HS-SPME-GC–MS Volatile Profile Characterization of Peach

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The volatile compounds of eight peach varieties (Prunus persica L.)—“Filina”, “Gergana”, “Ufo-4”, “July lady”, “Laskava”, “Flat Queen”, “Evmolpiya”, and “Morsiani 90”—growing in Bulgaria were analyzed for the first time. Gas chromatography–mass spectrometry (GC–MS) analysis and the HS-SPME technique revealed the presence of 65 volatile compounds; the main identified components were aldehydes, esters, and fatty acids. According to the provided principal component analysis (PCA) and hierarchical cluster analysis (HCA), the relative quantities of the identified volatile compounds depended on the studied peach variety. The results obtained could be successfully applied for the metabolic chemotaxonomy of peaches.

Prunus persica L. | gas chromatography–mass spectrometry (GC–MS) | volatile compounds | principal component analysis (PCA) | hierarchical cluster analysis (HCA) | headspace-solid phase micro extraction (HS-SPME)

1. Introduction

Peaches and nectarines are aroma-dense fruits with a specific, pleasant, and recognizable aroma [1]. Nectarines (*Prunus persica* var. nectarina) may have developed from peach seeds, but their origin is still unknown. The peach (*Prunus persica* L. Batsch), also known as Persian apple, is native to China and Iran. Subsequently, it has spread worldwide. Peaches have a large number of commercial varieties with different shapes, sizes, flesh colors (red, white, or yellow), skin types, seeds, among other variable aspects in relation to this popular fruit, representing a diverse international germplasm [2][3]. The largest producer is China, followed by Italy, Spain, and the United States [2].

The peach is a widely appreciated fruit for consumption, but has not yet been fully studied. The chemical composition of peaches depends on several factors, such as genotype, geographical and climatic conditions, seasonal and meteorological conditions, agronomic practices, stage of maturity, storage conditions, and processing methods [4]. In addition, it has been shown, over the years, that the phytochemicals are not evenly distributed in the fruit tissue; most are concentrated in the rind, particularly in the epidermal and subepidermal layers [5][6][7].

The peach species holds remarkable characteristics. More than a hundred volatiles have been identified in different peach varieties, with C_6 compounds, esters, benzaldehyde, linalool, C_{13} norisoprenoids, and lactones being the most abundant [8][9]. In general, polyunsaturated fatty acids (PUFAs), such as linoleic acid (18:2) and linolenic acid (18:3), are the main precursors for aroma-related volatiles of peach fruit generated via the lipoxygenase (LOX) pathway or β-oxidation [10]. β-Oxidation leads to the production of the primary aroma in fruits, whereas the LOX system may account for the widest assortment of lipid-derived precursors of aroma compounds in disrupted plant tissues [11]. In addition to their contribution to fruit quality, peach volatiles are also important for the food and fragrance industry, where they are used as flavoring agents. A notable example of a sought-after industrial product with a peach-like aroma is γ-decalactone [12].

2. Gas Chromatography–Mass Spectrometry (GC–MS) Profiling of Volatile Compounds of Analyzed Peach Samples

The volatile profiles of eight peach varieties (four local and four introduced) grown in Bulgaria were analyzed by GC–MS (**Figure 1**).

