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HS-SPME-GC/MS Metabolomic Analysis for the Comparative Evaluation between a Plum–Apricot Hybrid and Its Parents

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Abstract: The "Stendesto" plumcot is the only successful Bulgarian plum–apricot hybrid having the "Modesto" apricot and the "Stanley" plum as parents. The current study reports on the metabolites and volatile organic compounds (VOCs) discovered in the three fruits. Forty-one metabolites in total, as represented by amino acids, organic acids, sugar acids and alcohols, phenolic acids, fatty acids, mono- and di-saccharides, and sterols, were identified in the samples. Additionally, sixty-five VOCs were profiled using the gas chromatography/mass spectrometry (GC/MS) analysis and HS-SPME technique. Among these VOCs, alcohols, aldehydes, esters, ketones, lactones, terpenoids, and benzene derivatives were the existing chemical classes. Not all metabolites were present in both apricot and plum, but the hybrid had managed to inherit all of the identified metabolites with the exception of γ-aminobutyric acid. This study is a first on the topic of plumcot fruit evaluation referencing its parental lines. Principal component (PCA) and hierarchical cluster (HCA) analyses further aided in revealing the differences and similarities between the "Stendesto" plum–apricot hybrid and its parents.

Keywords: untargeted metabolites; chromatography; volatolomics; principal component analysis; *Prunus armeniaca* L.; *Prunus domestica* L.; volatile compounds

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1. Introduction

Fruit consumption is highly associated with lower risks of non-communicable diseases due to the presence of various bioactive molecules [1]. The genus *Prunus* is widely known for its delicious fruits. The apricot fruit is characterized with not only a distinct flavor and odor but also a variety of health-enhancing compounds like phenolic compounds and carotenoids [2]. Worldwide, all cultivated apricots originate from only one species—the *Prunus armeniaca* L., and, according to authors, apricot varieties are categorized into four eco-geographical groups, namely Central Asia, Europe, Iran-Caucasus, and Dzhungar-Zailing [3]. The plum is another cherished representative that is used to boost intestinal health and is reported to contain many antioxidant substances in addition to organic acids, vitamins, minerals, and sugars [4]. The European plum (*Prunus domestica* L.) and the Japanese plum (*Prunus salicina* L.) are differentiated based on their origin and domination in contemporary commercial production [5]. The apricot and the plum fruits are successfully crossed in hybrids that can resemble either the plum or the apricot more. The "Stendesto" is currently the only successful Bulgarian plum–apricot hybrid. It is a plumcot, meaning it looks more like a plum on the outside. The "Stendesto" is currently not well studied and rarely promoted to countries outside Bulgaria.

In general, several partial studies on the topic of plum–apricot hybrids exist [6], but none of them gives a comprehensive evaluation of their primary metabolites and volatile

organic compounds (VOCs). In addition to this, a variety definition is not likely to be found in the majority of published research, which sets a number of limitations for further comparison and evaluation.

VOCs are very important to food components as they primarily attract the attention of the consumer. Aroma evaluation is also crucial to the development of new food products. Gas chromatography/mass spectrometry has proven to be a reliable technique for volatile evaluation. Fruit VOCs can widely differ, but some of the chemical classes reported in research include acids, alcohols, aldehydes, alkanes, alkenes, ketones, esters, furans, and others [7]. Additionally, apricots are rich in esters, terpenes, alcohols, lactones, and aldehydes, while esters, alcohols, aldehydes, ketones, acids, and terpenoids are usually present in plums [8]. VOCs not only distinguish different fruits but also indicate quality parameters like maturity and freshness [9]. Regardless of variety, the VOCs of freshly harvested and mature fruits are esters, alcohols, aldehydes, ketones, lactones, and terpenoids [10]. Several extraction techniques are used to better evaluate the volatile profile of fruit. Headspace solid-phase microextraction (HS-SPME) together with gas chromatography/mass spectrometry (GC/MS) analysis is commonly performed to report on the aroma of fruits [11]. HS-SPME is considered especially suitable for volatile and semi-volatile profiling [12].

Presently, volatile organic compounds are not only studied because of their aroma attributes but also because of their potential applications like biocontrol alternatives for postharvest diseases [13]. Authors state that VOCs can be a new eco-friendly alternative to different challenges like pathogen-associated diseases and promoting plant growth [13]. In the plant kingdom, the role of volatiles is mainly attributed to microorganism and herbivore defense, as well as intra-plant communication [14].

The current study aimed at reporting the volatile composition and metabolic profile of the "Stendesto", the first Bulgarian plum–apricot hybrid fruit, and providing comparable data relevant to both its parents. Using principal component analysis (PCA) and hierarchical cluster analysis (HCA), the differentiation and clustering of the results were presented in terms of the metabolites present in plum–apricot hybrid fruits with reference to their parental lines.

2. Materials and Methods

2.1. Materials

Fruits from the "Modesto" (apricot), "Stanley" (plum), and "Stendesto" (plum–apricot) varieties (Figure 1) were gathered in the year 2023 according to ripening periods from the same plantation. In total, sixty fruits per variety were harvested from the experimental fields of the Fruit Growing Institute (Plovdiv, Bulgaria) and transported in an air-conditioned vehicle to the University of Food Technologies, where the fruits (including the peel) were freeze-dried with a vacuum freeze dryer (BK-FD12 S, Biobase, Jinan, China), powdered, and kept prior to extraction.

"Modesto" apricot "Stanley" plum "Stendesto" plum–apricot

Figure 1. Fruit samples.

2.2. Headspace Solid-Phase Microextraction (HS-SPME) and Gas Chromatography/Mass Spectrometry Analysis (GC/MS)

A 2 cm SPME Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA) fiber assembly was employed for headspace sampling. An online integrated sampling procedure was automatically performed with a G1888 Network Headspace Sampler. An Agilent 7890 A GC unit coupled to an Agilent 5975 C MSD and a DB-5 ms (30 m × 0.25 mm × 0.25 µm) column were used to analyze the volatile compounds in all samples. The oven temperature program was the following: from 40 °C (hold 1 min) to 250 °C (hold 5 min) at 2 °C/min; carrier gas: helium with a flow rate of 1.0 mL/min; transfer line temperature: 270 °C; ion source temperature: 200 °C; EI energy: 70 eV; and mass range: 50 to 550 m/z at 1.0 s/decade.

Sample preparation and Gas Chromatography/Mass Spectrometry Analysis (GC/MS) of polar metabolites and fatty acids.

The HS-SPME extraction technique of the studied fruits followed the description of Mihaylova et al. [15]: 0.05 g of freeze-dried material was mixed with a 1.0 mL methanol/water (75:25, v/v) solution and 50.0 µL of each internal standard, followed by heating at 70 °C for 1 h in a laboratory thermo mixer (Analytik Jena AG, Jena, Germany). The solution, cooled to room temperature, was subjected to the following procedure: 500.0 mL chloroform and 200.0 mL water were added, and then the mixture was centrifuged (for 5 min at 22 °C at 13,000 rpm). The lower phase was designed for the analysis of non-polar substances; the upper phase was for the polar constituents. Prior to the gas chromatography/mass spectrometry (GC/MS) analysis, the two fractions were derivatized.

The AMDIS software (Automated Mass Spectral Deconvolution and Identification System, NIST, Gaithersburg, MD, USA) recorded the RIs of the compounds with a standard n-hydrocarbon calibration mixture ($C_{8–36}$, Restek, Teknokroma, Barcelona, Spain) and supported the reading of the mass spectra and the metabolite identification. The separated compounds were compared to the GC/MS spectra and Kovats retention index (RI) of reference compounds in the Golm Metabolome Database (http://csbdb.mpimp-golm.mpg.de/csbdb/gmd/gmd.html, accessed on 28 December 2023) and the NIST'08 database (NIST Mass Spectral Database, PC-Version 5.0, 2008 from the National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.3. Statistical Analysis

The results are presented as ± SD (standard deviation) plotted with MS Excel software 365 (n = 3). Further statistical analyses of the data were presented using one-way ANOVA and a Tukey–Kramer post hoc test (α = 0.05), as described by Assaad et al. [16]. The MetaboAnalyst 6.0, a web-based platform (www.metaboanalyst.ca, accessed on 7 January 2024), was used to plot the PCA and HCA of GC/MS data. Analyses were triplicated for each fruit. The concentrations of the identified compounds were employed for PCA. All zero values were replaced with a value (½ of the minimum positive values in the original data) assumed to be the detection limit. PCA (95% confidence level) was employed to calculate the eigenvector loading values and to identify the major statistically different components among the samples. The GC/MS data were additionally subjected to HCA, which produced a Ward dendrogram of hierarchical clustering and a Euclidean distance measurement between the analyzed samples. The values were normalized by $\log_{10}$ transformation.

