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Research article

Effects of cultivar, harvesting time and isolation techniques on the essential oil compositions of some lemon cultivars

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Abstract: In this study, Batem Pınarı, Interdonato, Meyer, and Ak Limon lemon cultivars were studied. Lemon peel's essential oils were obtained by two different methods (hydrodistillation and cold pressing) during four different harvest periods for each cultivar. Essential oil content, density, refractive index, optical activity, and composition were evaluated. The highest essential oil amount was found in the Interdonato cultivar (2.54%) and the lowest in Ak Limon (1.37%). The highest density value was 0.8471 g/mL (Ak Limon) and the lowest was 0.8423 g/mL (Meyer). Essential oil densities obtained by cold pressing were higher than those obtained by hydrodistillation. The highest refractive index values were determined for Batem Pınarı and Meyer (1.4747), and the lowest were determined for Ak Limon (1.4740). The refractive index values obtained by cold pressing were higher than those obtained by hydrodistillation. Optical activity values were found to be highest in Ak Limon and lowest in Batem Pınarı, and higher following hydrodistillation than cold pressing. The essential oil compositions of the samples showed significant differences depending on the cultivar and isolation method. Limonene, the highest component proportionally, composed 76.0%–89.0% of samples. The highest limonene content was determined for Ak Limon (88.7%), and the lowest for Batem Pınarı (76.7%). Limonene content did not change significantly between hydrodistillation (82.2%) and cold press (82.2%) isolation methods. Findings show that there is significant variation in quality parameters of lemon peel essential oils.

Keywords: *Citrus limon*; cultivar; peel essential oil; harvesting time; isolation method

1. Introduction

Essential oils are generally obtained from the leaves, fruits, bark, or roots of plants. These are natural products that are present in liquid form at room temperature, can easily crystallize, are usually colorless or light yellow in color, and have a strong and aromatic odor [1]. Essential oils consist of a complex mixture of fragrant and volatile components found in secondary plant metabolism. Most of the components found in their structures are terpenoids, monoterpenes, and sesquiterpenes [2–4]. *Citrus* oils have an important place among essential oils. It is reported that the global *Citrus* oil production is approximately 16,000 tons and the global price is approximately 14,000 USD/ton [5].

Although Turkey holds an important position in the production of citrus fruits, it generally relies on imports for *Citrus* peel oils. However, Turkey has the potential to produce these oils domestically. Citrus peel oils are among the most significant essential oils imported by Turkey. According to 2022 data, the total value of essential oil imports was 31,783,450 USD, with approximately 30% of this value consisting of citrus peel oils. When examining the total essential oil trade, excluding citrus fruits, the export value of 29,599,149 USD in 2022 surpasses the import value of 21,795,022 USD [6]. These data highlight the importance of domestic production of these products for the Turkish economy. Citrus essential oils are listed on the GRAS (Food Generally Recognized as Safe) list and are known for their broad-spectrum biological activities, including antimicrobial, antifungal, antioxidant, anti-inflammatory, and anxiolytic [7–10]. Citrus peel oils can be obtained by hydrodistillation or cold pressing method [11–13] and are used in many areas such as cosmetics, perfumery, pharmaceutics, production of cleaning products [14–17], and the food industry [18–21].

The most important feature of citrus (orange, mandarin, bergamot, bitter orange) peel essential oils is their high limonene content, which varies widely from 36.54% to 96.10% [12]. Limonene is used on an industrial scale in many areas such as food, medicine, and cosmetics [22].

In Turkey, which has a major potential in terms of raw materials, the production of such products is significant for the country's economy. In addition, the utilization of citrus peels, which can be seen as waste, can also contribute to the development of the producer and processing industry. The quality of the obtained product will be determined by the *Citrus* cultivar, the harvesting time, and the processing method.

The four cultivars studied in this experiment are very popular and appreciated in Turkey. For this reason, there is great interest in the physico-chemical composition of different parts of the fruit and its derivatives. The techniques and varieties of lemon cultivation were selected based on previous experiments, hoping to obtain a product with a more valuable organoleptic composition. This study aimed to reveal the characteristics and essential oil composition of lemon peel oils obtained by two different methods in four different harvest periods for a total of four cultivars, which have an important place among *Citrus* fruits.

2. Materials and methods

2.1. Plant material

This research was carried out between 2021 and 2023 in the Aksu-central unit of the Batı Akdeniz

Agricultural Research Institute (Antalya, Turkey). Four lemon (*Citrus limon*, L) cultivars were used in the research. Each commercial cultivar was harvested in two production seasons (2021–2022 and 2022–2023) covering four different harvest periods (Table 1). The products were obtained from the *Citrus* parcels of the Kayaburnu unit of the Batı Akdeniz Agricultural Research Institute. During the harvesting process, care was taken to take samples from four different components of each tree. The harvested fruit samples were brought to the Food Technology and Medicinal Plants Laboratory on the same day and analysis was started.

Table 1. Lemon cultivars and harvest times.

Harvest	Batem Pınarı	Interdonato	Meyer	Ak Limon
1	01 Sep 2021/2022	01 Sep 2021/2022	20 Oct 2021/2022	20 Feb 2022/2023
2	20 Sep 2021/2022	20 Sep 2021/2022	10 Nov 2021/2022	10 Mar 2022/2023
3	10 Oct 2021/2022	10 Oct 2021/2022	30 Nov 2021/2022	30 Mar 2022/2023
4	30 Oct 2021/2022	30 Oct 2021/2022	20 Dec 2021/2022	20 Apr 2022/2023

First, fruit weight and peel ratio were analyzed. For this purpose, 10 fruits were used for each repetition, and each fruit and its peels were weighed to an accuracy of 0.01 g. Fruit weight and peel ratio were given by taking the average of all measurement values.

2.2. Hydrodistillation (HD) process

Essential oil production from fruit peels was carried out using two different methods. For the hydrodistillation process, the Clevenger apparatus was used, according to TS EN ISO 6571 [23]. For this, 200 mL of distilled water was added to 50 g of fresh fruit peel. The mix was homogenized (1 min, 25 °C, 22,000 rpm) with a blender (Waring 8011ES, Model HGB2WTS3, USA) and then subjected to distillation using a Clevenger device (Isotex, Turkey) for 3 h. The amount of essential oils was given by the volume based on the weight of fresh fruit peel (mL/100 g, %). TS EN ISO 6571 Turkish Standard is identical to the relevant ISO standard.

