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*Research article*

## **The physico-chemical characteristics of peel essential oils of mandarin cultivars grown in Türkiye**

**Muharrem Gölükcü<sup>1</sup>, Burcu Bozova<sup>1</sup>, Angelo Maria Giuffrè<sup>2,\*</sup>**

<sup>1</sup> Republic of Türkiye Ministry of Agriculture and Forestry, Batı Akdeniz Agricultural Research Institute [BATEM], Food Technology Department, 07112 Antalya, Türkiye

<sup>2</sup> Department AGRARIA, University of Studies “*Mediterranea*” of Reggio Calabria, 89124 Reggio Calabria, Italy

\* **Correspondence:** Email: amgiuffre@unirc.it; Tel.: +393277022840.

**Abstract:** Essential oil (EO) content and its quality parameters of seven mandarin cultivars were evaluated with respect to harvest time and isolation methods. EOs were obtained by hydrodistillation (HD) and cold pressing (CP) methods. EO components of the oil were identified by gas chromatography-mass spectrometry (GC-MS/FID). The EO content ranged between 1.12–2.76% in samples. While the effect of isolation method on relative density, refractive index, and optical activity values was statistically important, the effect of cultivar was important ( $p < 0.05$ ) on refractive index and optical activity. The relative density of EOs obtained by CP (0.8440) was higher than that obtained by HD (0.8402). The refractive index value of the samples obtained by CP (1.4739) was higher than HD method (1.4723). On the other hand, the optical activity value of the samples obtained by HD ( $98.20^\circ$ ) was higher than CP ( $95.32^\circ$ ). Refractive index and optical activity value ranged between 1.4734 (Batem Göral)–1.4741 (Nova) and  $77.38$  (Yerli Apireno)– $96.13$  (Batem Göral) according to cultivars. EO components and their contents also showed some differences on the basis of cultivars, harvesting times and isolation methods, but these differences generally were not statistically significant. The most important component was determined to be limonene, and it ranged between 76.6% (Yerli Apireno) and 96.2% (Nova). While the effect of isolation method and harvesting time on limonene were not statistically significant ( $p > 0.05$ ), the effect of cultivar was significantly important ( $p < 0.05$ ). The second highest component was determined as  $\beta$ -myrcene and ranged between 1.9 and 2.1%. Cultivar and harvest time effects on  $\beta$ -myrcene content were statistically significant. Another important component was  $\gamma$ -terpinene for Batem İncisi (4.6%), Batem Yıldızı (4.4%), and Yerli Apireno (13.7%). Results showed that there were significant variations in composition and some quality parameters of the mandarin peel EOs according to cultivar and isolation methods. The research

findings indicated that the essential oil obtained from the Yerli Apireno was in compliance with international standards for limonene and  $\gamma$ -terpinene content. However, the Batem Göral, Clementine Fina, Nova, and Fortune cultivars, which have a high limonene content, were found to stand out in this regard.

**Keywords:** mandarin; peel essential oil; cultivar; harvest time; cold press; hydrodistillation

## 1. Introduction

Citrus fruits constitute the most widely produced fruit group, with an annual production amount of over 160 million tons and grown in more than 130 countries worldwide [1]. Citrus fruits are well-known for their nutritional advantages, primarily due to their high content of vitamin C, phenolic compounds, and dietary fiber [2]. Approximately 50–60% of citrus fruits are consumed as fresh fruit, while the remaining 40–50% are processed industrially. The processing of these fruits generates a significant amount of waste, approximately 50% of the total volume, which includes peels, pulps, and seeds with variations depending on the processing technology and fruit cultivar. Globally, the annual production of citrus product waste is estimated to be around 10 million metric tons. Notably, this citrus waste is rich in several beneficial components, including flavonoids, carotenoids, dietary fiber, soluble sugars and essential oils [3,4].

One of the most produced species among citrus fruits is mandarin, with approximately 52 million tons being produced annually worldwide. China is the main mandarin producing country (51%), followed by Türkiye, Spain, and Egypt. Türkiye realized 5.6% of the world's mandarin production in 2023 [1]. In view of the citrus production data, Türkiye has a notable potential in the production of citrus peel oils. Nevertheless, a substantial amount of citrus oil is supplied through imports. The total import value of citrus essential oils was 15,641,898 USD in 2023 for Türkiye [5]. Citrus peel oils can be obtained by cold pressing, hydrodistillation, and solvent extraction methods [6–8]. Citrus peel oil contains many bioactive components with different functional properties, such as limonene, myrcene,  $\alpha$ -pinene,  $\beta$ -pinene, sabinene, and linalool [9–11]. Citrus peel oils are used in many areas, such as the food industry, cosmetics, perfumery, pharmaceutical industry, and the production of cleaning products [12–15]. Citrus essential oils are generally recognized as safe (GRAS) list with their broad-spectrum biological activities, such as antimicrobial, antifungal, antioxidant, anti-inflammatory, and anxiolytic [12,17]. The most important feature of citrus peel essential oils is their high limonene content. It has been reported that the limonene content of citrus peel essential oils (orange, mandarin, bergamot, and bitter orange) is distributed in a very wide range, such as 36.54% to 96.10% [18]. It is stated that mandarin peel oil stands out with its high limonene content among citrus fruits [7]. The primary components of essential oils are mainly responsible for their biological properties. However, minor compounds can also significantly influence bioactivity, either by enhancing the effects of the major components or through antagonistic or additive interactions [19]. Limonene (p-mentha-1,8-diene) is used in many areas, such as food, medicine, and cosmetics, on an industrial scale. Its use in the cosmetics and pharmaceutical industry is due to its aromatic properties, as well as its high absorption. The easy absorption of biologically active compounds like limonene by the body will enable their potential health benefits to emerge more effectively. Limonene, which is widely used in the food industry due to its aromatic properties, is accepted as GRAS on the FDA list [20–21]. It has been reported that the composition of citrus peel oils may differ according to the type and cultivar, as well as the

isolation method (cold press, hydrodistillation) [22,23].

The majority of the literature on citrus essential oil focused on oranges. There are relatively few studies focused specifically on mandarins. In Türkiye, which has significant potential in terms of raw materials, the production of such products is important for the world and national economy. Mandarin cultivars are constantly increasing by breeding studies. Some of the mandarin cultivars studied in this research are rarely recognized in the fresh fruit market outside Türkiye. Furthermore, no prior reports on the oils of these cultivars were found in the literature. Therefore, the compositions of these oils may serve as valuable contributions to researchers, producers, and industry present in mandarin oils obtained through both cold-pressing and hydrodistillation processes. In addition, the evaluation of citrus peels, which are seen as waste, will also contribute to the development of the producer and processing industry. The citrus cultivars to be used in production, the harvest time of the material used, and the isolation method are among the determining factors on the quality of essential oil. Within the scope of the study, it was aimed to reveal some quality characteristics and essential oil compositions of peel essential oils obtained from seven mandarin cultivars with two different methods in four different harvest times.