3. Results and Discussion

The current study is considered to be a first on the topic of VOCs and on polar and non-polar metabolites in apricots, plums, and plum–apricot hybrids obtained by HS-SPME-GC/MS. In total, forty-one metabolites and another sixty-five volatile compounds were identified in the samples. Among the metabolites that were obtained by the analysis, amino acids, organic acids, sugar acids and alcohols, mono- and di-saccharides, phenolic acids, fatty acids, and sterols were identified as chemical groups (Table 1).

Table 1. Polar metabolites and lipids (mg/kg dw).

RT	RI	Class/Name	Modesto	Stanley	Stendesto
		Amino acids			
4.28	1106	Alanine	nd	0.13 ± 0.03	0.15 ± 0.03
5.22	1234	Valine	0.23 ± 0.05 ^{a}	0.15 ± 0.03 ^{a}	0.18 ± 0.04 ^{a}
5.74	1261	Leucine	0.29 ± 0.06 ^{a}	0.20 ± 0.04 ^{b}	0.22 ± 0.04 ^{b}
6.00	1298	Isoleucine	0.25 ± 0.05 ^{a}	0.17 ± 0.03 ^{ab}	0.19 ± 0.04 ^{b}
6.08	1303	Proline	0.36 ± 0.07 ^{a}	0.24 ± 0.05 ^{b}	0.27 ± 0.05 ^{a}
6.62	1340	Serine	0.40 ± 0.08 ^{a}	0.27 ± 0.05 ^{a}	0.31 ± 0.06 ^{a}
6.88	1361	Threonine	0.46 ± 0.09	nd	0.35 ± 0.07
8.04	1502	Aspartic acid	3.04 ± 0.61 ^{a}	2.03 ± 0.41 ^{a}	2.33 ± 0.47 ^{a}
8.22	1517	Pyroglutamic acid	0.27 ± 0.05 ^{a}	0.18 ± 0.04 ^{a}	0.21 ± 0.04 ^{a}
9.36	1616	Glutamic acid	0.32 ± 0.06 ^{a}	0.21 ± 0.04 ^{a}	0.25 ± 0.05 ^{a}
9.44	1625	Phenylalanine	0.40 ± 0.08 ^{a}	0.27 ± 0.05 ^{a}	0.31 ± 0.06 ^{a}
11.14	1775	Glutamine	0.54 ± 0.11 ^{a}	0.36 ± 0.07 ^{a}	0.41 ± 0.08 ^{a}
		Organic acids			
6.24	1314	Succinic acid	0.32 ± 0.03 ^{a}	0.13 ± 0.01 ^{c}	0.20 ± 0.02 ^{b}
6.56	1330	Fumaric acid	0.80 ± 0.08 ^{b}	0.37 ± 0.04 ^{c}	1.65 ± 0.16 ^{a}
7.83	1475	Mallic acid	2.53 ± 0.25 ^{b}	1.51 ± 0.15 ^{c}	4.25 ± 0.42 ^{a}
8.33	1530	γ-Aminobutyric acid	0.10 ± 0.01	nd	nd
11.15	1823	Quinicc acid	0.22 ± 0.02 ^{a}	0.26 ± 0.03 ^{a}	0.20 ± 0.02 ^{a}
12.00	1870	Citric acid	5.65 ± 0.56 ^{a}	2.46 ± 0.25 ^{c}	3.85 ± 0.38 ^{b}
		Sugar acids and alcohols			
5.78	1264	Glycerol	0.37 ± 0.04 ^{a}	0.21 ± 0.02 ^{b}	0.19 ± 0.02 ^{b}
8.52	1541	Eritrreonic acid	0.14 ± 0.01 ^{a}	0.08 ± 0.01 ^{c}	0.11 ± 0.01 ^{b}
10.36	1695	Xylitol	nd	0.24 ± 0.02	0.27 ± 0.03
10.43	1718	Arabitol	0.12 ± 0.01	nd	0.13 ± 0.01
		Mono- and di-saccharides			
11.76	1856	Fructose isomer	3.59 ± 0.36 ^{b}	4.79 ± 0.48 ^{a}	3.86 ± 0.39 ^{ab}
11.87	1868	Fructose isomer	5.20 ± 0.52 ^{a}	6.45 ± 0.64 ^{a}	5.98 ± 0.60 ^{a}
12.34	1877	Galactose isomer	nd	2.14 ± 0.21	1.63 ± 0.16
12.37	1879	Glucose isomer	1.18 ± 0.12 ^{b}	13.81 ± 1.38 ^{a}	2.38 ± 0.24 ^{b}
13.15	1898	Galactose isomer	nd	1.80 ± 0.18	4.30 ± 0.43
13.21	1903	Glucose isomer	8.85 ± 0.89 ^{b}	18.70 ± 1.87 ^{a}	20.15 ± 2.02 ^{a}
14.50	1960	Glucose 1-phosphate	10.70 ± 1.07 ^{a}	13.58 ± 1.36 ^{a}	12.27 ± 1.23 ^{a}
23.28	2620	Sucrose isomer	24.83 ± 2.48 ^{a}	11.59 ± 1.16 ^{b}	14.79 ± 1.48 ^{b}
24.29	2620	Sucrose isomer	28.20 ± 2.82 ^{a}	18.00 ± 1.80 ^{b}	21.33 ± 2.13 ^{b}
		Phenolic acids			
12.52	1835	Protocatechuic acid	0.42 ± 0.04	nd	0.29 ± 0.03
13.77	1940	trans-p-Coumaric acid	2.21 ± 0.22 ^{a}	0.40 ± 0.04 ^{c}	0.83 ± 0.08 ^{b}
16.36	2106	trans-Ferulic acid	nd	0.14 ± 0.01	0.16 ± 0.02
		Fatty acids			
29.16	1920	Palmitic acid	10.33 ± 1.55 ^{a}	7.23 ± 1.08 ^{a}	8.78 ± 1.32 ^{a}
32.11	2094	Linoleic acid	4.59 ± 0.69 ^{a}	3.21 ± 0.48 ^{a}	3.90 ± 0.59 ^{a}
32.22	2101	Oleic acid	4.17 ± 0.63 ^{a}	2.92 ± 0.44 ^{a}	3.54 ± 0.53 ^{a}
32.28	2106	Linolenic acid	1.61 ± 0.24 ^{a}	1.12 ± 0.17 ^{a}	1.36 ± 0.20 ^{a}
32.71	2128	Stearic acid	9.78 ± 1.47 ^{a}	6.85 ± 1.03 ^{a}	8.31 ± 1.25 ^{a}
		Sterols			
37.22	3197	Campesterol	14.20 ± 2.13 ^{a}	9.94 ± 1.49 ^{a}	12.07 ± 1.81 ^{a}
38.19	3297	β-Sitosterol	36.28 ± 5.44 ^{a}	25.40 ± 3.81 ^{a}	30.84 ± 4.63 ^{a}

RT—retention time; RI—retention index; nd—not detected. Different letters in the same row indicate statistically significant differences ($p < 0.05$), according to ANOVA and the Tukey test ($n = 3$).