2.3. Cold press (CP) process

The cold press method, which is also used in commercial production, was also used. The amount of essential oils was determined according to Kırbaslar et al. [24]. For this purpose, the flavedo part of the fruit peels, which is rich in essential oils, was grated and then subjected to manual pressing with a 10 cm diameter seven-hole kitchen-type hand press. The resulting water–essential oil (volatiles) mixture was then separated by centrifugation at 15,294 × g for 20 min at 20 °C. The amount of essential oils was given by the volume based on the weight of fresh fruit peel (mL/100 g, %).

2.4. Density, refractive index, and optical rotation

The essential oils obtained were analyzed for density, refractive index, optical rotation, and essential oil composition, which are among the basic quality analyses specified in the European Pharmacopoeia. Density analyzes of the samples were determined according to the Turkish Standards Institute method of determining the density of essential oils using a capillary tube TS ISO 279 [25].

Refractive index analyses were carried out according to TS ISO 280 [26]. Measurements were made at 20° using a digital refractometer (A. Krüss Optronic GmbH. DR6000). Optical rotation values were determined according to TS ISO 592 at 589.44 nm [27] using a polarimeter device (Optical Activity Ltd. PolAAR 31). TS ISO 279, TS ISO 280, and TS ISO 592 standards are identical to the relevant ISO standards and are used as the Turkish Standard.

2.5. Essential oil composition

The composition of essential oils (%) was determined by a gas chromatography (Agilent 7890A)-mass spectrometry (Agilent 5975C)-flame ionization detector (GC-MS/FID) device [28]. For this purpose, samples were diluted with hexane at a ratio of 1:50. Essential oil component analysis of the samples was performed using a capillary column (HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 µm). Helium was used as the carrier gas at a flow rate of 0.8 mL/min. Samples were injected at 1 µL with a split ratio of 50:1. The injector temperature was set to 250 °C. The column temperature program was set to 60 °C (10 min), 20 °C/min from 60 to 250 °C, and 250 °C (10.5 min). In line with this temperature program, the total analysis time was 60 min. For the mass detector, scanning range (m/z) 35–500 atomic mass units and electron bombardment ionization 70 eV were used. WILEY and OIL ADAMS libraries were used to identify the components of the essential oils. Relative retention indices (RRI) of the compounds were determined relative to the retention times of a series of C8–C40 n-alkanes (Sigma, USA). Relative ratio amounts (%) of the determined components were calculated from FID chromatograms without normalization.

2.6. Statistical analyses

The research was carried out with three replicates according to the randomized parcel trial design [29]. Analyses were carried out in two parallels and results were subjected to variance analysis (ANOVA) and Duncan multiple comparison test using the SAS package program. Results are given as mean ± standard error.

3. Results

The average values of fruit weights, fruit peel ratios, and peel essential oil amounts of the four lemon cultivars are given in Table 2. It was observed that the fruit weights generally increased, partially depending on the harvest time. According to fruit weight, the most suitable harvest time for Meyer and Interdonato cultivars was the third and fourth harvest period, while the fourth harvest time for Batem Pınarı and the second harvest time for Ak Limon were found to be the most suitable. Among lemon varieties, Ak Limon differs significantly from other varieties with its high peel rate (30.13%). Depending on the harvest time, the peel ratios varied among the varieties and were distributed within a narrower range.

The essential oil amounts of the samples were obtained by two different methods, hydrodistillation and cold pressing, and the essential oil amounts were evaluated based on the values determined by the first method (Table 2). The effects of cultivar and harvest time and the interaction between variety and harvest time on the essential oil amount were statistically significant. The Interdonato cultivar had the highest amount of essential oils, followed by Batem Pınarı, Meyer, and

Ak Limon varieties. The highest amount of essential oils was detected in the samples taken at the second harvest time. However, this differs depending on the cultivar. The highest essential oil content was detected in the Interdonato variety obtained in the third harvest period. Batem Pınarı and Meyer varieties had the highest essential oil content in the second harvest period, and Ak Limon in the fourth harvest period (Table 2).

Table 2. Fruit weight, peel ratio, and essential oil amounts of lemon cultivars according to harvest times (mean ± standard error).

Cultivar	Harvest	Fruit weight (g/fruit)	Peel ratio (%)	Essential oil content (%) by CP	Essential oil content (%) by HD
Batem Pınarı	1	103.13 ± 2.935	21.12 ± 0.770	0.21 ± 0.070	1.98^{c} ± 0.195
	2	119.53 ± 8.865	21.19 ± 1.910	0.24 ± 0.030	2.47^{b} ± 0.186
	3	130.41 ± 1.845	18.33 ± 1.325	0.26 ± 0.075	1.99^{c} ± 0.035
	4	170.12 ± 13.230	19.62 ± 0.385	0.24 ± 0.050	1.98^{c} ± 0.144
Interdonato	1	98.34 ± 2.940	19.43 ± 0.380	0.26 ± 0.030	2.60^{b} ± 0.236
	2	118.16 ± 10.365	19.56 ± 0.375	0.29 ± 0.000	2.72^{ab} ± 0.142
	3	149.08 ± 23.175	18.22 ± 1.780	0.31 ± 0.005	3.12^{a} ± 0.073
	4	148.95 ± 22.180	19.92 ± 0.080	0.18 ± 0.050	1.70^{cd} ± 0.058
Meyer	1	89.85 ± 10.075	19.71 ± 0.210	0.15 ± 0.005	1.23^{e} ± 0.023
	2	115.96 ± 18.100	19.30 ± 0.430	0.17 ± 0.040	1.71^{cd} ± 0.131
	3	139.42 ± 7.385	20.28 ± 0.850	0.16 ± 0.030	1.48^{de} ± 0.144
	4	139.96 ± 3.035	20.09 ± 0.920	0.18 ± 0.005	1.29^{de} ± 0.131
Ak Limon	1	108.29 ± 5.555	30.07 ± 2.400	0.32 ± 0.080	1.49^{de} ± 0.038
	2	149.69 ± 5.760	29.19 ± 2.855	0.28 ± 0.050	1.32^{de} ± 0.116
	3	126.93 ± 6.505	31.77 ± 4.500	0.22 ± 0.075	1.21^{e} ± 0.172
	4	137.57 ± 2.245	29.48 ± 1.915	0.24 ± 0.020	1.47^{de} ± 0.181
F-value					17.64
p-value					0.0001
CV*					13.03

Different letters in the same column indicate a difference between the means at the $p < 0.05$ level. *Coefficient of variation.

