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Volatiles Distinguishing the European 'Conference' and the Asian 'Yali' Pears Stored at Different Post-Harvest Temperatures

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Abstract: A total of 124 identical volatile aromatic compounds were identified during storage of the European 'Conference' and the Asian 'Yali' pear cultivars in different temperature conditions. Only 5 volatiles were statistically differentiated in both cultivars by means of successive multinomial logistic regression: 3-methylbutan-1-al, 2-methylpropyl acetate, 2-methoxy-4-vinylphenol, ethanol, and eugenol. Significant statistical data obtained by sequential multinomial logistic regression developed by the principal component analysis (PCA) procedure and distinguishing the different 'Conference' and 'Yali' pears storage regimes were dimensionless in themselves. The PCA components were expressed as linear combinations of selected variables necessary to distinguish the cultivars. The eigenvalues of the first three PCA components differentiated the storage regimes. For each principal components were selected volatiles with a probability higher than 0.4. Combinations created from PCA components were shown using clusters distinguishing the pear storage conditions used. Analytical data from SPME-GC/MS such as concentration (ng kg^{-1}) demonstrated multiple and order-of-magnitude differences between the 'Conference' and 'Yali' pears. The 'Yali' cultivar exhibited significantly higher concentrations of eugenol.

Keywords: European pear; Asian pear; storage; significant volatiles; eugenol

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1. Introduction

European pear cultivars are the third-most cultivated temperate climate fruit in the EU with an annual production of around 2.5 million tonnes, roughly 25% of which are the 'Conference' cultivar. This cultivar, like most other European pears, requires a cooling period after harvest to initiate autocatalytic ethylene production and thus make the fruit ripen [1,2].

China produces more Asian pears than any other country in the world, and the 'Yali' cultivar (*Pyrus bretschneideri* Rehder) is one of the main cultivars with an annual production of around 2.6 million tonnes [2,3]. The introduction of genetic resources and cultivars from regions with high genetic diversity can have a positive effect on fruit growing, especially in the context of a changing climate [4].

Late-maturing cultivars 'Conference' and 'Yali' belong to pears with higher storability under cold storage. The fruits of the two cultivars are easily distinguishable from each other by their shape and colour. The fruits of the 'Yali' cultivar are also pear-shaped, but they are more robust. The skin colour of the 'Conference' cultivar is completely brown, while 'Yali' pears are light yellow and tender and have a delicate aroma. Consumers most highly value pears that are tasty and have a penetrometer firmness ranging from 10 to 30 N. At this stage of softening, most European pear cultivars have a buttery consistency. In contrast, the fruits of the 'Yali' cultivar are juicy for a long time, but never acquire a buttery consistency, not even at the end of ripening [5–7].

The typical pear aroma is the result of a mixture of volatile compounds that are formed during different stages of fruit growth and depend on many genetic, environmental and

post-harvest factors. Aroma plays an important role in fruit quality and by examining various compositions of volatile compounds it is possible to distinguish between fruit species and cultivars. Different methods of post-harvest storage of pear fruits have different effects on their sensory and chemical characteristics, which can affect their sensory quality [8].

In addition to pear texture, aroma is a key component of pear flavour [9,10]. They identified important compounds such as alcohols and esters in particular. Just esters of short- to medium-chain alcohols, in particular ethyl and methyl esters, have been identified as the most important volatile compounds in pears [10]. Among the esters in pears of the 'Bartlett' cultivar, ethyl (2E,4Z)-deca-2,4-dienoate was identified as the 'character impact compound' [9,11], while hexyl acetate was identified as a 'contributory flavour compound' [12,13]. Similarly, Kahle et al. [14] identified hexyl acetate and decadienoate esters as characteristic compounds of European pears. In addition, hexanal, 2-methylpropyl acetate, ethyl acetate, 3-methylbutyl-2-methylbutanoate, ethylbutanoate, and butanol have also been identified in 'Conference' pears [15], the concentrations of which were largely influenced by fruit ripeness at harvest time and by post-harvest storage conditions.

Twenty-three batches of Asian 'Korla' pears collected from different areas in Xinjiang, China, were analyzed using GC-MS. The results of the analysis were combined with olfactometry to identify 26 common odour-active compounds as the characteristics of the 'Korla' pear flavour fingerprint [16]. The volatile profile of pear juice from the *Pyrus pyrifolia* cultivar was determined using GC-MS (Gas Chromatography-Mass Spectrometry) combined with solid phase microextraction (SPME). A total of twenty-eight volatile components consisting of esters, aldehydes, terpenes, ketones, benzene, and furan were identified in four juice samples. Esters were always the dominant component, accounting for more than 60% of total volatile compounds [17]. Esters also dominated qualitatively and quantitatively in the fruits of the 'Abate Fetel' cultivar (*Pyrus communis* L.), accounting for more than 90% of total volatiles. The main esters of the aromatic complex were acetates, represented mainly by butyl acetate (44–50%) and hexyl acetate (27–32%) [18].

Low temperature storage is a fundamental method for delaying the ripening of pears and extending their shelf life [19]. The lower storage temperature of pears suppressed the development of all volatiles compared to 1 °C [20]. In most European pears, low temperature conditioning plays a crucial role in the stimulation of ethylene biosynthesis during ripening at room temperature and the formation of pear aroma [21]. Low temperature conditioning (0 ± 0.5 °C) was an effective method for preventing loss of aroma-related esters from refrigerated 'Nanguo' pears during ripening in shelf-life conditions [22].

