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Article

Composition of the Scent in Some *Ophrys* Orchids Growing in Basilicata (Southern Italy): A Solid-Phase Microextraction Study Coupled with Gas Chromatography and Mass Spectrometry

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Abstract: Several methods have been used to determine the volatile organic compounds emitted by *Ophrys* orchids. The use of different methods results in incomparable data. Solid-phase microextraction (SPME) has not been used extensively on *Ophrys* orchids. The main components found in the SPME analysis of the scent in *Ophrys* orchids were as follows: *O. apifera*: benzyl benzoate and α-copaene; *O. crabronifera* subsp. *biscutella*: pentadecane, heptadecane, and nonadecane; *O. bertolonii* subsp. *bertolonii*: pentadecane and heptadecane; *O. passionis* subsp. *garganica*: *i*-propyl palmitate and heptadecane; *O. holosericea* subsp. *apulica*: α-copaene, pentadecane, and heptadecane; *O. lacaitae*: α-copaene, pentadecane, and heptadecane; *O. bombyliflora*: cyclosativene, pentadecane, and ethyl dodecanoate; *O. insectifera*: 8-heptadecene and pentadecane; *O. lutea*: heptadecane and docosane; *O. tenthredinifera* subsp. *neglecta*: α-copaene, caryophyllene, and i-propyl palmitate.

Keywords: *Ophrys*; scent composition; solid-phase microextraction; gas chromatography–mass spectrometry

Citation: D'Auria, M.; Lorenz, R.; Mecca, M.; Racioppi, R.; Romano, V.A. Composition of the Scent in Some *Ophrys* Orchids Growing in Basilicata (Southern Italy): A Solid-Phase Microextraction Study Coupled with Gas Chromatography and Mass Spectrometry. *Compounds* **2023**, *3*, 573–583. https://doi.org/10.3390/compounds3040041

Academic Editor: Pawel Pohl

Received: 6 October 2023
Revised: 8 November 2023
Accepted: 10 November 2023
Published: 14 November 2023

1. Introduction

Solid-phase microextraction (SPME) is a simple and cheap method to identify the composition of volatile organic compounds in natural matrices. SPME has proven to be an extremely powerful and versatile method. Considering only recently published articles, it is possible to find articles on natural matrices such as radish [1], *Camellia oleifera* [2], soy [3], and soybean flowers [4]. Furthermore, articles devoted to finding volatile organic compounds in microorganisms such as kombucha fermentation broth [5], *Kosakonia cowani* [6], and *Bacillus aryabhattai* AYG1023 [7] have been published. Finally, there have been several articles related to the chemical characterization of food and beverages, such as grapevine chips for wine production [8], virgin olive oil [9], oranges [10], coffee [11], rice milk [12], Jiang-flavor baijiu liquor [13], and meat [14].

Some years ago, a systematic study was initiated on the scent of spontaneous orchid species growing in Basilicata (Southern Italy). The aim of this study was to create a homogeneous picture of the composition of the aroma of these species using the same methodology for all of the species. In particular, we decided to use solid-phase microextraction (SPME) [15]. This study allowed us to identify the components of the scent of *Platanthera bifolia* subsp. *osca* [16], *Platanthera chlorantha* [17], *Cephalanthera* orchids [18], *Orchis* [19], *Serapias* [20], *Himantoglossum* [21]. *Barlia robertiana* [22], *Dactylorhiza* [23], *Gymnadenia* [24], *Neotinea* [25], and *Anacamptis* orchids [26].

Ophrys orchids are distributed between the two subgenera Fuciflorae and Ophrys, and they belong to different sections and subsections (*O. apifera* Huds. 1762, *O. bertolonii* subsp.

bertolonii Moretti 1823, *O. crabronifera* subsp. *biscutella* (O. Danesch and E. Danesch) Kreutz and Kreutz 2013, *O. bombyliflora* Link (1779) 1800, *O. holosericea* subsp. *apulica* (O. Danesch and E. Danesch) Buttler 1986, *O. insectifera* L. 1753, *O. lacaitae* Lojac. 1909, *O. lutea* subsp. *lutea* Cav. 1793, *O. passionis* subsp. *garganica* E. Nelson ex H. Baumann and R. Lorenz 2005, *O. tenthredinifera* subsp. *neglecta* (Parl.) E. G. Camus, Bergon and A. Camus 1908) (Table 1 and Figures 1–5).

Table 1. Taxonomic identification of the species utilized in this study. The nomenclature has been referred to [27].

