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Identification of volatile compounds in salep (*Serapias vomeracea*) tubers and effects of harvest time and drying method on composition variation

Identificação de compostos voláteis em tubérculos de salepo (*Serapias vomeracea*) e efeitos do momento da colheita e do método de secagem na variação da composição

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ABSTRACT

Serapias vomeracea is an economically valuable plant belonging to the Orchidaceae family; particularly, its tubers are consumed as hot drinks. Past studies on *S. vomeracea* have focused on volatile components only in the aerial parts of this orchid. This study is the first to investigate the volatile compounds present in *S. vomeracea* tubers, which have a high commercial value. Additionally, we determined the effect of harvest time and drying method on the volatile compound profile. The tubers were harvested on two different days (May 17, 2022, and June 6, 2022) and subjected to four different drying conditions (shade drying, oven drying, shade drying after boiling, and lyophilizer drying). To determine the volatile compound profile, sample extracts were prepared by solid-phase microextraction (SPME) and analyzed by gas chromatography–mass spectrometry. Overall, 22 volatile compounds were identified: 3 esters, 3 aldehydes, 3 alcohols, 3 terpenes, 3 alkanes, 3 carboxylic acids, 1 phenol, 1 ketone, 1 lactone, and 1 furan. The results revealed that p-cresol was the main compound. During harvesting and subsequent drying, some compounds were lost, and some new compounds were formed. Moreover, harvest time had quite limited effects on volatile compounds in the lyophilizer method. Cluster analysis revealed that the combination of harvest time and drying was effective in the distribution of volatile compounds in salep powder.

Index terms: Orchid; solid-phase microextraction; GC-MS.

RESUMO

Serapias vomeracea é uma planta economicamente valiosa pertencente à família Orchidaceae; particularmente, seus tubérculos são consumidos como bebidas quentes. Estudos anteriores sobre *S. vomeracea* têm se concentrado apenas nos componentes voláteis das partes aéreas desta orquídea. Este estudo é o primeiro a investigar os compostos voláteis presentes nos tubérculos de *S. vomeracea*, que possuem alto valor comercial. Além disso, determinamos o efeito do momento da colheita e do método de secagem no perfil de compostos voláteis. Os tubérculos foram colhidos em dois dias diferentes (17 de Maio de 2022 e 6 de Junho de 2022) e submetidos a quatro condições diferentes de secagem (secagem à sombra, secagem em forno, secagem à sombra após fervura e secagem por liofilização). Para determinar o perfil de compostos voláteis, foram preparados extratos de amostras por microextração em fase sólida (SPME) e analisados por cromatografia gasosa acoplada à espectrometria de massa. No total, 22 compostos voláteis foram identificados: 3 ésteres, 3 aldeídos, 3 álcoois, 3 terpenos, 3 alcanos, 3 ácidos carboxílicos, 1 fenol, 1 cetona, 1 lactona e 1 furano. Os resultados revelaram que o p-cresol foi o composto principal. Durante a colheita e a subsequente secagem, alguns compostos foram perdidos e alguns novos compostos foram formados. Além disso, o momento da colheita teve efeitos bastante limitados nos compostos voláteis no método de liofilização. A análise de agrupamento revelou que a combinação de momento da colheita e secagem foi eficaz na distribuição de compostos voláteis no pó de salepo.

Termos para indexação: Orquídea; microextração em fase sólida; GC-MS.

INTRODUCTION

The salep plant belongs to the Orchidaceae family (Christenhusz; Byng, 2016). This family includes plants with tuberous and tuberless roots (Clifford; Lavarack, 1974). *Serapias* is a member of the genus comprising plants with tuberous roots (Caliskan; Kurt; Odabas, 2020). *Serapias vomeracea* (Burm.f.) Briq. is an important species and an emerging crop that is increasingly being used in Turkey. Harvested tubers are dried and ground into a fine powder before use (Turgay; Çınar, 2017). Salep

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is an important raw material in the pharmaceutical and food industries owing to the presence of glucomannan. In Turkey, "salep" refers to the traditional beverage prepared from dried and fine-ground tubers (Tamer; Omeroglu; Copur, 2019). Salep powder is used as a raw material to prepare Maraş ice cream (Sezik, 2002). Hossain (2011) reported that orchids are used as medicines to cure many diseases, such as paralysis, stomach ailments, asthma, inflammation, tuberculosis, rheumatism, and chest pain since prehistoric times.

Understanding the diversity and proportional values of fragrance compounds is essential in revealing the differences between products and applications. Therefore, solid-phase microextraction (SPME) is used for this purpose. SPME (Xu et al., 2016) is proven as an effective tool available within a short time; it is also an inexpensive and solvent-free extraction technique and can be used for flavor analysis in foods (Souza-Silva; Gionfriddo; Pawliszy, 2015). This method has been widely used in many studies. Several studies have focused on the detection of volatile compounds in the aerial parts (parts present above the ground) of various orchid species. Erik et al. (2020) investigated the profile of volatile compounds present in solvent extracts of *Serapias orientalis* subsp. *orientalis* plants. The major compound identified in that study was limonene (76.5%), followed by heptanal (10.4%), pelargonaldehyde (7%), and capronaldehyde (2.1%). Moreover, components whose levels proportionally increased and decreased were determined by solvent extraction. The authors reported that new compounds were formed, such as α-methoxy-p-cresol (52.9%) in water extract, decanal (7.5%), and 2(E)-heptenal (2.1%) in methanolic extract, and octanal (3.6%) in hexane extract.