## 2. Materials and methods

### 2.1. Plant material

This research was carried out between 2021–2023 in the Aksu-central unit of the Batı Akdeniz Agricultural Research Institute (Antalya, Türkiye). Seven mandarin (*Citrus reticulata* [L.]) cultivars were used in the research. Each commercial cultivar was harvested in two production seasons (2021–2022, 2022–2023) covering four different harvest times (Table 1) at commercial maturity stage. The materials used within the scope of the research were obtained from the citrus parcels of the Kayaburnu unit of the Batı Akdeniz Agricultural Research Institute. During the harvesting process, fruit samples were randomly taken from four different sides of each tree. The harvested fruit samples were brought to the Food Technology and Medicinal Plants Laboratory on the same day, and the analyses were started.

**Table 1.** Mandarin cultivars and harvest times.

Cultivars	1 <sup>th</sup> Harvest	2 <sup>nd</sup> Harvest	3 <sup>th</sup> Harvest	4 <sup>th</sup> Harvest
Batem İncisi	1 Oct 2021	20 Oct 2021	10 Nov 2021	30 Nov 2021
Batem Yıldızı	1 Oct 2021	20 Oct 2021	10 Nov 2021	30 Nov 2021
Batem Göral	10 Nov 2021	30 Nov 2021	20 Dec 2021	10 Jan 2022
Clementine Fina	10 Nov 2021	30 Nov 2021	20 Dec 2021	10 Jan 2022
Nova	10 Nov 2021	30 Nov 2021	20 Dec 2021	10 Jan 2022
Fortune	2 Jan 2022	20 Jan 2022	10 Feb 2022	2 Mar 2022
Yerli Apireno	2 Jan 2022	20 Jan 2022	10 Feb 2022	2 Mar 2022

Firstly, fruit weight and peel content were analyzed on the samples brought to the laboratory. For this purpose, 10 fruits were used for each repetition. Each fruit and its peels were weighed to an accuracy of 0.01 g. Fruit weight and peel content were given by taking the average of the measurement values taken from ten fruits in each repetition.

## 2.2. Hydrodistillation (HD) process

Essential oil in the samples was extracted by hydro-distillation method with a Clevenger-type apparatus according to ISO 6571 [24]. For this purpose, 200 mL of distilled water was added to 50 g of fresh fruit peel, homeogenized with a blender (Waring 8011ES, USA), and then subjected to distillation using a Clevenger device (Isotex, Türkiye). The amount of essential oil was given by volume based on the weight of fresh fruit peel (mL/100 g, %).

## 2.3. Cold press (CP) process

Cold pressing essential oil was collected according to Kırbaşlar et al. [25]. For this purpose, the flavedo part of the fruit peels was grated, and then this part, which is rich in essential oil, was subjected to manual pressing with a 10 cm diameter seven-hole kitchen type apparatus. The water-essential oil emulsion was collected and then separated by centrifugation. This process was applied at  $15,294 \times g$  at 20 °C for 20 minutes. The amount of essential oil was given by volume based on the weight of fresh fruit peel (mL/100 g, %).

## 2.4. Relative density, refractive index, and optical rotation

Physico-chemical characteristics are an important criterion of the quality and purity of essential oils. The essential oils obtained were analyzed for relative density, refractive index, optical rotation and essential oil composition, which are among the basic quality analyzes specified in the European Pharmacopoeia. Relative density analyzes were determined according to ISO 279 [26]. Refractive index analyzes were carried out according to ISO 280 [27]. Measurements were made at 20 °C using a digital refractometer (A. Krüss Optronic GmbH, DR6000). Optical rotation values of lemon peel essential oils were determined according to ISO 592 [28] by polarimeter device (Optical Activity Ltd, PolAAR 31).

## 2.5. Essential oil composition

The EO composition was analyzed by gas chromatography (Agilent 7890A)-mass spectrometry (Agilent 5975C)-flame ionization detector (GC-MS/FID device) Özek et al. [29]. Samples were diluted with hexane at a ratio of 1:50 to be analyzed. EO component analysis of the samples was performed using a capillary column (HP Innowax Capillary; 60.0 m x 0.25 mm x 0.25 µm). GC-MS spectra was obtained using following conditions: carrier gas Helium; flow rate of 0.8 mL/min; injection volume 1 µL with a split ratio of 50:1. The injector temperature was set at 250 °C. The column temperature program was set as 60 °C (10 minutes), 20 °C/minute from 60 °C to 250 °C, and 250 °C (10.5 minutes). In line with this temperature program, the total analysis time was 60 minutes. 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 oil. Relative retention indices (RRI) of the compounds on column were determined in relation to the retention times of a series of C8–C40 n-alkanes (Sigma, USA). Relative amounts (%) of the determined components were calculated from FID chromatograms.

## 2.6. Statistical analyses

The research was carried out with three replicates according to the randomized parcel trial design [30]. The analyses were carried out in two parallels and the results were subjected to variance analysis (ANOVA) and Duncan multiple comparison test using the SAS package program. Results were given as mean  $\pm$  standard error.

## 3. Results and discussion

The ANOVA and Duncan multiple comparison test results for the fruit weights, peel content of the fruit, and peel essential oil content of the mandarin cultivars were evaluated within the scope of the study given in Table 2 and Table 3. The effect of the cultivar (C) and harvest time (HT) on the fruit weight of the mandarin samples were statistically significant, while the effect of the C  $\times$  HT interaction was not significant. The Fortune cultivar had the highest average fruit weight among the varieties evaluated within the scope of the study (Table 3). The average fruit weight of the examined mandarin cultivars generally increased from the first harvest to the last harvest time. The effect of the cultivar on the peel content was also significant, while the effects of the harvest time and C $\times$ HT interaction were not significant. It was observed that the peel content of Fortune and Nova was lower than other studied cultivars.