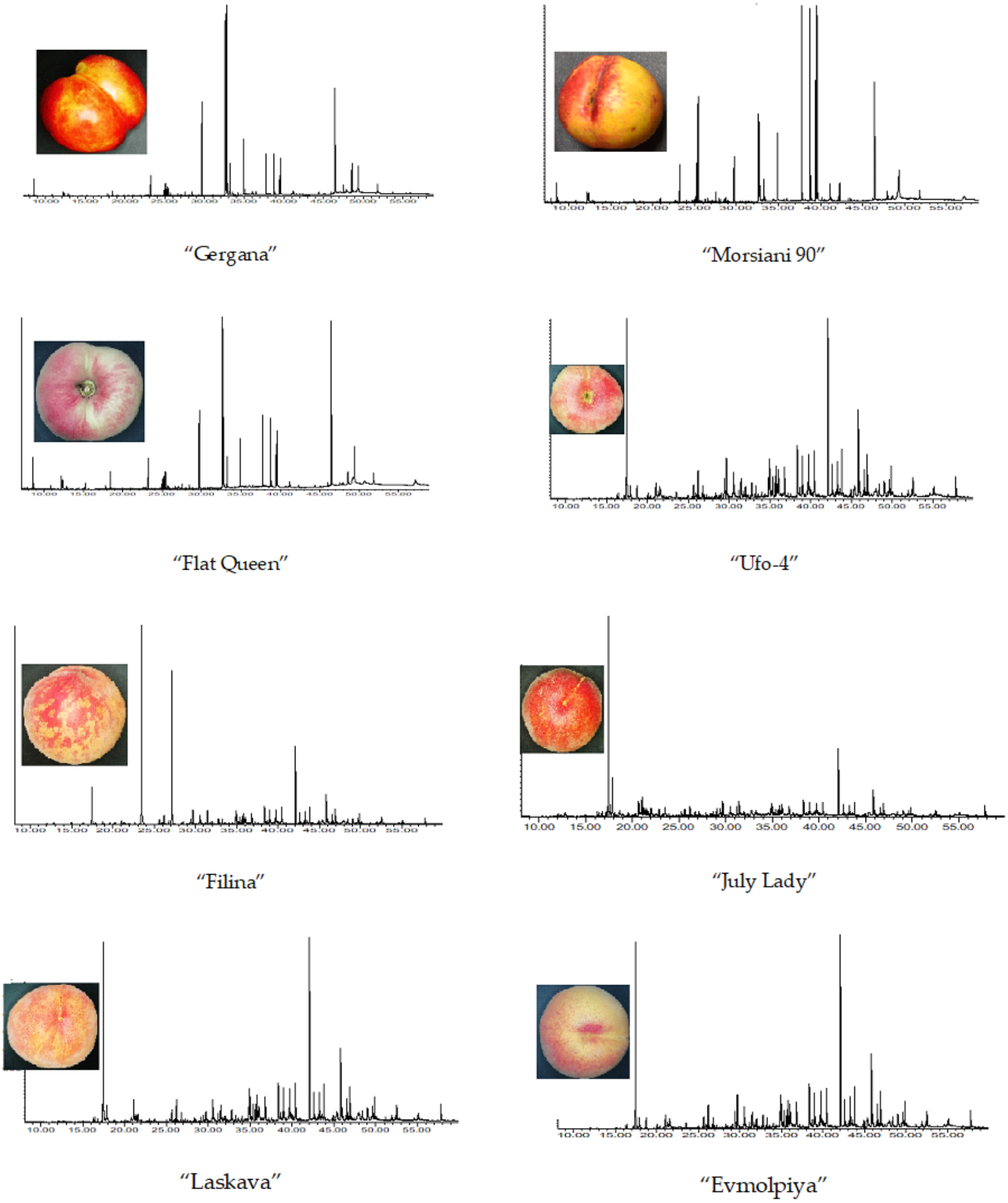

Figure 1. TIC chromatogram obtained for peach and nectarine samples.

Table 1 is a visual presentation of the results; sixty-five volatile compounds, belonging to seven chemical classes (aldehydes, ketones, alcohols, fatty acids, esters, hydrocarbons, and terpenes), were identified.

	RI	„Filina"	„Gergana"	„Ufo-4"	„July lady"	„Laskava"	„Flat Queen"	„Evmolpiya"	„Morsiani 90"
Aldehydes									
Pentanal	738	0.70*	1.17	0.25	1.09	1.14	1.54	0.80	0.99
Hexanal	800	1.95	6.55	3.20	7.13	4.40	2.68	2.24	5.57
(E)-2-Hexenal	849	2.83	4.00	1.35	2.04	7.36	5.30	3.26	6.40
Heptanal	907	4.35	1.47	1.58	1.38	1.14	3.95	3.00	1.25
Benzaldehyde	948	0.71	0.49	0.53	0.46	0.48	0.65	0.82	0.42
(E)-2-Heptenal	960	0.51	0.35	1.64	1.42	1.49	0.46	0.58	1.30
Octanal	999	1.06	0.73	0.79	0.68	0.71	0.97	1.22	0.62
(E)-2-Octenal	1051	1.43	0.98	0.59	3.12	3.26	1.30	1.65	2.83
2-methyl-Benzaldehyde	1070	0.83	0.57	0.53	0.46	1.71	0.76	0.96	1.49
4-methyl-Benzaldehyde	1084	0.24	0.16	1.44	1.25	1.31	0.21	0.27	1.14
Nonanal	1102	3.89	2.21	2.37	2.06	1.99	3.54	1.48	1.88
(E)-2-Nonenal	1160	1.57	1.07	2.42	2.10	2.20	1.42	1.80	1.91
Decanal	1204	0.39	0.27	0.28	0.25	0.26	0.35	0.44	0.23
(E)-2-Decenal	1250	0.31	0.22	1.50	1.30	1.36	0.29	0.36	1.18
Total aldehydes		*20.77*	*20.24*	*18.47*	*24.74*	*28.81*	*23.42*	*18.88*	*27.21*
Ketones									
3-Octanone	975	0.71	0.49	0.52	0.45	0.25	0.64	0.82	0.41
2-Octanone	991	0.56	0.38	0.41	0.36	0.14	0.51	0.64	0.32
γ-hexalactone	1045	0.29	0.20	0.21	0.19	0.42	0.26	0.33	0.17
2-Nonanone	1090	0.64	0.44	0.47	0.41	0.14	0.58	0.73	0.37
γ-octalactone	1250	1.78	1.22	0.58	2.24	2.80	1.62	1.04	2.04
γ-decalactone	1461	1.11	1.52	1.63	1.42	1.48	1.01	1.28	1.29
γ-dodecalactone	1673	1.47	2.52	4.00	3.48	3.64	1.34	1.69	3.16
Total ketones		*6.56*	*6.77*	*7.82*	*8.55*	*8.87*	*5.96*	*6.53*	*7.76*
Alcohols									
Pentanol	770	1.63	1.12	1.21	1.05	1.10	1.49	1.88	0.95
Hexanol	851	0.49	0.34	0.36	0.32	0.13	0.45	0.57	0.29
Heptanol	920	0.74	0.51	0.55	0.47	0.50	0.67	0.85	0.43