Species	Subgenus	Section	Subsection
O. apifera	Fuciflorae	Apiferae	
O. crabronifera subsp. *biscutella*	Fuciflorae	Araniferae	Sphegodes
O. bertolonii subsp. *bertolonii*	Fuciflorae	Araniferae	Bertoloniorum
O. passionis subsp. *garganica*	Fuciflorae	Araniferae	Sphegodes
O. holosericea subsp. *apulica*	Fuciflorae	Fuciflorae	
O. lacaita	Fuciflorae	Fuciflorae	
O. bombyliflora	Ophrys	Bomyliflorae	
O. insectifera	Ophrys	Ophrys	
O. lutea subsp. *lutea*	Ophrys	Pseudophrys	Fusci-luteae
O. tenthredinifera subsp. *neglecta*	Ophrys	Tenthrediniferae	

Figure 1. *Ophrys apifera* (**left**); *Ophrys crabronifera* subsp. *biscutella* (**right**) (photos of V. A. R.).

Figure 2. *Ophrys bertolonii* subsp. *bertolonii* (**left**); *Ophrys passionis* subsp. *garganica* (**right**) (photos of V. A. R.).

Figure 3. *Ophrys holosericea* subsp. *apulica* (**left**); *Ophrys lacaitae* (**right**) (photos of V. A. R.).

Figure 4. *Ophrys bombyliflora* (**left**); *Ophrys insectifera* (**right**) (photos of V. A. R.).

Figure 5. *Ophrys lutea* subsp. *lutea* (**left**); *Ophrys tenthredinifera* subsp. *neglecta* (**right**) (photos of V. A. R.).

It is known that *Ophrys* flowers imitate the mating signals of some insect species and are pollinated by sexually excited males who mistake the flower for a female of the same species and pollinate it during a "pseudocopulation".

Sexually deceptive orchids are unique in their exclusive and effective use of male insects, primarily aculeate Hymenoptera, but also other Hymenoptera and some Diptera [28].

In most of the European *Ophrys* species studied so far, male copulation attempts can only be elicited by a scent identical to the female sex pheromone of the pollinating species; visual cues appear to be less important [29].

The differences in odor between similar orchid species are often small. Small variations have been found between the bouquets of *Ophrys fusca* and *O. bilunulata*, as well as between the similar *O. sphegodes* and *O. exaltata* [29,30].

Sexually deceptive orchid species typically exploit one or a few specific species of pollinators and may have different pollinators in different regions. A single insect species can also pollinate more than one sexually deceptive orchid species in different regions [30].

All of the species examined in this work are sexually deceptive, with the sole exception of *O. apifera*, which is notoriously an autogamous (i.e., self-pollinating) species.

With this work, we wanted to use a rapid method (SPME) to test species belonging to different sections and subsections of the *Ophrys* genus in order to verify whether the scents they emit are very similar or different within the different groups that they belong to.

The scent of *Ophrys* orchids has been extensively studied. Most of the studies have been performed though the identification of the components of the extracts of labella. Thus, *O. insectifera* showed the presence of pentacosane, tetracosane, nonanoic acid, and nonanal as its main components in a study conducted in 1987 [31], as well as tricosene, pentacosane, 9-heptacosene, and 9-nonacosene in a study conducted in 2017 [32]. However, the absorption of the scent of *O. insectifera* subsp. *insectifera* on Porapak Q showed the main components to be pentadecane, heptadecane, and cyclosativene [33], then alkanes with a lower molecular weight than those determined in the other studies on orchids, and a terpene. In the scent of *O. sphegodes*, pentacosane and tricosane were found in labella extracts [34], while tricosane, pentacosane, and *p*-cresol were the main components of the scent obtained via steam distillation of the flowers [35]. The scent of *O. lupercalis* and *O. iricolor* was due to the presence of tricosane, pentacosane, and heptacosane [36]. Together with the same compounds, nonanal was found in *O. lupercalis*, *O. bilunulata*, and *O. fabrella* [37]. SPE collection of the scent of *O. normanii* showed the presence of octadecanal, tricosane, tricosene, and pentacosene [38]. SPE absorption of the scent of *O. apifera* showed the presence of butanol, butyl ether, and caryophyllene [39]. Pentacosene and tricosene were found in the labella extracts of *O. holosericea* [40], while nonanal was the main component of the scent of *O. lutea* [41]. These studies showed that the extraction of the labella led to the identification of high-molecular-weight alkanes and alkenes as the main components of the aroma of these orchids. However, when SPME was used to determine the composition of the scent of *O. bertolonii* subsp. *Benacensis*, 4-methyl tetradecane, nonanal, decanal, dodecanal, 3,5-octadiene-2-one, and caryophyllene were found as the main components of the aroma [42].

In this work, the scents emitted by ten species of spontaneous orchids growing in Basilicata (Italy), belonging to the *Ophrys* genus, were determined using solid-phase microextraction. This method allows us to define a homogeneous framework of the scent of several species in the genus.