**Table 2.** ANOVA results for fruit weight, peel content and essential oil content values of mandarins.

	Fruit weight		Peel content		Essential oil content	
	Statistic F	p-Value	Statistic F	p-Value	Statistic F	p-Value
Cultivar (C)	17.10	0.0001	16.31	0.0001	17.62	0.0001
Harvest time (HT)	7.55	0.0007	1.20	0.3291	1.63	0.1935
C $\times$ HT	0.74	0.7414	0.84	0.6401	0.95	0.5233
Coefficient of variation	13.4054		10.9520		19.4309	

The changes in the essential oil content of the samples according to the cultivar and harvest time were subjected to statistical evaluation based on the hydrodistillation method data. While the effect of the cultivar on the essential oil content of the samples was statistically significant, the effect of the harvest time and the C  $\times$  HT interaction remained not significant. Among the varieties, the peel's essential oil content of the Fortune and Yerli Apireno varieties was higher than the other varieties. Among the analyzed varieties, the one with the lowest essential oil content was Batem İncisi. When a general evaluation was made according to the harvest time, the highest essential oil amount was determined in the Batem İncisi, Batem Yıldızı, Clementine Fina, Fortune varieties in the first harvest time, in the Batem Göral cultivar in the second harvest, in the Yerli Apireno in the third harvest, and in the Nova cultivar in the fourth harvest time. When an evaluation was made according to the extraction method, it was seen that the essential oil amount obtained by hydrodistillation was higher than the essential oil content obtained by cold pressing. Bourgou et al. [31] found that the amount of mandarin peel essential oil varies according to harvest time (0.22–2.70%). In the study conducted by Sulzbach et al. [32], it was reported that the amount of mandarin peel essential oil decreased with the advancement of harvest time. Frizzo et al. [33] also reported that cold press and hydrodistillation methods cause partial differences in terms of essential oil yield. In a study where Lota et al. [34] determined the essential oil yield and the compositional changes of 15 mandarin varieties with the cold

press method, they found that the amount of essential oil in the samples ranged from 0.05% to 0.60%. Our study findings differed from the literature findings, and it was thought that this is due to the difference in the materials studied.

**Table 3.** Duncan test results of fruit weight, peel content and essential oil content of the samples according to cultivar and harvesting time (mean  $\pm$  standard error).

Cultivar	Harvest	Fruit weight (g/fruit)	Peel content (%)	Essential oil (CP, %)	Essential oil (HD, %)
Batem İncisi	1	89.61 $\pm$ 7.43	23.84 $\pm$ 0.395	0.16 $\pm$ 0.010	1.43 $\pm$ 0.033
	2	93.80 $\pm$ 4.22	23.47 $\pm$ 0.380	0.13 $\pm$ 0.010	1.12 $\pm$ 0.165
	3	95.43 $\pm$ 5.22	24.84 $\pm$ 1.820	0.14 $\pm$ 0.020	1.22 $\pm$ 0.095
	4	107.50 $\pm$ 15.90	23.64 $\pm$ 2.795	0.13 $\pm$ 0.020	1.18 $\pm$ 0.189
	mean	96.58 <sup>cd</sup> $\pm$ 4.35	23.95 <sup>ab</sup> $\pm$ 0.670	0.14 $\pm$ 0.008	1.24 <sup>d</sup> $\pm$ 0.068
Batem	1	100.96 $\pm$ 12.87	24.55 $\pm$ 0.355	0.20 $\pm$ 0.015	1.63 $\pm$ 0.033
Yıldızı	2	108.00 $\pm$ 11.35	21.40 $\pm$ 0.490	0.15 $\pm$ 0.020	1.56 $\pm$ 0.099
	3	120.86 $\pm$ 13.32	23.37 $\pm$ 2.520	0.13 $\pm$ 0.020	1.36 $\pm$ 0.149
	4	120.44 $\pm$ 15.98	23.21 $\pm$ 1.045	0.14 $\pm$ 0.010	1.53 $\pm$ 0.122
	mean	112.56 <sup>b</sup> $\pm$ 6.02	23.13 <sup>ab</sup> $\pm$ 0.678	0.15 $\pm$ 0.011	1.52 <sup>cd</sup> $\pm$ 0.056
Batem Göral	1	79.16 $\pm$ 0.59	2078 $\pm$ 1.675	0.27 $\pm$ 0.025	1.87 $\pm$ 0.414
	2	74.29 $\pm$ 6.41	22.11 $\pm$ 0.035	0.28 $\pm$ 0.010	1.98 $\pm$ 0.188
	3	100.69 $\pm$ 17.05	24.31 $\pm$ 0.500	0.29 $\pm$ 0.015	1.83 $\pm$ 0.127
	4	96.93 $\pm$ 16.67	26.21 $\pm$ 0.745	0.28 $\pm$ 0.020	1.52 $\pm$ 0.031
	mean	87.76 <sup>d</sup> $\pm$ 6.32	23.35 <sup>ab</sup> $\pm$ 0.863	0.28 $\pm$ 0.008	1.80 <sup>bc</sup> $\pm$ 0.113
Clementine	1	71.15 $\pm$ 7.89	21.62 $\pm$ 2.060	0.52 $\pm$ 0.060	2.01 $\pm$ 0.358
Fina	2	73.89 $\pm$ 0.25	20.74 $\pm$ 0.045	0.44 $\pm$ 0.050	1.70 $\pm$ 0.326
	3	89.35 $\pm$ 4.65	20.96 $\pm$ 1.985	0.34 $\pm$ 0.035	1.77 $\pm$ 0.067
	4	94.38 $\pm$ 1.98	22.26 $\pm$ 0.725	0.30 $\pm$ 0.020	1.46 $\pm$ 0.172
	mean	82.19 <sup>d</sup> $\pm$ 4.13	21.39 <sup>b</sup> $\pm$ 0.602	0.40 $\pm$ 0.037	1.74 <sup>bc</sup> $\pm$ 0.125
Nova	1	89.05 $\pm$ 8.39	15.79 $\pm$ 0.545	0.53 $\pm$ 0.050	1.98 $\pm$ 0.188
	2	90.58 $\pm$ 7.93	16.02 $\pm$ 0.010	0.39 $\pm$ 0.030	1.89 $\pm$ 0.182
	3	107.06 $\pm$ 20.49	16.86 $\pm$ 0.635	0.39 $\pm$ 0.040	1.79 $\pm$ 0.331
	4	128.51 $\pm$ 8.40	20.28 $\pm$ 4.555	0.56 $\pm$ 0.055	2.10 $\pm$ 0.282
	mean	103.80 <sup>bc</sup> $\pm$ 4.75	17.23 <sup>c</sup> $\pm$ 1.109	0.47 $\pm$ 0.034	1.94 <sup>b</sup> $\pm$ 0.113
Fortune	1	141.94 $\pm$ 6.41	17.98 $\pm$ 0.675	0.60 $\pm$ 0.045	2.68 $\pm$ 0.153
	2	126.89 $\pm$ 0.02	17.76 $\pm$ 0.410	0.53 $\pm$ 0.045	2.67 $\pm$ 0.175
	3	131.26 $\pm$ 4.70	15.13 $\pm$ 0.155	0.58 $\pm$ 0.055	2.34 $\pm$ 0.007
	4	167.97 $\pm$ 11.06	15.43 $\pm$ 0.120	0.49 $\pm$ 0.020	2.15 $\pm$ 0.125
	mean	142.01 <sup>a</sup> $\pm$ 6.56	16.57 <sup>c</sup> $\pm$ 0.516	0.55 $\pm$ 0.023	2.46 <sup>a</sup> $\pm$ 0.088
Yerli	1	90.29 $\pm$ 6.28	25.09 $\pm$ 1.310	0.41 $\pm$ 0.010	2.43 $\pm$ 0.193
Apireno	2	94.87 $\pm$ 1.32	25.70 $\pm$ 0.950	0.35 $\pm$ 0.040	1.93 $\pm$ 0.064
	3	92.23 $\pm$ 0.52	23.07 $\pm$ 4.645	0.37 $\pm$ 0.005	2.76 $\pm$ 0.332
	4	93.97 $\pm$ 4.20	26.89 $\pm$ 0.280	0.39 $\pm$ 0.015	2.42 $\pm$ 0.371
	mean	92.84 <sup>cd</sup> $\pm$ 1.60	25.19 <sup>a</sup> $\pm$ 1.068	0.38 $\pm$ 0.012	2.39 <sup>a</sup> $\pm$ 0.145

Different letters on the same column indicate significant difference between the means for cultivars at the  $p < 0.05$  level. HD: Hydrodistillation, CP: Cold-pressed.

