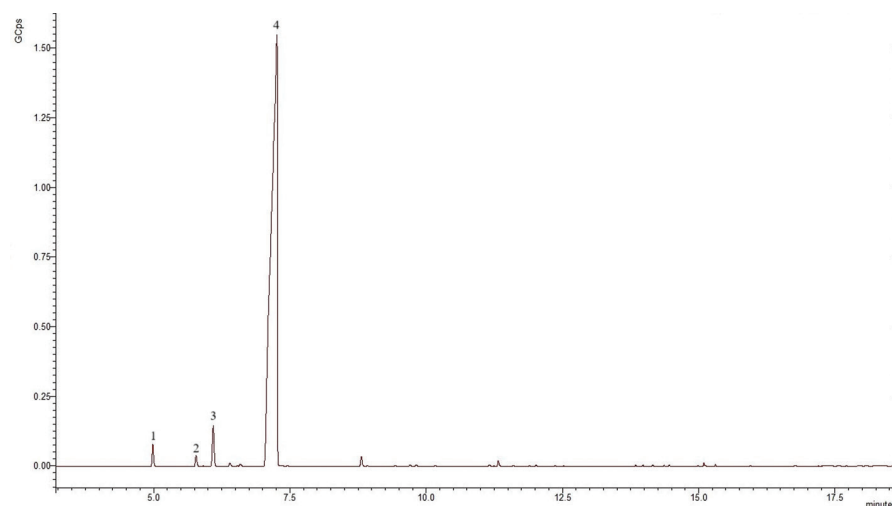


Figure 3. GC-MS chromatogram of Sample 2 (S2) EO, where MCps is Mega Counts per second, and the numbers refer to the following compounds: 1- α -pinene; 2-sabinene; 3- β -pinene; 4-D-limonene.

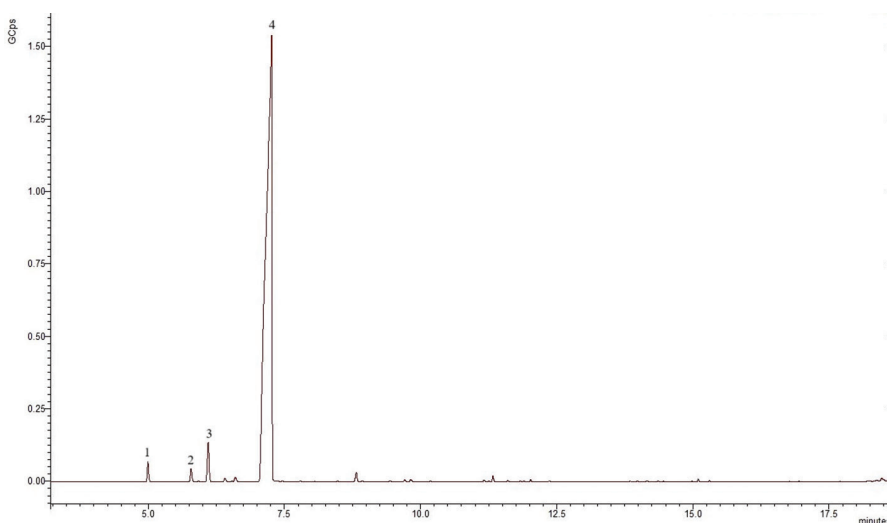


Figure 4. GC-MS chromatogram of Sample 3 (S3) EO, where MCps is Mega Counts per second, and the numbers refer to the following compounds: 1- α -pinene; 2-sabinene; 3- β -pinene; 4-D-limonene.