The aim of our study was to compare the effect of fruit storage at different post-harvest temperatures on the formation of volatile substances in European and Asian pear cultivars.

2. Materials and Methods

2.1. Plant Materials

The European 'Conference' and the Asian 'Yali' cultivar came from one single row of trees that were grown in the research plot of the Mendel University located in Lednice, Czech Republic. Each cultivar was harvested in the amount of 50 kg at the same stage of ripeness, which physiologically corresponded to just a few days before the onset of the climacteric development stage. Healthy fruits were then immediately selected based on skin colour, uniformity, and size. The fruit was harvested and removed from the experimental orchard with minimal delay and transported to the post-harvest laboratory in approximately 2 h. The pears of the two cultivars were sorted according to uniformity of colour development and defective pieces were excluded. During the post-harvest storage, the fruits were exposed to three different temperatures: 20 °C (samples SL) for 14, 22, and 30 days, 5 °C (samples LT5) for 25, 55, and 70 days, and 1 °C (samples LT1) for 25, 55, and 70 days. Pears were sampled for the determination of volatiles at the beginning of storage (IN samples) and specified days of storage: 14, 22, 30 and 25, 55, 70. Fruits stored at 1 °C and 5 °C were additionally exposed to shelf-life conditions at 20 °C for 10 days (samples SL1, respective SL5) and then also followed the determination of volatiles. The

initial matrix consisted of the average values of the concentration of volatile compounds obtained from three fruits from each collected sample (n = 3).

2.2. Determination of Volatile Compounds in Pear Fruits

The SPME method was used to determine the volatile compounds. Fresh pear cultivars were homogenized and stored at −27 °C until the analyses were performed. A total of 2 g of the homogenate was quickly transferred to a 4 mL vial and the sample was kept at 50 °C for 30 min. Manual SPME was performed using a fibre coated with a 85 µm layer of poly-dimethylsiloxane (PDMS) purchased from Supelco (Supelco, PA, USA), which was used to absorb the volatile components from the fruit sample. First, the SPME extraction fibre was conditioned in the GC-MS injection space at 250 °C for 5 min, then it was pierced by the septum of the vial in the SPME absorbent device and exposed in the vial gas space for 30 min at 50 °C to the volatile compounds, which were already in the equilibration phase at the time. The needle was then removed from the sample vial. Finally, the needle was manually inserted into a gas chromatograph injector in which the analytes were thermally desorbed and analysed in a capillary column. The desorption time was 5 min at 250 °C. GC-MS measurements were performed on a gas chromatograph (Agilent Technologies 7890A, Inc., Santa Clara, CA, USA) connected to a quadrupole mass spectrometer (Agilent GC MSD 597) and using the NIST 98 library containing mass spectra. The analytes were separated in a 30 m × 0.25 mm DW WAX capillary column with a phase thickness of 0.25 µm. The capillary column was made by J&W Scientific (Agilent technologies Inc., Santa Clara, CA, USA). The capillary column was inserted directly into the MS ion source. The compounds were preliminarily identified by searching the NIST Mass Spectral Search Program (Version 2.2, 2014 distributed by the Standard Reference Data Program of the National Institute of Standards and Technology (Gaitherburg, MD, USA), and the identity of these compounds was confirmed by comparing their mass spectra and retention times with those obtained for the standard. All standards were purchased from Sigma-Aldrich and Fluka (Darmstadt, Germany). Diluted standards were separately injected into the gas chromatograph as native samples.

2.3. Statistical Analysis

All statistical analyses were performed using a SAS statistical software package—version 9.2. (SAS Institute Inc., Cary, NC, USA). For each pear cultivar and each volatile compound identified, the mean ± standard deviation was calculated for each sampling date. Statistically significant differences were evaluated by means of ANOVA and Tukey test. A stepwise log regression model was used to explore the effect of all volatiles. Principal component analysis (PCA) was used to distinguish the two cultivars, which were always sampled on the same dates. The first three principal compounds were selected as linear combinations of the observed variables, which were chosen so that the resulting compounds represented the maximum amount of variation in the set of volatile variables. Before proceeding with the statistical analysis, the data matrix was standardized by setting mean values at zero.

3. Results and Discussion

3.1. Aromatic Compounds Identified in Pears Stored in Different Regimes

A total of 124 identical volatile compounds were identified in both studied pear cultivars: 25 alcohols, 16 aldehydes, 47 esters, 4 lactones, 17 terpenes, 10 ketones, 2 organic acids, and 3 hydrocarbons. Name of volatiles and retention indexes according the NIST Mass Spectral Search Program Version 2.2 are listed in the Table 1. Some volatile compounds were found in high concentrations in both varieties without statistically significant differences. The lowest contents were found for just a few compounds. At the mean storage time at 20 °C, both butanoates (ethyl 2-ethyl-3-hydroxybutanoate and hexyl 2-methylbutanoate) were at very low concentrations in both cultivars (on the order of a few ng kg^{-1}). In contrast, 3-methylbutan-1-ol, n-butanol, hexanal, acetaldehyde, and ethanol were present in very high concentrations in both cultivars (on the order of more than 100,000 ng kg^{-1}).