Di Vaio et al. [30] found that the amount of essential oils in 18 lemon varieties ranged from 1.90% to 2.28%. Bourgou et al. [31] found that the amount of lemon peel essential oil varied (0.48%–1.30%) according to the harvest time. Vekiari et al. [32] also reported that the harvest time had a significant effect on the amount of lemon peel essential oils. Our research findings have shown a significant effect of cultivar and harvest time. Regarding the isolation method, the essential oil rate in the peels obtained by the hydrodistillation method was considerably higher (1.86%) than by the cold press method (0.23%). This was expected; Ferhat et al. [33] have shown that although there were differences depending on species and varieties, the yield obtained by cold pressing was significantly lower than by hydrodistillation. Mahato et al. [14] also reported that the hydrodistillation method is quite advantageous compared to the cold press method in terms of efficiency in the production of *Citrus* peel oil. In fact, as seen in the analyses, a significant amount of essential oils remains in the peel waste obtained from industrial cold press applications.

The density, refractive index, and optical activity values of essential oils obtained from lemon peels were also analyzed. ANOVA and Duncan test results for the four lemon varieties evaluated in the study, according to different harvest times and isolation methods, are given in Table 3 and Table 4. While the effect of the isolation method on the density values of lemon peel oils was statistically significant, the effect of cultivar, harvest time, and interactions was not. The density values ranged between 0.8341 and 0.8532 g/mL. Among the cultivars, the highest average density value was for Ak Limon (0.8471 g/mL) and the lowest was for Meyer (0.8423 g/mL). This difference between varieties may also be related to the chemical composition of the oils. There were some differences in the density values according to harvest times; however, these differences remained statistically insignificant. Density values showed the most significant difference according to isolation methods (Table 3 and Table 4). The density values of essential oils obtained by cold pressing were higher than those obtained by hydrodistillation. This may be due to the fact that oils obtained by cold pressing partially contain components with higher molecular weights, especially carotenoids and chlorophyll. In fact, Gonzalez-Mas et al. [34] reported that there are components such as flavonoids, coumarins, diterpenoids, sterols, and fatty acids in the non-volatile parts of citrus oils. The density value range for lemon peel oil obtained by cold pressing is reported as 0.850–0.858 g/mL in the European Pharmacopoeia [35] and 0.845–0.858 g/mL in ISO standards [36]. While the values obtained by cold pressing were compatible with the limit values, the density of the oils obtained by hydrodistillation was below these values. This may be due to the fact that the oils obtained by hydrodistillation consist only of volatile components.

The refractive index values of the samples showed partial differences among the varieties. The refractive index value of the Ak Limon variety was statistically significantly lower than the other three varieties. The effect of harvest time on the refractive index remained insignificant ($p > 0.05$). Essential oil isolation methods had a significant effect on the refractive index ($p < 0.05$); it was significantly higher for the cold pressing method than hydrodistillation (Table 4). This may be due to the differences in the components in the oil content. The refractive index value for lemon peel essential oils obtained by cold pressing is 1.473–1.476 according to the European Pharmacopoeia [35] and 1.473–1.479 according to ISO standards [36]. The refractive index values of the peel oils obtained by two different methods from four lemon cultivars in four different harvest periods were compatible with the reference values.

Table 3. Analysis of variance results for density, refractive index, and optical activity values.

	Density		Refractive index		Optical activity	
	Statistic F	p-value	Statistic F	p-value	Statistic F	p-value
Cultivar (C)	2.54	0.0740	6.50	0.0015	118.55	0.0001
Harvest time (HT)	0.74	0.5381	0.23	0.8751	0.65	0.5889
İsolation method (IM)	33.34	0.0001	389.30	0.0001	38.40	0.0001
C × HT	0.91	0.5259	1.00	0.4631	0.78	0.6379
C × IM	0.50	0.6833	3.14	0.0389	0.52	0.6700
HT × IM	0.83	0.4876	0.43	0.7350	0.21	0.8909
C × HT × IM	0.19	0.9932	0.45	0.8983	0.11	0.9991
Coefficient of variation	0.6627		0.0350		3.0184	

Table 4. Duncan multiple comparison test results of density, refractive index, and optical activity values of lemon peel essential oils according to cultivar, harvest time, and isolation method (mean ± standard error).

	Batem Pınarı	Interdonato	Meyer	Ak Limon
Density (g/mL)	0.8455 ± 0.0016	0.8429 ± 0.0017	0.8423 ± 0.0015	0.8471 ± 0.0018
Refractive index	1.4747[a] ± 0.0004	1.4744[a] ± 0.0004	1.4747[a] ± 0.0003	1.4740[b] ± 0.0003
Optical activity (°)	72.24[d] ± 0.640	75.38[c] ± 0.714	82.59[b] ± 0.678	86.44[a] ± 0.764
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Density (g/mL)	0.8427 ± 0.0020	0.8447 ± 0.0021	0.8449 ± 0.0013	0.8454 ± 0.0012
Refractive index	1.4745 ± 0.0004	1.4744 ± 0.0003	1.4745 ± 0.0003	1.4744 ± 0.0004
Optical activity (°)	79.52 ± 1.744	79.60 ± 1.699	78.96 ± 1.526	78.58 ± 1.457
	Hydrodistillation		Cold-pressed	
Density (g/mL)	0.8404[b] ± 0.0010		0.8485[a] ± 0.0009	
Refractive index	1.4732[b] ± 0.0001		1.4757[a] ± 0.0001	
Optical activity (°)	81.02[a] ± 1.025		77.31[b] ± 1.118	

Different letters on the same line indicate a significant difference between the means at the $p < 0.05$ level.

The optical activity values varied between 70.20° and 90.40° depending on the cultivar, harvest time, and isolation methods. The highest optical activity value was determined in Ak Limon, followed by Meyer, Interdonato, and Batem Pınarı cultivars. Regarding the harvest times, differences remained statistically insignificant. The optical activity of essential oils obtained by the hydrodistillation method was higher than those obtained by cold pressing. The optical activity of lemon peel essential oils, which is one of the most important quality criteria of essential oils, should range between +57° and +70° according to the European Pharmacopoeia when obtained by cold pressing [35] and between +66° and +78° for three different lemon types (obtained by cold-press) in ISO standards. In our study, the optical activity values for the Batem Pınarı and Interdonato varieties were compatible with these reference values, while the Meyer and Ak Limon varieties remained above these range values. This may be closely related to the composition of the peel oils. In fact, Ak Limon and Meyer varieties attract attention for their higher limonene content compared to the other two varieties.