2. Materials and Methods

2.1. Plant Material

The sample of *O. apifera* was collected at Piani del Mattino (Pz) on 8 June 2017. The sample of *O. crabronifera* subsp. *biscutella* was collected at Valico Faggeto in the municipality of Moliterno (Pz) on 11 March 2018. The sample of *O. bertolonii* subsp. *bertolonii* was collected at Monte Grosse (Pz) on 18 April 2018. The sample of *O. bombyliflora* was collected at Contrada Macchia Orsino in the municipality of Tolve on 9 April 2018. The sample of *O. holosericea* subsp. *apulica* was collected at Scalo di Grassano on 19 April 2018. The sample of *O. insectifera* was collected at Monte Zaccana in the municipality of Castelluccio Superiore on 2 May 2018. The sample of *O. lacaitae* was collected at Contrada l'Aia Antica

in the municipality of Calvello on 6 June 2018. The sample of *O. lutea* subsp. *lutea* was collected at Scalo di Albano on 11 April 2018. The sample of *O. passionis* subsp. *garganica* was collected at Scalo di Campomaggiore on 16 April 2018. The sample of *O. tenthredinifera* subsp. *neglecta* was collected at Torrente Serrapotomo in the municipality of Laurenzana on 8 April 2018. The plants were collected by Vito Antonio Romano.

The plants were harvested taking all the clod of earth, taking care not to damage the root system. All of the plants had closed flowers to avoid using flowers that were already fertilized but not visible because they were at the beginning of fertilization. The plants were planted in special pots in the greenhouse of the University of Basilicata (Potenza 650 m. a.s.l.), in closed boxes, with transparent cloth to avoid fertilization (even if occasional). The correct classification of the species was carried out on the flowering plants. The plants were tested when the flowers were all open except for the last two.

The plants were tested whole, without being damaged, under a cylindrical glass bell (12 cm × 45 cm), in which only the inflorescence and the SPME probe were inserted [16–26].

To avoid contamination, the interior of the bell was isolated from the external environment with appropriate closing and sealing systems during the 24 h of the test (from eight in the morning to eight the following day).

In order to be sure that the internal environment of the bell was isolated from the external environment, various blank tests were carried out.

After the tests, the plants remained closed in the boxes to verify that at the end of flowering there were no fertile ovaries, and for this reason no herbarium samples were taken. The earthen breeds with the bulbs were brought back to the site.

In view of the fact that the investigated taxa are rare wild plants, in order to preserve the species, we chose to use a single plant for our analysis.

2.2. Analysis of Volatile Organic Compounds

The SPME analysis of ten different samples of *Ophrys* was performed. In this way, the identified plants were collected and placed in glass jars for 24 h, which also contained the fiber (DVB/CAR/PDMS) and SPME syringe. After this time, the fiber was desorbed in a gas chromatographic apparatus equipped with a quadrupole mass spectrometer detector. A 50/30 µm DVB/CAR/PDMS module with 1 cm fiber (57328-U, Supelco, Milan, Italy) was employed to determine the VOCs. The SPME fiber was maintained in the bell jar for 24 h. The analytes were desorbed in the splitless injector at 250 °C for 2 min. Analyses were accomplished with an HP 6890 Plus gas chromatograph equipped with a Phenomenex Zebron ZB-5 MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm FT) (Agilent, Milan, Italy). An HP 5973 mass-selective detector in the range 0–800 m/z (Agilent) was utilized with helium at 0.8 mL/min as the carrier gas. The EI source was used at 70 eV. The analyses were performed using a splitless injector. The splitless injector was maintained at 250 °C, and the detector was maintained at 230 °C. The oven was held at 40 °C for 2 min and then gradually warmed at 8 °C/min up to 250 °C, where it was held for 10 min. Tentative identification of the aroma components was based on mass spectra and Wiley 11 and NIST 14 library comparisons. A single VOC peak was considered to have been identified when its experimental spectrum matched that present in the library with a score over 90%. All of the analyses were performed in triplicate.

3. Results and Discussion

The SPME-GC-MS analysis of *Ophrys* samples gave the results reported in Table 2. The main component of the scent of *O. apifera* was benzyl benzoate (22.52%), while other important components included a-copaene (9.11%), caryophyllene (8.07%), and cyclosativene (6.97%) (Table 2). The significant difference in the scent in comparison with that obtained using SPE absorption should be noted. In that case, butanol, butyl ether, and caryophyllene were the main components of the aroma [39,43]. The observed differences could be due to the different analyzed species, the different harvesting places (Basilicata and Catalonia), the different pollinator insects, and/or the different analytical procedures.

Table 2. Volatile organic compounds detected by using SPME-GC-MS in *Ophrys* species.

