



Research article

Characterization of aroma and aroma-active compounds of black carrot (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.) pomace by aroma extract dilution analysis

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ABSTRACT

The aim of this study was to characterize the aroma and aroma-active compounds of black carrot pomace, a valuable by-product of black carrot juice industry. Aroma compounds were analyzed using GC-MS-O and extracted via the SAFE technique. The extract samples were determined to represent the odor of black carrot pomace quite well according to the results of the representative test. Accordingly, the aromatic extract scored 77.7 mm for intensity and 87 mm for similarity on a 100 mm unstructured scale. A total of 47 volatile constituents were identified and quantified including terpenes (20), alcohols (11), acids (7), esters (4), ketones (4), and lactone (1). It was observed that terpenes were the major aroma group. The use of aroma extract dilution analysis (AEDA) revealed only 29 of these compounds as aroma-active constituents. Phenylethyl alcohol (FD:512, OAV: 2488, rose) and phenylethyl acetate (FD:256, OAV:280, rose) were the aroma substances providing the strongest rose odor. Following these compounds, acetic acid (vinegar), dimethyl-propanedioic acid (pungent), (*E*)- β -caryophyllene (salty cheese) and elemicin (spicy) were identified as other strong aroma-actives with FD values of 128 contributing to the characteristic odor of the black carrot pomace samples. The odor activity values (OAVs) ranged from 1 to 2488. The highest OAVs represent the high aromatic active compounds (FD \geq 128). The distinctive aroma and rich color of black carrot pomace make it a recommended choice for enhancing flavor and adding natural coloring to food products.

1. Introduction

Carrot (*Daucus carota* L.) is one of the primary root vegetables in the Apiaceae family and it has been cultivated for over 600 years [1,2]. Carrot is frequently preferred by consumers due to its sweetness, crispness, nutritional properties and characteristics aroma [3, 4]. The nutritional contents and sensory properties show variations in different carrot genotypes. In particular, the profile of volatile compounds is affected by the variety as well as growing and environmental conditions [5,6]. Its importance has been revealed especially in studies on the chemical composition of carrot genotypes with different colors [7,8]. Although orange carrots are

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Table 1
Aroma compounds of the black carrot pomace samples examined in the study.

No	LRI ^a	Compounds	Concentration ($\mu\text{g}/\text{kg}$) ^b	Identification ^c
Terpenes				
1	1038	α -Pinene	3075 \pm 177.75	LRI, MS, std
2	1096	β -Pinene	5811 \pm 323.61	LRI, MS, std
3	1167	β -Myrcene	5555 \pm 297.98	LRI, MS, std
4	1196	Limonene	15594 \pm 172.45	LRI, MS, std
5	1261	γ -Terpinene	5711 \pm 343.61	LRI, MS, std
6	1265	<i>o</i> -Cymene	5988 \pm 369.49	LRI, MS, std
7	1288	α -Terpinolene	44334 \pm 2919.61	LRI, MS, std
8	1433	<i>p</i> -Cymenene	1798 \pm 24.76	LRI, MS, std
9	1589	(<i>Z</i>)- β -Caryophyllene	19759 \pm 74.40	LRI, MS, std
10	1628	(<i>E</i>)- β -Caryophyllene	33620 \pm 1597.19	LRI, MS, std
11	1689	β -Citral	721 \pm 14.26	LRI, MS, std
12	1697	Terpineol	1607 \pm 37.43	LRI, MS, std
13	1704	(<i>E</i>)- β -Farnesene	22481 \pm 1101.48	LRI, MS, std
14	1736	(<i>Z</i>)- γ -Bisabolene	112912 \pm 4938.58	LRI, MS, tent
15	1790	(<i>E</i>)- γ -Bisabolene	20503 \pm 382.95	LRI, MS, tent
16	1844	<i>p</i> -Cymen-8-ol	7889 \pm 104.23	LRI, MS, std
17	2013	Caryophyllene oxide	2773 \pm 63.11	LRI, MS, tent
18	2215	Elemicine	470 \pm 6.22	LRI, MS, std
19	2228	α -Bisabolol	98.00 \pm 0.35	LRI, MS, std
20	2257	Myristicine	5668 \pm 261.63	LRI, MS, std
Total Terpenes			316367	
Alcohols				
21	1090	2-Methyl-1-propanol	6300 \pm 405.35	LRI, MS, std
22	1192	3-Hexanol	646 \pm 28.09	LRI, MS, std
23	1206	1-Pentanol	30757 \pm 1557.44	LRI, MS, std
24	1359	1-Hexanol	250 \pm 6.90	LRI, MS, std
25	1461	Heptanol	775 \pm 14.23	LRI, MS, std
26	1468	Sulcatol	486 \pm 19.63	LRI, MS, std
27	1494	2,3-Butanediol	2678 \pm 69.91	LRI, MS, std
28	1544	(<i>Z</i>)-2,3-Butanediol	16070 \pm 351.10	LRI, MS, std
29	1666	1-Nonanol	1250 \pm 8.60	LRI, MS, std
30	1877	Benzyl alcohol	230 \pm 17.96	LRI, MS, std
31	1922	Phenylethyl alcohol	9951 \pm 245.01	LRI, MS, std
Total Alcohols			69393	
Acids				
32	1424	Acetic acid	21187 \pm 77.69	LRI, MS, std
33	1486	Propanoic acid	1676 \pm 25.87	LRI, MS, std
34	1506	Dimethyl-propanedioic acid	6317 \pm 352.82	LRI, MS, std
35	1598	Butanoic acid	6106 \pm 266.94	LRI, MS, std
36	1810	Hexanoic acid	1546 \pm 88.13	LRI, MS, std
37	2030	Octanoic acid	1591 \pm 25.64	LRI, MS, std
38	2144	Nonanoic acid	292 \pm 22.88	LRI, MS, std
Total Acids			38715	
Esters				
39	996	Propyl acetate	265 \pm 15.77	LRI, MS, std
40	1552	Isobornyl acetate	8907 \pm 471.69	LRI, MS, tent
41	1785	Phenylethyl acetate	5325 \pm 315.72	LRI, MS, std
42	1983	Geranyl pentanoate	2731 \pm 144.94	LRI, MS, std
Total Esters			17228	
Ketones				
43	970	2,3-Butanedione	246 \pm 3.39	LRI, MS, std
44	1006	3-Methyl-3-buten-2-one	397 \pm 5.50	LRI, MS, std
45	1250	Acetoin	12072 \pm 363.35	LRI, MS, std
46	1341	6-Methyl-5-hepten-2-one	816 \pm 27.35	LRI, MS, std
Total Ketones			13531	
Lactone				
47	2050	γ - <i>n</i> -Amylbutyrolactone	144 \pm 0.20	LRI, MS, std
Total Lactone			144	
General Total			455378	