Essential oil compositions of the four lemon varieties were determined by the chromatographic method in order to reveal detailed quality characteristics according to harvest time and isolation method. Essential oil compositions for the lemon varieties Batem Pınarı, Interdonato, Meyer, and Ak Limon are given in Tables 5, 6, 7, and 8, respectively.

Among the lemon cultivars evaluated within the scope of the study, 25 different components of Batem Pınarı were identified. The main component of all essential oils in all analyzed cultivars was limonene, which has a monoterpene structure. Limonene content for this cultivar ranged between 76.0%–77.5%, depending on harvest time and isolation method. Batem Pınarı had the lowest average limonene content. It is known that limonene, which has different functional properties, is also determinant for the characteristic smell of *Citrus* fruits. Three other components that were proportionally higher in Batem Pınarı were γ-terpinene, β-pinene, and β-myrcene. Batem Pınarı (3.6%–5.1%) and Interdonato (2.7%–4.0%) varieties differed significantly from the other two regarding their high β-pinene content. There were some slight differences in the ratios of these components and other essential oil components depending on the harvest time and isolation method.

Table 5. Essential oil composition (%) of Batem Pınarı lemon variety according to harvest time and isolation method.

Compound	RRI	Harvest 1		Harvest 2		Harvest 3		Harvest 4	
		HD	CP	HD	CP	HD	CP	HD	CP
α-pinene	1030	1.4	1.1	1.6	1.2	1.5	1.2	1.5	1.3
α-thujene	1133	0.4	0.3	0.4	0.3	0.4	0.3	0.3	0.3
β-pinene	1122	4.8	4.1	4.7	3.6	4.7	4.0	5.1	4.8
Sabinene	1132	1.0	0.8	0.9	0.8	0.9	0.9	1.0	1.0
β-myrcene	1170	1.9	1.8	1.8	1.8	1.9	1.8	1.8	1.8
α-terpinene	1187	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Limonene	1214	76.0	76.2	76.4	77.5	77.3	76.9	76.5	76.7
β-phellandrene	1223	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
β-ocimene	1242	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.1
γ-terpinene	1260	9.4	9.5	9.3	9.3	8.9	9.4	9.1	9.2
p-cymene	1285	0.2	0.1	0.1	-	0.2	-	-	-
Terpinolene	1298	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4
Linalool	1549	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1
Bergamotene	1553	0.3	0.6	0.3	0.5	0.3	0.5	0.4	0.5
Terpinen-4-ol	1595	0.2	-	0.2	-	0.1	0.2	0.2	0.2
β-caryophyllene	1596	0.3	0.3	0.2	0.2	0.2	0.3	0.2	0.2
α-humulene	1661	0.3	0.4	0.3	0.4	0.1	0.2	0.1	0.1
(*E*)-β-farnesene	1663	0.3	0.3	0.3	0.3	0.1	0.1	0.1	0.1
α-terpineol	1709	-	-	-	-	0.1	0.1	0.1	0.1
Neryl acetate	1734	0.3	0.5	0.4	0.5	0.5	0.7	0.5	0.6
β-bisabolene	1746	0.5	1.0	0.5	0.9	0.5	0.9	0.6	0.9
Geranial	1748	0.5	1.0	0.5	0.9	0.4	0.7	0.4	0.6
Geranyl acetate	1764	0.2	0.3	0.2	0.2	0.3	0.4	0.4	0.4
Nerol	1804	0.3	0.2	0.2	0.1	0.3	0.1	0.2	0.1
Geraniol	1848	0.3	0.1	0.2	-	0.3	-	0.3	-
Unidentified	-	0.3	0.2	0.2	0.1	0.0	0.2	0.1	0.1

HD: Hydrodistillation, CP: Cold-pressed.

Table 6. Essential oil composition (%) of Interdonato lemon variety according to harvest time and isolation method.

Compound	RRI	Harvest 1		Harvest 2		Harvest 3		Harvest 4	
		HD	CP	HD	CP	HD	CP	HD	CP
α-pinene	1030	1.3	1.0	1.3	1.1	1.5	1.1	1.3	1.2
α-thujene	1133	0.3	0.3	0.4	0.3	0.4	0.3	0.3	0.3
β-pinene	1122	3.2	3.3	3.6	2.7	3.6	3.1	4.0	3.5
Sabinene	1132	0.6	0.7	0.7	0.6	0.7	0.7	0.8	0.7
β-myrcene	1170	2.0	1.8	1.9	1.9	2.0	1.9	1.9	1.9
α-terpinene	1187	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Limonene	1214	79.5	78.2	78.6	79.8	79.9	79.6	79.2	79.0
β-phellandrene	1223	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
β-ocimene	1242	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
γ-terpinene	1260	8.3	9.0	8.6	8.5	8.3	8.6	8.3	8.6
p-cymene	1285	0.1	0.1	0.1	-	0.2	-	-	-
Terpinolene	1298	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Linalool	1549	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Bergamotene	1553	0.3	0.4	0.2	0.3	0.1	0.3	0.2	0.3
Terpinen-4-ol	1595	0.1	-	0.2	-	0.2	0.2	0.2	0.2
β- caryophyllene	1596	0.3	0.5	0.2	0.2	0.1	0.4	0.2	0.3
α- Humulene	1660	0.3	0.2	0.3	0.4	0.1	0.1	0.1	0.1
(*E*)-β-farnesene	1663	0.3	0.3	0.3	0.3	-	0.1	0.1	0.1
Neryl acetate	1734	0.4	0.4	0.3	0.4	0.3	0.4	0.5	0.6
β-bisabolene	1746	0.5	0.6	0.4	0.6	0.3	0.7	0.5	0.8
Geranial	1748	0.5	0.9	0.5	0.8	0.4	0.6	0.3	0.5
Geranyl acetate	1764	0.3	0.7	0.4	0.5	0.3	0.5	0.3	0.4
Nerol	1804	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.1
Geraniol	1848	0.2	0.1	0.3	-	0.1	-	0.2	-
Unidentified	-	0.3	0.1	0.0	0.1	0.2	0.2	0.2	0.2

HD: Hydrodistillation, CP: Cold-pressed.