Compound	**r.t. [min.]**	**KI** [a]	**Area% ± 0.03**									
			O. apifera	***O. crabronifera* subsp. *biscutella***	***O. bertolonii* subsp. *bertolonii***	***O. passionis* subsp. *Garganica***	***O. holosericea* subsp. *apulica***	***O. lacaitae***	***O. bombyliflora***	***O. insectifera***	***O. lutea* subsp. *lutea***	***O. tenthredinifera* subsp. *neglecta***
Octanol	10.82	1072									4.69	
Undecane	11.31	1100		1.05								
Decanal	13.17	1195	0.70	0.74						0.48		
Dodecane	13.27	1200			0.58					0.45		0.53
Nonanoic acid	14.38	1272	2.00									
Isobornyl acetate	14.97	1285							1.65	0.96		1.27
2-Undecanone	15.01	1291			5.18							
Tridecane	15.05	1300		3.37	4.48		3.63	0.56	3.08	1.90	1.56	2.32
Decanoic acid	16.02	1335	3,30									
Cyclosativene	16.38	1344	6.97		5.15	6.79			10.09	0.96	1.09	
a-Copaene	16.52	1353	9.11	3.81			11.30	12.08	3.15			11.61
Tetradecane	16.74	1400	0.86	1.81	6.36	3.72	4.47	3.17	3.47	2.13	1.09	3.60
Dodecanal	16.86	1407		3.49								
Caryophyllene	17.28	1428	8.07	1.08	1.72	8.34	7.90	6.68	4.38		1.23	11.73
Geranylacetone	17.46	1451	0.88						1.62			
b-Farnesene	17.62	1454								3.58		
Alloaromadendrene	17.76	1456	0.86									
Epi-b-santalene	17.80	1460		0.74								
2,6-Di-*t*-butyl-*p*-benzoquinone	17.96	1458					1.97	1.69	1.77	0.80		1.06
1-Pentadecene	17.99	1489		6.43								
Pentadecane	18.30	1500	2.52	8.06	28.62	8.69	11.48	13.40	10.33	13.73	5.53	5.04
b-Cadinene	18.65	1507	2.52									
Methyl dodecanoate	18.68	1509			1.00					1.09		
d-Cadinene	18.82	1524						1.27	6.94			1.04
Dodecanoic acid	19.11	1559	1.78	0.71				0.98				
Cyclotridecane	19.46	1565								0.64		
Ethyl dodecanoate	19.75	1579		1.40	4.16	1.83	5.31		9.46	5.18	0.99	4.16
Hexadecane	19.80	1600		2.86	3.58	6.38	6.16	4.17	6.97	3.38	2.08	4.03
Tetradecanal	20.01	1611	1.63	2.03	4.10		3.72	2.93		1.02		
i-Propyl dodecanoate	20.06	1618	0.86									
Isolongifolen-5-one	20.17	1622		3.81								
Benzophenone	20.28	1625	1.97									
Unidentified	20.62								8.52			
Methyl dihydrojasmonate	20.66	1648		3.15						2.93		
8-Heptadecene	20.91	1664		3.12	2.20		4.61	3.91		18.88	7.25	
Heptadecane	21.22	1700	4.78	8.37	7.23	9.22	8.15	14.43	6.67	7.98	39.37	4.40
Pristane	21.30	1709	1.14	1.89		5.49	2.39	1.86	2.12	1.90		1.57
2-(Phenylmethylene)-octanal	21.87	1728	1.34									
Farnesal	21.89	1738									0.54	
Tetradecanoic acid	21.91	1761	1.89	1.60								
Benzyl benzoate	22.16	1768	22.52									

Table 2. *Cont.*

Compound	r.t. [min.]	KI [a]	Area% ± 0.03								
			O. apifera	*O. crabronifera* subsp. *biscutella*	*O. bertolonii* subsp. *bertolonii*	*O. passionis* subsp. *Garganica*	*O. holosericea* subsp. *apulica*	*O. lacaitae*	*O. bombyliflora*	*O. insectifera*	*O. lutea* subsp. *lutea*	*O. tenthredinifera* subsp. *neglecta*
3,5-Di-*t*-butyl-4-hydroxybenzaldehyde	22.24	1771								0.80		
Ethyl tetradecanoate	22.51	1774			0.90		1.08		1.53	1.49	0.59	1.47
Octadecane	22.57	1800	2.18	1.89	1.66	2.07	2.07	3.51	2.91	1.81	1.55	1.71
Phytane	22.64	1814								0.99		
Hexadecanal	22.82	1819	1.60		1.24		1.08	4.04		0.83		
Farnesyl acetaldehyde	23.18	1855		1.81						2.26		1.09
Nonadecane	23.85	1900	1.85	8.18	6.30	1.72	4.28	6.81	2.80	2.99	8.87	1.65
b-Springene	24.16	1922										1.60
Methyl hexadecanoate	24.20	1927										0.74
Hexadecanoic acid	24.58	1935		1.95						0.83		
a-Springene	24.78	1940										5.45
Ethyl 11-hexadecenoate	24.80	1974									2.58	
Eicosane	25.06	2000	1.82	1.15	1.32		1.18	1.57	1.06	1.56	0.74	
Geranyl-a-terpinene	25.15	2005										5.66
i-Propyl palmitate	25.40	2013	0.96	5.06	2.24	29.62	5.56			2.73	2.26	14.73
Heinecosane	26.25	2100		5.12	3.26	4.85	3.95	4.13	1.38			1.50
Ethyl oleate	26.98	2169	0.92							1.81		
Docosane	27.38	2200	1.52			1.30		0.87		1.81	9.94	0.71
1-Heneicosyl formate	28.10	2250						0.77				
9-Tricosene	28.14	2270		4.26								
Tricosane	28.47	2300	1.04	2.97				1.44		2.45	0.62	1.74