Mecca et al. (2022) conducted a study on the volatile compounds present in the flowers of *Dactylorhiza* species belonging to the Orchidaceae family, and the highest area values were attributed to verbenone (28.86%) in *Dactylorhiza viridis*, β-ocimene (18.69%) in *Dactylorhiza romana*, pentadecane (28.40%) in *Dactylorhiza incarnata*, caryophyllene (17.38%) in *Dactylorhiza saccifera*, and β-sesquiphellandrene (32.16%) in *Dactylorhiza sambucina*.

Past studies have paid attention to the volatile components present in the aerial parts of orchids obtained from natural flora. Although the tuber is the main part of the salep plant that is consumed and used, only a few studies have determined the volatile compounds present in salep tubers by SPME analysis. Salep plant harvesting in Turkey is usually carried out during the full flowering period of the plants or later for various reasons. Harvested tubers are traditionally cleaned, boiled, and then dried in the shade.

Different drying methods can be applied to tubers. To the best of our knowledge, the present study is the first to determine the volatile compounds present in *S. vomeracea* tubers. Moreover, given that the influence of late harvest on the volatile compound profile remains unknown, this study determined the effects of harvest time and drying method on the composition variation of volatile compounds in salep tubers by SPME and *gas chromatography–mass spectrometry* (GC-MS) analysis.

MATERIAL AND METHODS

Material preparation for analysis

This study was conducted in the Pazar District of Rize Province. *S. vomeracea* tubers were used in this study. The seedlings that emerged from tubers were transplanted on December 2, 2021, in plots filled with media comprising field soil, stream sand, and tea fiber (in a 2:1:1 ratio, respectively). Before transplanting the seedlings, a basal dose of 50-kg NPK per hectare (15–15–15) was applied to the plots.

Tubers from half of the grown plants were harvested on May 17, 2022 (first harvest time: H1), during the full flowering period. The remaining plants were harvested on June 6, 2022, which was 20 days after the first harvest (H2). The harvested tubers were washed to remove dust, after which they were dried. Ten tubers were dried. Drying was carried out in four different methods: shade drying, oven drying, shade drying after boiling, and lyophilizer drying.
(1) Shade drying: The samples were dried under room conditions and in the shade at 25 ±1 °C for 20 days.
(2) Oven drying: The samples were dried in an oven at 40 °C for 10 days.
(3) Shade drying after boiling: This method is traditionally used in Turkey. First, the samples were boiled for 7 min, after which they were filtered and dried in the shade at 25 ±1 °C under room conditions for 10 days.
(4) Lyophilizer drying: Labconco Lyophilizer (0.12 mbar vacuum and adjusted to −84 °C) was used for the drying process. In this method, the drying process took 42 h.
The dried tubers were ground into a fine powder and preserved in a refrigerator at 4 °C until further analysis.

The extract of volatile compounds was prepared by SPME using fiber (50/30-µm film thickness, 2-cm length, divinylbenzene/carboxen/polydimethylsiloxane). After samples were weighed to 0.1 g, they were placed in vials and placed on a hotplate at 65 °C for 5 min. Before the analysis, fiber was desorbed into the GC-MS injector at 65 °C for 5 min. SPME analyses were carried out in two replications.

The volatile compounds present in the samples were identified using a GC-MS system (PQ2010 Ultra; Shimadzu, Japan). Volatile compounds were separated using a fused-silica capillary column Rxi-5Sil MS (30.0 m, 0.25 mm ID, 0.25-µm thickness). The injection was carried out in split mode (1:25), and the column temperature was 250 °C. The GC-MS interface and ionization source temperatures were 210 °C and 250 °C, respectively. The column oven temperature was set at 40 °C. The carrier gas used was high-purity helium. Its flow rate was 1.00 mL min^{-1}. Detection was realized in electronic ionization in the pulse mode, and the ionization voltage was 70 eV. All compounds were identified by the NIST 1992 and Wiley mass spectral libraries. The relative intensity of each compound was calculated as the ratio between the area of the specific molecule and the sum of the areas of all identified peaks (% peak area normalization method) in the chromatogram (Kafkas et al., 2006).

Statistical analysis

The data of volatile compounds were obtained using SPME/GC-MS from the library and confirmed against references. Graphs were constructed using the obtained data with Microsoft Excel 2016. Hierarchical cluster analysis was performed using SPSS 20.0 software. Additionally, before PC analysis, the relative area value of each compound was recalculated using Log+1 transformation using the SPSS program. Cluster analysis (Ward method with squared Euclidean distances) was used for classifying a dataset into homogeneous groups. The results of processed data are displayed as a diagram, called a dendrogram, which belongs to hierarchical clustering.

RESULTS AND DISCUSSION

Twenty-two volatile compounds were detected in eight samples and identified by SPME/GC-MS analysis (Table 1). The compounds and their groups are listed in Table 1. Only three (phenol, aldehyde, and alcohol) of the ten compound groups have an area ratio of more than 5% (Figure 1). The areal distribution of volatile compounds (above 3%) according to harvest time and drying is shown in Figure 2. The data of the variation in the volatile compounds of the salep tuber are evaluated below.