Table 7. Essential oil composition (%) of Meyer variety according to harvest time and isolation method.

Compound	RRI	Harvest 1		Harvest 2		Harvest 3		Harvest 4	
		HD	CP	HD	CP	HD	CP	HD	CP
α-pinene	1030	1.2	1.0	1.3	1.0	1.3	1.0	1.3	1.1
α -thujene	1133	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.3
β-pinene	1122	0.7	0.6	0.8	0.7	0.7	0.6	0.8	0.7
Sabinene	1132	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
β-myrcene	1170	2.0	1.9	2.0	1.9	2.0	1.9	1.9	1.9
α-terpinene	1187	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.2
Limonene	1214	84.4	84.3	84.3	84.0	84.4	84.2	83.6	84.1
1,8-cineole	1222	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
β-phellandrene	1223	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2
β-ocimene	1242	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
γ-terpinene	1260	6.8	6.7	6.6	6.9	6.7	6.9	7.0	7.1
p-cymene	1285	1.0	1.0	1.1	1.1	1.1	1.1	1.3	0.8
Terpinolene	1298	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.4
p-cymenene	1443	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2
Citronellal	1481	-	0.1	-	0.1	0.1	0.1	0.1	0.1
Linalool	1549	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2
Bergamotene	1553	0.1	0.2	0.1	0.3	0.2	0.2	0.2	0.2
β-elemen	1585	0.3	0.4	0.2	0.5	0.3	0.3	0.2	0.2
Terpinen-4-ol	1595	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2
β-caryophyllene	1596	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
(*E*)-β-farnesene	1663	0.1	0.1	0.1	0.1	-	0.1	-	-
α-terpineol	1709	0.2	0.2	0.2	0.2	0.1	0.13	0.1	0.1
Neryl acetate	1734	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2
β-bisabolene	1746	0.2	0.3	0.2	0.3	0.2	0.3	0.1	0.3
Geranial	1748	-	0.1	0.4	0.1	0.1	0.2	0.1	0.2
Unidentified	-	0.8	0.9	0.4	0.7	0.6	0.7	0.8	0.7

HD: Hydrodistillation, CP: Cold-pressed.

Table 8. Essential oil composition (%) of Ak Limon cultivar according to harvest time and isolation method.

Compound	RRI	Harvest 1		Harvest 2		Harvest 3		Harvest 4	
		HD	CP	HD	CP	HD	CP	HD	CP
α-pinene	1030	0.8	0.8	0.8	0.8	0.8	0.7	0.8	0.8
α-thujene	1133	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
β-pinene	1122	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.4
Sabinene	1132	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.2
β-myrcene	1170	1.9	1.9	1.8	1.9	1.9	1.8	1.8	1.8
Limonene	1214	89.0	88.7	88.8	88.6	89.0	89.0	88.8	87.6
β-phellandrene	1223	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
γ-terpinene	1260	3.0	2.9	2.8	3.0	3.0	2.7	3.1	3.2
p-cymene	1285	1.0	0.9	1.0	0.9	1.1	0.9	1.1	1.1
Terpinolene	1298	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
p-cymenene	1443	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4
Citronellal	1481	0.9	1.3	1.1	1.3	1.0	1.2	0.9	1.3
Linalool	1549	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2
α-bergamotene	1553	0.2	0.3	0.2	0.3	0.2	0.3	0.2	0.4
β-caryophllene	1596	0.1	0.1	0.1	0.1	-	-	0.1	0.1
(*E*)-β-farnesene	1663	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
α-terpineol	1709	0.1	-	-	-	-	-	-	-
Germacrene D	1726	0.2	0.3	0.1	0.3	0.2	0.3	0.2	0.3
β-bisabolene	1746	0.4	0.5	0.4	0.5	0.3	0.5	0.4	0.6
Geranial	1748	0.3	0.3	0.2	0.3	0.1	0.2	0.1	0.3
Citronellol	1772	0.3	0.2	0.3	0.2	0.4	0.2	0.4	0.2
Unidentified	-	0.2	0.4	0.4	0.4	0.2	0.4	0.3	0.5

HD: Hydrodistillation, CP: Cold-pressed.

Proportionally, the second most important component in lemon peel oils was γ-terpinene. After analyzing the ANOVA and Duncan multiple comparison test results, the γ-terpinene ratio showed a significant variation regarding cultivars (Table 9, Table 10). Batem Pınarı was the standout cultivar with its γ-terpinene content of 9.3%. This was followed by Interdonato, Meyer, and Ak Limon cultivars. There was an inverse proportion between the rate of this component and limonene content. The effect of harvest time and isolation method on this component ratio remained limited.

There were some differences in other essential oil components of the cultivars, depending on the applications. Some other components found in proportionally high levels were α-pinene, β-pinene, and sabinene. Their proportions also varied depending on the cultivar, harvest time, and isolation method. Among these components, α-pinene showed a distribution in the range of 0.7–1.6; the highest was detected in the Batem Pınarı and the lowest in the Ak Limon variety. β-pinene was also detected at significant levels in lemon peel oils; it was highest in Batem Pınarı (3.63%–5.13%) and lowest in Ak

Limon cultivars (0.3%–0.4%). Sabinen, which is proportionally important in lemon peel oils, was widely distributed from 0.1% to 1.0%. The lemon cultivars examined within the scope of this research were generally rich in terms of components. While 25 components were identified in Batem Pınarı and Meyer varieties, 24 components were identified in Interdonato, and 21 components were identified in Ak Limon. In addition to the components evaluated, some differences were observed in their proportions depending on the cultivar, harvest time, and isolation method. However, these were very low, especially in the essential oil composition depending on the isolation method.

Reference values for some component ratios have been specified in the European Pharmacopoeia. These components include limonene and γ-terpinene, with reference values of 56%–78% and 6%–12%, respectively [35]. In ISO standards, limonene and γ-terpinene limit values are reported to be 60%–80% and 6%–12%, respectively [36].