[a] KI: Kovats index [44].

The analysis of the scent of *O. crabronifera* subsp. *biscutella* showed that the aroma is mainly due to the presence hydrocarbon compounds, as in several of the species reported above. However, the presence of pentadecene (6.43%), pentadecane (8.06%), heptadecane (8.37%), nonadecane (8.18%), and heinecosane (5.12%) was detected. Furthermore, *i*-propyl palmitate was detected at a relevant amount (5.06%) (Table 2). In other *Ophrys* species, the scent analysis performed through the labella extraction gave high-molecular-weight hydrocarbons (i.e., more than thirty carbon atoms), while, in our determination, the main component of the scent had seventeen carbon atoms. Unfortunately, other analyses of the same species are not available.

The scent of *O. bertolonii* subsp. *bertolonii* gave a similar result. The main component was pentadecane (28.62%), while other significant compounds included heptadecane (7.23%) and nonadecane (6.30%). Also, in this case, a significant difference was observed considering the results obtained in the SPME analysis of *O. bertolonii* subsp. *benancensis* [28]. The observed differences could be due to the different subspecies, the different harvesting places (Basilicata and Lecco), or the different pollinators.

When a sample of *O. passionis* subsp. *garganica* was analyzed, the main component of the scent was *i*-propyl palmitate (29.62%), while other components included caryophyllene (8.34%), pentadecane (8.69%), and heptadecane (9.22%). In this case, this is the first reported analysis of this species.

In the case of *O. holosericea* subsp. *Apulica*, the main components were a-copaene (11.30%) and pentadecane (11.48%), while other compounds found in the scent included caryophyllene (7.90%) and heptadecane (8.15%). The analysis of the labella extracts gave some alkenes as main components of the scent [40]. In this case, the difference in the analytical procedures was responsible for the observed differences.

The same trend was observed in the analysis of *O. lacaitae*: the main components were a-copaene (12.08%), pentadecane (13.40%), and heptadecane (14.43%), while caryophyllene was found in a relevant amount (6.68%).

The scent of *O. bombyliflora* showed the presence of cyclosativene (10.09%), pentadecane (10.33%), and ethyl dodecanoate (9.46%). The analysis of the scent of *O. insectifera* gave the following results: the main component was 8-heptadecene (18.88%), followed by pentadecane (13.73%) and heptadecane (7.98%). Previous results obtained from labella extracts showed the presence of high-molecular-weight compounds [41,42]. The compounds detected through absorption on Porapak Q were quite similar to the results presented here, with the exception of caryophyllene [33]. Finally, it is noteworthy that all of the high-molecular-weight hydrocarbons detected in the labella were solid and had a very low vapor pressure. As a result of these properties, it is very unlikely that they would be present in the scent.

Nonanal was the compound detected in a previous work in *O. lutea* [41]. SPME analysis showed the presence of heptadecane (39.37%), 8-heptadecene (7.25%), nonadecane (8.87%), and docosane (9.94%). These results are consistent with the trend of SPME analysis on *Ophrys* orchids, where, with some differences for different orchid species, the compounds that we detected were very similar. The differences from previous results can depend on the analytical procedure. Finally, the scent of *O. tenthredinifera* subsp. *neglecta* included a-copaene (11.61%), caryophyllene (11.73%), and *i*-propyl palmitate (14.73%), showing another case beyond *O. apifera* where hydrocarbons are not present in relevant amounts in the scent.

4. Conclusions

In this study, the composition of the aroma of some orchids belonging to the *Ophrys* genus was determined. The results were obtained using SPME coupled with GC-MS as an analysis technique. In the ten samples analyzed, 62 compounds were found; however, the compounds present in greater quantities were almost always the same, with variations (sometimes substantial) between species. It is important to note that there was never any correspondence between our analyses and those obtained through the chemical extraction

of plant labels. In this case, high-molecular-weight hydrocarbons are always recovered, although they are unlikely to be constituents of any aroma, being solid compounds with a low vapor pressure.

This article is part of a complex study with the aim of analyzing all of the orchid species that can be recovered in Basilicata. This work will be completed in the near future.