Harvest time

Volatile compounds showed a wide variation in number and percentage area depending on the harvest time. The oven drying method, followed by shade drying after boiling, shade drying, and lyophilizer drying, yielded the highest number of compounds (18 compounds) in tubers at the first harvest time (H1). In all drying conditions, the main compound obtained was p-cresol (minimum: 64.96%, maximum: 87.32%; Table 1). In the second harvest time (H2), shade drying, followed by oven drying, shade drying after boiling, and lyophilizer drying, yielded the highest number of compounds. Moreover, p-cresol values (minimum: 70.71%, maximum: 90.21%) showed increases in all the methods. Therefore, p-cresol was the primarily volatile compound in *S. vomeracea* tubers.

In H1, the average percentage area of the phenolic group was the highest at 74.53%, followed by the average percentage area of the aldehyde and alcohol groups at 8.15% and 5.90%, respectively. In H2, the ranking was similar, and the corresponding average values of these groups were 83.17%, 5.73%, and 5.39% (Figure 1).

Depending on the interaction of the drying × harvest time, the number of volatile compounds with an area ratio of above 3% was 10 (Table 1). Except for 2-ethyl hexanol, nine compounds (acetoin, ethyl lactate, capronaldehyde, p-methyl anisole, phenylmethanol, p-cresol, pelargonaldehyde, capraldehyde, and ethylene brassylate) were effective in H1. In H2, the number of volatile compounds with an area ratio of above 3% was only 5 in at least one sample. These compounds were capronaldehyde, 2-ethyl hexanol, phenylmethanol, p-cresol, and pelargonaldehyde.

In general, late harvest by 20 days caused an increase in the ratio of p-cresol, which had an animal, ink, or feces-like odor (Beauchamp; Zardin, 2017) and was the only phenolic volatile compound obtained in the study. Among the drying conditions, shade drying after boiling yielded the highest increase in this compound. p-Cresol exists at a high concentration mostly in animal products such as milk (Faulkner et al., 2017), cheese (Kilcawley et al., 2018), meat (Begić; Forto; Krvavica, 2022), and yogurt (Cheng et al., 2022). Interestingly, this compound also exists in a significant amount in coffee, depending on the roasting process (Ayseli et al., 2021). p-cresol concentrations in essential oils from flowers of different salep species were 38.10% in *Anacamptis morio*, 15.28% in *Himantoglossum robertianum*, 12.75% in *Ophrys sphegodes*, and 12.99% in Orchis purpurea (Robustelli della Cuna et al., 2022). Dalar et al. (2015) reported that the p-cresol ratio in the extracts of *Dactylorhiza chuhensis* flowers was 4.4%, which was much lower than that obtained in this study. This is because both the investigated species and the analyzed plant part are different from each other.

Table 1: Results of identification harvest time and drying conditions, retention index, and a chemical class of the volatile compounds.

				First harvest time				Second harvest time			
SN	Retention index	Compounds	Group of compound	SD	OD	SDB	LD	SD	OD	SDB	LD
1	709	Acetoin	Ketone	0.31 ± 0.15	5.99 ± 0.28	0.95 ± 0.17	0.41 ± 0.21	1.73 ± 0.31	0.84 ± 0.30	0.25 ± 0.13	0.38 ± 0.191
2	799	Ethyl lactate	Ester	1.11 ± 0.56	0.77 ± 0.44	3.80 ± 1.9	-	0.11 ± 0.05	-	-	-
3	803	Capronaldehyde	Aldehyde	0.92 ± 0.21	2.55 ± 0.27	6.19 ± 2.21	1.38 ± 0.25	2.84 ± 0.36	1.57 ± 0.20	0.71 ± 0.35	4.19 ± 1.26
4	849	Isovaleric acid	Carboxylic Acid	-	1.04 ± 0.22	-	-	0.17 ± 0.08	0.44 ± 0.07	-	-
5	874	n-Hexanol	Alcohol	1.02 ± 0.24	1.91 ± 0.34	0.71 ± 0.36	0.54 ± 0.27	1.99 ± 0.39	0.55 ± 0.00	0.21 ± 1.11	1.34 ± 0.24
6	993	3-Methylvaleric acid	Carboxylic Acid	-	-	2.45 ± 1.11	-	-	-	-	-
7	995	Valeric acid	Carboxylic Acid	1.81 ± 0.18	1.29 ± 0.65	-	-	0.78 ± 0.39	0.68 ± 0.34	-	-
8	997	2-Pentylfuran	Furan	-	0.43 ± 0.10	1.06 ± 0.30	-	-	-	0.30 ± 0.15	-
9	1006	Ethyl hexanoate	Ester	-	-	-	-	0.41 ± 0.16	-	-	-
10	1025	p-Methyl anisole	Terpene	-	3.62 ± 0.98	-	-	0.21 ± 0.11	-	-	-
11	1033	Limonene	Terpene	-	0.58 ± 0.16	0.92 ± 0.04	1.60 ± 0.58	0.62 ± 0.20	0.62 ± 0.15	1.20 ± 0.05	2.65 ± 0.49
12	1036	1,8-Sineol	Terpene	0.37 ± 0.01	-	-	-	0.37 ± 0.18	-	-	-
13	1037	2-Ethyl hexanol	Alcohol	0.77 ± 0.07	0.66 ± 0.03	1.27 ± 0.21	2.88 ± 0.12	1.42 ± 0.13	0.90 ± 0.01	1.91 ± 0.13	5.36 ± 1.24
14	1042	Phenylmethanol	Alcohol	1.50 ± 0.30	0.96 ± 0.11	3.62 ± 0.72	7.77 ± 1.28	1.38 ± 0.05	1.00 ± 0.08	1.20 ± 0.32	4.31 ± 1.47
15	1090	p-Cresol	Phenol	87.3 ± 0.22	75.11 ± 1.22	64.96 ± 0.52	70.70 ± 4.94	83.47 ± 1.48	90.21 ± 1.43	88.30 ± 0.53	70.71 ± 7.48
16	1111	Pelargonaldehyde	Aldehyde	1.69 ± 0.30	2.02 ± 0.32	4.27 ± 2.30	3.32 ± 0.41	1.51 ± 0.18	0.97 ± 0.04	1.56 ± 0.25	3.69 ± 0.72
17	1208	Dodecane	Alkane	-	-	-	-	-	-	-	1.16 ± 0.43
18	1213	Capraldehyde	Aldehyde	1.61 ± 0.34	1.11 ± 0.22	4.02 ± 1.89	3.50 ± 0.87	1.20 ± 0.06	0.80 ± 0.1	1.44 ± 0.01	2.43 ± 0.84
19	1410	Tetradecane	Alkane	0.71 ± 0.02	0.37 ± 0.01	1.56 ± 0.08	0.94 ± 0.47	0.54 ± 0.00	0.38 ± 0.00	1.27 ± 0.63	2.77 ± 1.02
20	1608	Diethyl phthalate	Ester	0.51 ± 0.25	0.50 ± 0.25	0.45 ± 0.23	1.43 ± 0.85	0.38 ± 0.04	0.46 ± 0.23	0.43 ± 0.22	-
21	1611	Hexadecane	Alkane	0.35 ± 0.18	0.29 ± 0.14	0.66 ± 0.30	1.91 ± 0.77	0.32 ± 0.16	0.58 ± 0.17	1.47 ± 0.17	1.01 ± 0.16
22	1648	Ethylene brassylate	Lactone	-	0.77 ± 0.20	3.11 ± 1.34	3.62 ± 2.20	0.55 ± 0.27	-	-	-
Total of volatile compounds				14	18	16	13	19	14	13	12