The statistical analysis of limonene, β-myrcene, and γ-terpinene components, which are proportionally higher in all samples according to cultivar, harvest time, and isolation method, are reported in Table 9 and Table 10. While the effect of variety and isolation method on the β-myrcene ratio of these components was significant, the effect of harvest time was not. Among the varieties, Meyer had the highest β-myrcene ratio, followed by Interdonato, Ak Limon, and Batem Pınarı cultivars. When the isolation methods were evaluated, the β-myrcene ratio obtained by the hydrodistillation method was higher than that by cold pressing. Regarding harvest times, there were almost no differences in β-myrcene ratios. Limonene had the highest proportion in lemon peel oils, being the characteristic component of *Citrus* peel essential oils. While the effect of the cultivar on the limonene content was statistically significant, the effect of harvest time and isolation method was not (Tables 9 and 10). Limonene content significantly changed depending on the varieties; Ak Limon had the highest limonene content, followed by Meyer, Interdonato, and Batem Pınarı. No significant changes were detected in limonene, β-myrcene, and γ-terpinene ratios according to harvest time. According to the isolation method, the β-myrcene ratio was significantly higher in the essential oils obtained by hydrodistillation.

Table 9. Analysis of variance results for β-myrcene, limonene, and γ-terpinene contents.

	β-myrcene		Limonene		γ-terpinene	
	Statistic F	p-value	Statistic F	p-value	Statistic F	p-value
Cultivar (C)	41.85	0.0001	739.79	0.0001	1079.53	0.0001
Harvest time (HT)	2.33	0.0930	1.94	0.1435	0.65	0.5898
Isolation method (IM)	17.32	0.0002	0.15	0.7043	2.20	0.1474
C × HT	0.86	0.5673	0.51	0.8533	0.65	0.7499
C × IM	3.50	0.0264	0.57	0.6414	0.54	0.6560
HT × IM	3.96	0.0165	0.80	0.5028	0.13	0.9419
C × HT × IM	1.32	0.2665	0.68	0.7178	0.70	0.7042
Coefficient of variation	1.7127		0.9533		4.9586	

Table 10. Duncan's multiple comparison test results of β-myrcene, limonene, and γ-terpinene contents (%) of lemon peel essential oils according to cultivar, harvest time, and isolation method (mean ± standard error).

Compound	Batem Pınarı	Interdonato	Meyer	Ak Limon
β-myrcene	$1.8^{b} \pm 0.011$	$1.9^{a} \pm 0.012$	$1.9^{a} \pm 0.011$	$1.8^{b} \pm 0.006$
Limonene	$76.7^{d} \pm 0.204$	$79.2^{c} \pm 0.244$	$84.2^{b} \pm 0.109$	$88.7^{a} \pm 0.149$
γ-terpinene	$9.3^{a} \pm 0.080$	$8.5^{b} \pm 0.105$	$6.8^{c} \pm 0.054$	$3.0^{d} \pm 0.065$
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
β-myrcene	1.9 ± 0.019	1.9 ± 0.016	1.9 ± 0.013	1.9 ± 0.012
Limonene	82.0 ± 1.281	82.3 ± 1.178	82.5 ± 1.189	81.9 ± 1.164
γ-terpinene	6.9 ± 0.649	6.9 ± 0.643	6.8 ± 0.634	6.9 ± 0.602
	Hydrodistillation		Cold-pressed	
β-myrcene	$1.89^{a} \pm 0.011$		$1.86^{b} \pm 0.009$	
Limonene	82.2 ± 0.857		82.2 ± 0.819	
γ-terpinene	6.8 ± 0.431		7.0 ± 0.449	

Different letters on the same line for each application indicate a difference between the averages at $p < 0.05$ level.

Benoudjit et al. [37] found that the major components of lemon peel essential oils obtained by cold pressing were limonene (64.75%), γ-terpinene (11.72%), and β-pinene (11.24%). Kırbaslar et al. [38] determined that peel oils from cold-pressed Turkish lemons (*Citrus limon* (L.) Burm. f. contained high amounts of monoterpene hydrocarbons (89.9%), and its major components are limonene (61.8%), γ-terpinene (10.6%), and β-pinene (8.1%). Paw et al. [39] determined that the major components in the chemical composition of essential oils obtained by hydrodistillation from the peel of *Citrus limon* L. Burmf grown in North East India are limonene (55.40%) and neral (10.39%) compounds. Owolabi et al. [40] determined that limonene (85.9%), sabinene (3.9%), and myrcene (3.1%) were the dominant components in the essential oils obtained by hydrodistillation of *Citrus* lemon dry peels grown in Nigeria. Aboubi et al. [41] determined that the main components of essential oils obtained by hydrodistillation in three different regions of Morocco were limonene (48.56%–53.44%), β-pinene (17.78%–17.37%), and γ-terpinene (12.81%–12.33%). Dao et al. [42] determined that the major components in the chemical composition of the essential oils obtained by hydrodistillation of lemon peel were limonene (62.17%), γ-terpinene (12.35), and β-pinene (11.72). Gök et al. [43] extracted the peel of Cyprus lemon (*Citrus limon* (L.) Burm. f.) by supercritical CO_2 extraction (SFE), cold pressing (CP), and hydrodistillation (HD) methods. Limonene, γ-terpinene, and β-pinene were the major compounds in lemon extracts obtained by all three methods. Akarca and Sevik [44] determined the main components of Kütdiken lemon peel essential oil to be limonene (68.65%), γ-terpinene (10.81%), and β-pinene (7.74%). According to long-term data, it was reported that the limonene content of lemon peel oil was 59.57%–79.15% [45]. Brahmi et al. [10] analyzed lemon peel oils obtained by hydrodistillation and microwave-assisted hydrodistillation extraction methods; there were significant changes in the ratio of all components, including the main components limonene and γ-terpinene, depending on the extraction method.

These data show that Batem Pınarı and, partly, the Interdonato varieties comply with the standards. Meyer and Ak Limon varieties attract attention with their higher limonene content. The data show that there is a significant variation. It has been reported that many factors, such as genotype, origin, environment, extraction method, and degree of maturity, may be effective in this difference [5,13,46]. This reveals that the essential oils obtained by both methods may show similar functional properties. The data show that it would be useful to conduct studies depending on the intended use.