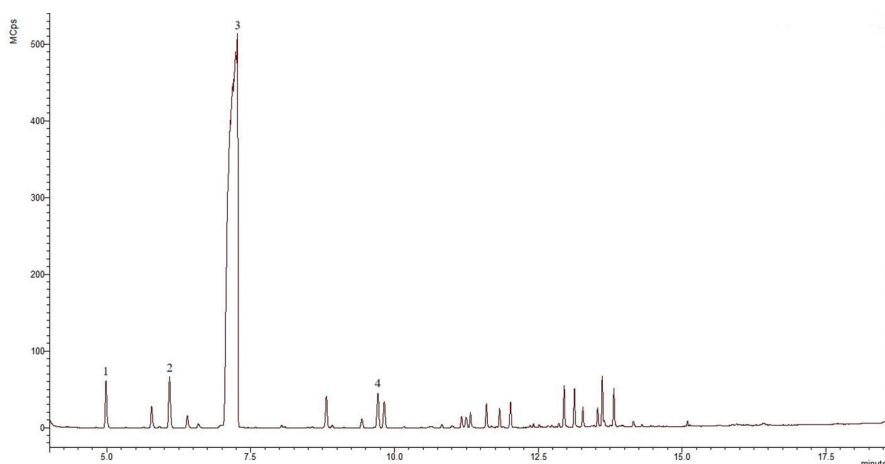


Figure 5. GC-MS chromatogram of Sample 4 (S4) EO, where MCps is Mega Counts per second, and the numbers refer to the following compounds: 1- α -pinene; 2- β -pinene; 3-D-limonene; 4-*trans-p*-menthae-2,8-dienol.

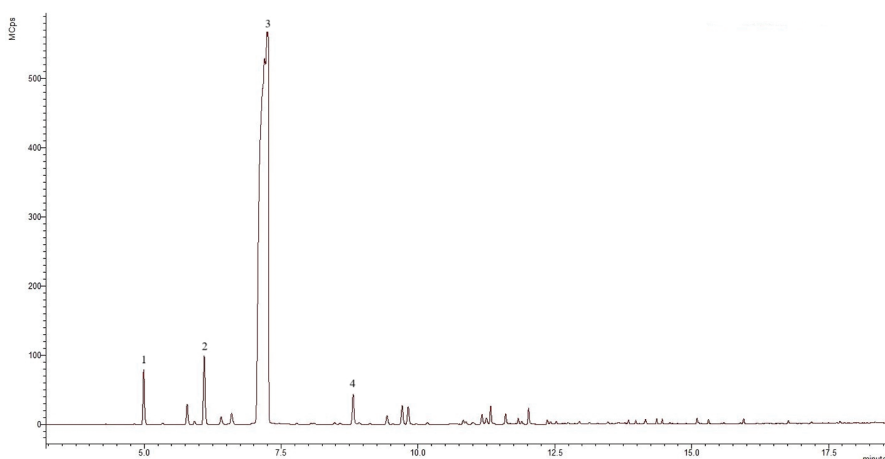


Figure 6. GC-MS chromatogram of the Sample 5 (S5) EO, where MCps is Mega Counts per second, and the numbers refer to the following compounds: 1- α -pinene; 2- β -pinene; 3-D-limonene; 4-linalool.

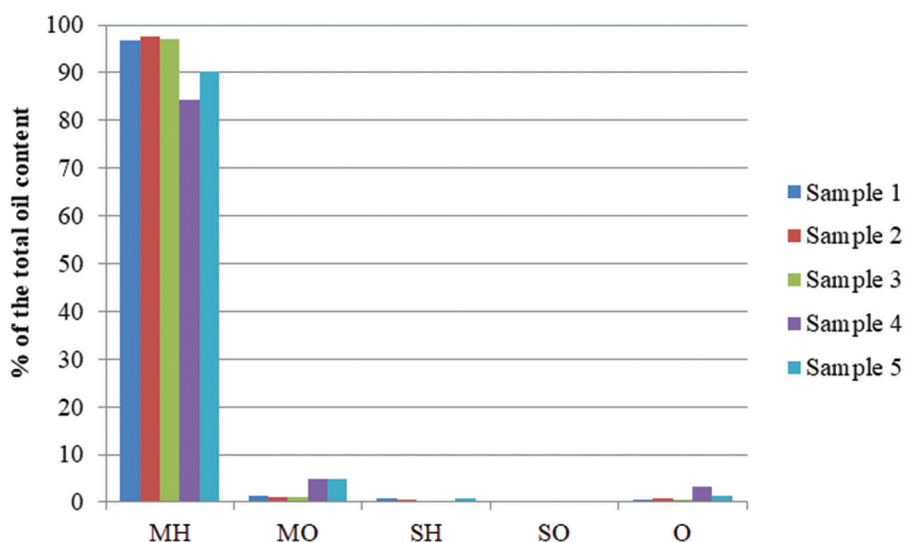


Figure 7. Main classes of volatile compounds in the analyzed samples, where MH: monoterpene hydrocarbons; MO: oxygenated monoterpenes; SH: sesquiterpene hydrocarbons; SO: oxygenated sesquiterpenes; O: others.