SD: shade drying, OD: oven drying, SDB: shade drying after boiling, LD: lyophilizer drying

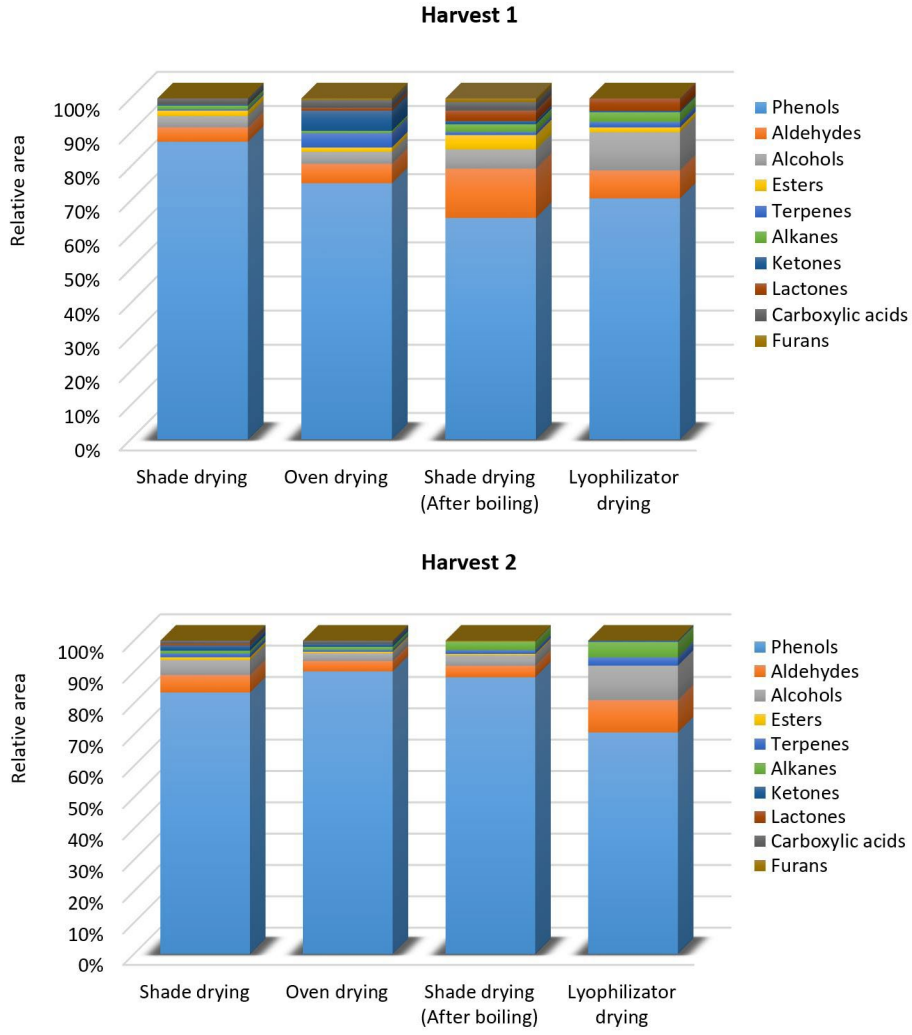

Figure 1: Areal distribution of compound groups depending on drying conditions in the first harvest time (A) and second harvest time (B).