Table 1. Identified volatile compounds in peach varieties analyzed by GC–MS. The results are given as % of Total

Ion Current *.

Aldehydes comprise 21% of the identified compounds, with the dominance of hexanal, (E)-2-hexenal, and nonanal in all peach varieties (**Table 1**). Some aldehyde compounds are formed in the event of frost damage: octanal, heptanal, and pentanal [13]. The different amounts in the studied samples prove that chilling injuries are variety-dependent, and do not follow the ripening period of the peach.

C_6 aldehyde compounds are desired, especially in not fully ripe pears, plums, and apples. Such compounds decrease in quantity during the process of full ripening of the fruit [14]. The melon-like flavor of the “Laskava”, “Ufo-4”, and “July Lady” varieties could be related to (E)-2-nonenal detection [15]. Hexanal is reported in literature [16] as a major compound in the volatile analysis of nectarines, which is further supported in the currently established results for the “Gergana” and “Morsiani 90” varieties. It is associated with a sweet, fruity taste [17]. Heptanal, 2-hexenal, and octanal, typical for peach varieties, were found to contribute to the fresh odor [18].

Lactones possess high aromatic values in peaches due to their low odor threshold. Lactones, as intramolecular esters of 4- and 5-hydroxy acids, shape the basic peach aroma [19], and have high aroma effects in stone fruits, in general. γ-Octalactone and γ-dodecalactone, that give peach-like aroma, act in association with aldehydes, alcohols and terpenoids, which are responsible for the spicy, floral and fruity features in the peach [20]. γ-Decalactone and γ-octalactone are characteristic volatile compounds for peaches. The compound γ-octalactone, which confers a sweet herbaceous, coconut-like odor and taste, was predominant in the “July Lady” and “Laskava” varieties [21]. γ-Decalactone was most abundant in the two nectarine varieties object of analysis, which supports the literature stating that this is the most common compound identified in the pulp of nectarines [22]. It has to be noted that lactone identification is highly dependent on the extraction conditions, which can be identified as a limitation in every study on the subject. The absence or presence of certain lactones can be due to the assessment methodology being used [23][24]. For example, other authors have managed to identify more than ten C_5-C_{10} γ-lactones [8][25]. It has been suggested that lactones in peaches are a result of the β-oxidation pathway of fatty acids [26].

The biosynthesis of fatty acids has been reported to being highly influential on the volatile profile. Eight fatty acids were identified, with hexanoic acid (1.22–6.84% of TIC) being the principle one (**Table 1**). Nonanoic and dodecanoic acid were the second most abundant of the investigated peach fruits. Fatty acids are important as they serve as carriers for some lipophilic vitamins and bioactive compounds present in fruits, and the presence of essential fatty acids is believed to play an important role in the prevention of cardiovascular diseases [27][28]. Acids most likely contribute little to the aroma profile though, because they normally have high odor detection thresholds [29].

Alcohols represent approximately 8% of the total identified compounds, with pentanol and nonanol predominating. Pentanol, which is responsible for the bouquet and astringent aroma description [30], was the main alcohol in all the studied samples (in the range from 0.95% to 1.88% of TIC). Benzyl alcohol, found in the highest concentrations in “Ufo-4”, is described as having a floral aroma [31]. Alcohol dehydrogenase in the fruit mesocarp accumulates throughout ripening [32], and alcohols are usually left undetectable by the consumers. Relatively low alcohol quantities suggest that the fruit is not overripe [33]. Fruit juice pH effectively converts alcohols and aldehydes into flavoring agents [33].