Twelve amino acids were present in the studied samples, with aspartic acid being the most abundant. The plum–apricot hybrid had inherited all amino acids from its parents, even though alanine is only present in the plum, and threonine in the apricot. Aspartic acid is a non-essential amino acid with a role in brain development and hypothalamus regulation [17]. The low presence of glutamic and aspartic acids in the human body was

reported as a prerequisite to diabetic retinopathy [18]. In this regard, it might be suggested that the consumption of *Prunus* fruit may act as a dietary prevention for non-communicable diseases like diabetes type II and its complications. Among the identified amino acids, five are essential (valine, isoleucine, leucine, phenylalanine, and threonine).

The presence of organic acids in fruits is not unusual and is associated with their sour taste [19]. The organic acids in the studied samples are presented by six compounds, with citric and malic acids being predominant. The only organic acid that the plum–apricot hybrid has not managed to synthetize in comparison with its parents is the γ-aminobutyric acid. The anions of citric and malic acids are Krebs cycle intermediates. During storage, their quantity decreases by being dissimilated/metabolized [19]. Some authors point out that the metabolism of the organic acids—malic and citric in particular—might be affected by the vacuole expansion due to fruit maturation and size change [20].

Fruit sugar accumulation mainly accounts for the presence of fructose, glucose, sucrose, and several alcohols (sorbitol, mannitol, erythritol, and xylitol, among others) [21]. The proportion distribution of sugars is what accounts for the quality and flavor of fruits [22]. It was noticed that commonly found sugars like glucose, fructose, and sucrose appeared as several isomers in the studied fruit samples. The current results reveal the prevalence of sucrose isomers, along with smaller of quantities of glucose and fructose ones. The "Stendesto" plum–apricot hybrid had inherited all mono- and di-saccharides identified in the plum and the apricot alone.

Phenolic acids are commonly found in plants and fruit in particular [23]. Three phenolic acids were identified in the current study. Trans-*p*-coumaric acid was the one with the highest values. The "Stendesto" plum–apricot hybrid had all three identified phenolic acids. *P*-coumaric acid is a hydroxycinnamic acid that is reported to improve the antioxidant activity of its carrier, along with providing defense responses against pathogens [24].

Fatty acids are the main predecessor of aromatic compounds, i.e., alcohols, aldehydes, esters, ketones, and lactones [25]. The fatty acid profile of the studied samples comprised two polyunsaturated fatty acids, two saturated fatty acids, and one monounsaturated fatty acid. The saturated fatty acids (palmitic and stearic) were the ones that prevailed in their quantity in all three fruit samples. The "Stendesto" plum–apricot hybrid had all five identified fatty acids. Palmitic and stearic acids are most commonly reported in various fruits [26]. The current study is another confirmative example of this statement.

Phytosterols have several beneficial effect that have been reported in the literature [27]. Campesterol and β-sitosterol are the two sterols identified in the studied samples. Campesterol has been reported to exhibit cholesterol-lowering and anticarcinogenic effects [28], which implies that regular consumption of the "Modesto" apricot, the "Stanley" plum, and the "Stendesto" plum–apricot hybrid might act positively on the management of blood cholesterol levels.

The overall distribution of the different metabolite classes is shown in Figure 2.

The plum–apricot hybrid fruit had accumulated more mono- and di-saccharides compared with the apricot and less compared with the plum. Both apricot and hybrid fruits had relatively the same quantities of organic acids and sugar acids and alcohols. The sterols had the greatest values in the "Modesto" apricot, followed by the "Stendesto" hybrid. The distribution of each specific compound was different, and the quantity of each contributed differently not only to the specific chemical family but also to the fruit itself.

Three major chemical groups are reported to be commonly identified in various fruits: alcohols, aldehydes, and esters [14]. The investigated volatile compounds in the three studied fruits (plum, apricot, and hybrid) are presented in Table 2. The identified compounds were profiled as alcohols, aldehydes, esters, lactones, ketones, terpenoids, and benzene derivatives. All of the identified compounds were present in the plum–apricot hybrid fruit; this was not valid for the plum or for the apricot. Only a few benzene derivatives were identified in the "Modesto" apricot, and none of the three lactones was present in the "Stanley" plum.

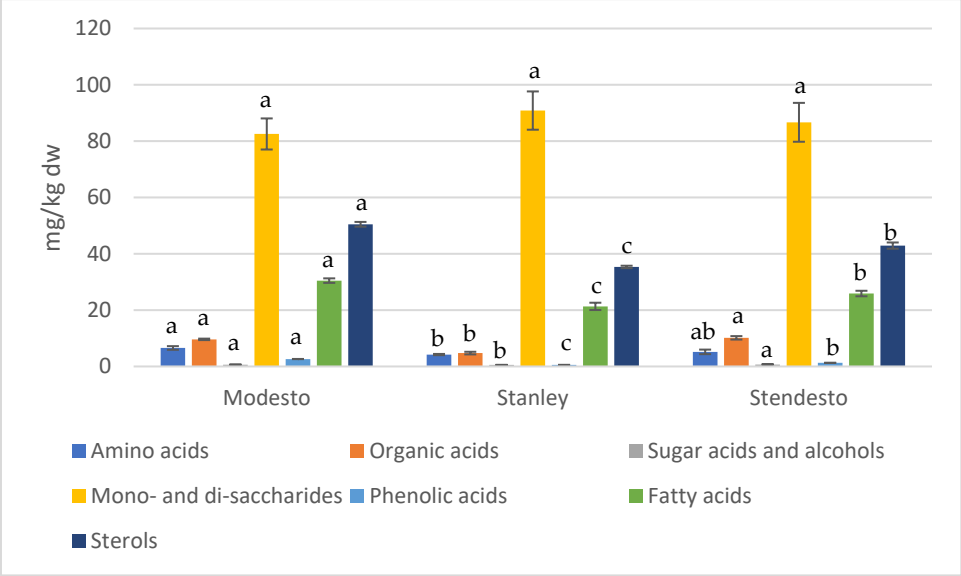


Figure 2. Distribution of chemical classes in studied fruit samples (mg/kg dw). Different letters in the same chemical class indicate statistically significant differences ($p < 0.05$), according to ANOVA and the Tukey test ($n = 3$).

Table 2. Identified fruit VOCs (%TIC) studied by HS-SPME-GC/MS.