Drying conditions

Shade drying

A total of 14 volatile compounds were detected in salep tubers that were shade-dried after harvesting during full bloom, 7 of which were at a concentration of below 1%. In this method, the first compound in the ranking was p-cresol (87.32%), followed by valeric acid (1.81%), pelargonaldehyde (1.69%), capraldehyde (1.61%), and others. Compounds with a high percentage area obtained at the second harvest time were listed as p-cresol (83.47%), capronaldehyde (2.84%), n-hexanol (1.99%), acetoin (1.73%), and others. Area values of 11 of 19 compounds detected in this period remained below 1% (Table 1).

Shade drying after boiling

The p-cresol ratio was 64.96% in tubers that were harvested during full flowering and then shade-dried after boiling, followed by capronaldehyde (6.19%), pelargonaldehyde (4.27%), capraldehyde (4.02%), ethyl lactate (3.80%), and others. As for the percentage area, 5 of 16 compounds existed at a concentration below 1%. In tubers belonging to the second harvest period, the total number of compounds detected decreased to 13. In this method, while the p-cresol ratio increased to 88.30%, the ratios of other compounds decreased. As for the ranking of compounds, p-cresol was the highest, followed by 2-ethyl hexanol (1.91%), pelargonaldehyde (1.56%), hexadecane (1.47%), and others (Table 1).

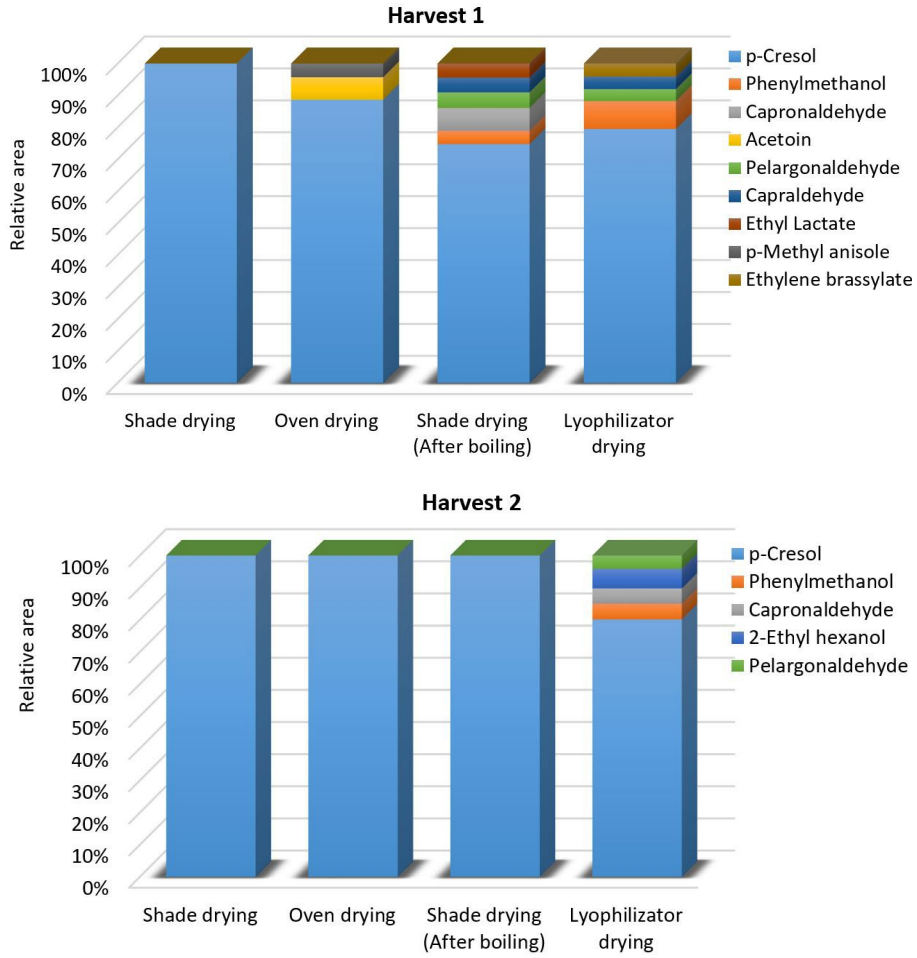

Figure 2: Areal distribution of volatile compounds (above 3%) depending on the drying conditions in the first harvest time (A) and second harvest time (B).

Oven drying

In this method, p-cresol (75.14%) was the main volatile compound obtained in the first harvest time tubers, followed by acetoin (5.99%), p-methyl anisole (3.62%), capronaldehyde (2.55%), pelargonaldehyde (2.02%), and others. The p-cresol ratio increased to 90.21% in the second harvest time. This compound was followed by capronaldehyde (1.57%), phenylmethanol (1.00%), pelargonaldehyde (0.97%), and others.

Lyophilizer drying

Compared with other methods, samples dried by lyophilization yielded the least number of volatile compounds at both harvest times. p-Cresol ratio at the first harvest time was 70.70%. This compound was followed by phenylmethanol (7.77%), ethylene brassylate (3.62%), capraldehyde (3.50%), and others. p-Cresol ratio was 70.71% in the tubers at the second harvest time. This compound was followed by 2-ethyl hexanol (5.36%), phenylmethanol (4.31%), pelargonaldehyde (3.69%), and others (Table 1).