Esters are the main VOCs produced by horticultural crops. Esters, especially straight chain esters, are generally metabolized from fatty acids [34]. The higher the amount of esters, the more pronounced the aroma and the taste of the fruit [35]. Esters, accounting for 25% of the identified compounds, represented the largest group. The composition of esters differed both qualitatively and quantitatively among the peach samples. It has to be noted that the ester distribution is reported to be different within the part of the fruit [25]. The total ester content varied between 25.51% and 34.61% of TIC. Esters contribute to the fruity aroma of peaches [36]. Ethyl hexanoate (in the range from 1.38% to 5.76% of TIC) and ethyl tiglate (between 2.78% and 5.4% of TIC) were present at the highest relative concentrations among the estimated esters. Although the amount of esters was predominant in all eight varieties, the relative TIC was two times smaller than the reported literature average [37]. This is most likely due to the specificity of orchard location, light availability, temperature, and season specificity, as well as ecological location.

Seven hydrocarbons were identified, of which tetradecane and tridecane were dominant. The total content of hydrocarbons was in the range from 7.23 to 10.20% of the TIC among the peach varieties. Lu et al. [38] reported the presence of several hydrocarbons in peaches. Tetradecane is considered a creamy descriptor, whereas dodecane is a woody descriptor [39]. The researchers reported the presence of (E)-2-nonen-1-ol, 2-methylpropyl acetate, ethyl butanoate, butyl acetate, 3-methyl-1-butyl acetate, ethyl pentanoate, ethyl hexanoate, hexyl acetate, methyl octanoate, and hexyl hexanoate [38]. Other researchers differentiate ethyl acetate as the major ester compound in both peaches and nectarines [40].

Limonene, linalool, and p-cymene are listed as key flavor compounds that form the characteristic aroma profile [14][41]. The predominant compound in the nectarine varieties was β-mycrene, while linalool was the most abundant in the peach varieties, bringing out the floral aroma in them. Terpenes contribute to a floral flavor of fruits [42], and a sweetening taste [32], and are the principal components in plant essential oils. Among the eight identified terpenes, limonene (0.60–3.26% TIC) and linalool (1.1–2.75% TIC) were in the highest relative concentrations. Linalool was also the major terpenoid compound in other peaches and nectarines [22]. Linalool is reported to possess a floral and citrus-like aroma [43]. Mycrene is a precursor of linalool and is reported as an usual representative in peaches and nectarines, characterizing their aroma with a woody note. The currently established results also prove that the more the linalool, the less the myrcene. Other authors also advocate the thesis that myrcene is found in higher amounts in nectarines [24], which is further supported by the current values.

Terpene compounds (i.e., linalool) and alcohols (i.e., 1-hexanol) are reported to be less abundant than aldehydes in apricots and were detected to decrease with ripening [44]. The relative presence of terpenes in the eight studied varieties was lower compared to the aldehydes, in accordance with the results mentioned above.

Based on the odor descriptors, volatile compounds in peaches can be divided into several sensory groups, including green, fruity, and peach-like aromas [22]. To obtain a clear picture of the overall contribution of the identified compounds on the general flavor of the studied peaches, several figures were created. **Figure 2** shows the odor/taste distribution [47] in the nectarine varieties based on the VOCs identified in the studied samples.

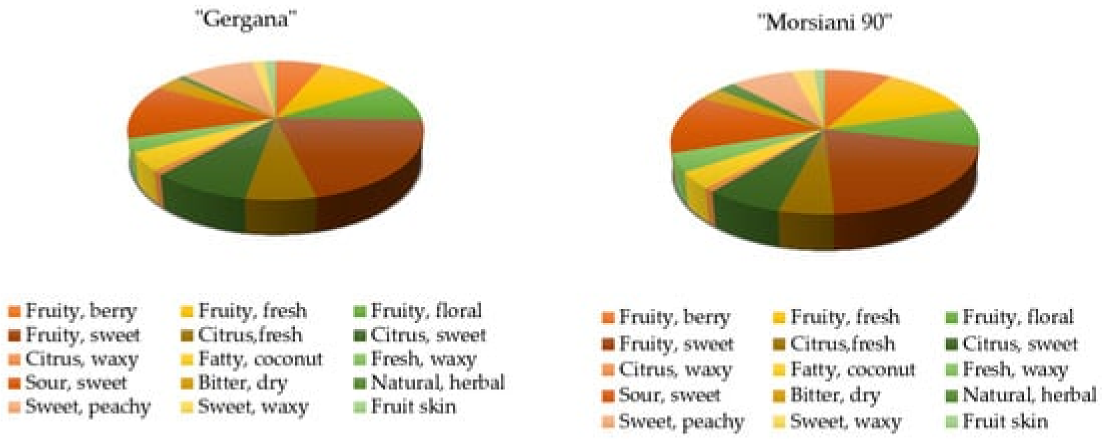

Figure 2. Flavor component distribution (%) in nectarine varieties.