Finally, the main problem connected with the use of SPME is the different absorption of the scent component on the fiber [45]. An important study should be performed in order to obtain calibration curves for the main components of the scent.

Author Contributions: Conceptualization, M.D. and V.A.R.; investigation, M.M. and R.R.; data curation, R.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zhong, Y.; Jia, Z.; Zhou, H.; Zhang, D.; Li, G.; Yu, J. Comparative Analysis of Volatile Compounds from Four Radish Microgreen Cultivars Based on Ultrasonic Cell Disruption and HS-SPME/GC–MS. *Int. J. Mol. Sci.* **2023**, *24*, 14988. [CrossRef] [PubMed]
2. Zeng, H.; Chen, M.; Zheng, T.; Tang, O.; Xu, H. Metabolomics Analysis Reveals the Accumulation Patterns of Flavonoids and Volatile Compounds in Camellia oleifera Petals with Different Color. *Molecules* **2023**, *28*, 7248. [CrossRef]
3. Li, J.; Ye, S.; Zongo, A.W.-S.; Li, J.; Li, B. Basic amino acids treatment prior to spray drying improved the functional properties and flavor attributes of soy protein isolate. *LWT Food Sci. Technol.* **2023**, *188*, 115447. [CrossRef]
4. Magalhães, S.P.; Guimarães da Silva, J.M.; Perez da Graça, J.; de Oliveira Nunes, E.; Zocolo, G.J.; Hoffmann-Campo, C.B.; Zeraik, M.L. Identification of volatile organic compounds in purple and white soybean flowers by HS-SPME/GC-MS. *Nat. Prod. Res.* **2023**. [CrossRef]
5. Kang, W.; Lin, H.; Ahmad, W.; Li, H.; Chen, Q. Determination of active constituents in kombucha fermentation broth using nano-composite colorimetric sensor based on selected volatile markers determined by GC–MS. *Microchem. J.* **2023**, *195*, 109493. [CrossRef]
6. Flores, J.L.H.; Martínez, Y.J.; López, M.A.R.; Gutierrez, C.S.; Reyes, A.A.; Rosales, M.M.A.; Pérez, M.J.C.; Mendoza, M.F.; Ramírez, J.R.; Zavala, G.R.; et al. Volatile Organic Compounds Produced by Kosakonia cowanii Cp1 Isolated from the Seeds of Capsicum pubescens R & P Possess Antifungal Activity. *Microorganisms* **2023**, *11*, 2491.
7. Song, C.; Zhang, Y.; Zhao, Q.; Chen, M.; Zhang, Y.; Gao, C.; Jia, Z.; Song, S.; Guan, J.; Shang, Z. Volatile organic compounds produced by Bacillus aryabhattai AYG1023 against Penicillium expansum causing blue mold on the Huangguan pear. *Microbiol. Res.* **2024**, *278*, 127531. [CrossRef]
8. D'Eusanio, V.; Morelli, L.; Marchetti, A.; Tassi, L. Aroma Profile of Grapevine Chips after Roasting: A Comparative Study of Sorbara and Spergola Cultivars for More Sustainable Oenological Production. *Separations* **2023**, *10*, 532. [CrossRef]
9. García-González, D.L.; Casadei, E.; Aparicio-Ruiz, R.; Romero, C.O.; Valli, E.; Brereton, P.; Koidis, A.; Korytkowska, M.; Servili, M.; Selvaggini, R.; et al. Multianalyte analysis of volatile compounds in virgin olive oils using SPME-GC with FID or MS detection: Results of an international interlaboratory validation. *Eur. J. Lipid Sci. Technol.* **2023**. [CrossRef]
10. Liu, S.; Lou, Y.; Li, Y.; Zhao, Y.; Feng, X.; Capozzi, V.; Laaksonen, O.; Yang, B.; Li, P.; Gu, Q. Comparison of anthocyanin and volatile organic compounds in juices and fruit wines made from blood oranges (*Citrus sinensis* L. Osbeck) at different maturity stages. *Food Biosci.* **2023**, *56*, 103194. [CrossRef]
11. Chen, S.; Xiao, Y.; Tang, W.; Jiang, F.; Zhu, J.; Zhou, Y.; Ye, L. Evaluation of Physicochemical Characteristics and Sensory Properties of Cold Brew Coffees Prepared Using Ultrahigh Pressure under Different Extraction Conditions. *Foods* **2023**, *12*, 3857. [CrossRef] [PubMed]
12. Zor, M.; Bulut, M.; Karagöz, S.G.; Çetintaş, Y.; Alwazeer, D. Use of Hydrogen-Rich water in rice milk preparation improves the nutritional and sensory properties of product. *Food Chem.* **2024**, *437*, 137821. [CrossRef] [PubMed]
13. Gong, J.; Ma, Y.; Li, L.; Cheng, Y.; Huang, Y. Comparative characterization and contribution of key aroma compounds in the typical base liquor of Jiang-flavor Baijiu from different distributions in the Chinese Chishui River basin. *Food Chem. X* **2023**, *20*, 100932. [CrossRef] [PubMed]
14. Ahamed, Z.; Seo, J.-K.; Eom, J.-U.; Yang, H.-S. Optimization of volatile compound extraction on cooked meat using HS-SPME-GC-MS, and evaluation of diagnosis to meat species using volatile compound by multivariate data analysis. *LWT Food Sci. Technol.* **2023**, *188*, 115374. [CrossRef]
15. Pawliszyn, J. *Applications of Solid Phase Microextraction*; Royal Society of Chemistry: London, UK, 1999.