Esters of short- to medium-chain alcohols and their corresponding acids with or without a degree of unsaturation were always present as the primary group of compounds in both cultivars. Straight chain alcohols with 2 to 8 carbons were present as the second largest group in the profile [10]. They state in their study that esters accounted for 60 to 98% of the total pear volatiles. Chen et al. [6] evaluated the aroma composition of ripe fruits from 12 cultivars of European pears. They identified a total of 335 volatiles, mainly esters, alcohols, alkanes, acids, ketones, terpenes and aldehydes. The concentration of total aroma was highest in cultivar 'Alexandrine Douillard' (18.73 μg g^{-1}), while the lowest total concentration was found in cultivar 'Bartlett-Max Red' (0.33 μg g^{-1}). Wang et al. [23] identified a total of 241 volatile compounds in five pear cultivars of European and Asian origin, with the predominant volatile compounds being esters (101 compounds), followed by alcohols (20 compounds) and aldehydes (28 compounds). This was the first time when volatile compounds such as sesquirosefuran and anethole were identified in pears.

Table 1. Determined volatile compounds in 'Conference' and 'Yali' pear cultivars.

Volatile Compound	RI	Volatile Compound	RI	Volatile Compound	RI
Alcohols		*Aldehydes*		*Terpenoids*	
ethanol	443	acetaldehyde	381	alpha-pinene	931
2-propanol	495	3-methylbutan-1-al	628	cymene	1011
1-propanol	524	2-methylbutan-1-al	632	limonene	1020
1-butanol	660	hexanal	769	linalool	1081
3-methyl-1-butanol	706	furfural	794	alpha-cyclocitral	1096
(2Z)-penten-1-ol	743	(E)-2-hexenal	822	thujone	1123
2-methyl-2-buten-1-ol	746	heptanal	882	(-)-terpinen-4-ol	1160
4-methyl-2-pentanol	760	5-methylfurfural	924	(R)-(+)-alpha-citronellol	1211
3-methyl-2-buten-1-ol	762	benzaldehyde	927	(Z)-citral	1214
2-furanmethanol	819	(2E)-octenal	1034	nerol	1215
2-methyl-1-pentanol	822	benzeneacetaldehyde	1048	(E)-geraniol	1232
3-methyl-1-pentanol	826	nonanal	1081	alpha-ionene	1255
(2Z)-hexen-1-ol	827	(2E,6Z)-nonadienal	1125	eugenol	1337
1-hexanol	852	(E)-2-nonenal	1133	damascenone	1361
2-heptanol	877	decanal	1183	(D)-nerolidol	1522
phenol	955	hydrxoymethylfurfural	1208	(Z)-nerolidol	1545
2-ethyl-1-hexanol	1010			(-)-menthol	1596
(3Z)-octen-1-ol	1041				
2-ethyl-2-hexen-1-ol	1051	*Hydrocarbons*		*Lactones*	
1-octanol	1054	1-methyl-1-cyclopentene	641	gama-octanolactone	1220
(3E)-octen-1-ol	1066	2-pentylfuran	977	gama-nonanolactone	1325
phenethyl alcohol	1082	(Z)-alpha-farnesene	1499	gama-decanolactone	1414
(2E)-decenol	1254			alpha-dodecalactone	1670
2-methoxy-4-vinylphenol	1272	*Organic acids*			
farnesyl alcohol	1658	hexoic acid	973		
		2-ethylhexoic acid	1965		
Esters		*Esters*		*Ketones*	
methyl acetate	517	methyl octanoate	1108	diacetyl	558
ethyl acetate	577	ethyl benzoate	1141	2,3-hexanedione	757
methyl propionate	614	benzyl acetate	1142	2-heptanone	871
2-methylpropyl acetate	767	diethyl succinate	1149	3-methyl-2,4-pentanedione	897
butyl acetate	805	methyl (2E)-octenoate	1164	6-methyl-5-heptene-2-one	958
ethyl 2-butenoate	820	butyl hexanoate	1165	2-methyl-2-heptene-6-one	960

Table 1. *Cont.*

Volatile Compound	RI	Volatile Compound	RI	Volatile Compound	RI
ethyl 2-methylbutanoate	829	hexyl butanoate	1171	3-octanone	963
amyl acetate	859	ethyl octanoate	1175	2-octanone	964
methyl hexanoate	903	methyl 2-hydroxybenzoate	1176	acetophenone	1049
ethyl 3E-hexenoate	981	octyl acetate	1185	1-decen-3-one	1485
ethyl 3Z-hexenoate	986	hexyl 2-methylbutyrate	1223		
(3Z)-3-hexenyl acetate	987	2-phenethyl acetate	1224		
hexyl acetate	990	ethyl (2E)-octenoate	1275		
methyl (2E,4E)-hexadienoate	998	ethyl (4E)-decenoate	1291		
methyl heptanoate	1005	geranyl acetate	1360		
butyl 2-methylbutyrate	1013	ethyl decanoate	1367		
(E,E)-ethyl 2,4-hexadienoate	1071	hexyl hexanoate	1386		
amyl butyrate	1079	methyl (E,Z)-2,4-decadienoate	1388		
ethyl (2S)-ethyl-(3S)-hydroxybutyrate	1084	ethyl (2E)-decenoate	1389		
heptyl acetate	1085	ethyl (E,Z)-2,4-decadienoate	1446		
hexyl propionate	1089	ethyl undecanoate	1483		
2-methylbutyl 2-methylbutanoate	1090	diethyl phthalate	1543		
3-methylbutyl 3-methylbutanoate	1094	ethyl hexadecanoate	1968		
		ethyl linolenate	2166		

RI = retention index (NIST Mass Spectral Search Program, Version 2.2).