^a LRI (Linear retention index) denotes the retention indices on the DB-WAX capillary column.

^b Concentration mean values are based on two replications as $\mu\text{g}/\text{kg}$ with \pm standard deviations.

^c Identification methods: LRI (linear retention index), Std (chemical standard); MS (mass spectra); tent (tentatively).

commonly known, there are also carrots in different colors such as purple/black, red, yellow and white [8,9]. It has been reported that the first type of carrot grown was purple and later yellow and orange types were derived [10]. Black carrots (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.) are produced in the Asian countries such as Türkiye, Afghanistan, Egypt, and India. In comparison to yellow, white, and orange carrots, black carrots have noticeable quality and sensory differences such as aroma and flavor, as well as higher antioxidant content [11].

The total production of carrots (including turnips) worldwide was approximately 61 million tons on an area of 1.51 million hectares in 2022. The largest producing countries were China, Uzbekistan, and the Russian Federation with around 18.7, 3.9, and 1.3 million tons, respectively. Türkiye ranked the sixth place in the world with a production of 0.7 million tons [12].

Black carrots are typically consumed fresh although they are also used for the production of shalgam, a traditional fermented beverage, as well as for juice, concentrate, and powder [13]. The yield in black carrot juice production varies between 45 and 65 %. Black carrot pomace is a valuable by-product from the black carrot juice industry. It is treated as waste by juice producers and often sold as animal feed. However, it is a crucial material due to its volatile aroma components, dietary fiber, antioxidants, and coloring agents and its valorization is of a great interest [14].

Aroma is one of the most significant quality requirements for foods and greatly impacts consumer acceptance and preference [15]. These compounds, which easily evaporate at room temperature, are often altered, degraded, and/or lost during food processing. They affect the quality of the final product and therefore plays an important role in the acceptance of the product by consumers [3,4]. Hundreds of volatiles were reported in carrots and no particular compound is responsible for the distinctive carrot-like flavor [11]. Black carrots and/or their pomaces possess a large number of volatile compounds, some of which contribute to its characteristic odor. These compounds, responsible for their typical odor, are referred to as aroma-active compounds. Despite their presence in very low concentrations, they can be easily detected by human nose due to their high detection threshold values. Gas chromatography-olfactometry (GC-O) is the most effective method for detecting these essential key odorants [16]. Among volatiles, terpenes, especially monoterpenes and sesquiterpenes, comprise the majority of the black carrot volatiles [17]. These terpenes contribute significantly to the aroma of carrots both quantitatively and qualitatively [18,19], imparting woody, pungent, terpenic and greenish odors to the overall flavor profile of carrots [2].

According to the existing literature review, although a study examining the aroma compounds of black carrot pomace exists [13], there has been no research reported on the aroma-active compounds of black carrot pomaces using gas chromatography-mass spectrometry combined with olfactometry (GC-MS-O). Consequently, it is necessary to determine the odor profile of this valuable by-product, which has the valorization potential to be added to various food products as a coloring and/or flavoring additive. For this purpose, the aroma and aroma-active compounds of black carrot pomace were thoroughly investigated using GC-MS-O in the present study. Furthermore, odor activity values (OAVs) were provided to assess the potent aroma compounds present in black carrot pomace.