Name/Class	RI	Modesto	Stanley	Stendesto
		Alcohols		
1-Butanol	655	2.03 ± 0.30 ^{b}	3.63 ± 0.39 ^{a}	2.37 ± 0.36 ^{b}
3-Methyl-2-buten-1-ol	719	0.80 ± 0.12 ^{b}	1.95 ± 0.14 ^{a}	0.86 ± 0.13 ^{b}
2-Methyl-1-butanol	724	1.22 ± 0.18 ^{b}	2.59 ± 0.24 ^{a}	1.43 ± 0.21 ^{b}
1-methylcyclopentanol	796	0.37 ± 0.06 ^{a}	0.48 ± 0.07 ^{a}	0.20 ± 0.03 ^{b}
(2)-3-hexen-1-ol	849	0.56 ± 0.08 ^{a}	0.73 ± 0.11 ^{a}	0.66 ± 0.10 ^{a}
1-Hexanol	852	0.72 ± 0.11 ^{b}	1.34 ± 0.14 ^{a}	0.84 ± 0.13 ^{b}
2-ethylhexanol	1028	0.81 ± 0.12 ^{a}	0.75 ± 0.11 ^{ab}	0.50 ± 0.07 ^{b}
Benzyl alcohol	1035	3.20 ± 0.48 ^{a}	4.16 ± 0.62 ^{a}	3.75 ± 0.56 ^{a}
3.5,5-Trimethyl-2-cyclohexen-1-ol	1147	0.69 ± 0.10 ^{a}	0.90 ± 0.13 ^{a}	0.81 ± 0.12 ^{a}
2.3,5-Trimethyl-1.4-benzenediol	1210	0.95 ± 0.14 ^{a}	1.35 ± 0.20 ^{a}	1.20 ± 0.18 ^{a}
4-Methoxy-benzenemethanol	1249	1.13 ± 0.17 ^{a}	1.46 ± 0.22 ^{a}	1.32 ± 0.20 ^{a}
3.4,5-Trimethoxy-benzenemethanol	1426	1.42 ± 0.21 ^{a}	1.85 ± 0.28 ^{a}	1.66 ± 0.25 ^{a}
		Aldehydes		
Hexanal	802	2.15 ± 0.32 ^{a}	1.72 ± 0.26 ^{a}	2.41 ± 0.36 ^{a}
(E)-2-hexenal	848	3.22 ± 0.48 ^{a}	2.57 ± 0.39 ^{a}	3.60 ± 0.54 ^{a}
Heptanal	898	1.68 ± 0.25 ^{a}	1.34 ± 0.20 ^{a}	1.88 ± 0.28 ^{a}
Benzaldehyde	939	6.77 ± 0.99 ^{a}	8.05 ± 0.79 ^{a}	7.36 ± 1.10 ^{a}
(E)-2-heptenal	953	1.18 ± 0.18 ^{a}	0.94 ± 0.14 ^{a}	1.32 ± 0.20 ^{a}
(E,E)-2.4-heptadienal	998	0.83 ± 0.13 ^{a}	0.67 ± 0.10 ^{a}	0.94 ± 0.14 ^{a}
Phenylacetaldehyde	1045	1.76 ± 0.26 ^{a}	1.40 ± 0.21 ^{a}	1.97 ± 0.29 ^{a}
Nonanal	1105	2.04 ± 0.35 ^{a}	1.89 ± 0.28 ^{a}	2.64 ± 0.40 ^{a}
2-Undecenal	1159	1.38 ± 0.21 ^{a}	1.10 ± 0.17 ^{a}	1.54 ± 0.23 ^{a}
Tetradecanal	1207	0.39 ± 0.06 ^{a}	0.31 ± 0.05 ^{a}	0.44 ± 0.07 ^{a}
(E,E)-2.4-decadienal	1295	6.04 ± 0.96 ^{a}	5.14 ± 0.77 ^{a}	7.19 ± 1.08 ^{a}
(E,Z)-2.4-decadienal	1319	4.88 ± 0.69 ^{a}	2.07 ± 0.31 ^{b}	2.89 ± 0.43 ^{b}
3-Methoxy-4-hydroxybenzaldehyde	1355	0.64 ± 0.10 ^{a}	0.51 ± 0.08 ^{a}	0.71 ± 0.11 ^{a}

Table 2. *Cont.*

Name/Class	RI	Modesto	Stanley	Stendesto
		Esters		
Butyl acetate	813	1.41 ± 0.21 ^{b}	2.83 ± 0.28 ^{a}	3.10 ± 0.17 ^{a}
(Z)-3-Hexenyl acetate	1005	1.13 ± 0.17 ^{ab}	1.47 ± 0.22 ^{a}	0.88 ± 0.13 ^{b}
Hexyl acetate	1010	6.02 ± 0.90 ^{a}	5.22 ± 0.78 ^{a}	3.13 ± 0.47 ^{b}
(Z)-3-hexenyl butanoate	1183	2.04 ± 0.31 ^{b}	2.94 ± 0.44 ^{a}	1.76 ± 0.26 ^{b}
(E)-2-hexenyl butanoate	1191	3.51 ± 0.53 ^{ab}	4.57 ± 0.69 ^{a}	2.74 ± 0.41 ^{b}
Ethyl octanoate	1196	1.83 ± 0.28 ^{ab}	2.38 ± 0.36 ^{a}	1.43 ± 0.21 ^{b}
1-Octen-3-yl-butanoate	1280	3.15 ± 0.47 ^{a}	4.09 ± 0.61 ^{a}	3.46 ± 0.37 ^{a}
(2 E)-Octenyl butanoate	1388	2.80 ± 0.42 ^{ab}	3.63 ± 0.55 ^{a}	2.18 ± 0.33 ^{b}
		Ketones		
3-Hydroxy-2-butanone	680	2.64 ± 0.40 ^{a}	1.85 ± 0.28 ^{b}	1.11 ± 0.17 ^{b}
3-Hexanone	784	1.38 ± 0.21 ^{a}	0.96 ± 0.14 ^{b}	0.58 ± 0.09 ^{b}
2-Hexanone	789	2.36 ± 0.35 ^{a}	1.65 ± 0.25 ^{b}	0.99 ± 0.15 ^{c}
5-Ethyl-2(H)-furanone	954	1.35 ± 0.20 ^{a}	0.94 ± 0.14 ^{b}	0.57 ± 0.08 ^{b}
6-Methyl-5-hepten-2-one	984	1.12 ± 0.17 ^{a}	0.78 ± 0.12 ^{b}	0.47 ± 0.07 ^{b}
5-Ethyl-2(H)-furanone	954	1.03 ± 0.15 ^{a}	0.72 ± 0.11 ^{b}	0.43 ± 0.06 ^{c}
6-Methyl-5-hepten-2-one	984	1.44 ± 0.22 ^{a}	1.01 ± 0.15 ^{b}	0.61 ± 0.09 ^{b}
2.2,6-Trimethylcyclohexanone	1036	0.21 ± 0.03 ^{b}	0.15 ± 0.02 ^{b}	0.32 ± 0.05 ^{a}
Acetophenone	1065	1.86 ± 0.28 ^{ab}	1.30 ± 0.20 ^{b}	2.38 ± 0.36 ^{a}
2-Ethylcyclohexanone	1158	0.67 ± 0.10 ^{ab}	0.47 ± 0.07 ^{b}	0.83 ± 0.12 ^{a}
4-Acetyl-1.2,3.5,5-pentamethyl-2-cyclopenten-l-one	1216	0.37 ± 0.06 ^{ab}	0.26 ± 0.04 ^{b}	0.48 ± 0.07 ^{a}
5-Butyldihydro-2(3 H)-furanone	1264	0.19 ± 0.03 ^{ab}	0.13 ± 0.02 ^{b}	0.25 ± 0.04 ^{a}
Tetrahydro-6-pentyl-2 H-pyran-2-one	1435	0.42 ± 0.06 ^{ab}	0.29 ± 0.04 ^{b}	0.55 ± 0.08 ^{a}
5-Hexyldihydro-2(3 H)-furanone	1461	1.17 ± 0.18 ^{ab}	0.82 ± 0.12 ^{b}	1.52 ± 0.23 ^{a}
		Lactones		
γ-Octalactone	1255	0.59 ± 0.09	nd	0.84 ± 0.13
γ-Nonalactone	1362	0.38 ± 0.06	nd	0.70 ± 0.10
γ-Dodecalactone	1413	0.79 ± 0.12	nd	0.96 ± 0.14
		Terpenoids		
p-Cymene	1026	1.12 ± 0.17 ^{a}	1.45 ± 0.22 ^{a}	1.68 ± 0.25 ^{a}
Limonene	1031	1.49 ± 0.22 ^{b}	1.93 ± 0.29 ^{ab}	2.23 ± 0.33 ^{a}
alfa-Terpineol	1199	0.90 ± 0.14 ^{b}	1.18 ± 0.18 ^{ab}	1.36 ± 0.20 ^{a}
Geraniol	1221	0.87 ± 0.13 ^{a}	1.13 ± 0.17 ^{a}	1.30 ± 0.20 ^{a}
Bomeol	1234	1.05 ± 0.16 ^{a}	1.36 ± 0.20 ^{a}	1.57 ± 0.24 ^{a}
Nerol	1251	1.77 ± 0.27 ^{a}	0.30 ± 0.04 ^{b}	1.25 ± 0.40 ^{a}
Hexyl acetate	1268	2.92 ± 0.44 ^{a}	0.80 ± 0.57 ^{b}	2.39 ± 0.66 ^{a}
Bomyl acetate	1287	0.35 ± 0.05 ^{b}	0.45 ± 0.07 ^{b}	0.72 ± 0.11 ^{a}
Geranyl acetate	1377	0.50 ± 0.08 ^{b}	0.65 ± 0.10 ^{ab}	0.85 ± 0.13 ^{a}
		Benzene derivatives		
1.4-Dimethylbenzene	865	0.41 ± 0.06 ^{a}	0.29 ± 0.04 ^{a}	0.33 ± 0.05 ^{a}
Naphthalene	1186	nd	0.51 ± 0.08	0.28 ± 0.09
2-Oxo-1-methyl-3-isopropylpyrazine	1225	0.23 ± 0.03 ^{a}	0.16 ± 0.02 ^{a}	0.19 ± 0.03 ^{a}
1.2,3.4-Tetrahydro-1.5,7-trimethylnaphthalene	1310	nd	0.42 ± 0.06	0.38 ± 0.07
1-Methyl-4-(methylthio) benzene	1316	0.29 ± 0.04 ^{a}	0.20 ± 0.03 ^{b}	0.23 ± 0.03 ^{ab}
1.2,3.4-Tetrahydro-1.1,6-trimethylnaphthalene	1349	nd	0.62 ± 0.09	0.70 ± 0.11

RI—retention index; nd—not detected. Different letters in the same row indicate statistically significant differences ($p < 0.05$), according to ANOVA and the Tukey test.