The two nectarine varieties had a dominant fruity, sweet vibe, as well as the sour sweet and sweet peachy descriptors. The “Gergana” variety can be seen as more citrus sweet and the “Morsiani 90” variety is more fruity fresh. Both varieties are considered sweet, but not as floral but fruity.

The results for the two flat peach samples are given in **Figure 3**. The two flat peach samples are mostly fruity sweet, and floral. The “Ufo-4” variety possesses more sweet citrus scents than the “Flat Queen”, which is probably the reason for the more sour sweetness of “Ufo-4” compared to “Flat Queen”. This is quite distinctive for the white flesh peaches, as they are usually described as mildly acidic with a distinct sweet taste.

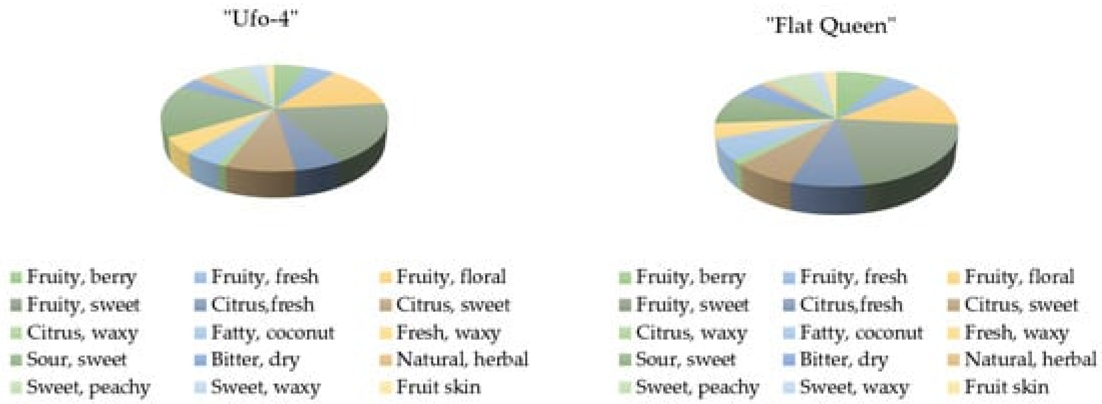

Figure 3. Flavor component distribution (%) in flat peach varieties.

When characterizing the four peach samples (**Figure 4**), it is evident that they are mainly fruity sweet.