The ANOVA and Duncan multiple comparison test results for relative density, refractive index, and optical activity values of mandarin varieties according to harvest times and extraction methods were given in Table 4 and Table 5. The relative density value of mandarin varieties according to harvest times and extraction methods was distributed in the range of 0.8339–0.8556. ANOVA data showed that the extraction method had a significant effect on the relative density value. It was determined that the relative density values of the samples obtained by hydrodistillation were lower than the relative density values of the essential oil obtained by the cold press method. The density value range for mandarin peel oil obtained by cold pressing method in the European Pharmacopoeia is 0.848–0.855 g/mL [35], while the density value for peel oil for Italian type mandarins is stated as 0.844–0.855 g/mL in ISO standards [36]. Javed et al. [37] also determined the density value of mandarin peel oil obtained by hydrodistillation method as 0.844 g/mL. While those obtained by cold pressing were in line with the limit values, the density values of the samples obtained by hydrodistillation were generally lower than the literature values. It was thought that this difference may be due to factors, such as cultivar, harvest time, climate, and cultural process differences, in addition to the chemical composition of the oils.

**Table 4.** Analysis of variance results for relative density, refractive index and optical activity values.

	Relative density		Refractive index		Optical activity	
	Statistic F	p-Value	Statistic F	p-Value	Statistic F	p-Value
Cultivar (C)	0.84	0.5425	2.80	0,0189	266,61	0,0001
Harvest time (HT)	2.36	0.0807	1.46	0,2355	1,11	0,3547
Isolation method (IM)	34.15	0.0001	530.47	0,0001	188,79	0,0001
C × HT	0.73	0.7627	1.11	0,3661	1,17	0,3146
C × IM	0.66	0.6793	3.96	0,0023	2,01	0,0794
HT × IM	014	0.9336	0.12	0,9437	0,02	0,9968
C × HT × IM	0.67	0.8271	0.53	0,9335	0,29	0,9974
Coefficient of variation	0.8671		0.0389		1.7307	

The refractive index value of the mandarin samples ranged from 1.4717 to 1.4761. When an evaluation was made based on varieties, the highest refractive index value was found in the Nova and Batem İncisi varieties, while the lowest was found in the Batem Göral cultivar. While the change detected according to harvest times was statistically insignificant, the effect of the extraction method on the refractive index value was statistically significant. This is likely to be due to different chemical components such as carotenoids in the structure of the essential oil rather than the essential oil composition. Indeed, it is obvious that the oils obtained with these two methods have some differences that can be observed by sensory observations, such as color. The refractive index limit value for mandarin peel oil obtained by cold pressing is stated as 1.474–1.478 in the European Pharmacopoeia [35] and as 1.4722–1.4758 in ISO standards [36]. In the study conducted by Javed et al. [37], the refractive index value of mandarin peel essential oil obtained by hydrodistillation method was found to be 1.465. It was observed that the refractive index values of the peel oils obtained by two different methods from the seven mandarin varieties evaluated within the scope of our study were compatible with the limit values, but higher than the value obtained in the study conducted by Javed et al. [37]. It was thought that this may be due to the differences in the characteristics of the cultivar studied in the literature and the region where it is grown.

**Table 5.** Relative density, refractive index and optical activity values of mandarin peel essential oils according to cultivar, harvest time, and isolation method (mean  $\pm$  standard error).

Factors		Relative density	Refractive index	Optical activity (°)
Cultivar	Batem İncisi	0.8408 $\pm$ 0.0017	1.4738 <sup>abc</sup> $\pm$ 0.0004	88.95 <sup>b</sup> $\pm$ 0.639
	Batem Yıldızı	0.8448 $\pm$ 0.0016	1.4740 <sup>ab</sup> $\pm$ 0.0003	89.81 <sup>b</sup> $\pm$ 0.621
	Batem Göral	0.8442 $\pm$ 0.0015	1.4734 <sup>c</sup> $\pm$ 0.0003	96.13 <sup>a</sup> $\pm$ 0.600
	Clementine Fina	0.8453 $\pm$ 0.0014	1.4736 <sup>bc</sup> $\pm$ 0.0004	95.15 <sup>a</sup> $\pm$ 0.843
	Nova	0.8427 $\pm$ 0.0023	1.4741 <sup>a</sup> $\pm$ 0.0004	95.42 <sup>a</sup> $\pm$ 0.833
	Fortune	0.8429 $\pm$ 0.0023	1.4737 <sup>abc</sup> $\pm$ 0.0003	96.03 <sup>a</sup> $\pm$ 0.693
	Yerli Apireno	0.8453 $\pm$ 0.0028	1.4739 <sup>ab</sup> $\pm$ 0.0002	77.38 <sup>c</sup> $\pm$ 0.543
Harvest Time	1 <sup>th</sup> harvest	0.8450 $\pm$ 0.0015	1.4737 $\pm$ 0.0003	91.00 $\pm$ 1.256
	2 <sup>nd</sup> harvest	0.8451 $\pm$ 0.0015	1.4738 $\pm$ 0.0003	90.96 $\pm$ 1.452
	3 <sup>th</sup> harvest	0.8441 $\pm$ 0.0014	1.4737 $\pm$ 0.0003	91.63 $\pm$ 1.320
	4 <sup>th</sup> harvest	0.8406 $\pm$ 0.0015	1.4740 $\pm$ 0.0003	91.47 $\pm$ 1.235
Isolation	HD	0.8397 <sup>b</sup> $\pm$ 0.0009	1.4725 <sup>b</sup> $\pm$ 0.0001	93.43 <sup>a</sup> $\pm$ 0.928
Method	CP	0.8477 <sup>a</sup> $\pm$ 0.0009	1.4750 <sup>a</sup> $\pm$ 0.0001	89.10 <sup>b</sup> $\pm$ 0.823

Different letters on the same column for each factors indicate significant difference between the means at the  $p < 0.05$  level.