Among the 22 volatile compounds, 10 (acetoin, capronaldehyde, n-hexanol, 2-ethyl hexanol, phenylmethanol, p-cresol, pelargonaldehyde, capraldehyde, tetradecane, and hexadecane) were detected at both harvest times and under all drying conditions. The remaining compounds differed depending

on the harvest time and drying method. This trend of volatile compounds revealed in the present study is consistent with the findings of Ye et al. (2019) for *Anoectochilus roxburghii* from the Orchidaceae family, those of Díaz-Maroto, Pérez-Coello, and Cabezudo (2022) for *Laurus nobilis* (Mr. Leaf) from the Lauraceae family, those of Zhang et al. (2016) for *Capsella bursa-pastoris* from the Brassicaceae family, and those of Polat et al. (2022) for *Daucus carota* (black carrot) from the Apiaceae family, indicating that volatile compounds differed depending on the drying conditions.

Some volatile compounds in salep tubers were specifically seen only in the harvest time–drying method combination. 3-Methylvaleric acid, ethyl hexanoate, and dodecane are examples of such compounds (Table 1). 3-Methylvaleric acid was obtained only by boiling and shade-drying of the tubers harvested at full bloom time. Ethyl hexanoate compound was detected only in tubers harvested at H2 and shade-dried. Dodecane was detected only in tubers harvested at H2 and dried in the lyophilizer.

In the shade drying method, five new volatile compounds (isovaleric acid, ethyl hexanoate, p-methyl anisole, limonene, and ethylene brassylate) were identified in the late harvest (Table 1). In the shade drying method after boiling, three compounds, namely, ethyl lactate, 3-methylvaleric acid, and ethylene brassylate, were seen in tubers from the first harvest time but disappeared in tubers from the second harvest time. This effect of harvest time may have resulted particularly from ontogenetic variability. Similarly, many researchers, such as Stefanakis et al. (2022), Li and Zidorn (2022), and Haghighi et al. (2019), have pointed out the importance of seasonal variation regarding harvest time.

The drying method in an oven at 40 °C caused the loss of four compounds (ethyl lactate, 2-pentylfuran, p-methyl anisole, and ethylene brassylate) in tubers from the second harvest time. By contrast, in the lyophilizer drying method, two compounds (diethyl phthalate and ethylene brassylate) were lost in tubers of the second harvest time, but one new compound (dodecane) appeared (Table 1). Díaz-Maroto, Pérez-Coello and Cabezudo (2002) reported the least change in the volatile composition of *Petroselinum crispum* from the Apiaceae family when the plants were dried at an ambient temperature; additionally, they reported that oven drying (at 45 °C) and freeze drying led to a decrease in most of the volatile compounds. In a similar approach, according to Ding et al. (2012), drying at higher temperatures may result in the decomposition of components, whereas drying at lower temperatures may inhibit the appearance of new compounds. The appearance of new compounds after drying and the realization of losses in some compounds may have been caused by glycoside hydrolysis and esterification (Bhatt et al., 2018).

Hierarchical clustering of volatile compounds considering harvest time and drying conditions. The clustering of volatile compounds based on the drying conditions applied to the tubers harvested at H1 (first harvest time) and H2 (second harvest time) was performed using the hierarchical clustering Ward method.

The results of the dendrogram obtained with the examined traits showed the formation of two separate clusters (Figure 3). One of these clusters was separated into two sub-clusters, wherein one of the subgroups consisted of H1-Lyophilizer and H2-Lyophilizer. The other cluster formed two sub-clusters within itself and a total of three different subgroups. The first subgroup consisted of H1-Shade and H2-Etuv, while the second subgroup consisted of H1-Etuv, H2-Shade, and H1-ShadeB. In the third subgroup, H2-ShadeB was found alone. H1-Shade and H2-Etuv had highly similar properties in the cluster. Finally, a total of 4 clusters were formed, namely, A, B, C, and D.

The volatile compounds and area values obtained from samples dried in the lyophilizer were not affected much by the harvest time, unlike the situation in other drying conditions. The results of the SPSS cluster analysis indicated that, generally, the differences among the drying conditions in terms of the volatile compounds obtained varied depending on the harvest time.

Hierarchical clustering analysis of compound groups depending on the harvest time and drying conditions. In the clustering analysis considering the compound groups, initially, two clusters had formed (Figure 4). Phenol group alone formed a cluster, whereas other groups were divided into two subgroups. Aldehyde and alcohol groups formed one of these clusters. The other cluster was divided into two more subgroups. Finally, a total of 4 clusters, namely, A, B, C, and D, had formed. Cluster analysis was performed to reveal the relationships between volatile component groups present in the tubers of *S. vomeracea*. The dendrogram of the hierarchical clustering analysis showing the component groups acting together is illustrated in Figure 4.

The first of the four clusters, i.e., Cluster A, consisted of the aldehyde and alcohol groups. The second of the four clusters (Cluster B) consisted of ester, lactone, carboxylic acid, furan, terpene, and alkane groups. The third one (Cluster C) consisted of only the ketone group, and the last cluster (Cluster D) consisted of the phenol group alone (Figure 4).