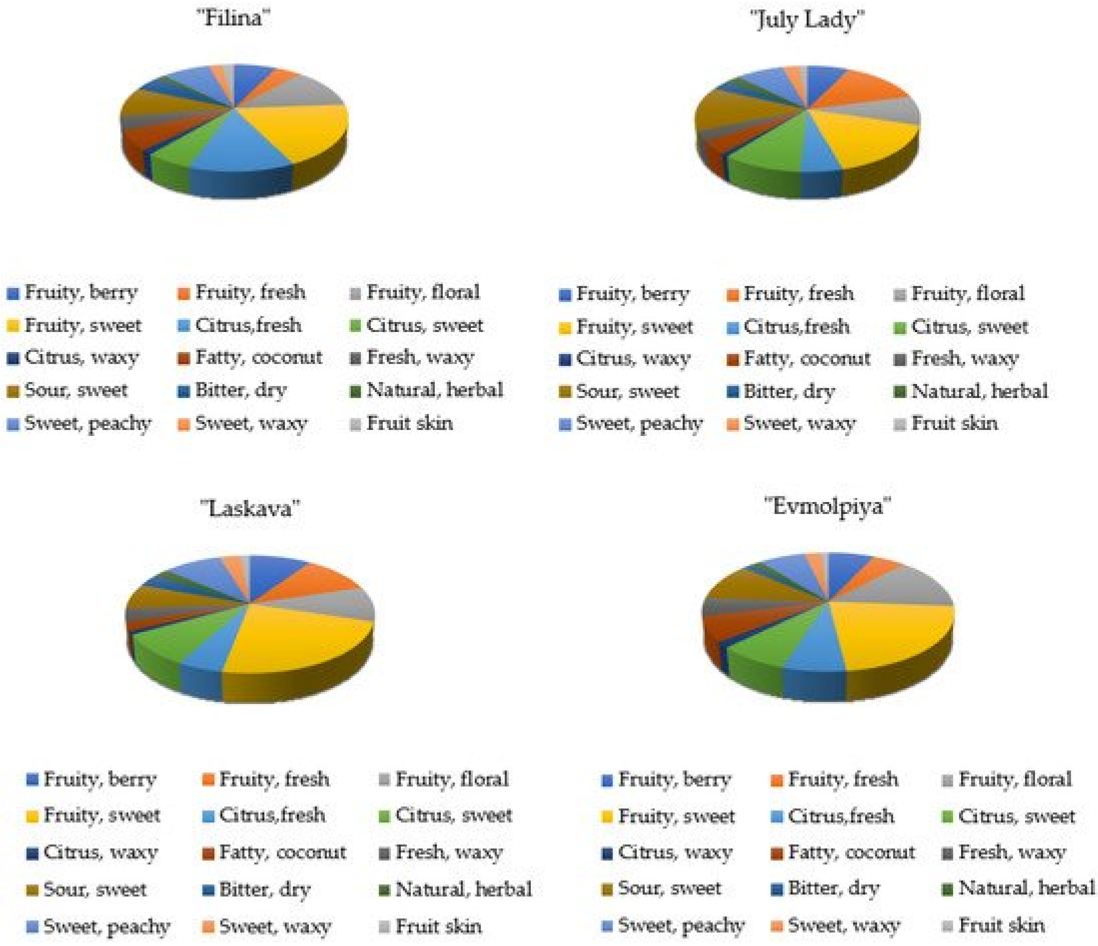

Figure 4. Flavor component distribution (%) in peach varieties.

The "Filina" variety has a moderate citrus fresh scent contributing to its overall flavor, while "July Lady" has a citrus sweet scent. "Evmolpiya" is more sour sweet than "Laskava", while "Laskava" has a specific sweet waxy flavor.

3. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) of GC–MS Data

PCA is an exploratory technique that is typically used as a descriptive analysis for variable selection in a propensity model. The result quality fluctuates significantly by the number of factors or the factors-to-variables ratio. Considering the variables-to-observations ratio is not a good way to determine the required number of observations [48].

In order to confirm sample differences or similarities, principal component analysis (PCA) and hierarchical cluster analysis (HCA) of the volatile compounds identified were applied. According to the PCA plot obtained, the first two principal components PC1 (47.4%) and PC2 (17.6%) accounted for 65% of the total variance of all identified volatile compounds in the analyzed peach varieties (**Figure 5**).

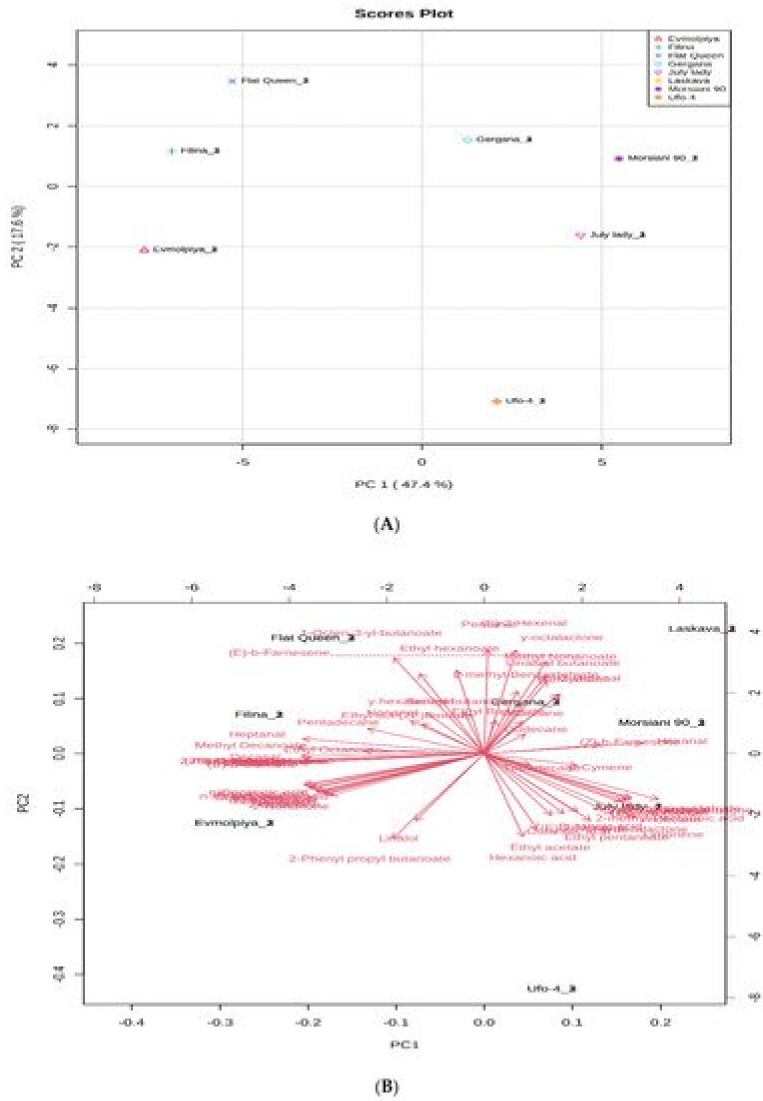

Figure 5. Principal component analysis (PCA) of GC–MS data for volatile compounds of peach (*Prunus persica* L.) varieties. (**A**) Principal component score plot for the eight peach varieties. (**B**) Eigenvector loading values of compounds identified in the eight peach varieties.