HD: Hydrodistillation, CP: Cold-pressed.

The optical activity value can be used as one parameter in the authentication of essential oils. The effect of cultivar and extraction method on optical activity values of mandarin peel essential oils were found to be statistically significant. Especially the Yerli Apireno cultivar showed significant differences from other varieties in this sense. It was thought that this may be due to the difference in essential oil composition. Similarities were observed between Batem İncisi and Batem Yıldızı, and between Batem Göral, Clementine Fina, Nova, and Fortune varieties in this sense. Slight differences were also detected in this quality feature according to the harvest times of each cultivar. Significant differences were detected in optical activity value according to the extraction method, and the optical activity value of the essential oils obtained by hydrodistillation were higher than those obtained by cold pressing. The reference range specified in the European Pharmacopoeia for mandarin peel essential oil varies between  $+64^\circ$  and  $+75^\circ$  [35], and in ISO standards, it varies between  $+69^\circ$  and  $+79^\circ$  for oils obtained by cold pressing to include different maturity stages of Italian type mandarin [36]. In the study conducted by Javed et al. [37], the optical activity value for mandarin peel essential oil obtained by hydrodistillation method was determined as  $+86^\circ$ . In the study conducted by Ikarini et al. [38], the optical activity value of mandarin peel essential oil obtained by hydrodistillation method was determined as 103.4. Main component of citrus essential oil was limonene, and its optical activity value was mentioned as  $+96^\circ$ . This showed that citrus essential oil optical activity value was significantly affected from limonene concentration. Additionally, optical activity value of citrus oils was varied according to extraction method based on their chemical composition. The optical activity value of distilled oil was higher than those obtained by cold-press method [20]. Similarly, Gonzalez-Mas et al. [7] also reported that the optically active value of citrus essential oils can vary with the chemical composition of the oil. The nonvolatile residue could be found in cold-pressed oil, such as flavonoids, coumarins, diterpenoids, sterols, and fatty acids. While the Yerli Apireno cultivar evaluated within the scope of our study was compatible with these limit values, the data determined in other varieties were higher than these limit values. It was thought that this difference may be due to factors



such as cultivar characteristics, climate and soil structure of the region where the samples are grown, as well as harvest time. In fact, literature values have also shown that there may be differences in this sense.

The average value of the composition of the essential oils obtained from the peels of the mandarin varieties evaluated within the scope of the study was given in Table 6, Table 7, Table 8, Table 9, Table 10, Table 11, Table 12, respectively, for Batem İncisi, Batem Yıldızı Batem Göral, Clementine Fina, Nova, Fortune, and Yerli Apireno varieties.

**Table 6.** EO composition (%) of Batem İncisi according to harvest time and isolation method. The highest limonene content was found at the third and fourth harvests after hydrodistillation and at the fourth harvest after cold pressed extraction.

Compound	RRI	1. Harvest		2. Harvest		3. Harvest		4. Harvest	
		HD	CP	HD	CP	HD	CP	HD	CP
$\alpha$ -pinene	1030	1.0	0.8	1.0	0.9	1.0	0.9	1.0	1.0
$\alpha$ -thujene	1033	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
$\beta$ -pinene	1122	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Sabinene	1132	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
$\beta$ -myrcene	1170	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
$\alpha$ -terpinene	1187	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Limonene	1214	89.5	89.6	89.7	89.4	90.3	89.8	90.0	90.2
$\beta$ -phellandrene	1223	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
$\gamma$ -terpinene	1260	4.7	4.9	4.7	4.9	4.3	4.6	4.5	4.5
p-cymene	1285	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Terpinolene	1298	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Octanal	1304	0.1	0.1	0.1	0.1	udl	udl	udl	udl
Decanal	1491	udl	udl	0.1	0.1	0.1	0.1	0.1	0.1
Linalool	1549	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1
$\beta$ -elemene	1585	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1
Terpinen-4-ol	1595	0.1	0.1	0.1	0.1	udl	0.1	0.1	Udl
( <i>E</i> )- $\alpha$ -farnesene	1663	0.2	0.3	0.2	0.3	0.2	0.4	0.2	0.2
$\alpha$ -terpineol	1709	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
$\beta$ -bisabolene	1746	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.1
Unidentified	-	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1

HD: Hydrodistillation, CP: Cold-pressed, udl: under detection level.

The composition of the essential oil of Batem İncisi mandarin peel, registered under the name of the Western Mediterranean Agricultural Research Institute, differed from the composition of the essential oil of other mandarin varieties. The number of components detected in this cultivar was 18, and the main component for this cultivar was limonene, a monoterpene structure that is a characteristic component of citrus peel essential oils. Limonene has anti-inflammatory, antidiabetic, anticancer, antioxidant, and lipid lowering activities [9]. The limonene content ranged between 89.4–90.5% depending on the harvest time and extraction method. The second component in the essential oil obtained from Batem İncisi was  $\gamma$ -terpinene (4.3–4.9%), followed by  $\beta$ -myrcene (2.0%) and  $\alpha$ -pinene (0.8–1.0%) (Table 6). In this sense, it can be said that it was partially similar to the Yerli Apireno cultivar. However, the amount of  $\gamma$ -terpinene detected in this cultivar was proportionally much lower than that detected

in the Yerli Apireno cultivar. These mandarin cultivars attract attention with their high  $\gamma$ -terpinene content. The effects of harvest time and extraction method on the essential oil composition were limited.

The composition of the peel essential oil of the Batem Yıldızı cultivar, which was the second mandarin cultivar evaluated in the study and registered under the name of the Western Mediterranean Agricultural Research Institute, shows significant similarities with the composition of the peel essential oil of Batem İncisi. A total of 19 components were identified in this cultivar, and the first four components with the highest proportions were limonene,  $\gamma$ -terpinene,  $\beta$ -myrcene and  $\alpha$ -pinene, and these components were distributed in the ranges of 89.6–91.0%, 3.9–4.8%, 2.0–2.1% and 0.8–1.0%, respectively. Other components that remained below 1% proportionally also showed partial variations according to the harvest time and extraction method (Table 7). The amount of limonene, the main component of Batem Göral peel essential oil, did not show a significant change according to the harvest time and extraction method. The second component that was higher compared to the other components in Batem Göral peel essential oil was  $\beta$ -myrcene (Table 8). The amount of limonene, the main component in the essential oil of the Clementine Fina cultivar peel, ranged from 95.4% to 96.1% (Table 9).

**Table 7.** EO composition (%) of Batem Yıldızı according to harvest time and isolation method. The highest limonene content was found at the second, third and fourth harvests after hydrodistillation and at the fourth harvest after cold pressed extraction.