Table 2. Volatile constituents of commercial products containing orange EOs as a percentage of the total EO

Nº	Compound	RI	Formula	Class of compound	S1	S2	S3	S4	S5
1	α -Pinene	933	C ₁₀ H ₁₆	MH	0.96	1.11	1.06	1.73	2.28
2	Sabinene	972	C ₁₀ H ₁₆	MH	0.61	0.63	0.75	0.88	0.91
3	β -Pinene	983	C ₁₀ H ₁₆	MH	2.41	2.55	2.50	2.26	3.45
4	Octanal	1002	C ₈ H ₁₆ O	O	0.29	0.24	0.25	0.52	0.40
5	α -Phellandrene	1007	C ₁₀ H ₁₆	MH	0.04	0.05	0.05	-	-
6	3-Carene	1010	C ₁₀ H ₁₆	MH	0.47	0.18	0.33	0.21	0.58
7	D-Limonene	1036	C ₁₀ H ₁₆	MH	92.08	92.91	92.22	79.1	82.83
8	γ -Terpinene	1058	C ₁₀ H ₁₆	MH	0.03	-	0.03	-	-
9	1-Octanol	1068	C ₈ H ₁₈ O	O	-	-	-	1.36	0.06
10	α -Terpinolene	1083	C ₁₀ H ₁₆	MH	0.04	0.02	0.04	-	0.09
11	Linalool	1099	C ₁₀ H ₁₈ O	MO	0.72	0.60	0.59	0.11	1.45
12	<i>n</i> -Nonanal	1101	C ₉ H ₁₈ O	O	-	0.04	0.04	0.39	0.08
13	<i>trans-p</i> -menthae-2,8-dienol	1112	C ₁₀ H ₁₆ O	MO	0.07	0.05	0.06	1.47	0.42
14	Limonene oxide	1118	C ₁₀ H ₁₆ O	MO	0.15	0.09	0.12	1.13	0.88
15	Citronellal	1134	C ₁₀ H ₁₈ O	MO	0.03	0.06	0.05	-	0.09
16	Terpinen-4-ol	1177	C ₁₀ H ₁₈ O	MO	-	-	-	-	0.10
17	α -Terpineol	1196	C ₁₀ H ₁₈ O	MO	0.11	0.09	0.10	0.46	0.40
18	<i>n</i> -Decanal	1206	C ₁₀ H ₂₀ O	O	0.18	0.32	0.31	0.78	0.68
19	<i>cis</i> -Carveol	1223	C ₁₀ H ₁₆ O	MO	0.07	0.04	0.06	0.05	0.43
20	Citronellol	1228	C ₁₀ H ₂₀ O	MO	-	-	-	0.04	-
21	Neral	1241	C ₁₀ H ₁₆ O	MO	0.04	0.03	0.04	-	0.10
22	Carvone	1249	C ₁₀ H ₁₄ O	MO	0.12	0.06	0.10	0.07	0.58
23	Geranial	1270	C ₁₀ H ₁₆ O	MO	0.03	0.04	0.06	0.11	0.20
24	Perillal	1280	C ₁₀ H ₁₄ O	MO	0.03	0.03	0.02	0.07	0.10
25	Perillol	1302	C ₁₀ H ₁₆ O	MO	-	-	-	1.27	-
26	Undecanal	1307	C ₁₁ H ₂₂ O	O	-	0.02	-	-	-
27	α -Copaene	1382	C ₁₅ H ₂₄	SH	0.04	0.06	0.04	-	0.09
28	β -Cubebene	1393	C ₁₅ H ₂₄	SH	0.03	0.06	0.04	-	0.11
29	Dodecanal	1407	C ₁₂ H ₂₄ O	O	0.02	0.06	0.05	0.06	0.13
30	Caryophyllene	1422	C ₁₅ H ₂₄	SH	0.03	0.04	0.03	-	0.13
31	β -Copaene	1449	C ₁₅ H ₂₄	SH	0.43	0.05	0.06	0.12	0.12
32	Valencene	1476	C ₁₅ H ₂₄	SH	0.13	0.14	0.02	0.03	0.12
33	δ -Cadinene	1492	C ₁₅ H ₂₄	SH	0.03	0.06	0.04	-	0.10
34	Caryophyllene oxide	1598	C ₁₅ H ₂₄ O	SO	-	0.03	0.02	-	0.16
Terpene classes									
Monoterpene hydrocarbons (MH)					96.64	97.45	96.98	84.18	90.14
Oxygenated monoterpenes (MO)					1.37	1.09	1.2	4.78	4.75
Sesquiterpene hydrocarbons (SH)					0.69	0.41	0.23	0.15	0.67
Oxygenated sesquiterpenes (SO)					-	0.03	0.02	-	0.16
Others					0.49	0.68	0.65	3.11	1.35
Total identified (%)					99.19	99.66	99.08	92.22	97.07