Among the twelve identified alcohols, 1-butanol and benzyl alcohol held the highest %TIC. Alcohols are associated with a green-like aroma in *Prunus* fruits [10]. Butanal is often associated with a nutty flavor note [29]. Aldehydes are usually part of the volatile profile of fruits [30]. Aldehydes are produced as a result of the enzymatic degradation of lipids and/or unsaturated fatty acids (linoleic and linolenic acids) or from amino acids [31].

Aldehydes further convert to their corresponding alcohols by alcohol dehydrogenase, or they convert to acids [32]. Thirteen different aldehydes were identified in the current study with hexanal, E-2- hexenal, benzaldehyde, nonanal, and decadienal holding the highest %TIC. Hexanal and its isomers attribute to a greenish sensory association in fruit and vegetables [33]. Hexanal has been particularly recorded as important to the fresh plum volatiles [34]; and (E)-2-hexenal holds a mixture of sweet, floral, and fruity odors [35]. Nonanal is reported to have a woody-like aroma and is typically found in ripe plums of different varieties [34]. The existence of decadienal is presented as a floral, herbaceous, and woody scent [36]. Eight esters were identified in the studied fruit samples, with butyl acetate, hexyl acetate, and 1-octen-3-yl butanoate being the most representative. Esters are usually key odorants in the *Prunus* fruit family [37]. Fourteen ketones were identified in the three fruit samples, with acetophenone, 2-hexanone, and 3-hydroxy-2-butanone holding the highest %TIC. The presence of acetophenone is linked to a sweet perception of taste [38]. Only three lactones were identified in the current study with relatively small %TIC. A total of nine terpenoids were identified in the plum, apricot, and plum–apricot fruit. Hexyl acetate and limonene were the most present in the total ion content. Hexyl acetate is often identified in various *Prunus* fruits [37].

Figure 3 reveals the differences between the hybrid fruit and those of the plum and apricot in terms of %TIC predominance and variety dependance.

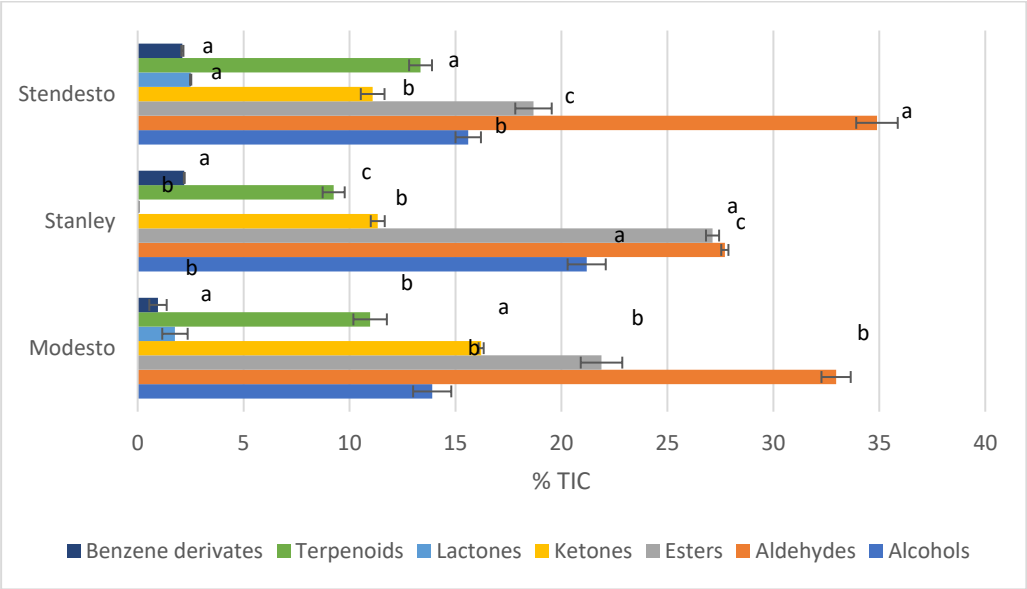


Figure 3. Distribution of VOC chemical classes in studied fruit samples (%TIC). Different letters in the same chemical class indicate statistically significant differences ($p < 0.05$), according to ANOVA and the Tukey test ($n = 3$).

Aldehydes are the most abundant among the identified compounds. The plum–apricot hybrid had more aldehydes compared with the plum and apricot. Esters, alcohols, and ketones were also widely represented. The "Stendesto" fruit had more terpenoids compared with its parents. Lactones were not identified in the plum, but they were available in the apricot and plum–apricot. Figure 4 is a visual presentation of the odor description of each fruit part of the current study based on the VOCs present in them.

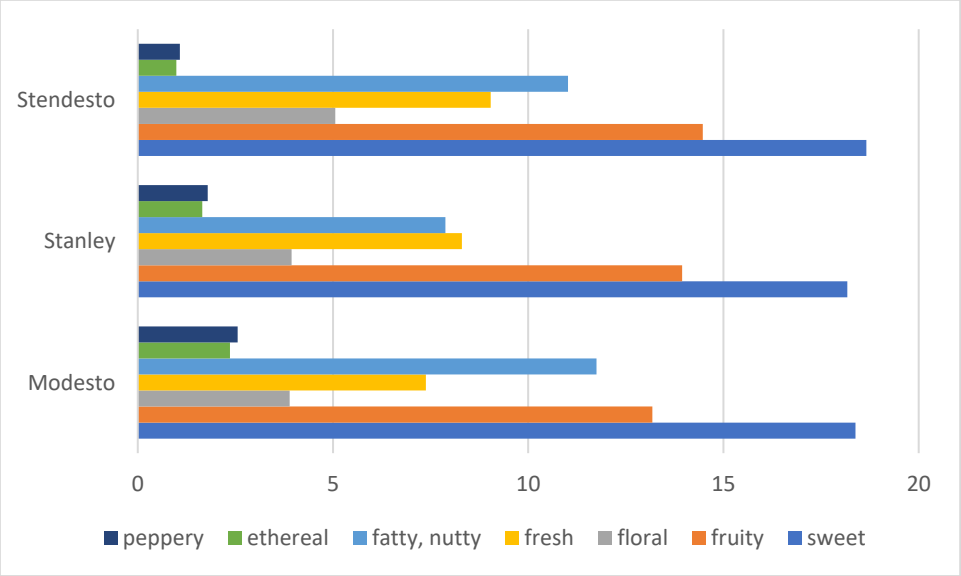


Figure 4. Odor description attribution of VOCs in studied fruit samples (https://foodb.ca, accessed on 29 December 2023).