Compound	RRI	1. Harvest		2. Harvest		3. Harvest		4. Harvest	
		HD	CP	HD	CP	HD	CP	HD	CP
$\alpha$ -pinene	1030	1.0	0.9	0.9	0.9	1.0	0.8	1.0	0.9
$\alpha$ -thujene	1033	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
$\beta$ -pinene	1122	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3
Sabinene	1132	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
$\beta$ -myrcene	1170	2.1	2.0	2.0	2.0	2.0	2.0	2.0	2.0
$\alpha$ -terpinene	1187	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Limonene	1214	89.7	89.6	90.1	89.8	90.3	89.5	90.6	90.2
$\beta$ -phellandrene	1223	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
$\gamma$ -terpinene	1260	4.6	4.8	4.3	4.4	4.3	4.6	3.9	4.2
p-cymene	1285	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Terpinolene	1298	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Octanal	1304	0.1	udl	0.1	0.1	udl	udl	0.1	udl
Citronellal	1481	udl	0.1	udl	0.1	udl	udl	udl	0.1
Decanal	1491	udl	udl	0.1	0.1	0.1	0.1	0.1	0.1
Linalool	1549	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.1
$\beta$ -elemene	1585	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.2
$\alpha$ -terpineol	1709	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1
$\beta$ -bisabolene	1746	0.1	0.1	0.1	0.2	0.1	0.3	0.1	0.2
Geranial	1748	0.2	0.3	0.3	0.3	0.2	0.6	0.2	0.4
Unidentified	-	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1

HD: Hydrodistillation, CP: Cold-pressed, udl: under detection level.

**Table 8.** EO composition (%) of Batem Göral according to harvest time and isolation method. In all harvests, the highest limonene content was found after cold pressed extraction.

Compound	RRI	1. Harvest		2. Harvest		3. Harvest		4. Harvest	
		HD	CP	HD	CP	HD	CP	HD	CP
$\alpha$ -pinene	1030	0.6	0.5	0.6	0.5	0.6	0.5	0.6	0.5
Sabinene	1132	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.4
$\beta$ -myrcene	1170	2.0	2.0	2.0	2.0	2.0	1.9	2.0	2.0
Limonene	1214	95.6	95.7	95.5	95.8	95.7	95.9	95.8	95.9
$\beta$ -phellandrene	1223	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Octanal	1304	0.3	0.2	0.2	0.2	0.3	0.2	0.2	0.2
Decanal	1491	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.2
Linalool	1549	0.5	0.4	0.5	0.4	0.4	0.3	0.4	0.3
$\alpha$ -sinensal	1765	udl	0.1	0.1	0.1	udl	0.1	udl	0.1
Unidentified	-	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

HD: Hydrodistillation, CP: Cold-pressed, udl: under detection level.

**Table 9.** EO composition (%) of Clementine Fina according to harvest time and isolation method. In all harvests, the highest limonene content was found after cold pressed extraction. At the second harvest the hydrodistillation method showed the same limonene content of cold-pressed extraction.

Compound	RRI	1. Harvest		2. Harvest		3. Harvest		4. Harvest	
		HD	CP	HD	CP	HD	CP	HD	CP
$\alpha$ -pinene	1030	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sabinene	1132	0.5	0.4	0.3	0.3	0.3	0.3	0.2	0.2
$\beta$ -myrcene	1170	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Limonene	1214	95.3	95.5	95.7	95.7	95.7	95.9	96.0	96.1
$\beta$ -phellandrene	1223	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Octanal	1304	0.2	0.2	0.3	0.2	0.3	0.2	0.2	0.1
Decanal	1491	0.4	0.3	0.2	0.3	0.2	0.2	0.2	0.2
Linalool	1549	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.2
$\alpha$ -terpineol	1709	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
$\alpha$ -sinensal	1765	0.1	0.3	0.1	0.2	0.1	0.1	0.1	0.2
Unidentified	-	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1

HD: Hydrodistillation, CP: Cold-pressed.

The main component of Nova peel essential oil was limonene (95.4–96.2%), and the content of this component showed small differences according to the harvest time and extraction method. The lowest content of this component was determined in the first harvest time. When an evaluation was made between the extraction applications, it was found to be slightly higher in those obtained by cold pressing. However, this difference was quite low. Another component above 1% in the samples was  $\beta$ -myrcene, which is also a monoterpene structure. The content of other components in the peel essential oil composition obtained from Nova cultivar remained below 1% (Table 10). The peel

essential oil of Fortune mandarin cultivar evaluated within the scope of the study also stands out with its high limonene content (95.2–95.7%). No significant difference was detected in the limonene content of this cultivar according to the harvest time and extraction method. The second most important component in the peel essential oil obtained from this cultivar was  $\beta$ -myrcene, which is a monoterpene structure. The statistical evaluations of these two components were also made for seven mandarin cultivars according to cultivar, harvest time, and extraction method. The content of the other components detected remained below 1% (Table 11).

**Table 10.** EO composition (%) of Nova according to harvest time and isolation method. The highest limonene content was found at the second and fourth harvests.

Compound	RRI	1. Harvest		2. Harvest		3. Harvest		4. Harvest	
		HD	CP	HD	CP	HD	CP	HD	CP
$\alpha$ -pinene	1030	0.6	0.5	0.6	0.5	0.6	0.5	0.6	0.5
Sabinene	1132	0.4	0.3	0.2	0.2	0.3	0.3	0.2	0.2
$\delta$ -3-carene	1156	0.1	0.1	udl	udl	0.1	0.1	udl	udl
$\beta$ -myrcene	1170	2.1	2.1	2.1	2.0	2.1	2.0	2.0	2.0
Limonene	1214	95.4	95.7	96.1	96.1	95.7	95.7	96.2	96.1
$\beta$ -phellandrene	1223	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
$\gamma$ -terpinene	1260	0.3	0.3	0.1	0.1	0.3	0.3	0.2	0.2
Decanal	1491	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.2
Linalool	1549	0.6	0.5	0.4	0.3	0.4	0.3	0.3	0.2
Unidentified	-	0.1	0.1	0.1	0.3	0.1	0.3	0.1	0.3

HD: Hydrodistillation, CP: Cold-pressed, udl: under detection level.

**Table 11.** EO composition (%) of Fortune according to harvest time and isolation method. The highest limonene content was found at the first harvest after hydrodistillation extraction.