16. D'Auria, M.; Lorenz, R.; Racioppi, R.; Romano, V.A. Fragrance components of *Platanthera bifolia* subsp. *osca*. *Nat. Prod. Res.* **2017**, *31*, 1612–1619. [CrossRef]
17. D'Auria, M.; Lorenz, R.; Mecca, M.; Racioppi, R.; Romano, V.A.; Viggiani, L. Fragrance components of *Platanthera bifolia* subsp. *osca* and *Platanthera chlorantha* collected in several sites in Italy. *Nat. Prod. Res.* **2020**, *34*, 2857–2861. [CrossRef]
18. D'Auria, M.; Lorenz, R.; Mecca, M.; Racioppi, R.; Romano, V.A. Aroma components of *Cephalanthera* orchids. *Nat. Prod. Res.* **2021**, *35*, 174–177. [CrossRef]
19. Mecca, M.; Racioppi, R.; Romano, V.A.; Viggiani, L.; Lorenz, R.; D'Auria, M. Volatile organic compounds from *Orchis* species found in Basilicata (Southern Italy). *Compounds* **2021**, *1*, 83–93. [CrossRef]
20. D'Auria, M.; Lorenz, R.; Mecca, M.; Racioppi, R.; Romano, V.A. The composition of the aroma of *Serapias* orchids in Basilicata (Southern Italy). *Nat. Prod. Res.* **2021**, *35*, 4068–4072. [CrossRef]
21. Mecca, M.; Racioppi, R.; Romano, V.A.; Viggiani, L.; Lorenz, R.; D'Auria, M. The scent of *Himantoglossum* species found in Basilicata (Southern Italy). *Compounds* **2021**, *1*, 164–173. [CrossRef]
22. Romano, V.A.; Rosati, L.; Fascetti, S.; Cittadini, A.M.R.; Racioppi, R.; Lorenz, R.; D'Auria, M. Spatial and temporal Variability of the floral scent emitted by *Barlia robertiana* (Loisel.) Greuter, a Mediterranean food-deceptive orchid. *Compounds* **2022**, *2*, 37–53. [CrossRef]
23. Mecca, M.; Racioppi, R.; Romano, V.A.; Viggiani, L.; Lorenz, R.; D'Auria, M. Volatile organic compounds in *Dactylorhiza* species. *Compounds* **2022**, *2*, 121–130. [CrossRef]
24. D'Auria, M.; Lorenz, R.; Mecca, M.; Racioppi, R.; Romano, V.A.; Viggiani, L. Fragrance components of *Gymnadenia conopsea* and *Gymnadenia odoratissima* collected at several sites in Italy and Germany. *Nat. Prod. Res.* **2022**, *36*, 3435–3439. [CrossRef] [PubMed]
25. D'Auria, M.; Lorenz, R.; Mecca, M.; Racioppi, R.; Romano, V.A.; Viggiani, L. The scent of *Neotinea* orchids from Basilicata (Southern Italy). *Nat. Prod. Res.* **2022**, *36*, 3741–3743. [CrossRef] [PubMed]
26. D'Auria, M.; Emanuele, L.; Lorenz, R.; Mecca, M.; Racioppi, R.; Romano, V.A.; Viggiani, L. HS-SPME-GC-MS determination of the scent of *Anacamptis* taxa (fam. Orchidadìceae) from Basilicata (Southern Italy). *Nat. Prod. Res.* **2023**. [CrossRef]
27. GIROS. *Orchidee d'Italia. Guida Alle Orchidee Spontanee*; Il Castello: Milan, Italy, 2016.
28. Schiestl, F.P. On the success of a swindle: Pollination by deception in. orchids. *Sci. Nat.* **2005**, *92*, 255–264. [CrossRef]
29. Ayasse, M.; Stökl, J.; Francke, W. Chemical ecology and pollinator-driven speciation in sexually deceptive orchids. *Phytochemistry* **2011**, *72*, 1667–1677. [CrossRef]
30. Gaskett, A.C. Orchid pollination by sexual deception: Pollinator perspectives. *Biol. Rev.* **2011**, *86*, 33–75. [CrossRef]
31. Borg-Karlson, A.-K.; Bergström, G.; Kullenberg, B. Chemical basis for the relationship between *Ophrys* orchids and their pollinators. I. Volatile compounds of *O. insectifera* and *O. speculum* as insect mimetic attractans/excitans. *Chemica Scripta* **1987**, *27*, 303–311.
32. Gervasi, D.D.L.; Selosse, M.-A.; Sauve, M.; Francke, W.; Vereecken, N.J.; Cozzolino, S.; Schiestl, F.P. Floral scent and species divergence in a pair of sexually deceptive orchids. *Ecol. Evol.* **2017**, *7*, 6023–6034. [CrossRef]