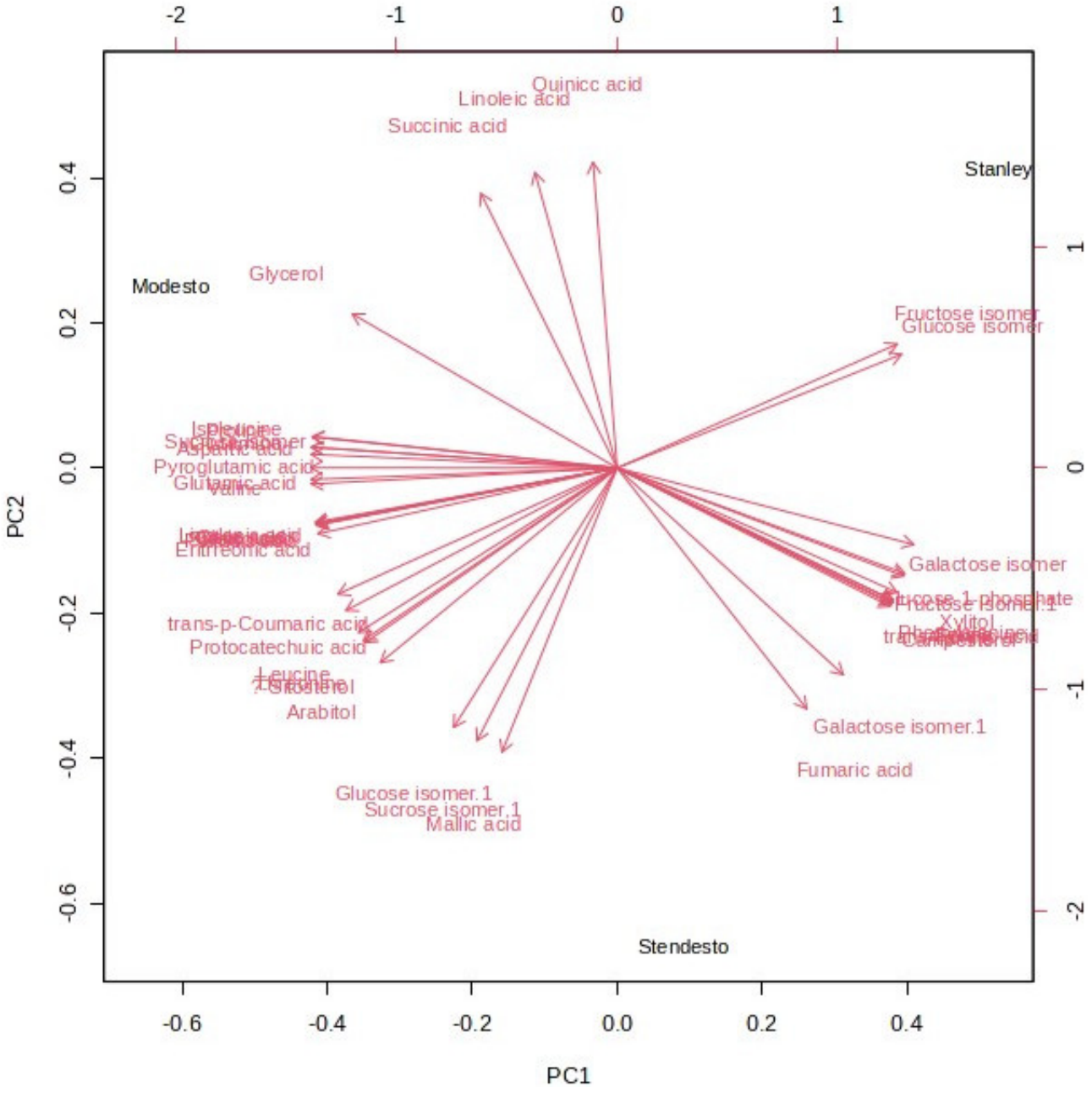
The "Modesto" apricot was characterized with predominantly sweet volatiles, followed by fruity and fatty/nutty associations. The "Stanley" plum was mainly sweet and fruity, with fresh sensory relations. The "Stendesto" plum–apricot hybrid appeared to have a more distinct fruity, fresh, and floral profile compared with its parents. It had fewer ethereal, peppery, and fatty/nutty notes. The quantity of fruity volatiles was comparable in all three fruit samples.

This study can be seen as a pioneer on the topic of metabolite identification in "Modesto" (apricot), "Stendesto"(plum–apricot hybrid), and "Stanley" (plum) fruit and on providing core data for future evaluations and comparison.

Principal Component Hierarchical Cluster and Correlation Analyses of HS-SPME-GC/MS Data

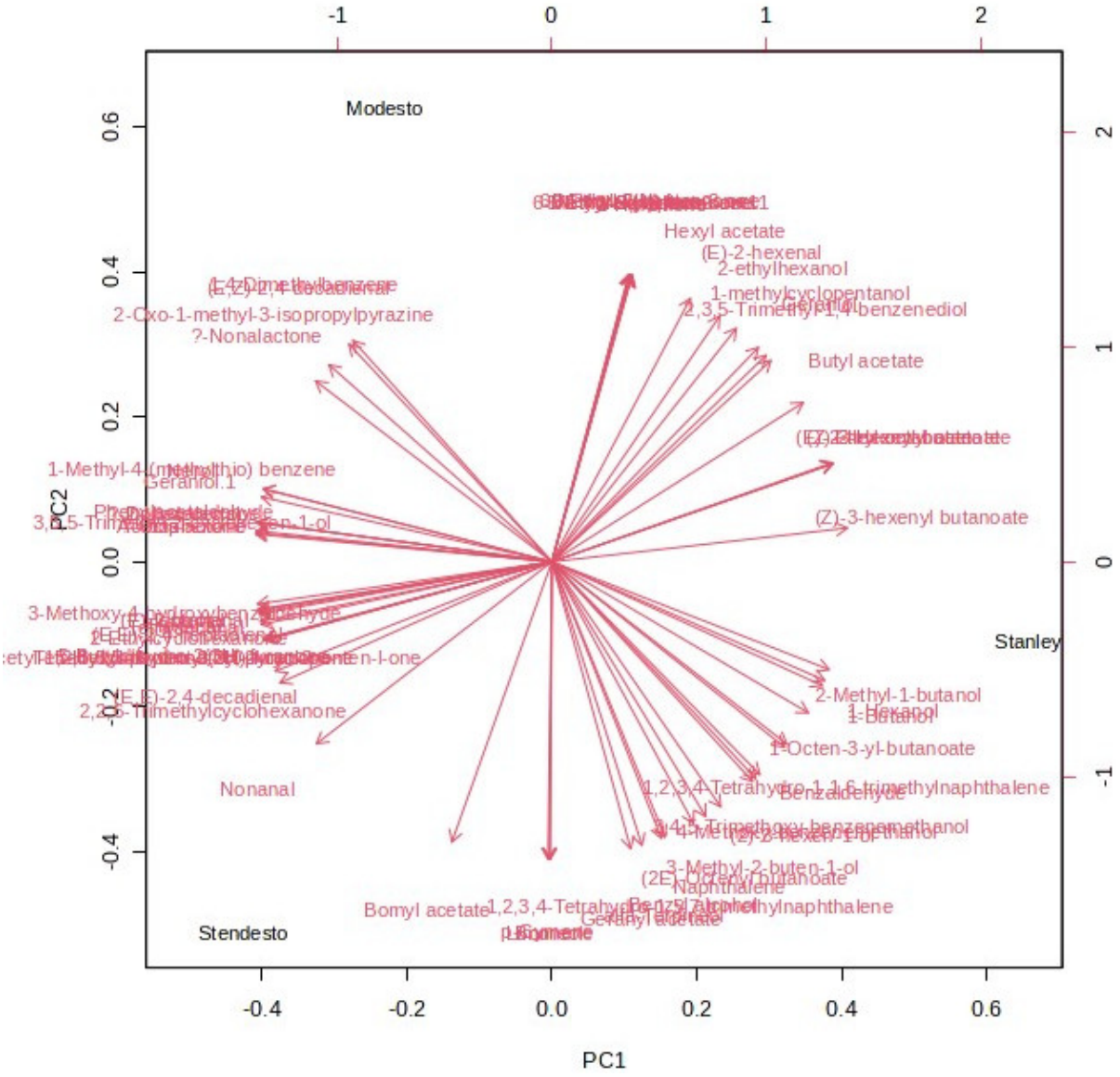
The chemical and volatile compositions were evaluated using principal component analysis (PCA) and further studied to observe separate groups using hierarchical cluster analysis (HCA). As shown in Figure 5A (metabolites), two principal components were generated in the PCA with an eigenvalue of greater than one, accounting for 74.6% (PC1) and 25.4% (PC2) of the total variance, whereas in Figure 5B (VOCs), the distribution for PC1 was 54.5% and for PC2—45.5%. Hexyl acetate, 2-ethyl hexanol, and (E)-2-hexenal are arranged most positively, whereas nonanal and 2.2,6-trimethylcyclohexanone contributed most negatively. Additionally, fructose and glucose isomers contributed most positively, compared with fumaric acid and galactose isomer contributing most negatively.