2. Materials and methods

2.1. Black carrot pomace

Black carrot pomace (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.) was obtained from a carrot juice concentrate manufacturer located in Eregli district of Konya province, Türkiye in December 2023. About 5 kg of black carrot pomace was transported to the laboratory under cold storage conditions and analyzed quickly upon arrival.

2.2. Chemicals

Dichloromethane, 4-nonanol (internal standard), and anhydrous sodium sulfate were acquired from Merck (Darmstadt, Germany). High purity standard aroma compounds (referred as std in Table 1) used in the study were supplied by Sigma Aldrich (Steinheim, Germany).

2.3. Analysis of the volatile compounds

2.3.1. Extraction of the volatile compounds

The aromatic compounds from the black carrot pomace samples were extracted using the solvent-assisted flavor evaporation (SAFE) method. In this procedure, 6.00 g of the sample were mixed with 120 ml of water in a 500 ml Teflon container. Subsequently, 100 ml of diethyl ether was introduced into the container and the mixture was stirred on a magnetic stirrer at 200 rpm for 40 min. Afterward, the sample underwent centrifugation at 2500 rpm for 15 min. This entire process, involving diethyl ether, was carried out twice resulting in a total aroma extract volume of approximately 300 ml. Following centrifugation, the supernatant was subjected to the SAFE process at 40 °C within a vacuum (10^{-3} Pa). The sample was then concentrated to a volume of 5 ml using a Vigreux concentrator. Then, further concentration to 0.5 ml was achieved with a gentle flow of nitrogen before ultimately injected into the gas chromatography-mass spectrometry device (GC-MS) [20].

2.3.2. GC-MS analysis of the aroma extraction

For the identification and quantification of the aroma substances in the black carrot pomace extracts, a Shimadzu GC-MS-QP2020 NX mass spectrometer was employed as connected to a Shimadzu Nexis GC-2030 gas chromatograph with a flame ionization detector (FID) in Central Research Laboratory of Cukurova University (CUMERLAB, Adana, Turkey). A DB-WAX capillary column (30 m × 0.25

mm \times 0.25 μ m) was used to separate the aroma compounds. The injector and detector temperatures were set at 270 and 280 $^{\circ}$ C, respectively. The column temperature started at 60 $^{\circ}$ C for 3 min, then increased to 220 $^{\circ}$ C at a rate of 2 $^{\circ}$ C/min, followed by a further increase to 245 $^{\circ}$ C at 3 $^{\circ}$ C/min, and remained constant at 245 $^{\circ}$ C for 20 min. A 3 μ l injection volume was used in pulsed splitless mode while helium served as the carrier gas with a flow rate of 1.5 ml/min. The MS conditions were as follow: an ionization energy of 70 eV, a quadrupole temperature of 120 $^{\circ}$ C, and an ion source temperature of 250 $^{\circ}$ C. Scanning ranged from m/z 29 to 350 at a rate of one scan per second. The volatiles were identified through a combination of retention index, standard aroma compounds, and mass spectra, utilizing a commercial spectral database (Wiley 10, Flavor 2L, NIST-11). The retention indices for the individual compounds were calculated using the retention data from a series of linear n-alkanes (C₅–C₃₀). Quantification was carried out using 4-nonanol as an internal standard. Response factors were determined by calculating the intensity ratio of each constituent to 4-nonanol and peak area ratios were adjusted using the response factor specific to each volatile compound. Consequently, quantification was calculated by the below equation. Subsequently, means and standard deviations were quantified based on the duplicate GC analyses [9].

$$C_i = (A_i/A_{std}) \times C_{std} \times RF \times CF$$

C_i : concentration of aroma compound; A_i : peak area of aroma compound; A_{std} : peak area of internal standard; C_{std} : concentration of internal standard (41.5 mg/kg); RF: response factor; and CF: calculation factor.

2.4. Aroma extract dilution analysis (AEDA) and odor activity value (OAV)

GC-MS-Olfactometry analyses of the extracts were conducted using a GC system (Shimadzu Nexis GC-2030) coupled with olfactometry with the same operating conditions as mentioned above. The GC system incorporated a Deans switch to divide the effluent as 1:1:1 between the detectors and a sniffing port. This allowed simultaneous signal acquisition for the FID (quantification), olfactometer (sniffing), and MS (identification). AEDA was executed on the SAFE extract samples from the black carrot pomace samples. During this procedure, the aromatic extracts were screened for the key odorants by two experienced sniffers and the means of the results were calculated. To determine the flavor dilution (FD) factor of the identified aroma-active compounds, the concentrated aromatic extract (200 μ l) of black carrot pomace was stepwise diluted with dichloromethane as the solvent in the rates of 1:1, 1:2, 1:4, 1:8, 1:16, ..., 1:512. The panelists sniffed the samples in the GC-O until no odor was detected. The aroma compound with the highest FD value was identified as the most potent key odorant [21]. The concentrations of aroma compounds were divided by their odor threshold value to determine the odor activity values (OAVs). Compounds that were found to individually contribute to the aroma of black carrot pomace were limited to those with an OAV greater than 1 [22].