Volatile esters were the main volatiles produced during the ripening of 'Nanguoli' pears (*Pyrus ussuriensis* Maxim), in particular ethyl butanoate, ethyl hexanoate, and hexyl acetate esters, followed by aldehydes and terpenes, the main components of which were hexanal, E-2-hexenal, and alpha-farnesene. The ripening and post-harvest storage stages affected the production and relative composition of esters and terpenes [24]. Yi et al. [25] conducted a volatile profiling of two Asian pear genotypes. Of the many volatiles identified, ethyl 2-methylbutyrate and ethyl hexanoate were the most potent and pleasant odorants in intact fruit and fruit pulp.

3.2. Statistically Significant Volatile Substances Distinguishing the Cultivars and Post-Harvest Storage Regimes

Subsequent application of multinomial logistic regression determined just 5 of the original 124 volatile compounds that were common to both cultivars during the temperature changes over the 80 days post-harvest period. At one degree of freedom (DF 1), these selected volatile compounds achieved a high Chi-Square value and were all significant ($p < 0.0001$) (Table 2). Compounds such as 3-methylbutan-1-al, 2-methylpropyl acetate, 2-methoxy-4-vinylphenol, ethanol, and eugenol were monitored over time. The volatile substances detected by the logistic regression differed significantly depending on the cultivar and post-harvest maturation under the fluctuating temperature conditions over the 80 days. Eugenol showed the largest differences in production between the two cultivars. The PCA components were expressed as linear combinations of selected variables to distinguish cultivars. Table 3 shows the variables with greater contribution to the PCA components (compounds with contribution with absolute value of >0.4 are highlighted). In 'Conference' pears, the first principal component (Comp1) accounted for 43.2% of the variance and showed a high correlation with 3-methylbutan-1-al, 2-methylpropyl acetate and ethanol. 2-methylpropyl acetate and 2-methoxy-4-vinylphenol were decisive for the first principal component of the 'Yali' cultivar, which accounted for 43.5% of the variance.

The cumulative sum of the three calculated components generated eigenvalues of 91.7% and 89.3% as variables for the 'Conference' and 'Yali' cultivars respectively.

Table 2. Significant volatile substances distinguishing the different storage regimes of pear cultivars 'Conference' and 'Yali'—selected by stepwise multinomial logistic regression.

Variable	D.F.	Chi-Square	Pr > ChiSq
3-Methylbutan-1-al	1	30.8592	<0.0001
2-Methylpropyl acetate	1	26.9671	<0.0001
2-Methoxy-4-vinylphenol	1	16.0979	<0.0001
Ethanol	1	16.9146	<0.0001
Eugenol *	1	53.6200	<0.0001

D.F. = degree of freedom; ChiSq = Chi-Square; * the component showing the highest differences in production between the two cultivars.

Table 3. Eigenvalues of the first three principal components and their cumulative value distinguishing the different storage regimes of pear cultivars 'Conference' and 'Yali'.

Cultivar	'Conference'			'Yali'		
Variable	Comp1	Comp2	Comp3	Comp1	Comp2	Comp3
3-Methylbutan-1-al	−0.6609	−0.0049	0.4368	−0.3678	0.5979	0.7059
2-Methylpropyl acetate	0.5287	−0.2910	0.7974	0.6046	0.3706	−0.0923
2-Methoxy-4-vinylphenol	−0.1735	0.7720	0.3965	0.6083	0.3564	0.1088
Ethanol	0.5036	0.5651	−0.1273	0.3593	−0.6149	0.6938
Comp. cumulative (%)		91.7			89.3	

Comp = Component.

Qin et al. [11] also divided pear cultivars into several groups based on the PCA of 108 volatile compounds. The first group of cultivars was characterized by a high ester concentration and low hydrocarbon content. In another group, cultivars with a high concentration of hydrocarbons and low concentration of esters were identified, and another one contained cultivars with a high concentration of aldehydes. Chen et al. [5] determined by GC-olfactometry that the volatile compounds identified by SPME-GC/MS were responsible for the aroma of 'Yali' pears. The dominant components were ethyl butanoate, ethyl hexanoate, alpha-farnesene, hexanal, ethyl acetate, hexyl acetate, and ethanol. Taiti et al. [26] evaluated ripe 'Williams' pears and two Asian cultivars ('Hosui' and 'Yali') ready for direct consumption by analysing their volatile compounds. The greatest intensity of the signals of certain compounds that create the typical pear scent were found in the 'Yali' pears, followed by 'Williams' and then 'Hosui'. The 'Williams' pears, however, differed from the Asian cultivars by having the highest intensity of the signals of terpenes, sesquiterpenes and butanal.

3.3. Concentration Changes of Significant Volatiles under Various Storage Conditions

Pears could be distinguished at the beginning of storage according to orders of magnitude lower concentrations of eugenol (172 ± 13 ng kg^{-1}) in the 'Conference' cultivar compared to cultivar 'Yali' (6571 ± 740 ng kg^{-1}). In contrast, the input ethanol content (4,236,735 ± 868,662 ng kg^{-1}) and 2-methoxy-4-vinylphenol (2222 ± 308 ng kg^{-1}) was statistically significantly higher in 'Conference' pears (Table 4). The documented higher concentrations of ethanol were the result of a rapid change in fruit temperature that stimulated anaerobic changes in the fruit, which can be an indicator of anaerobic processes that are a source of reduced storage capacity and that occur during common technological procedures that take place in cold storage. Similar differences in ethanol production were observed by Zlatić et al. [20] during storage of pears at temperatures of −1 °C and +1 °C and after exposed to shelf-life conditions.