Compound	RRI	1. Harvest		2. Harvest		3. Harvest		4. Harvest	
		HD	CP	HD	CP	HD	CP	HD	CP
$\alpha$ -pinene	1030	0.6	0.5	0.6	0.5	0.6	0.5	0.6	0.5
Sabinene	1132	0.3	0.3	0.3	0.4	0.4	0.3	0.5	0.4
$\beta$ -myrcene	1170	2.0	2.0	2.0	1.9	1.9	1.9	1.9	1.9
Limonene	1214	95.7	95.4	95.3	95.3	95.4	95.4	95.3	95.3
$\beta$ -phellandrene	1223	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
$\gamma$ -terpinene	1260	0.5	0.4	0.4	0.5	0.5	0.5	0.6	0.5
Linalool	1549	0.3	0.4	0.5	0.4	0.6	0.4	0.3	0.2
$\alpha$ -terpineol	1709	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
$\alpha$ -sinensal	1765	0.1	0.2	0.1	0.18	0.1	0.2	0.1	0.3
Unidentified	-	0.1	0.4	0.4	0.4	0.1	0.4	0.3	0.5

HD: Hydrodistillation, CP: Cold-pressed.

The composition of the peel essential oil of the Yerli Apireno cultivar evaluated within the scope of the research showed a significant difference from other mandarin varieties. This cultivar also

differed from other varieties in terms of the number of components. The main component in this cultivar was limonene, but the rate of this component was quite low compared to other mandarin cultivars. The limonene content detected in this cultivar varied between 76.6% and 78.9% depending on the harvest time and extraction method. Among the mandarin cultivars Yerli Apireno draws attention with its high  $\gamma$ -terpinene content. The  $\gamma$ -terpinene rate detected in this cultivar varied between 13.2% and 14.4%. This rate has some similarities with lemon varieties. The other two components over 1% in the samples were  $\alpha$ -pinene and  $\beta$ -myrcene, and the rate of these components ranged between 1.7% and 1.9% and 1.9% and 2.0%, respectively (Table 12).

**Table 12.** EO composition (%) of Yerli Apireno according to harvest time and isolation method. The highest limonene content was found at the first and fourth harvest after hydrodistillation extraction and at the fourth harvest after cold-pressed extraction.

Compound	RRI	1. Harvest		2. Harvest		3. Harvest		4. Harvest	
		HD	CP	HD	CP	HD	CP	HD	CP
$\alpha$ -pinene	1030	1.8	1.7	1.9	1.7	1.9	1.7	1.8	1.7
$\alpha$ -thujene	1033	0.6	0.6	0.7	0.6	0.7	0.6	0.7	0.6
$\beta$ -pinene	1122	1.2	1.1	1.3	1.2	1.2	1.1	1.2	1.1
Sabinene	1132	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
$\beta$ -myrcene	1170	1.9	1.9	1.9	1.9	2.0	1.9	1.9	1.9
$\alpha$ -terpinene	1187	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.3
Limonene	1214	78.3	77.9	76.6	77.3	77.8	77.8	78.7	78.2
$\beta$ -phellandrene	1223	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2
$\gamma$ -terpinene	1260	13.2	13.6	14.4	14.3	13.5	13.8	13.2	13.6
p-cymene	1285	0.6	0.4	0.6	0.5	0.6	0.6	0.5	0.4
Octanal	1304	0.7	0.5	0.7	0.7	0.7	0.7	0.6	0.7
Decanal	1491	0.1	0.5	0.1	0.1	0.1	0.1	0.1	0.1
Linalool	1549	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Terpinene-4-ol	1595	0.1	0.1	0.1	udl	0.1	udl	udl	0.1
$\beta$ -caryophyllene	1596	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
( <i>E</i> )- $\alpha$ -farnesene	1663	0.1	0.1	0.2	0.2	0.1	0.2	0.1	0.1
$\alpha$ -terpineol	1709	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
$\alpha$ -sinensal	1765	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2
Unidentified	-	0.2	0.4	0.4	0.3	0.2	0.3	0.1	0.3

HD: Hydrodistillation, CP: Cold-pressed, udl: under detection level.

The ANOVA and Duncan test results for  $\beta$ -myrcene, limonene, and  $\gamma$ -terpinene components, which were found at higher levels than other components in the peel essential oil composition of mandarin samples, were given in Table 13 and Table 14. The effects of cultivar and isolation method on  $\beta$ -myrcene content were statistically significant, while the effect of harvest time remained not significant (Table 13). Nova had the highest  $\beta$ -myrcene content among the cultivars, while Yerli Apireno had the lowest.  $\beta$ -myrcene is an important component for citrus essential oil, which has antifungal and embryofetotoxicity activity [9]. Batem İncisi, Batem Yıldızı, Batem Göral and Clementine Fina cultivars were in the same group in terms of  $\beta$ -myrcene content. Fortune cultivar was in the same group with the Yerli Apireno cultivar in this sense. The average  $\beta$ -myrcene content of

mandarin peel essential oils obtained by hydrodistillation method was higher than the  $\beta$ -myrcene content of essential oils obtained by cold press method. While the effect of cultivar on limonene content of samples was significant ( $p < 0.01$ ), the effect of harvest time, extraction method and interaction remained not statistically significant. Batem Göral, Clementine Fina, Nova, and Fortune varieties were prominent varieties with high limonene content and showed similarity with orange varieties with this feature. In this sense, Batem İncisi and Batem Yıldızı showed similarity. The Yerli Apireno cultivar showed significant difference from other varieties in terms of this feature. These data showed that cultivar selection was quite important in terms of essential oil composition during the production of mandarin peel oils. The difference determined according to harvest times and extraction methods remained limited (Table 14).

**Table 13.** Analysis of variance results for  $\beta$ -myrcene, limonene, and  $\gamma$ -terpinene contents.

	$\beta$ -myrcene		Limonene		$\gamma$ -terpinene	
	Statistic F	p-Value	Statistic F	p-Value	Statistic F	p-Value
Cultivar (C)	17.39	0.0001	1180.86	0.0001	804.55	0.0001
Harvest time (HT)	1.90	0.1393	2.25	0.0921	1.06	0.3825
Isolation method (IM)	18.66	0.0001	0.24	0.6265	1.19	0.2864
C×HT	1.07	0.4041	0.65	0.8397	0.54	0.7740
C×IM	0.12	0.9928	0.24	0.9608	0.02	0.9780
HT×IM	0.05	0.9856	0.17	0.9129	0.04	0.9897
C×HT×IM	0.37	0.9882	0.16	1.000	0.06	0.9990
Coefficient of variation	1.9726		0.8397		9.8701	

**Table 14.** Duncan results of  $\beta$ -myrcene, limonene, and  $\gamma$ -terpinene contents (%) of mandarin peel essential oil according to cultivar, harvest time, and isolation method (mean±standard error).