2.5. Sensory descriptive analysis and representativeness test of the aromatic extract

2.5.1. Sensory panel

The sensory panel consisted of seven evaluators (4 female and 3 males between 22 and 35 years of age) from the Department of Food Engineering, University of Cukurova, Adana, Türkiye. The panelists were familiar with the aroma of black carrot pomace and had previously received training in odor recognition and sensory evaluation techniques, and had experience in GC-MS-O analyses. As for the training sessions on black carrot pomace descriptors, the first session was held in an ordinary room to generate descriptors for black carrot pomace. The panel generated descriptors for black carrot pomace, resulting in a list of seven consensus odor descriptors (carrot, pungent, salted cheese, floral, spicy, herbal-green, and terpene-like). Subsequent sessions took place in a sensory room with isolated booths, under natural light at room temperature.

2.5.2. Sample preparation and presentation

Cardboard sniffing strips (Granger-Veyron, Lyas, France) were employed to appraise the aroma extracts obtained through the SAFE method. In previous research, cardboard strips were used to test the representativeness of Turkish coffee extracts [20] and black cumin seed extracts [18]. To conduct the representativeness tests, 15 g of black carrot pomace sample was placed in a brown-coded flask (25 ml). The aroma extract from the sample was adsorbed on the cardboard strips. After 1 min during which dichloromethane evaporation occurred, the strips were placed in dark-colored coded flasks (25 ml). After 15 min, the flasks were presented to the panelists for sniffing.

2.5.3. Similarity and intensity tests

A similarity assessment was used to determine the degree of similarity between the extract's odor and that of black carrot pomace (the reference sample). The panelists identified the similarities between the odors of the black carrot pomace and the aromatic extract. A 100-mm unstructured scale was used with a mark of "very different from the reference" on the left side and "similar to the reference" on the right side (Supplementary Material). The sample's position on the unstructured scale was measured in mm from the left anchor. The panelists were required to evaluate the odor intensity of the extract. A 100-mm unstructured measure was utilized with "no odor" note on the left side and "very strong odor" note on the right side. The sample's position on the unstructured scale was quantified as a distance in mm from the left anchor [21].

2.5.4. Descriptive analysis

Seven descriptors including carrot, pungent, salted cheese, floral, spicy, herbal-green, and terpene-like that define the samples'

decisive aroma were assigned by the trained panel evaluators. The aromatic extract and reference sample (black carrot pomace) were presented to the assessors and then, they were asked to appraise the odor properties of each sample by determining the intensity of each given descriptor on an unstructured scale of 100 mm with “no odor” mark at the left-hand side and “very strong odor” mark at the right-hand side (Supplementary Material). Each panelist’s scores were recorded on the scale and the average odor intensity was determined in millimeters and the results were given using a spider web diagram [22].

2.5.5. Data analysis

The statistical method employed was the independent-samples analysis of variance test (*t*-test), which compares the sensorial scores of the reference sample (black carrot pomace) and its aromatic extract. All data obtained during the aroma analysis were presented as averages of duplicate. The statistical analysis was performed using SPSS Statistics software version 22.0 (Chicago, IL, USA).

3. Results and discussion

3.1. Sensory analysis

3.1.1. Odor comparison of the black carrot pomace and its extract

Seven panelists compared the aromatic extract on the sniffing strip to the black carrot pomace sample (reference sample). Fig. 1 represents the mean intensity scores for the reference (pomace) sample and its extract sample, plotted on a spider graph with seven descriptors (carrot, pungent, salted cheese, floral, spicy, herbal-green, and terpene-like). Keskin et al. [9] utilized descriptors of black carrot and determined the sensory properties of fresh and dried black carrots. The descriptors with the highest scores were floral, pungent, terpene-like, and herbal-green while salted cheese and spicy received the lowest scores [9]. Fig. 1 clearly shows that the panelists assessed the sensory properties of the aromatic extract as similar to the original (reference sample) pomace sample in the present study. There was no statistical difference ($p > 0.05$) between the reference sample and its aromatic extract for the six of the seven descriptors while a substantial difference was found only for the “carrot” descriptor which was rated higher than that of the aromatic extract. The highest descriptive score was attributed to floral and in line with the sensory analysis, the results confirmed the phenylethyl alcohol as the most dominant key odorant, followed by phenylethyl acetate. These were identified as responsible for the rose odor in black carrot pomace.

3.1.2. Similarity and intensity evaluation of the aromatic extract

The intensity score of the aromatic extract on the smelling strip was found to be 77.7 mm on a 100 mm unstructured scale while the similarity score of the aromatic extract was 87 mm on the same scale in the present study. The aim of both the similarity and intensity evaluation tests was to compare the representativeness of the odor of the aromatic extract with that of the reference sample (black carrot pomace). Our sensory analysis results suggested that the extract from the black carrot pomace was considered representative of the olfactometric analysis. Kesen et al. [22] reported similarity and intensity scores of 79.0 mm and 71.0 mm, respectively, for black cumin seed extract. In another previous study, the similarity scores of the raw champignon and oyster mushroom extracts were 77 and 81 mm, respectively, while the intensity scores were 80 and 74 mm [21].