33. Borg-Karlson, A.-K.; Growth, I.; Ågren, L.; Kullenberd, B. Form-specific fragances from *Ophrys insectifera* L. (Orchisaceae) attract species of different pollinator genera. Evidence of sympatric speciation? *Chemoecology* **1993**, *4*, 39–45. [CrossRef]
34. Ayasse, M.; Schiestl, F.P.; Paulus, H.F.; Löfstedt, C.; Hansson, B.; Ibarra, F.; Francke, W. Evolution of reproductive strategies in the sexually deceptive orchid *Ophrys sphegodes*: How does flower -specific variation of odor signals influence reproductive success? *Evolution* **2000**, *54*, 1995–2006. [PubMed]
35. Robustelli della Cuna, F.S.; Cortis, P.; Esposito, F.; De Agostini, A.; Sottani, C.; Sanna, C. Chemical composition of essential oil from four sympatric orchids in NW-Italy. *Plants* **2022**, *11*, 826. [CrossRef]
36. Stökl, J.; Schlüter, P.M.; Stuessy, T.F.; Paulus, H.F.; Assum, G.; Ayasse, M. Scent variation and hybridization cause the displacement of a sexually deceptive orchid species. *Am. J. Bot.* **2008**, *95*, 472–481. [CrossRef] [PubMed]
37. Stökl, J.; Schlüter, P.M.; Stuessy, T.F.; Paulus, H.F.; Fraberger, R.; Erdmann, D.; Schulz, C.; Francke, W.; Assum, G.; Ayasse, M. Speciation in sexually deceptive orchids: Pollinator-driven selection maintains discrete odour phenotypes in hybridizing species. *Biol. J. Linnean Soc.* **2009**, *98*, 439–451. [CrossRef]
38. Gögler, J.; Stökl, J.; Sramkova, A.; Twele, R.; Francke, W.; Cozzolino, S.; Cortis, P.; Scrugli, A.; Ayasse, M. Ménage à trois—Two endemic species of deceptive orchids and one pollinator species. *Evolution* **2009**, *63*, 2222–2234. [CrossRef] [PubMed]
39. Gallego, E.; Gelabert, A.; Roca, F.J.; Perales, J.F.; Guardino, X. Identification of volatile organic compounds (VOC) emitted from three European orchid species with different pollination strategies: Two deceptive orchids (*Himantoglossum robertianum* and *Ophrys apifera*) and rewardin orchid (*Gymnadenia conopsea*). *J. Biodiv. Environ. Sci.* **2012**, *2*, 18–29.
40. Robustelli della Cuna, F.S.; Calevo, J.; Bari, E.; Giovannini, A.; Boselli, C.; Tava, A. Characterization and antioxidant activity of essential oil of four sympatric orchid species. *Molecules* **2019**, *24*, 3878. [CrossRef]
41. Borg-Karlson, A.-K.; Bergström, G.; Growth, I. Chemical basis for the relationship between *Ophrys* orchids and their pollinators. II. Volatile compounds of *Ophrys lutea* and *O. fusca* as insect mimetic attractants/excitants. *Chemica Scriptai* **1985**, *25*, 283–294.
42. Manzo, A.; Panseri, S.; Vagge, I.; Giorgi, A. Volatile fingerprint of Italian population of orchids using solid phase microextraction and gas chromatography coupled with mass spectrometry. *Molecules* **2014**, *19*, 7913–7936. [CrossRef]
43. Borg-Karlson, A.-K. Chemical and ethological studies of pollination in the genus *Ophrys* (Orchidaceae). *Phytochemistry* **1990**, *29*, 1359–1387. [CrossRef]

44. Kovats, E. Gas-chromatographische Charakterisierung organischer Verbindungen, Teil 1: Retentionsindices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone. *Helv. Chim. Acta* **1958**, *41*, 1915–1932. [CrossRef]
45. D'Auria, M.; Racioppi, R. Characterization of volatile fraction of mastic oil and mastic gum. *Nat. Prod. Res.* **2022**, *36*, 3460–3463. [CrossRef] [PubMed]