Table 4. Initial contents of significant volatiles (ng kg^{-1}) distinguishing the storage regimes of pear cultivars 'Conference' and 'Yali' at 20 °C.

Variable	'Conference'		'Yali'	
	Mean	±S.D. *	Mean	±S.D. *
3-Methylbutan-1-al	553,025 [a]	25,109	507,430 [a]	33,282
2-Methylpropyl acetate	57 [b]	10	117 [a]	18
2-Methoxy-4-vinylphenol	2222 [a]	308	1344 [b]	181
Ethanol	4,236,735 [a]	868,662	876,435 [b]	75,366
Eugenol	172 [b]	13	6571 [a]	740

Different letters in columns indicate statistically significant differences (Tukey, $p < 0.05$). * Standard deviation (n = 3).

Increase of ethanol content was always associated with storage temperature 20 °C or the transfer of the fruit to the shelf-life at 20 °C (Figure 1). These ethanol changes for both cultivars were identical, but differed in nominal value, which was always lower for 'Yali' pears. Figure 2 shows the compound eugenol as a derivative of guaiacol with an allyl chain on the benzene ring, which was found in cultivar 'Yali' at a concentration at least ten times higher than in 'Conference' pears, in which only tens to hundreds of ng kg^{-1} were formed. This finding was confirmed in all compared storage regimes in our study. Differences in eugenol may be related to the higher production of phenylpropanoids, which is produced in plants in response to biotic and abiotic environmental factors [27].

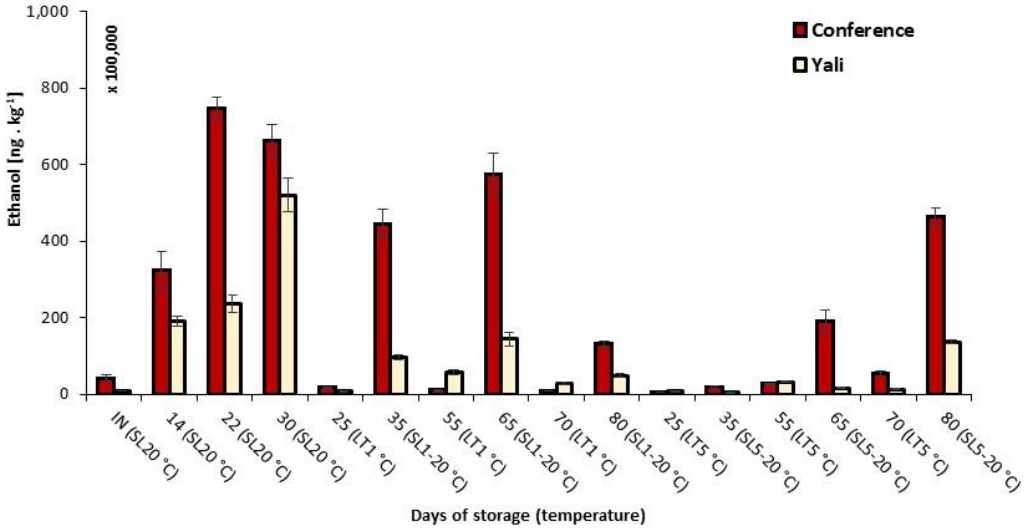

Figure 1. Concentration changes of ethanol in 'Conference' and 'Yali' pear cultivars depending on time and storage regimes. (SL20 °C—shelf-life at 20 °C; LT—low storage at 1 or 5 °C; SL1-20 °C, SL5-20 °C—shelf-life at 20 °C after storage at 1 or 5 °C).

Figures 3 and 4 show clear similarities as well as differences in the production of 3-methylbutan-1-al and 2-methylpropyl acetate as a result of the fruit's reaction to temperature changes, which express detailed characteristics of the cultivar. At the beginning of storage, a high concentration of 3-methylbutan-1-al was evident in both cultivars and was clearly maintained in particular in cultivar 'Conference' stored at 1 °C. The production of 2-methylpropyl acetate as in the case of ethanol has always been greatly suppressed at both pear cultivars by the lower storage temperature (1 °C or 5 °C). After the fruit was removed from low storage temperatures to the shell-life conditions, the cultivar 'Yali' produced more of this volatile substance than the 'Conference' pears. It can be seen from Figure 5 that the development of 2-methoxy-4-vinylphenol of both cultivars was almost identical during 30 days of storage at 20 °C, but content always increased after transferring of 'Yali' pears from 1 °C to a temperature of 20 °C.