3.2. Volatile compounds of the black carrot pomace

The aroma compounds identified in the black carrot pomace samples are listed in Table 1. A diverse ranges of compounds were detected in the samples. Based on the GC-MS analysis results, a total of 47 volatile constituents including terpenes (20), alcohols (11), acids (7), esters (4), ketones (4), and lactone (1) were identified (Table 1). The aroma group identified with the highest number and concentration in black carrot pomace was terpenes. This was followed, in order, by alcohols, acids, esters, and ketones. The compound with the lowest concentration was a lactone (γ -*n*-amylbutyrolactone). The total aroma concentration was determined to be 455378 $\mu\text{g}/$

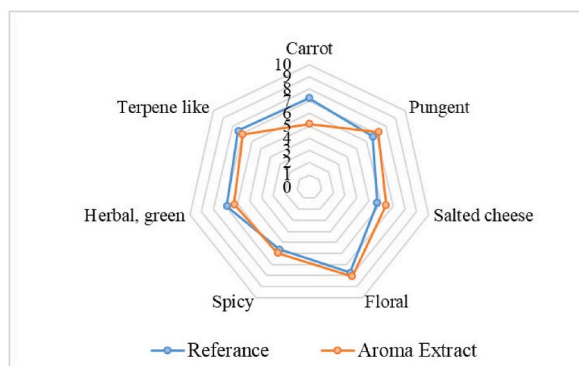


Fig. 1. Odor sensory profiles of the black carrot pomace (reference) and its aromatic extract.

kg (Table 1). According to previous studies, many colored carrot varieties have also been reported to contain significant amounts of the identified aroma compounds [11,23–25].

3.2.1. Terpenes

Terpenes were identified as the dominant aroma group in the black carrot pomace samples in the present study. A total of 20 terpenes were determined and the total terpene concentration was 316367 $\mu\text{g}/\text{kg}$ (Table 1). Similarly, in previous studies, terpenes were identified as the major aroma group in both fresh black carrots [9] and black carrot pomaces [13]. Similar to our findings, in some studies examining the aroma compounds of carrots with different colors (purple, red, orange, yellow, and white), terpenes were the most dominant aroma group in all carrot varieties [6,11,23,24,26]. In these studies, terpenes were reported to give carrots their characteristic aroma and thus, they are considered the most important volatiles. Similarly, the dominant aroma group in yellow-purple Polignano carrots was terpenes [27].

Terpenes are secondary metabolites consisting of five-carbon isoprene units in their structure and classified according to the number of isoprene in their structure [28]. Monoterpenes contain two isoprene units while sesquiterpenes contain three of them. Plants use two biosynthetic pathways for the synthesis of terpenes [29]. The first is the mevalonate pathway in the cytosol and sesquiterpenoids are synthesized there. Another is the methylerythritol phosphate pathway, which is independent of the mevalonate pathway occurring in plastids. In this way, monoterpenoids are synthesized [7,30]. It was determined in the current study that the terpene with the highest quantity was (*Z*)- γ -bisabolene, followed by α -terpinolene and (*E*)- β -caryophyllene (Table 1). Similarly, the previous studies on the aroma compounds of carrots with different colors stated that (*E*)- γ -bisabolene and terpinolene were the main terpenes (6,11, 23). Some previous studies reported terpinolene and (*E*)-caryophyllene as abundant terpenes in carrots [4,17,25,31]. In the current study, the α -terpinolene was the second most abundant terpene. In addition, terpineol was present in very low amounts. In an earlier study, it was highlighted that terpinene-4-ol and terpineol could be produced easily from α -terpinolene by oxidative processes that attack the tetrasubstituted double bond, which is common when carrots are blended [7,32]. Other important terpenes identified in the existent study include the monoterpene compound of limonene and the sesquiterpene compounds of (*E*)- β -farnesene, (*E*)- γ -bisabolene, and (*Z*)- β -caryophyllene. These results are consistent with the terpenes reported in carrots in some previous investigations [9,11,32,33]. In the current study, elemicine and myristicine were also among the compounds identified in the black carrot pomace samples. Previous research highlighted terpenes such as elemicine and myristicine in orange carrots. These compounds were reported as phenylpropanoids and exist as secondary metabolites in plants [18,32]. In a previous study on the volatile compounds of carrots with different colors, myristicin was detected only in black and red carrot varieties [6]. Another study conducted on yellow-purple Polignano carrots reported that myristicin was the most abundant sesquiterpene [27]. Myristicin plays a role in the formation of bitter taste in carrots [34]. Genetic diversity, environmental and storage conditions affect the chemical composition of carrots, thereby influencing the qualitative and quantitative aspects of their volatiles [24,31].