(E)-2-Hexanal, pentanal, γ-octalactone, methyl nonanoate, dodecane, ethylbenzoate, hexanal, linalool, 2-methyl-benzaldehyde, β-myrcene, and (Z)-β-farnesene showed high positive loading scores in PC1 that distinguished the "Laskava", "Morsiani 90", and "Gergana" varieties from the other five. Volatile compounds with high negative scores in PC1 were 1-octen-3-yl-butanoate, ethyl hexanoate, (E)-β-farnesene, pentadecane, heptanal, methyl decanoate, nonanal, γ-hexalactone, and ethyl octanoate, which distinguished the "Flat Queen" and "Filina" varieties from the others. The "Evmolpiya" variety appeared clearly different from the other peach varieties, shown by the high negative loading values in PC2 of (E)-2-decenal, n-decanoic acids, 2-nonanone, linalool, and 2-phenyl propyl butanoate. Tetradecane, p-cymene, butanoic acid, 2-methyl-pentanoic acid, ethyl pentanoate, ethyl acetate, hexanoic acid, and limonene clearly differentiated the "July lady" and "Ufo-4" varieties from the other six.

No clear distinguishment between nectarines, peaches, and flat fruits could be stated based on the results. The early season fruits were quite similar to the late season and to the mid-season, and in reverse. Moreover, when clustering the metabolites in polar fractions (phenolic acids, amino acids, organic acids, sugar alcohols, carbohydrates, and saturated and unsaturated fatty acids [49]), different phytochemical similarities were reported.

HCA was performed to understand the relationships between the analyzed varieties. According to the dendrogram and heatmap obtained (**Figure 6** and **Figure 7**), the “Filina” variety had the highest phytochemical similarity to “Flat Queen” and these were grouped in one cluster, with “Morsiani 90” and “July lady” grouped in another cluster. The observed clusters can be explained by the similar quantities of the identified metabolites. HCA also showed the highest diversity among the “Evmolpiya” and “Morsiani 90” varieties because of the significant differences in the quantities of the identified metabolites. According to the results obtained by PCA and HCA, the relative amounts of the identified volatile compounds differed between the studied varieties. In addition, many studies [22][50][42][51][52] also report that the volatile composition of peaches depends on the variety.