maining terpene classes is significantly lower: oxygenated monoterpenes (MO) – 1.09%–4.78%, sesquiterpene hydrocarbons (SH) – 0.15%–0.69%, oxygenated sesquiterpenes (SO) – 0.02%–0.16%, and other compounds – 0.49%–3.11%.

Some of the most important international reference standards for assessing the quality of orange essential oil are currently ISO 3140:2019^[10] and the European Pharmacopoeia^[11]. In this study, the quality of the analyzed samples was assessed by comparing them to the specifications outlined in the reference standards.

According to the International Organization for Standardization (ISO) requirements, the major compound of orange EO should be D-limonene (MH) in an amount over 93.0%.^[10] Sample 1 (S1), Sample 2 (S2), and Sample 3 (S3) almost meet these requirements, as shown in **Table 2**. However, the amount of D-limonene in Sample 4 (S4) and Sample 5 (S5) is significantly lower—79.1% and 82.83%, respectively. Other MH present in the EOs composition are α -pinene, sabinene, β -pinene, and *n*-octanal. Accordingly, the content of α -pinene in all five analyzed essential oils exceeds the permissible amount, while the content of β -pinene is considerably above the levels specified in the ISO standards requirements.^[10]

Linalool is the main MO in the composition of S1 (0.72%) and S5 (1.45%), while in S4 the main MO is *trans-p*-menthae-2,8-dienol (1.47%). The concentrations of other MO, such as neral and geranial, are in accordance with the requirements.

The representative of SH in a higher percentage in S2 is valencene – 0.14%, while in S1, S3 and S4 is β -copaene – 0.06%–0.43%, respectively. In S5, β -copaene and valencene were found in the highest percentage and in equal amounts – 0.12%. The limit for nonanal (O), conforming to ISO, is up to 0.06%^[10], with S4 and S5 having a higher amount (0.39% and 0.08%). Another characteristic compound of orange EO is *n*-decanal (O), the content of which is from 0.18% to 0.78%.

The permissible content of orange EO components in the composition, according to the European Pharmacopoeia^[11], as well as their comparison with the examined commercial products, is presented in **Table 3**.

Table 3 shows that the percentage content of α -pinene and β -pinene does not meet the required specifications. These structural isomers are associated with different biological activities, such as antioxidant, anti-inflammatory, analgesic, antimicrobial, antiviral, and fungicidal activity.^[12] However, pinenes are characterized by irritant effects and also could cause sedation and induce anesthesia but do not irritate the respiratory system. The D-enantiomers of pinenes exhibit different potencies and are considered one of the most sensory irritants known.^[13]

The results of the current study reveal the need for obligatory analytical control before marketing of products containing EOs. Although the regulation of essential oils (EOs) in cosmetic products within the EU is already complex and detailed, there is still room for improvement to enhance consumer safety. For instance, requiring obligatory chemical composition analyses for each batch in accordance with Good Laboratory Practice (GLP) standards would ensure higher product consistency and safety. While these enhanced quality control measures would undoubtedly benefit consumers, they would also lead to increased production costs for manufacturers.^[14]