3.2.2. Alcohols

Alcohols were found to be the aroma group with the highest concentration after terpenes in the present study (Table 1). Similarly, Polat et al. [13] reported the alcohols as the most dominant aroma group following terpenes in black carrot pomaces. In another study, Keskin et al. [9] examined the aroma profile of black carrots and similarly reported that alcohols were the most abundant aroma group after terpenes and also identified the compound 3-hexanol. In the current study, the total alcohol concentration was determined as 69393 $\mu\text{g}/\text{kg}$ (Table 1). Among the alcohols, 1-pentanol was the most dominant followed by (*Z*)-2,3-butanediol and phenylethyl alcohol, respectively. 1-Pentanol was also detected in microwave-dried orange-colored carrots in another previous study [4]. Alcohols are formed by the alcohol dehydrogenase (ADH) enzyme, which is commonly found in plants and responsible for the formation of volatile alcohols that give flavor to foods [22].

3.2.3. Acids

After terpenes and alcohols, the other most dominant aroma group was found to be acids (Table 1). A total of 14 acids were determined with a total concentration of 38715 $\mu\text{g}/\text{kg}$. Among them, the most abundant one was acetic acid followed by dimethylpropanedioic acid, and butanoic acid. Acetic, hexanoic, octanoic, and nonanoic acids were also reported in black carrot pomaces in a previous study [13]. Similarly, hexanoic acid was detected in the samples of shalgam, a fermented beverage produced from black carrots [35]. In another study on carrots, acetic acid was also among the major volatile components [17].

3.2.4. Esters

Four esters as propyl acetate, isobornyl acetate, phenylethyl acetate, and geranyl pentanoate were identified and their total concentration was 17228 $\mu\text{g}/\text{kg}$ (Table 1). Among them, isobornyl acetate was detected in the highest quantity while propyl acetate had the lowest concentration. Similar to our findings, isobornyl acetate was determined with the highest concentration among esters in fresh black carrots and black carrot pomaces in previous studies [9,13]. Similarly, this substance was also previously reported in carrot seed essential oil [36]. In a study on shalgam, a fermented beverage produced from black carrots, the bornyl acetate was detected as originated from black carrots [35]. Phenylethyl acetate was similarly detected in black carrot pulp in an earlier study [13].

3.2.5. Ketones

A total of four ketones were quantified in the black carrot pomace samples, including 2,3-butanedione, 3-methyl-3-buten-2-one, acetoin, and 6-methyl-5-hepten-2-one, with a total quantity of 13531 $\mu\text{g}/\text{kg}$. Among these, acetoin was the most abundant (Table 1). In a previous study, acetoin was similarly detected in both raw and dehydrated carrot samples [17]. In the same study, 2,3-butanedione

was also determined. The 6-methyl-5-hepten-2-one was also identified in other previous studies in orange carrots as one of the degradation products of carotenoids [18,32]. In an earlier study on the volatile profile of carrots with different colors, 6-methyl-5-hepten-2-one was detected in dark-colored (black and red) carrots [6].

3.2.6. Lactone

One lactone compound (γ -*n*-amylbutyrolactone) was identified in the samples with the lowest concentration of 144 $\mu\text{g}/\text{kg}$ (Table 1). Lactones are formed by the β -oxidation of saturated fatty acids [27].

3.3. Aroma-active compounds of the black carrot pomace

In the present study, aroma extract dilution analysis (AEDA) was employed along with GC-MS-O to assess the aroma-active compounds that form the characteristic odor of the black carrot pomace samples. The aroma-actives identified in the samples, their odor descriptions and their FD values were given in Table 2. This table also shows the compounds with an OAV greater than 1. FD factors of aroma-active compounds ranged between 2 and 512, while OAVs were in the range of 1–2488. A total of 29 aroma-actives were identified including terpenes (13), alcohols (6), acids (4), esters (3), ketone (1), lactone (1), and unknown (1). This unknown compound was detected by GC-O but not identified by GC-MS due to the very small areas of the peaks and/or their mass/charge (m/z) ratio. An unknown compound (LRI: 1489), may contribute to the global aroma of black carrot pomace with a cucumber, and herbs notes. Similar to the aroma profile described above, terpenes dominated the key odorants of the samples, with 13 compounds having FD values ranging between 2 and 128. Although terpenes were quantitatively more abundant in aroma-actives, it was determined that the compounds providing the strongest aroma to the black carrot pomace samples were an alcohol (phenylethyl alcohol, FD: 512, OAV: 2488) and an ester (phenylethyl acetate, FD: 256, OAV: 280) (Table 2). These compounds had the highest aroma dilution values and OAV providing rose odor.

Terpenes had the largest number of aroma-active compounds providing a citrusy, terpenic, and spicy odor (Table 2). Terpenes make a substantial contribution to the aroma of various types of carrots and are generally considered potent aroma compounds. They were reported to be responsible for the harsh or bitter flavor in carrots [23]. Many authors also indicated that terpenes are primarily responsible for the unique flavor of carrots [11,18,23,49]. In the current study, among the terpenes, (*E*)- β -caryophyllene (FD: 128, salted cheese, spicy), elemicine (FD: 128, spicy), and myristicine (FD: 64, spicy, sweet) were the most potent key odorants (Table 2).