		$\beta$ -myrcene	Limonene	$\gamma$ -terpinene
Cultivar	Batem İncisi	1.99 <sup>b</sup> ± 0.001	89.9 <sup>b</sup> ± 0.106	4.64 <sup>b</sup> ± 0.063
	Batem Yıldızı	2.00 <sup>b</sup> ± 0.009	90.2 <sup>b</sup> ± 0.137	4.40 <sup>b</sup> ± 0.071
	Batem Göral	2.00 <sup>b</sup> ± 0.011	95.8 <sup>a</sup> ± 0.058	-
	Clementine Fina	2.00 <sup>b</sup> ± 0.010	95.8 <sup>a</sup> ± 0.067	-
	Nova	2.04 <sup>a</sup> ± 0.015	95.9 <sup>a</sup> ± 0.134	-
	Fortune	1.94 <sup>c</sup> ± 0.007	95.3 <sup>a</sup> ± 0.071	-
	Yerli Apireno	1.92 <sup>c</sup> ± 0.008	77.8 <sup>c</sup> ± 0.368	13.70 <sup>a</sup> ± 0.257
Harvest Time	1 <sup>th</sup> harvest	2.00 ± 0.012	91.4 ± 1.160	7.63 ± 1.245
	2 <sup>nd</sup> harvest	1.98 ± 0.008	91.3 ± 1.241	7.85 ± 1.406
	3 <sup>th</sup> harvest	1.99 ± 0.010	91.6 ± 1.183	7.53 ± 1.317
	4 <sup>th</sup> harvest	1.97 ± 0.010	91.8 ± 1.143	7.31 ± 1.297
Isolation Method	HD	2.00 <sup>a</sup> ± 0.007	91.6 ± 0.826	7.46 ± 0.912
	CP	1.97 <sup>b</sup> ± 0.007	91.5 ± 0.831	7.70 ± 0.911

Different letters on the same column (for each factors) indicate significant difference between the means at the  $p < 0.05$  level.

Another striking feature of the cultivars analyzed within the scope of the research on Batem İncisi,

Batem Yıldızı, and Yerli Apireno was that they have high  $\gamma$ -terpinene content.  $\gamma$ -Terpinene is a well-known natural product used in perfume and flavoring and has some potential biological activities, especially antioxidant and antibacterial properties [39]. While there was no significant difference according to harvest times, it was generally seen that the  $\gamma$ -terpinene content of the essential oils obtained by cold pressing was slightly higher than those obtained by hydrodistillation. However, it was seen that the real difference here is based on the cultivar. It will be seen that the Yerli Apireno cultivar stands out in this sense.

A study was conducted by Frizzo et al. [33] on the composition of essential oils obtained from two mandarin varieties by cold press and hydrodistillation methods in four harvest times. Limonene,  $\gamma$ -terpinene, and  $\beta$ -myrcene contents were determined as the main components in Cai and Montenegrina varieties varied between 67.14–76.41%, 13.67–19.76%, and 1.33–1.78%, respectively. The findings showed that the two varieties have similarities in terms of essential oil composition, and the harvest time and isolation method caused partial differences in the peel oil composition. It was observed that the oil compositions of Cai and Montenegrina varieties were similar to the Yerli Apireno cultivar evaluated within the scope of our research. Sulzbach et al. [32] performed essential oil component analyses of green mandarin peel oils obtained by cold press and hydrodistillation methods in four different times. It was determined that the essential oil compositions of the samples differed according to the isolation method. In the study, it was determined that the content of the main components (limonene,  $\gamma$ -terpinene and  $\beta$ -myrcene) was distributed in the range of 66.16–72.20%, 10.67–19.89%, 1.08–1.82%, respectively. Bozova et al. [10] also investigated the effect of harvest time and extraction method on the essential oil composition of five orange varieties. The study found some difference in essential oil composition among the varieties, but no statistically significant difference was found based on the isolation method. In the study conducted by Shin et al. [40], the main components, limonene,  $\gamma$ -terpinene 8.09%, and  $\beta$ -myrcene 1.68%, were determined in the composition of satsuma mandarin peel essential oil obtained by hydrodistillation method. Dugo et al. [41] obtained mandarin peel oils taken from two different regions where citrus cultivation is widespread in Italy at different harvest times by two different cold press methods (Screw press and Brown type extractor), and reported that harvest time, region and extraction method were partially effective on the peel essential oil composition. Limonene,  $\gamma$ -terpinene,  $\alpha$ -pinene and  $\beta$ -myrcene were determined as the main components in the samples and it was found that these components were distributed in the ranges of 68.51–77.59%, 13.13–20.70%, 1.55–2.14%, 1.29–1.55%, respectively. In the study conducted by Viuda Martos et al. [42] in Spain, the main components of mandarin peel essential oil obtained by cold pressing method were limonene (74.7%),  $\gamma$ -terpinene (15.7),  $\alpha$ -pinene (2%), p-cymene (2%), and myrcene (1.4%). In the European Pharmacopoeia, limit rates were set for  $\alpha$ -pinene, sabinene,  $\beta$ -pinene,  $\beta$ -myrcene, p-cymene, limonene, and  $\gamma$ -terpinene from the essential oil components of mandarin peel oil. The reference ranges for limonene,  $\gamma$ -terpinene, and  $\beta$ -myrcene, which were the prominent components in proportion, were given as 65–75%, 16–22%, and 1.5–2.0%, respectively [35]. The limit values for limonene,  $\gamma$ -terpinene, and  $\beta$ -myrcene in ISO standards are also reported as 65–75%, 16–22%, and 1.4–2.0%, respectively [36]. When these limit data were evaluated, it was seen that the varieties evaluated within the scope of the study, except for the Yerli Apireno, were incompatible with the limit values. This also reveals that our country's mandarin resources show a wide variation.

#### 4. Conclusions

As a result of the research; when the analysis findings obtained were evaluated, it was determined that the mandarin peel essential oil content showed significant differences according to the cultivar and harvest time. It was observed that the relative density, refractive index, and optical activity properties showed significant differences especially according to the essential oil extraction method. It was determined that the essential oil composition could show significant differences according to the cultivar, partial differences during harvesting time, and the extraction method. However, these differences generally remained insignificant at a statistical level. Limonene and  $\beta$  myrcene were the main components in all of the evaluated samples.  $\gamma$ -Terpinene was an important component for Batem İncisi, Batem Yıldızı, and Yerli Apireno cultivars. According to the findings obtained as a result of the study, it was shown that all of these cultivars can be used in the production of mandarin peel oils. Yerli Apireno cultivar complied with international standards for limonene and  $\gamma$ -terpinene content. The cultivars within the scope of our study attracted attention with their generally high limonene content according to international standards. The peel essential oil of Batem Göral, Clementine Fina, Nova, and Fortune cultivars stands out with its high limonene. Cold press application is a widely preferred method in citrus peel essential oil on the industrial scale. On the other hand, an evaluation was made on the basis of two isolation methods, especially in terms of limonene, which is the functional component of citrus oils, it was determined that the production of mandarin peel oils by the hydrodistillation method did not cause any negative effects in terms of limonene content.

#### 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.

#### Authors contributions

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 B.B.; resources, B.B. and M.G.; data curation, B.B., M.G. and A.M.G.; writing—original draft preparation, M.G., B.B.; 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.



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