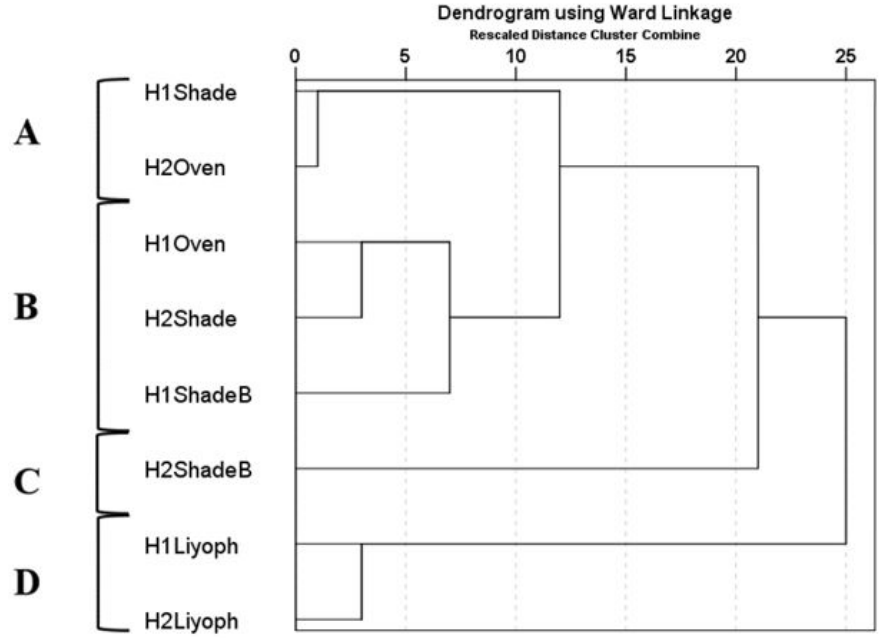

Figure 3: Hierarchical clustering analysis of volatile compounds considering harvest time × drying method. Clusters A, B, C, and D were identified at cutting point 10. Eight combinations were obtained: H1 Shade = shade drying in the first harvest time, H2Etuv = oven drying in the second harvest time, H1Etuv = oven drying in the first harvest time, H2Shade = shade drying in the second harvest time, H1ShadeB = shade drying in the first harvest time, H2ShadeB = shade drying after boiling in the second harvest time, H1Lyoph = lyophilizer drying in the first harvest time and H2Lyoph = Lyophilizer drying in the second harvest time.

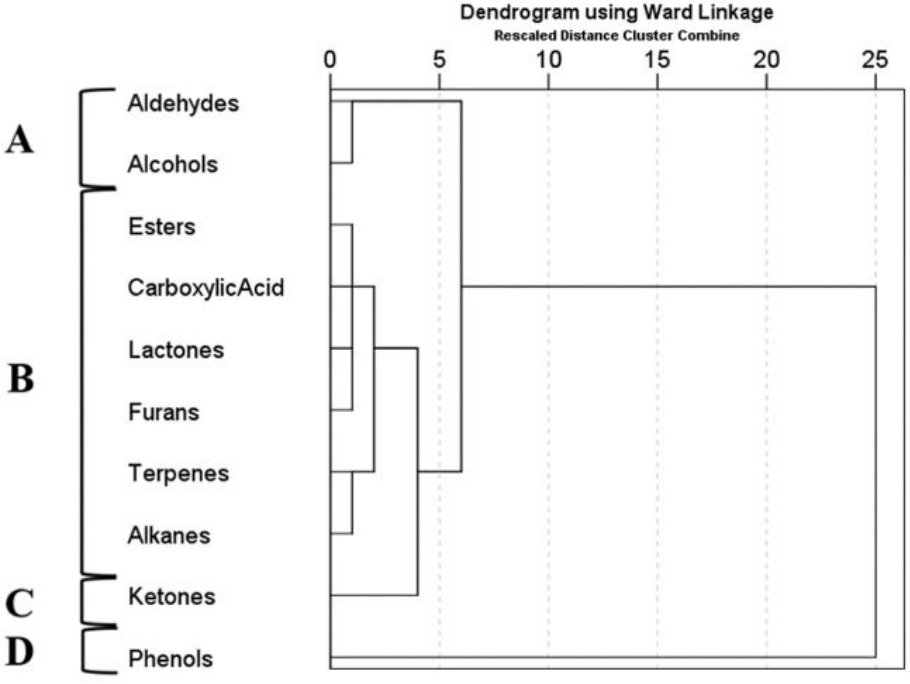

Figure 4: Hierarchical clustering of compound groups. Clusters A, B, C, and D were identified at cutting point 3.

CONCLUSIONS

This study demonstrated that the type of volatile compounds present in the tubers of *S. vomeracea* grown under field conditions changed depending on the harvest time and drying conditions. Some compounds were lost or new compounds appeared depending on the application. This study presents original information about the formation and diversity of volatile compounds under different drying conditions. The findings of this study can serve as an important resource for researchers in obtaining more specific salep powder.

AUTHOR CONTRIBUTION

Conceptual idea: Şavşatlı, Y.; Methodology design: Şavşatlı, Y.; Data collection: Şavşatlı, Y.; Data analysis and interpretation: Şavşatlı, Y. and Writing and editing: Şavşatlı, Y.

REFERENCES

AYSELI, M. T.; KELEBEK, H.; SELLI, S. Elucidation of aroma-active compounds and chlorogenic acids of Turkish coffee brewed from medium and dark roasted *Coffea arabica* beans. Food chemistry, 338:127821, 2021.

BEAUCHAMP, J.; ZARDIN, E. Odorant detection by on-line chemical ionization mass spectrometry. Part C analytics, sensor technology and human-sensory evaluation. In: BUETTNER, A. (Ed.). Springer Handbook of Odor. Springer dordrecht heidelberg. London New York, p.355-405, 2017.

BEGİĆ, M. A. G.; FORTO, A.; KRVAVİCA, M. Volatile flavour compounds of herzegovinian dry smoked goat meat. Agriculture & Forestry, 68(3):255-1275, 2022.