Table 2

Aroma-active compounds of the black carrot pomace samples examined in the study.

No	LRI ^a	Compounds	Odor descriptions ^b	FD ^c	OT ^d ($\mu\text{g}/\text{L}$)	OAV ^e	Ref.
1	1090	2-Methyl-1-propanol	Green	2	7000	1	[37]
2	1096	β -Pinene	Terpen like, pine	4	140	42	[38]
3	1206	1-Pentanol	Cheesy	4	4000	8	[37]
4	1196	Limonene	Citrus	8	230	24	[39]
5	1250	Acetoin	Creamy	4	800	15	[40]
6	1261	γ -Terpinene	Fruity	2	3260	2	[38]
7	1288	α -Terpinolene	Sweet	2	200000	<1	[41]
8	1424	Acetic acid	Vinegar	128	25	847	[42]
9	1433	<i>p</i> -Cymenene	Green, leaf	8	–	–	
10	1489	Unknown	Cucumber, herb	32	100	17	[43]
11	1494	(<i>E</i>)-2,3-Butanediol	Green pepper	16	10000	<1	[40]
12	1506	Dimethyl-propanedioic acid	Sharp	128	–	–	
13	1544	(<i>Z</i>)-2,3-Butanediol	Fresh, alcohol	4	10000	2	[40]
14	1552	Isobornyl acetate	Fresh	2	–	–	
15	1598	Butanoic acid	Cheesy	64	100	61	[43]
16	1628	(<i>E</i>)- β -Caryophyllene	Salted cheese, spicy	128	64	525	[44]
17	1697	Terpineol	Spicy	2	250	6	[38]
18	1704	(<i>E</i>)- β -Farnesene	Fresh, floral	32	–	–	
19	1785	Phenylethyl acetate	Rose	256	19	280	[43]
20	1810	Hexanoic acid	Fresh	4	40	39	[40]
21	1844	<i>p</i> -Cymen-8-ol	Green	4	–	–	
22	1877	Benzyl alcohol	Floral	2	100	2	[45]
23	1922	Phenylethyl alcohol	Rose	512	4	2488	[46]
24	1983	Geranyl pentanoate	Fruity	16	–	–	
25	2013	Caryophyllene oxide	Sweet	16	–	–	
26	2050	γ - <i>n</i> -Amylbutyrolactone	Fruity	32	–	–	
27	2215	Elemicine	Spicy	128	–	–	[47]
28	2228	α -Bisabolol	Floral	32	–	–	
29	2257	Myristicine	Spicy, sweet	64	30	189	[48]

^a LRI (Linear retention index) denotes the retention indices on the DB-WAX capillary column.

^b Odor descriptions as sensed by the panelists through olfactometry.

^c FD (Flavor dilution) factor is the highest dilution of the extract sample at which an odorant was determined by the aroma extract dilution analysis.

^d OT: Odor threshold values in water ($\mu\text{g}/\text{L}$) were taken from the literature [37–48].

^e The odor activity values (OAV): Odor activity values were calculated by dividing the concentration by the odor threshold.

Similarly, among these compounds, (*E*)- β -caryophyllene (OAV: 525) and myristicine (OAV: 189) were calculated to have high OAV values (Table 2). Likewise, a study reported that myristicin provided sweet, spicy odors to orange-colored carrots [32]. The terpenes such as γ -terpinene, α -terpinolene, (*E*)- β -farnesene, caryophyllene oxide, and α -bisabolol were responsible for the fruity, sweet, and floral odors of black carrot pomace (FD: 2–32). While the OAVs of γ -terpinene and caryophyllene oxide compounds were found to be similarly low, it can be inferred that α -terpinolene (FD:2) does not significantly contribute to the odor, as its OAV was determined to be less than 1. Similarly, Kjeldsen et al. [32] reported that limonene, γ -terpinene and terpinolene provided citrus-like, fruity and sweet odors, respectively, in orange carrots. Besides, other terpenes such as β -pinene, limonene, *p*-cymenene, terpineol, and *p*-cymen-8-ol ensured terpene-like, greenish, and citrus-like odors to black carrot pomace with FD values ranging from 2 to 8 (Table 2). Similarly, Kjeldsen et al. [32] stated that α -pinene, β -myrcene, and *p*-cymene provided the carrot with a terpene-like, pine, and green odors in orange carrots. In another study by Kreutzmann et al. [23], a relationship between terpenoid and bitterness properties with *p*-cymene, γ -terpinene, limonene, (*E*)- β -farnesene, α -phellandrene and terpinolene was reported. The same authors also reported that β -myrcene, α -terpinene and β -phellandrene were associated with terpenoid and greenish odors. It was stated in another study that terpenes were responsible for the characteristic aroma of carrots as the most important volatiles providing greenish and earthy odors in carrots [18]. Another study reported that β -myrcene (FD: 128, fresh green), α -pinene (FD: 16, herbaceous), α -terpinene (FD: 8, carrot top) and α -phellandrene (FD: 4, terpinene-like) were responsible for the typical carrot odors and that terpinolene, γ -terpinene and caryophyllene oxide provided fruity aroma [49]. Fukuda et al. [5] assessed the key odorants of different carrot varieties and reported that β -myrcene and terpinolene had the highest odor intensity values providing greatest contribution to carrot flavor. In a study on the aroma-actives of sweet purple potatoes, *p*-cymen-8-ol and α -terpineol were similarly detected with a floral and green-floral odor, respectively [50].