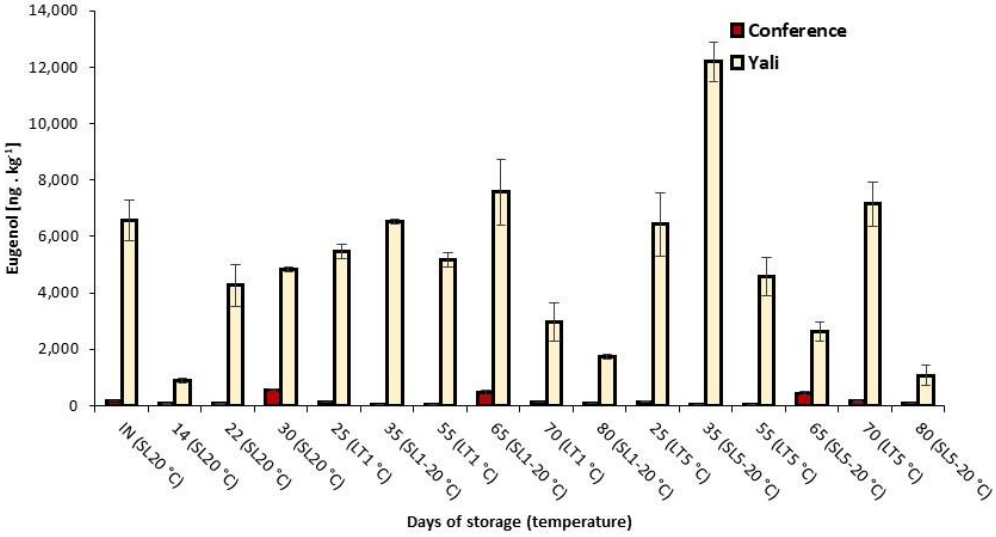

Figure 2. Concentration changes of eugenol in 'Conference' and 'Yali' pear cultivars depending on time and storage regimes. (SL20 °C—shelf-life at 20 °C; LT—low storage at 1 or 5 °C; SL1-20 °C, SL5-20 °C—shelf-life at 20 °C after storage at 1 or 5 °C).

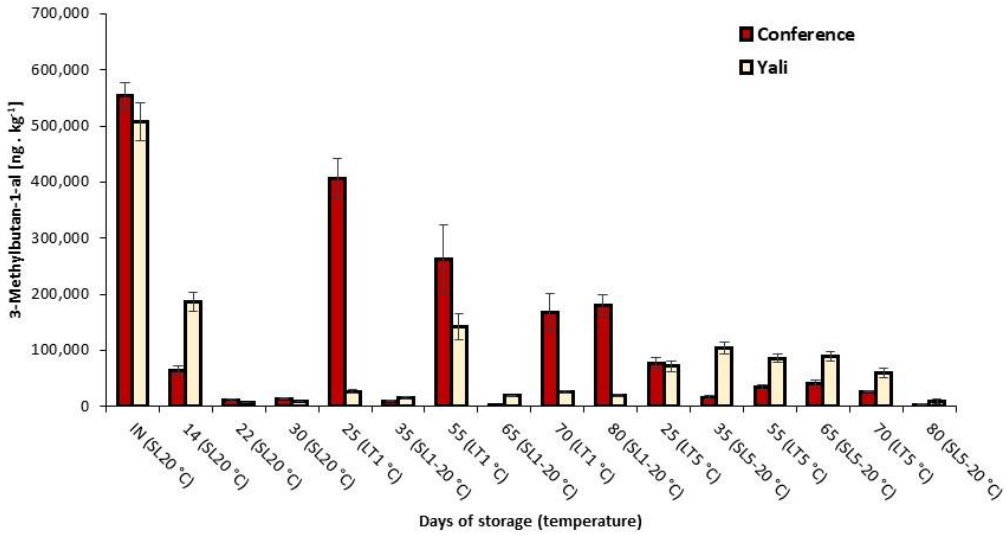

Figure 3. Concentration changes of 3-methylbutan-1-al in 'Conference' and 'Yali' pear cultivars depending on time and storage regimes. (SL20 °C—shelf-life at 20 °C; LT—low storage at 1 or 5 °C; SL1-20 °C, SL5-20 °C—shelf-life at 20 °C after storage at 1 or 5 °C).

A study by Liang et al. [28] additional also investigated the effects of slow and rapid cooling methods on the post-harvest physiology of 'Yali' pears during storage. Their results showed that the slow cooling treatment delayed the occurrence of the peak values of respiration rate, ethylene production, and the activity of other volatile components. Terregrosa et al. [7] observed that the fruits of cultivar 'Conference', after being placed in shelf-life conditions, generally released volatile substances of the ester type, in particular butyl acetate and ethyl (2E,4Z)-deca-2,4-dienoate.