BHATT, S. et al. Impact of drying methods on essential oil composition of *Ocimum americanum* L. from kumaun Himalayas. Journal of Essential Oil Bearing Plants, 21(5):1385-1396, 2018.

CALISKAN, Ö.; KURT D.; ODABAS, M. O. Agronomic characteristics of *S. vomeracea* (Burm. f.) Briq. salep orchids. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 48(1):245-260, 2020.

CHENG, Z. et al. Sensorial, cultural and volatile properties of milk, dairy powders, yoghurt and butter: A review. International Journal of Dairy Technology, 75(4):761-790, 2022.

CHRISTENHUSZ, M. J. M.; BYNG, J. W. The number of known plants species in the world and its annual increase. Phytotaxa, 261:201-217, 2016.

CLIFFORD, H. T.; LAVARACK, P. S. The role of vegetative and reproductive attributes in the classification of the Orchidaceae. Biological Journal of the Linnean Society, 6(2):97-110, 1974.

DALAR, A. et al. Health attributes of an endemic orchid from Eastern Anatolia, *Dactylorhiza chuhensis* Renz&Taub: *In vitro* investigations. Journal of Herbal Medicine, 5:77-85, 2015.

DÍAZ-MAROTO, M. C.; PÉREZ-COELLO, M. S.; CABEZUDO, M. D. Effect of drying method on the volatiles in bay leaf (*Laurus nobilis* L.). Journal of Agricultural and Food Chemistry, 50(16):4520-4524, 2002.

DÍAZ-MAROTO, M. C.; PÉREZ-COELLO, M. S.; CABEZUDO, M. D. Effect of different drying methods on the volatile components of parsley (*Petroselinum crispum* L.). European Food Research and Technology, 215:227-230, 2022.

DING, S. H. et al. Effect of drying methods on volatiles of Chinese ginger (*Zingiber officinale* Roscoe). Food and Bioproducts Processing, 90(3):515-524, 2012.

ERIK, I. et al. Chemical composition, antimicrobial, and lipase enzyme activity of essential oil and solvent extracts from *S. orientalis* subsp. *orientalis*. Turkish Journal of Chemistry, 44:1655-1662, 2020.

FAULKNER, H. et al. Effect of different forage types on the volatile and sensory properties of bovine milk. Journal of Dairy Science, 101:1034-1047, 2017.

HAGHIGHI, T. M.; SAHARKHIZ, M. J.; NADDAF, F. Ontogenetic variability of *Vitex pseudo-negundo* essential oil and its phytotoxic activity. Scientia Horticulturae, 257:108735, 2019.

HOSSAIN, M. M. Therapeutic orchids: Traditional uses and recent advances: An overview. Fitoterapia, 82:102-140, 2011.

KAFKAS, E. et al. Identification of volatile aroma compounds of strawberry wine using solid-phase microextraction techniques coupled with gas chromatography–mass spectrometry. Flavour and Fragrance Journal, 21(1):68-71, 2006.

KILCAWLEY, K. N. et al. Factors influencing the flavour of bovine milk and cheese from grass based versus non-grass based milk production systems. Food, 7(3):37, 2018.

LI, Y.; ZIDORN, C. Seasonal variations of natural products in European herbs. Phytochemistry Reviews, 21:1549-1575, 2022.

MECCA, M. et al. Volatile organic compounds in *Dactylorhiza* species. Compounds, 2:121-130, 2022.

POLAT, S. et al. Comparative elucidation of colour, volatile and phenolic profiles of black carrot (*Daucus carota* L.) pomace and powders prepared by five different drying methods. Food Chemistry, 369:130941, 2022.

ROBUSTELLI DELLA CUNA, F. S. et al. Chemical composition of essential oil from four sympatric orchids in NW-Italy. Plants, 11(6):826, 2022.

SEZIK, E. Turkish orchids and salep. Acta Pharmaceutica Turcica, 44:151-157, 2002.

SOUZA-SILVA, E. A.; GIONFRIDDO, E.; PAWLISZY.N, J. A critical review of the state of the art of solid-phase microextraction of complex matrices II. Food analysis. Trends in Analytical Chemistry, 71:236-248, 2015.

STEFANAKIS, M. K. et al. Seasonal variation of aromatic plants under cultivation conditions. Plants, 11:2083, 2022.

TAMER, C. E.; OMEROGLU, P. Y.; COPUR, O. U. 15-Functional and traditional nonalcoholic beverages in Turkey. In: GRUMEZESCU, A. M.; HOLBAN, A. M. (Ed.). Nonalcoholic beverages. Volume Six in The Science of Beverages series. Woodhead Publishing: United Kingdom, p.483-521, 2019.

TURGAY, O.; CINAR, I. Salep: The common name of the plant, powder, hot beverage, food ingredient. Kahramanmaras Sutcu Imam University Journal of Engineering Sciences, 20(3):68-71, 2017.

XU, C. H. et al. Applications of solid-phase microextraction in food analysis. Trends in Analytical Chemistry, 80:12-29, 2016.

YE, S. et al. Sensory qualities, aroma components, and bioactive compounds of *Anoectochilus roxburghii* (Wall.) Lindl. as affected by different drying methods. Industrial Crops & Products, 134:80-88, 2019.

ZHANG, L. et al. Effects of drying methods on the aroma components and qualıty of *Capsella bursa-pastoris* L. Journal of Food Process Engineering, 39(2):107-120, 2016.