As illustrated in Figure 6, two clusters are formed for both metabolites and VOCs. Interestingly, the plum–apricot hybrid is more similar to the plum when metabolites are being evaluated and, respectively, to the apricot when volatile compounds are being assessed. The results from the PCA and HCA were useful for preliminarily distinguishing the samples. Literature states that plum–apricot hybrids can be either more similar to the plum and they are referred to as plumcots or to the apricots when apriums apply [39].



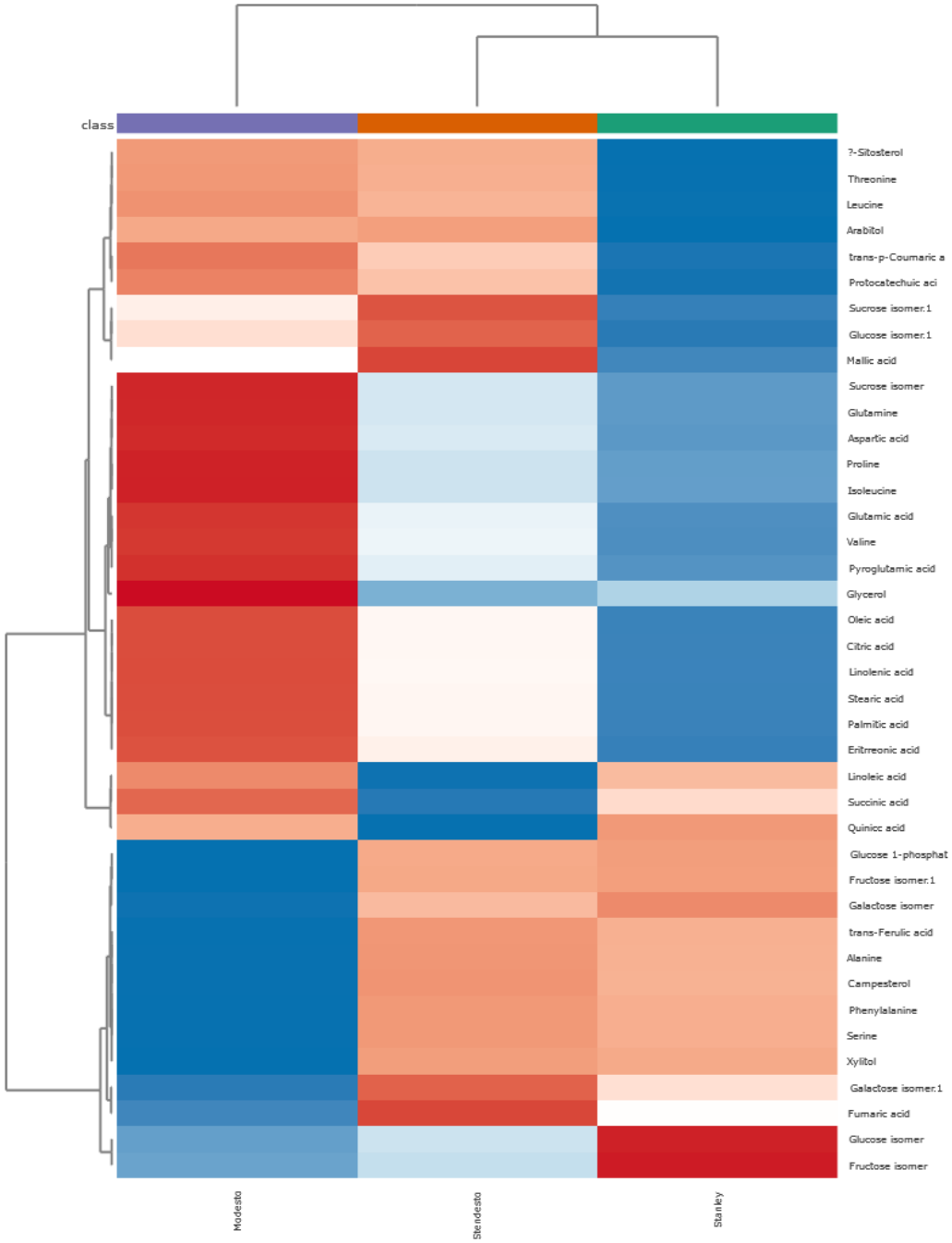
(**A**)

Figure 5. *Cont.*



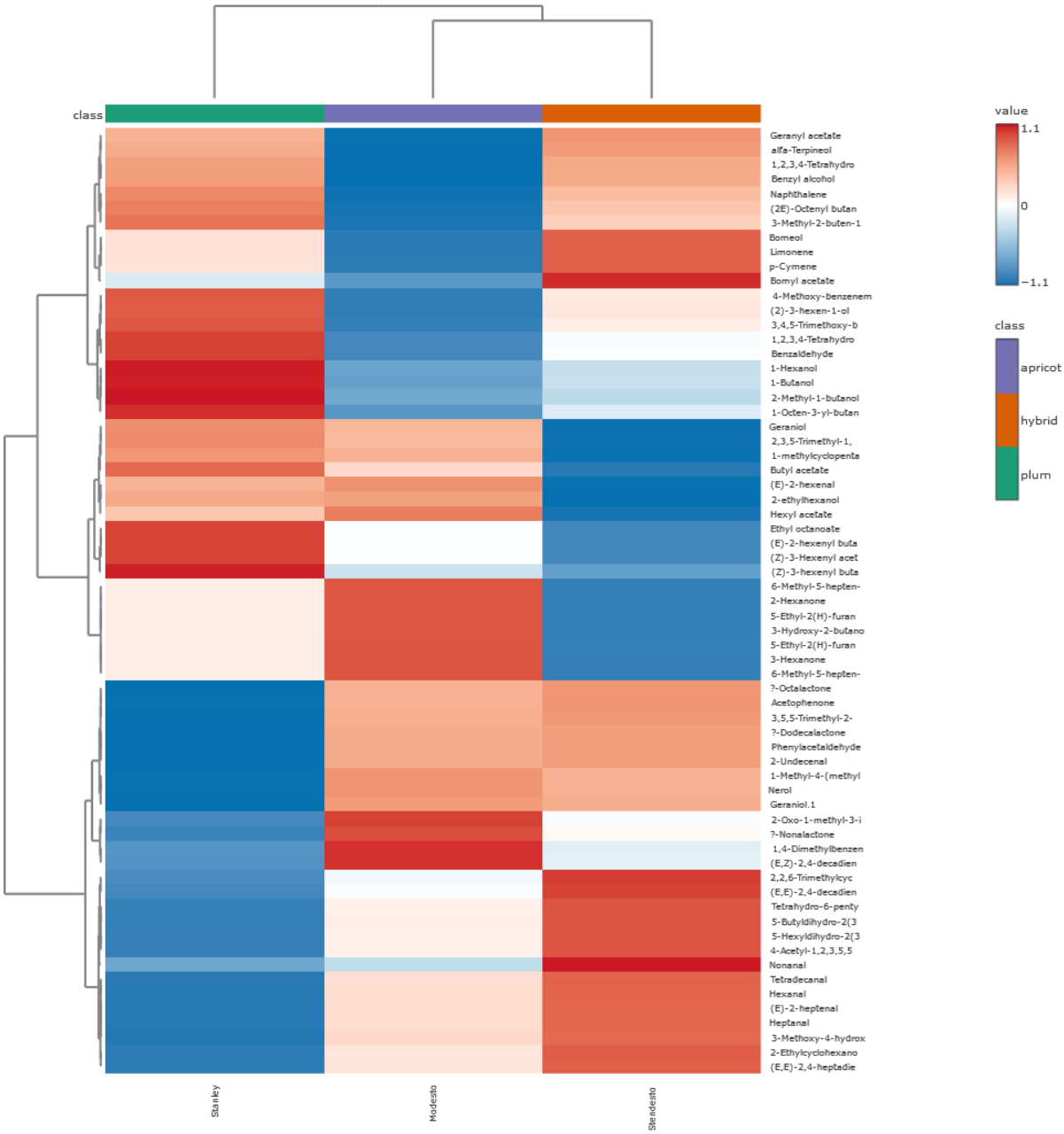
(**B**)