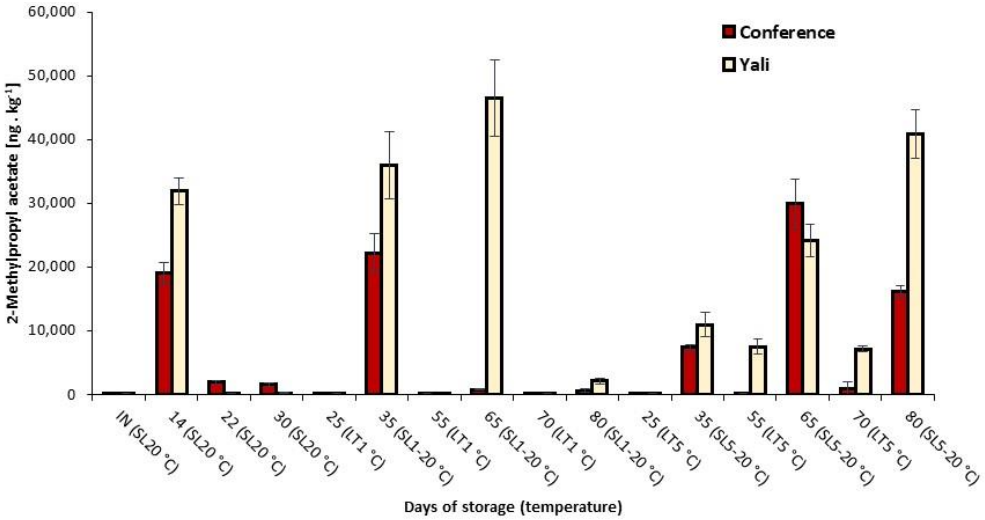

Figure 4. Concentration changes of 2-methylpropyl acetate in 'Conference' and 'Yali' pear cultivars depending on time and storage regimes. (SL20 °C—shelf-life at 20 °C; LT—low storage at 1 or 5 °C; SL1-20 °C, SL5-20 °C—shelf-life at 20 °C after storage at 1 or 5 °C).

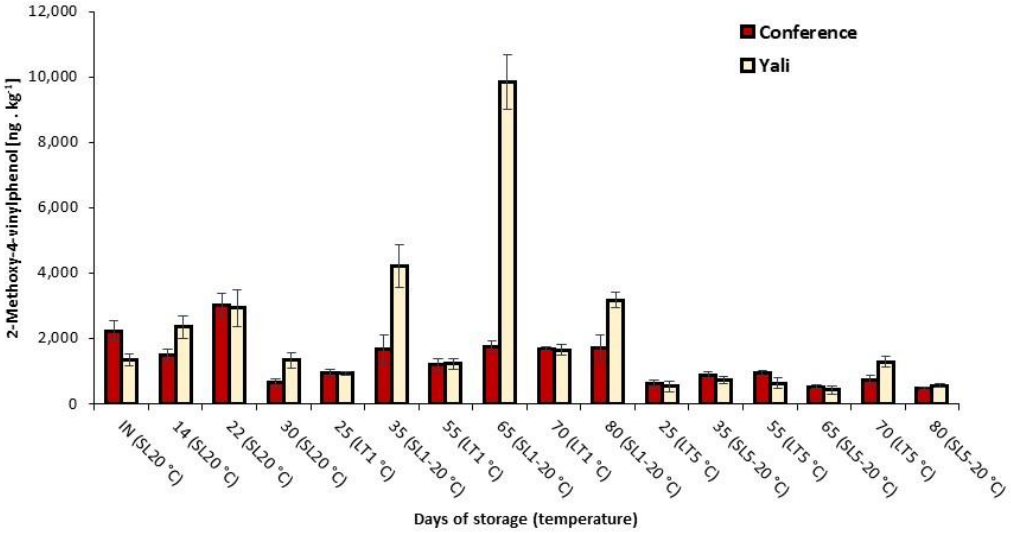

Figure 5. Concentration changes of 2-methoxy-4-vinylphenol in 'Conference' and 'Yali' pear cultivars depending on time and storage regimes. (SL20 °C—shelf-life at 20 °C; LT—low storage at 1 or 5 °C; SL1-20 °C, SL5-20 °C—shelf-life at 20 °C after storage at 1 or 5 °C).

3.4. Graphic Expression of Principal Components Distinguishing the Different Storage Regimes

Figures 6 and 7 highlight the combinations of the main PC1 and PC2 components and the clusters formed by them that differentiate the fruits of the cultivars studied. When calculating the eigenvalues for PC1 and PC2, the proportion of variance accounted for by the first two components was 75.7% for the 'Conference' cultivar and 70.6% for the 'Yali' cultivar. This usually made it possible to show most often 6 clusters for each cultivar separately. Their spatial arrangement arose from pre-programmed temperatures that were always the same for the storage of the two pear cultivars. Clusters for the 'Conference' cultivar and the 'Yali' cultivars at the beginning of storage, designated as K_IN and Y_IN, respectively, were clearly differentiated from other storage conditions. Similar clear separation was observed for 'Conference' pears stored for 14 and 22 days at 20 °C (K14_SL, K22_SL) and also after being removed from 1 °C and placed in shelf-life conditions (K35_SL1). In the 'Yali' cultivar, there was significant differentiation between fruit stored for 14 days at 20 °C (Y14_SL) and fruit removed from 1 °C and placed in shelf-life conditions (Y35_SL1 and Y65_SL1). In contrast, high frequencies in the clusters corresponded to the frequency

metabolism of the production of volatile compounds of the fruit, which could not keep up with the changes in the production of volatile compounds. Therefore, there is at least one multiple cluster in each cluster which does not distinguish the temperature change of shelf life. Similarly, Zlatić et al. [20] observed that long-term cold storage of pears can significantly modify the formation of volatile pear compounds. Hendges et al. [29] reported that storage of 'Conference' pears under ULO conditions reduced the production of ethyl (2E,4Z)-deca-2,4-dienoate regardless of the maturity stage of the fruit at harvest. In contrast, pentyl and hexyl acetates were only reduced in fruits of higher harvest maturity compared to fruits stored under refrigerated conditions.