4. Conclusions

This research revealed that the lemon peel essential oil composition and some of its properties may vary depending on the cultivar, harvest time, and isolation method. When the essential oil amounts of the samples were evaluated, there were significant differences between cultivars—the Interdonato variety had the highest value. This also varied depending on the harvest time of the samples. The density, refractive index, and optical activity values of the analyzed essential oils showed significant differences depending on the variety and isolation method. Regarding the essential oil composition, the number of components detected and their ratio differed depending on the cultivar and harvest time. When a general evaluation was made, the differences in component ratios determined according to the isolation method remained statistically insignificant. The main component in all samples was limonene, ranging in proportion from 76.0% to 89.0%. The limonene content of Batem Pınarı and Interdonato varieties was generally consistent with the literature. On the other hand, the limonene content of Meyer and Ak Limon varieties differed from the literature and standard data (ISO, European Pharmacopeia). In particular, Ak Limon had higher limonene content than the other varieties and standard reference values. Therefore, Ak Limon may make a difference in terms of functional properties depending on the area of use. The findings obtained here show that there is a variation in lemon peel oils. Particular attention should be paid to cultivar selection and isolation methods, depending on the area of use.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Plant authority, E.T.; conceptualization, M.G.; methodology, M.G.; software, M.G. and A.M.G.; validation, B.B., M.G. and A.M.G.; formal analysis, B.B. and M.G.; investigation, M.G. and O.Ç., D.Y.T., H.T.; resources, B.B. and M.G.; data curation, B.B., M.G., O.Ç., D.Y.T., H.T., E.T. and

A.M.G.; writing—original draft preparation, M.G., B.B. O.Ç., D.Y.T., H.T., E.T.; writing—review and editing, B.B., M.G. and A.M.G.; visualization, B.B., M.G. and A.M.G.; supervision, B.B., M.G. and A.M.G.; project administration, B.B., M.G. and A.M.G.; funding acquisition, M.G. All authors have read and agreed to the published version of the manuscript.

References

1. Kılıç A (2008) Methods of essential oil production. *J Bartin Fac For* 10: 37–45.
2. Evren M, Tekgüler B (2011) Antimicrobial properties of essential oils. *Elektronik Mikrobiyoloji Dergisi* 9: 28–40.
3. Gioffrè G, Ursino D, Labate MLC, et al. (2020) The peel essential oil composition of bergamot fruit (*Citrus bergamia*, Risso) of Reggio Calabria (Italy): A review. *Emirates J Food Agric* 32: 835–845. https://doi.org/10.9755/ejfa.2020.v32.i11.2197
4. Bozova B, Gölükcü M, Giuffrè AM (2024) The effect of different hydrodistillation times on the composition and yield of bergamot (*Citrus bergamia*, Risso) peel essential oil and a comparison of the cold-pressing method. *Flavour Fragrance J* 39: 263–270. https://doi.org/10.1002/ffj.3789
5. Singh B, Singh JP, Kaur A, et al. (2021) Insights into the chemical composition and bioactivities of citrus peel essential oils. *Food Res Int* 143: 110231. https://doi.org/10.1016/j.foodres.2021.110231
6. Turkish Statistical Institute (TUİK) (2023) Bitkisel üretim istatistikleri. Available from: http://Tuikapp.Tuik.Gov.Tr/Bitkiselapp/Bitkisel.Zul.
7. Fisher K, Phillips C (2008) Potential antimicrobial uses of essential oils in food: Is citrus the answer? *Trends Food Sci Technol* 19: 156–164. https://doi.org/10.1016/j.tifs.2007.11.006
8. Hosni K, Zahed N, Chrif R, et al. (2010) Composition of peel essential oils from four selected Tunisian *Citrus* species: Evidence for the genotypic influence. *Food Chem* 123: 1098–1104. https://doi.org/10.1016/j.foodchem.2010.05.068
9. Palazzolo E, Laudicina VA, Germanà MA (2013) Current and potential use of citrus essential oils. *Curr Org Chem* 17: 3042–3049. https://doi.org/10.2174/13852728113179990122
10. Brahmi F, Mokhtari O, Legssyer B, et al. (2021) Chemical and biological characterization of essential oils extracted from citrus fruits peels. *Mater Today Proc* 45: 7794–7799. https://doi.org/10.1016/j.matpr.2021.03.587
11. Osman A (2019) Citrus oils. *Fruit oils: Chemistry and functionality,* 521–540. https://doi.org/10.1007/978-3-030-12473-1_26
12. Shahidi F, Zhong Y (2012) Citrus oils and essences. In: Shahidi F (Ed.), *Bailey's Industrial Oil and Fat Products,* Six Edition, John Wiley & Sons, Inc. USA, 49–66. https://doi.org/10.1002/0471238961.citrshah.a01
13. Schmidt E (2010) Production of essential oils (Chapter 4). In: Başer KHC, Buchbauer G (Eds.), *Handbook of Essential Oils Science, Technology and Applications,* Florida, USA: Crc Pres Taylor & Francis Group, 83–119. https://doi.org/10.1201/9781420063165-c4
14. Mahato N, Sharma K, Koteswararao R, et al. (2019) Citrus essential oils: Extraction, authentication and application in food preservation. *Crit Rev Food Sci Nutr* 59: 611–625. https://doi.org/10.1080/10408398.2017.1384716
15. Steuer B, Schulz H, Läger E (2001) Classification and analysis of citrus oils by NIR spectroscopy. *Food Chem* 72: 113–117. https://doi.org/10.1016/S0308-8146(00)00209-0