Figure 5. Principal component analysis of fruit samples. Eigenvector loading values of compounds: (**A**) primary metabolites and (**B**) VOCs.



(**A**)

Figure 6. *Cont.*



(**B**)

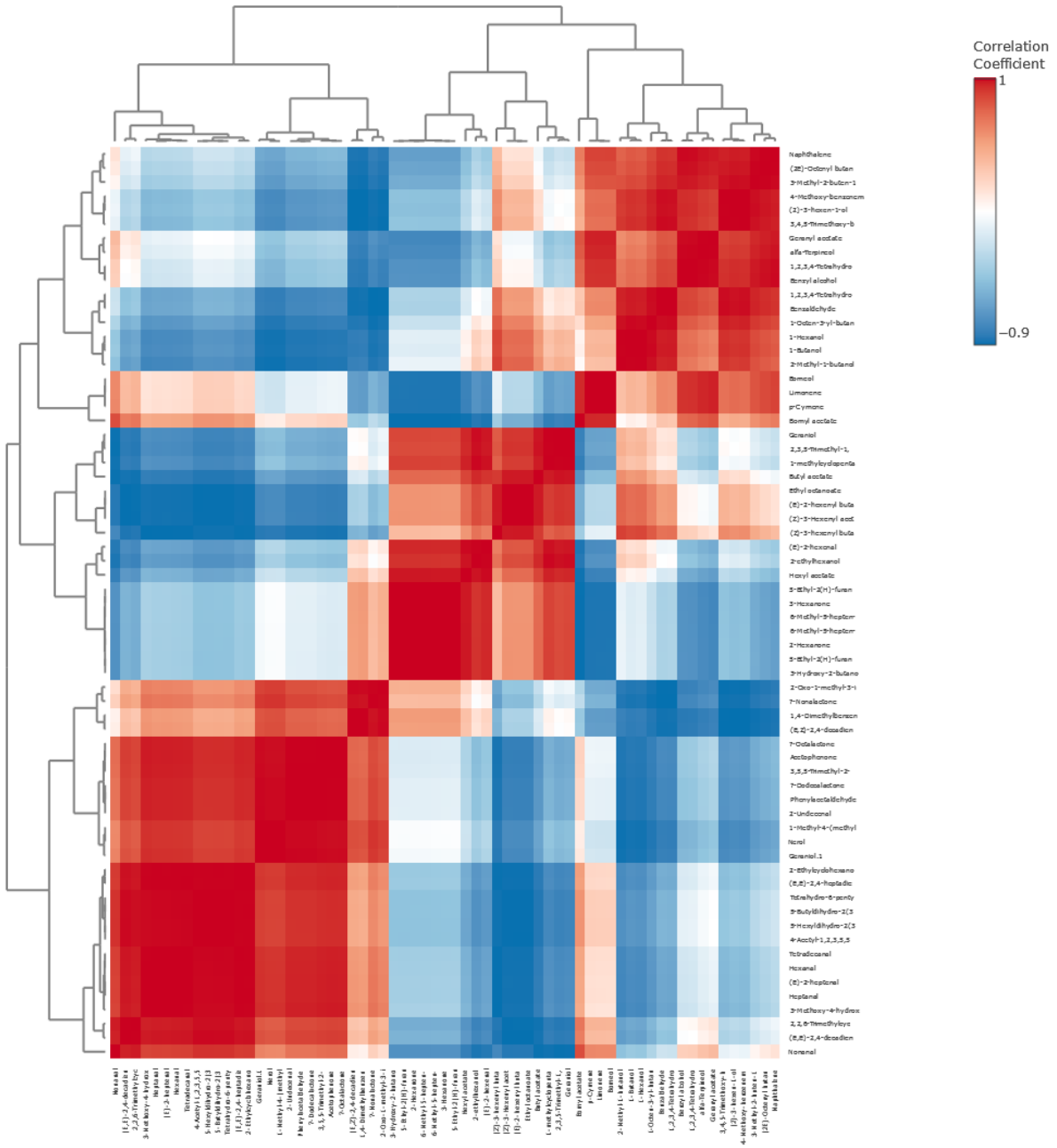
Figure 6. Clustering results of fruit samples shown as heatmap: (**A**) primary metabolites and (**B**) VOCs. The values were normalized by $\log_{10}$ transformation.

The estimated HCA placed the apricot as different from the other two concerning the available metabolites. However, when comparing the VOCs, the plum appeared to be different from the other two and was placed in a separate cluster.

A correlation analysis of the data is presented in Figure 7. A positive correlation has been established between 1-butanol and ten other structures, including 1-hexanol, 2-methyl-1-butanol, 1-octen-3-yl-butan, and benzaldehyde, among others. Additionally, benzyl alcohol was positively correlated with fifteen other structures (geranyl acetate, limonene, p-cymene, benzaldehyde, and others). Hexanal was positively correlated with nineteen compounds, while a positive correlation between (E)-2-hexenal and sixteen other components was established.

(**A**)

Figure 7. *Cont.*



(**B**)

Figure 7. Pearson's correlation heatmaps of the different compounds in studied fruits: (**A**) primary metabolites and (**B**) VOCs. The values were normalized by $\log_{10}$ transformation.

Nonanal was positively correlated with thirteen metabolites, including tetradecanal, hexanal, and heptanal, among others. Butyl acetate established a positive relationship with ten other metabolites. Aspartic acid had a positive correlation with thirteen other compounds (glutamine and sucrose isomer having the highest correlation values). Mallic acid had a positive correlation with sucrose and glucose isomers, along with another seventeen compounds. Palmitic acid was positively correlated with stearic acid, citric acid, and linolenic acid, among others. Campesterol was positively correlated with ten metabolites (alanine and trans-ferulic acid having the highest correlation values).

4. Conclusions

This is a core comprehensive evaluation of the subject on primary metabolites and VOCs of fruit samples from the "Modesto" (apricot), "Stanley" (plum), and "Stendesto" (plum–apricot) varieties. In total, forty-one metabolites were identified belonging to the following chemical groups: amino acids, organic acids, sugar acids and alcohols, mono- and di-saccharides, phenolic acids, fatty acids, and sterols. The most abundant were the mono- and di-saccharides and the sterols. The hybrid fruit had generally inherited all metabolites present in the parental ones with the exception of γ-aminobutyric acid. Sixty-five VOCs were identified from the three samples, with aldehydes being the most contributing for all evaluated fruits (plums, apricots, and plum–apricot hybrids). Considering the VOCs, the hybrid fruit had also managed to synthetize all identified compounds; this was not valid for the plum or for the apricot. Only a few benzene derivatives were identified in the "Modesto" apricot, and none of the three lactones was present in the "Stanley" plum.

The applied PCA placed the plum and plum–apricot fruits in the same group when the metabolites where being evaluated and the apricot and plum–apricot in the same group when VOCs were in question. The obtained results can successfully be used as a reference and stepping stone for future analyses.

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