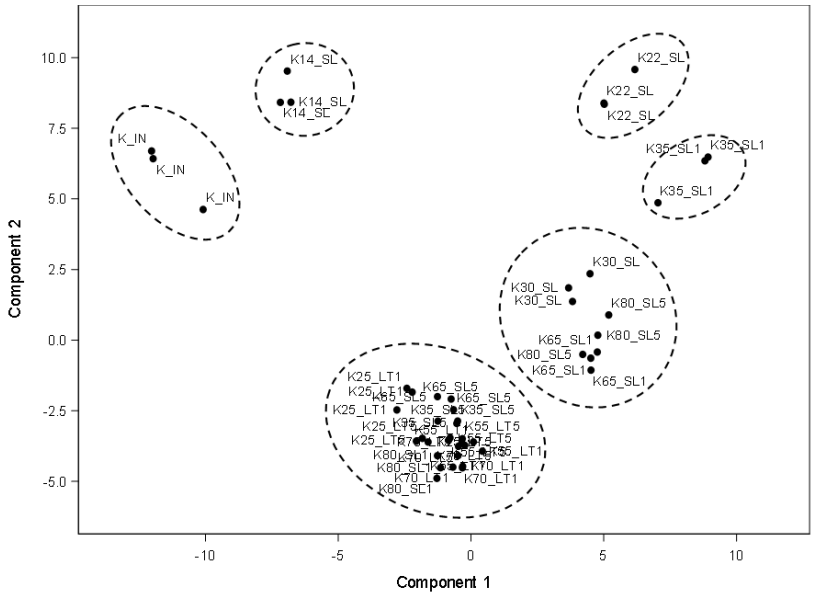

Figure 6. Score plot of PC1 and PC2 for cultivar 'Conference' ripening in different storage regimes. (K-'Conference'; IN-storage start; 14, 22, 25, 30, 35, 55, 65, 70, 80-storage days; LT1, LT5-low storage at 1 or 5 °C; SL-shelf life at 20 °C; SL1, SL5-shelf life at 20 °C after storage at 1 or 5 °C).

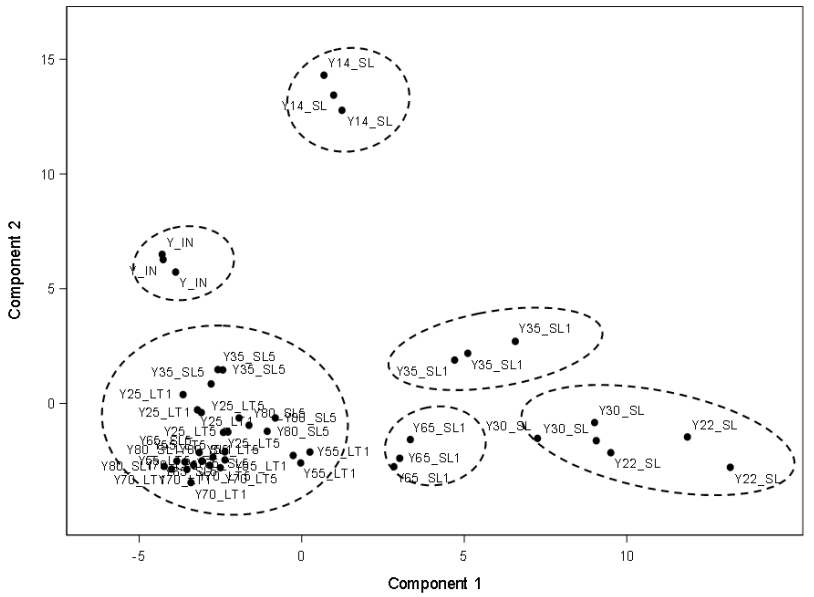

Figure 7. Score plot of PC1 and PC2 for cultivar 'Yali' ripening in different storage regimes. (Y-'Yali'; IN-storage start; 14, 22, 25, 30, 35, 55, 65, 70, 80-storage days; LT1, LT5-low storage at 1 or 5 °C; SL-shelf life at 20 °C; SL1, SL5-shelf life at 20 °C after storage at 1 or 5 °C).

4. Conclusions

In two pear cultivars, one European 'Conference' and the other Asian 'Yali', the temperature of post-harvest storage was programmatically changed over 80 days. A total of 124 volatile compounds were identified by analysis: 25 alcohols, 16 aldehydes, 47 esters, 4 lactones, 17 terpenoids, 10 ketones, 2 organic acids, and 3 hydrocarbons. The given compounds were identified in both cultivars, although they were found in them different concentrations. From the logistic regression output, only 5 compounds (3-methylbutan-1-al, 2-methylpropyl acetate, 2-methoxy-4-vinylphenol, ethanol, eugenol) had the most significant variability caused by differing storage-temperature regimes. The volatile substances detected by logistic regression differed significantly depending on the cultivar and post-harvest conditions. Statistically significant volatiles selected by the PCA and confirmed by the high Chi Square reached multiple and order-of-magnitude concentration differences between the 'Conference' and 'Yali' pears. Eugenol shows significantly higher content in the 'Yali' cultivar. The PCA components were expressed as linear combinations of selected variables necessary to distinguish the cultivars. The eigenvalues of the first three PCA components differentiated the storage regimes. The spatial arrangement of PC1 and PC2 is processed for each cultivar separately, but for each of them a division into 6 clusters is achieved. Clusters for the 'Conference' cultivar and the 'Yali' cultivar at the beginning of storage were clearly differentiated from other storage conditions. The period of SL—shelf life was included in the system as a time period of the fruit transition from the previous temperature. The reactions of the cultivars were quite clear, depending on the compounds tested and their concentration.

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