The main compound in all five EO samples is D-limonene, although its concentration in S4 and S5 is less than the required levels. D-Limonene (or 4-isopropenyl-1-methylcyclohexene) is an active form of limonene, which is present in high concentrations in citrus EOs and spices. However, the most common dietary source of D-limonene is sweet orange peel oil (90%–95%).^[15] D-limonene is regarded as a safe compound, making it commonly utilized as a flavoring agent in the food sector, as well as a fragrance agent in the cosmetic industry.^[15,16] However, irritation and rashes may be observed with topical application as a result of the oxidized forms of D-limonene.^[17] Also, the generated free

Table 3. Comparison of the sample's composition to the international standards requirements

Concentrations in the required range for:	S1	S2	S3	S4	S5
α -Pinene	x	x	x	x	x
β -Pinene	x	x	x	x	x
Sabinene	✓	✓	✓	✓	✓
β -Myrcene	Not detected	Not detected	Not detected	Not detected	Not detected
Limonene	✓	✓	✓	x	x
Octanal	✓	✓	✓	x	✓
Decanal	✓	✓	✓	x	x
Linalool	✓	✓	✓	✓	x
Neral	✓	✓	✓	Not detected	✓
Valencene	✓	✓	✓	✓	✓
Geranial	✓	✓	✓	✓	✓

radicals from the reactions that occur between limonene and ozone, with the formation of organic aerosols, can cause respiratory tract sensitivity.^[17] Hepatotoxicity has been observed with prolonged intake of limonene in high doses^[17], and nephrotoxicity was only found in male rats, due to α_2 -globulin (a specific protein)^[17].

Other research has also found lower levels of D-limonene, as well as higher levels of α -pinene, β -pinene, and myrcene. A study was conducted on the chemical composition of sweet orange EO extracted from store-bought fruits, with the main component being MH—91.74% of the total oil content. The amount of D-limonene was 77.49%, α -pinene – 1.49%, and β -pinene – 0.41%. From the MO, the quantity of linalool was 0.22%.^[5] Marjana Radünz et al. reported that the main components in commercial orange EOs were D-limonene (96.0%) and β -myrcene (4.0%).^[4] The results of the GC-MS analysis conducted by Araújo et al. showed D-limonene as a prevalent constituent—96.02%. Other compounds were also identified in concentrations under 2.23% (α -pinene, linalool, n-octanal, n-decanal, sabinene, etc.).^[8]

Conclusion

In recent years, a positive growth trend has been reported in the global orange oil market. The rising demand for natural ingredients in the food and beverage industry, especially for enhancing nutritional value, is driving this rapid growth. Moreover, the wide availability of orange essential oil, its expanding applications, and growing consumer awareness of its health and functional benefits are expected to further drive market demand.

The analytical control of orange essential oil is crucial to ensure consumer safety and product integrity. The present study aimed to evaluate the phytochemical profiles of commercial products containing orange EO. The analyses revealed that in most of the tested samples, the major constituent of the sweet orange peel EO, i.e., D-limonene, meets the requirements specified in the standardization documents. However, the content of some of the minor components of the EO, such as α -pinene and β -pinene, was higher than the percentage content set in the standards, such as those defined by ISO 3140:2019 and the European Pharmacopoeia. This may reflect negatively on the overall quality of these commercial products.

Conflicts of interest

The authors declare no conflicts of interest.

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Author contributions

Conceptualization: S.I. and Z.D.; methodology: S.I., Z.D., and Y.G.; software: S.I. and Z.D.; formal analysis: S.I. and Z.D.; investigation: S.I. and Z.D.; resources: S.I. and Z.D.; data curation: S.I. and Z.D.; writing—original draft preparation: Z.D., S.I., N.K., and T.D.; writing—review and editing: S.I. and Z.D.; visualization: S.I. and Z.D.; supervision: S.I. All authors have read and agreed to the published version of the manuscript.

Data availability statement

Data are contained within the article.

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