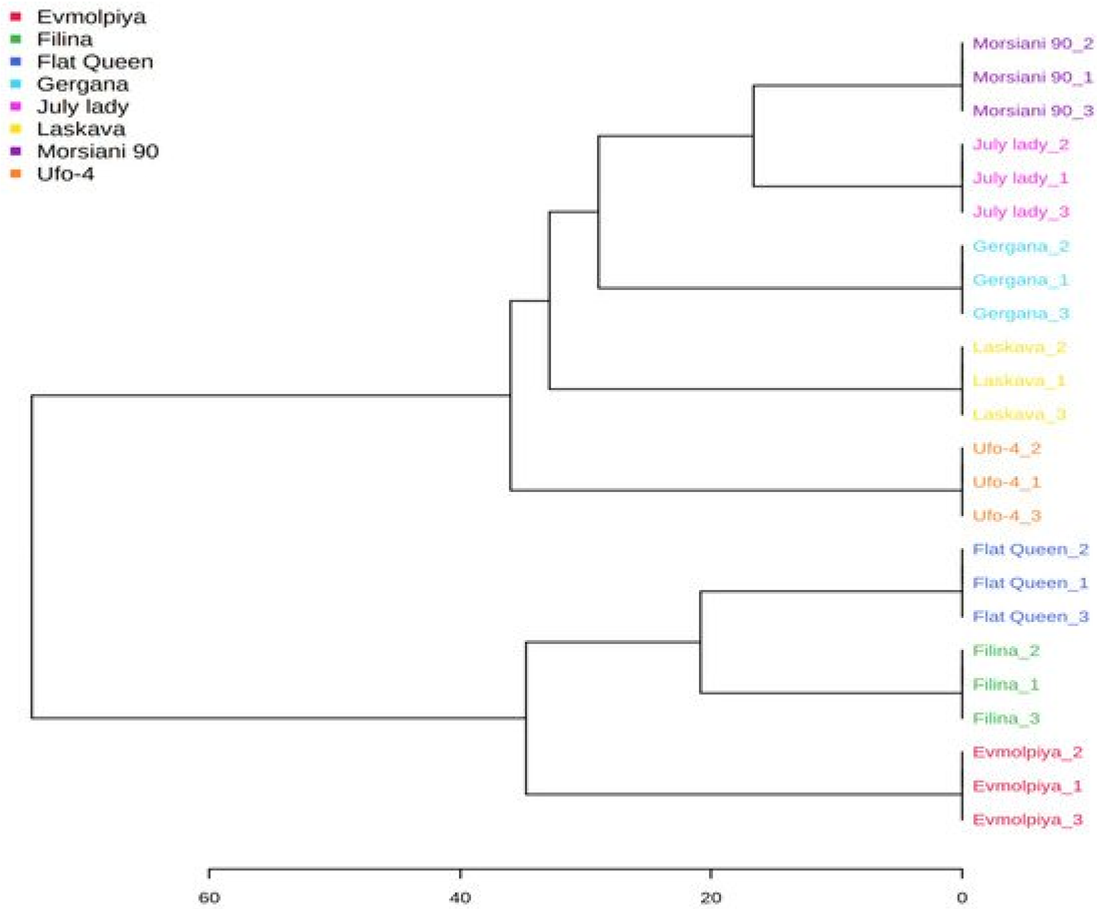

Figure 6. Clustering result of peach varieties shown as a dendrogram (by Euclidean distance measure, and Ward‘s clustering algorithm).

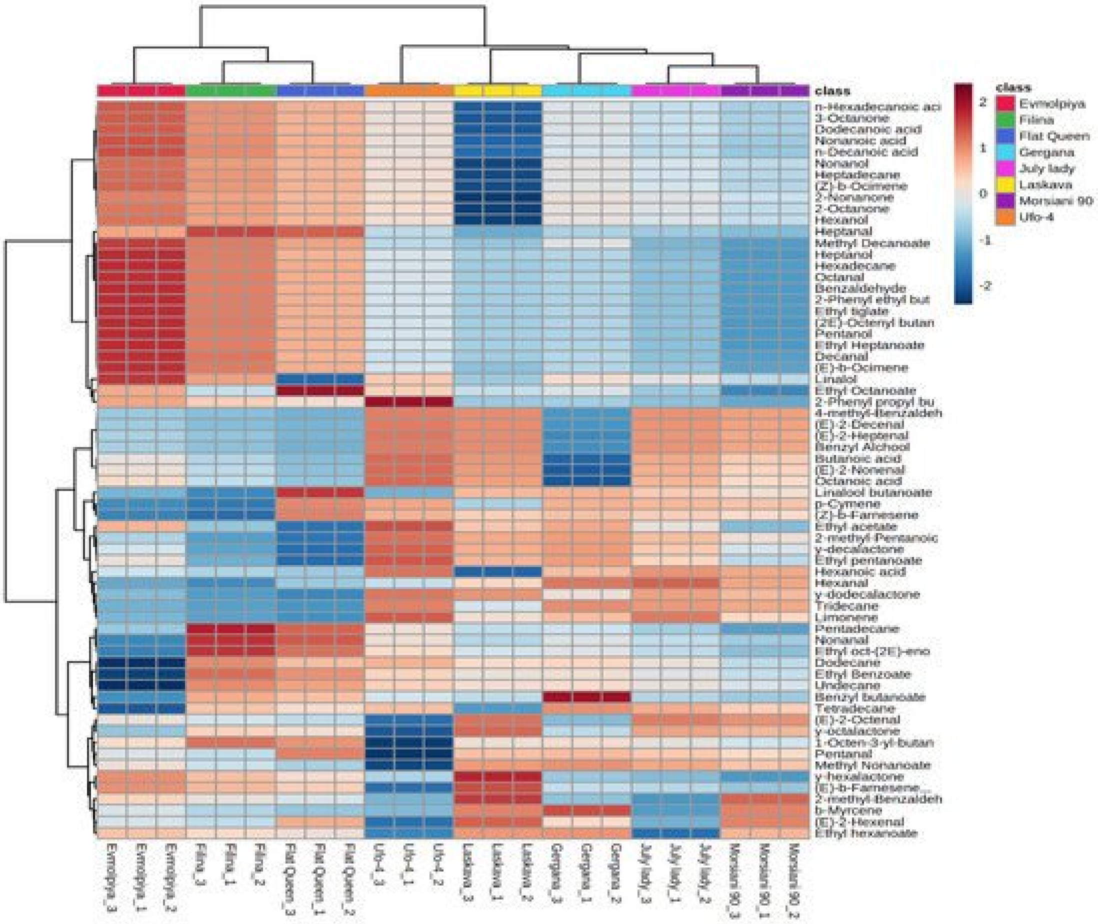

Figure 7. Clustering result of peach varieties shown as a heatmap. The color scale of the heat map ranges from dark brown (value, +2) to dark blue (value, −2). The values were normalized by $\log_{10}$ transformation.

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