Acids were the second important key odorants in the black carrot pomace samples in the present study (Table 2). Five acids were identified including acetic acid (FD: 128, vinegar), unknown (FD: 32, cucumber, herb), dimethyl-propanedioic acid (FD: 128, sharp), butanoic acid (FD: 64, cheesy), and hexanoic acid (FD: 4, fresh). Acetic acid (OAV: 847), which exhibits the highest dilution value among acid compounds, was also found to have the highest OAV among acids (Table 2).

After phenylethyl alcohol, other important alcohols that contribute to the typical odor of black carrot pomace included 2,3-butanediol (green pepper), 1-pentanol (cheesy), (*Z*)-2,3-butanediol (fresh, alcohol), benzyl alcohol (floral), and 2-methyl-1-propanol (green) with the FD values from 2 to 16 and OAV from 2 to 8 in the present study (Table 2). Apart from phenylethyl alcohol, these alcohols had minor effects on the odor of black carrot pomace.

Regarding the esters, three compounds were identified as the aroma active compounds in the black carrot pomace samples (Table 2). In general, esters are responsible for the fruity odor [35]. Phenyl acetate, with a high FD value (256), was determined as one of the strong scents attributed to rose odor in the present study. Likewise, the OAV of phenylethyl acetate was also found to be high, with a value of 280 (Table 2). After phenylethyl acetate, isobornyl acetate and geranyl pentanoate were identified as minor esters. Isobornyl acetate and geranyl pentanoate were determined to provide fresh and fruity odors with FD values of 2 and 16, respectively.

The other key odorants included a ketone (acetoin, creamy) and a lactone (γ -*n*-amylbutyrolactone, fruity) with FD values of 4 and 32, respectively (Table 2). While the OAV of acetoin was calculated as 15, the odor threshold value of γ -*n*-amylbutyrolactone could not be determined as it was not found in the literature. Acetoin was reported to be a volatile with a pleasant creamy odor and/or fatty butter flavor [51]. In a previous research on black cumin, acetoin was denoted as one of the aroma-actives contributing most to the characteristic aroma [22]. Lactones generally provide fruity, coconut-like, creamy, sweet or nutty odors [35,52].

In sum, the most important odorants in black carrot pomace samples were determined to be benzeneethanol from the alcohols (FD: 512, rose); phenylethyl acetate from the esters (FD: 256, rose); (*E*)- β -caryophyllene (FD: 128, salted cheese, spicy), and elemicine (FD: 128, spicy) from the terpenes; and from the acids, acetic acid (FD: 128, vinegar) and dimethyl-propanedioic acid (FD: 128, sharp). There have been no studies examining the key odorants of black carrot or its pomace, hence with this study, the aroma-active compounds of black carrot pomace were detected for the first time.

4. Conclusion

In the current study, the aroma and aroma-active compounds of black carrot pomace, a valuable by-product of black carrot juice industry often discarded as waste, were examined. As far as it is known, this was the first report on aroma and aroma-active compounds of black carrot pomace. A total of 47 aroma compounds were identified in the black carrot pomace samples, with terpenes being the most dominant aroma group, followed by acids. However, only 29 of those were identified as aroma-active compounds. The FD factors of aroma-active compounds varied between 2 and 512, while OAVs were in the range of 1–2488. Among them, phenylethyl alcohol (rose), phenylethyl acetate (rose), acetic acid (vinegar), dimethyl-propanedioic acid (sharp), (*E*)- β -caryophyllene (salted cheese, spicy), and elemicine (spicy) play a predominant role for the typical odor. This was consistent with the findings of sensory analyses of black carrot pomace (reference) and its aromatic extract. Contrary to its consideration as waste, the pomace generated after the production of black carrot juice can be recognized as a valuable by-product that can be valorized as an ingredient or colorant in various food products such as bakery and dairy products and beverages due to its strong aroma and coloring properties. Further research is needed to obtain more information about the aroma and key odorants of this valuable by-product and to evaluate the potential for use of its aroma in different food products.

Data availability statement

The data that has been used is confidential.

Ethical approval

Ethics approval was not required for this research as this article does not contain any studies with human participants or animals performed by any of the authors. Informed consent was obtained from all participants of sensory analysis.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Serkan Selli, the corresponding author, has currently an Associate Editor position in the journal Heliyon. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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