16. Chiralt A, Martınez-Monzo J, Chafer T, et al. (2002) Limonene from citrus. In: Shi J, Mazza G, Le Maguer M (Eds.), *Functional Foods: Biochemical and Processing Aspects,* Boca Raton, Fl.: Crc Press, 163–180.
17. Bousbia N, Vian MA, Ferhat MA, et al. (2009) A new process for extraction of essential oil from Citrus peels: Microwave hydrodiffusion and gravity. *J Food Eng* 90: 409–413. https://doi.org/10.1016/j.jfoodeng.2008.06.034
18. Sawamura M (2011) *Citrus essential oils flavor and fragrance*. Singapore: John Wiley & Sons, 398p. https://doi.org/10.1002/9780470613160
19. Jayaprakasha G, Murthy KC, Uckoo RM, et al. (2013) Chemical composition of volatile oil from Citrus limettioides and their inhibition of colon cancer cell proliferation. *Ind Crops Prod* 45: 200–207. https://doi.org/10.1016/j.indcrop.2012.12.020
20. Gölükcü M, Toker R, Tokgöz H, et al. (2015) Bitter orange (*Citrus aurantium* L.) peel essential oil compositions obtained with different methods. *Derim* 32: 161–170. https://doi.org/10.16882/derim.2015.15556
21. Bora H, Kamle M, Mahato DK, et al. (2020) Citrus essential oils (CEOs) and their applications in food: An overview. *Plants* 9: 357. https://doi.org/10.3390/plants9030357
22. Nikfar S, Behboudı, AF (2014) Limonene. *Encycl Toxicol* 3: 78–81. https://doi.org/10.1016/B978-0-12-386454-3.00628-X
23. Turkish Standard Institution (TSE) (2011) TSE EN ISO 6571- Spices, condiments and herbs - Determination of volatile oil content (hydrodistillation method).
24. Kirbaslar FG, Kirbaslar SI, Dramur U (2001) The compositions of Turkish bergamot oils produced by cold-pressing and steam distillation. *J Essent Oil Res* 13: 411–415. https://doi.org/10.1080/10412905.2001.9699710
25. Turkish Standard Institution (TSE) (2012a) TS ISO 279- Essential oils - Determination of relative density at 20 °C - Reference method (https://intweb.tse.org.tr/).
26. Turkish Standard Institution (TSE) (2009) TS ISO 280- Essential oils - Determination of refractive index (https://intweb.tse.org.tr/).
27. Turkish Standard Institution (TSE) (2012b) TS ISO 592- Essential oils - Determination of optical rotation (https://intweb.tse.org.tr/).
28. Özek G, Demirci F, Özek T, et al. (2010) Gas chromatographic-mass spectrometric analysis of volatiles obtained by four different techniques from Salvia rosifolia Sm., and evaluation for biological activity. *J Chromatogr A* 1217: 741–748. https://doi.org/10.1016/j.chroma.2009.11.086
29. Düzgüneş O, Kesici T, Kavuncu O, et al. (1987) Research and trial methods. Türkiye, Ankara University Faculty of Agriculture, Publication Number:1021, 381p.
30. Di Vaio C, Graziani G, Gaspari A, et al. (2010) Essential oils content and antioxidant properties of peel ethanol extract in 18 lemon cultivars. *Sci Hortic* 126: 50–55. https://doi.org/10.1016/j.scienta.2010.06.010
31. Bourgou S, Rahali FZ, Ourghemmi I, et al. (2012) Changes of peel essential oil composition of four Tunisian citrus during fruit maturation. *Sci World J* 2012: 528593. https://doi.org/10.1100/2012/528593
32. Vekiari SA, Protopapadakis EE, Papadopoulou P, et al. (2002) Composition and seasonal variation of the essential oil from leaves and peel of a Cretan lemon variety. *J Agric Food Chem* 50: 147–153. https://doi.org/10.1021/jf001369a

33. Ferhat MA, Boukhatem MN, Hazzit M, et al. (2016) Cold pressing, hydrodistillation and microwave dry distillation of citrus essential oil from Algeria: A comparative study. *Electron J Biol* S1: 30–41.
34. González-Mas MC, Rambla JL, López-Gresa MP, et al. (2019) Volatile compounds in citrus essential oils: A comprehensive review. *Front Plant Sci* 10: 433929. https://doi.org/10.3389/fpls.2019.00012
35. Anonymous (2008) Lemon oil limonis aetheroleum. *European Pharmacopeia*, 2246–2248. Available from: https://pheur.edqm.eu/home.
36. Anonymous (2003) Oil of lemon [*Citrus limon* (L.) Burm. F.], obtained by expression. International Standards ISO 855 (https://www.iso.org/standard/32042.html).
37. Benoudjit F, Maameri L, Ouared K (2020) Evaluation of the quality and composition of lemon (*Citrus limon*) peel essential oil from an Algerian fruit juice industry. *Alger J Environ Sci Technol* 6: 1575–1581.
38. Kirbaşlar ŞI, Boz I, Kirbaşlar FG (2006) Composition of Turkish lemon and grapefruit peel oils. *J Essent Oil Res* 18: 525–543. https://doi.org/10.1080/10412905.2006.9699161
39. Paw M, Begum T, Gogoi R, et al. (2020) Chemical composition of Citrus limon L. Burmf peel essential oil from North East India. *J Essent Oil Bear Plants* 23: 337–344. https://doi.org/10.1080/0972060X.2020.1757514
40. Owolabi MS, Avoseh ON, Ogunwande IA, et al. (2018) Chemical composition of *Citrus limon* (L.) Osbeck growing in Southwestern Nigeria: Essential oil chemotypes of both peel and leaf of lemon. *American J Essent Oils Nat Prod* 6: 36–40.
41. El Aboubi M, Hdech DB, Bikri S, et al. (2022) Chemical composition of essential oils of Citrus limon peel from three Moroccan regions and their antioxidant, anti-inflammatory, antidiabetic and dermatoprotective properties. *J Herbmed Pharmacol* 12: 118–127. https://doi.org/10.34172/jhp.2023.11
42. Dao TP, Tran NQ, Tran TT (2022) Assessing the kinetic model on extraction of essential oil and chemical composition from lemon peels (*Citrus aurantifolia*) by hydro-distillation process. *Mater Today Proc* 51: 172–177. https://doi.org/10.1016/j.matpr.2021.05.069
43. Gök A, İsmail Kirbaşlar Ş, Gülay Kirbaşlar F (2015) Comparison of lemon oil composition after using different extraction methods. *J Essent Oil Res* 27: 17–22. https://doi.org/10.1080/10412905.2014.982872
44. Akarca G, Sevik R (2021) Biological activities of *Citrus limon* L. and *Citrus sinensis* L. peel essential oils. *J Essent Oil Bear Plants* 24: 1415–1427. https://doi.org/10.1080/0972060X.2021.2022000
45. Dugo G, Cotroneo A, Bonaccorsi I, et al. (2011) Composition of the volatile fraction of citrus peel oils. In: Dugo G, Mondello L (Eds.), *Citrus Oils Composition, Advanced Analytical Techniques, Contaminants, and Biological Activity,* London, UK: CRC Press Taylor and Francis Group, 1–161. https://doi.org/10.1201/b10314
46. Li C, Li X, Liang G, et al. (2022) Volatile composition changes in lemon during fruit maturation by HS-SPME-GC-MS. *J Sci Food Agric* 102: 3599–3606. https://doi.org/10.1002/jsfa.11706