Research Article

The Pear Aroma in the Austrian Pinot Blanc Wine Variety: Evaluation by Means of Sensorial-Analytical-Typograms with regard to Vintage, Wine Styles, and Origin of Wines

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The perceived pear aroma in wines was associated with various short- and medium-chain ester compounds. A consumer study confirmed this assumption. In total, eight ester compounds from a series of 100 aromatic substances were associated with the pear aroma. In this study, a valid stable isotope dilution assay headspace solid-phase microextraction gas chromatography mass spectroscopy (SIDA-HS-SPME-GC-MS) method was developed for the analysis of these compounds, and 102 Austrian Pinot blanc wines of vintages 2013–2016 were analysed. They were assessed with regard to vintage and origin influences as well as wine styles. However, an attempt was made to capture the synergies of these compounds for the pear aroma. With the detection of ethyl (E,Z)-2,4-decadienoate and methyl (E)-geranoate, two volatile compounds were measured which had not previously been detected in Austrian wines. The eight analysed esters were perceived very differently. Therefore, specific odour activity values for the various sensations could be calculated with a mathematical combination of the analyses and the results of the sensory studies. A vintage influence on the sensorial descriptor “overripe pear” and a relationship between wine style and total pear aroma were determined.

1. Introduction

With a total cultivated area of 1914 hectares, Austria is the third largest Pinot blanc-producing country in the world (15,493 hectares) [1–3]. Typical Austrian Pinot blanc wines reveal aromas described with pear, apple, quince, banana, apricot, caramel, and citrus fruits. Mature wines are reminiscent of fresh bread and gain in density and structure through cask storage and longer bottle ageing. The Pinot blanc lacks intensity, but its aromas are perfectly expressed in young wines [4, 5].

The pear aroma is described as particularly typical for Pinot blanc wines. This aroma in wine has previously been associated with various short- and long-chain ester compounds. These include isoamyl acetate [6], ethyl pentanoate [7], hexyl acetate [8, 9], ethyl isobutyrate [10], methyl (E)-geranoate ((E)-3,7-dimethyl-2,6-octadienoic acid methyl ester) [9], ethyl octanoate [8], isoamyl octanoate [9], and butyl butyrate [11, 12]. Ethyl (E,Z)-2,4-decadienoate is an essential ingredient of the typical

aroma in pears and their products and has not been previously described in wine and grapes from the *Vitis vinifera* [13]. In grapes from the *Vitis labrusca* vines (Concord and Niagara) and Elvira (*Vitis labrusca* and *Vitis riparia*), concentrations between 10 and 300 µg/L ethyl (E,E)-2,4-decadienoate and ethyl (E,Z)-2,4-decadienoate have been found so far [14].

Modern sensory studies are generally conducted with the help of trained panels because they are usually more reliable than individual people. Accordingly, the quality of the tasting results depends on the selection and training of the tasters [15]. On the other hand, extensive consumer studies have been conducted in order to assess overall attractiveness [15–17]. A preliminary by Philipp et al. [18] with 81 consumers (3 age groups, 32 females) and by six experts has shown that ethyl (E,Z)-2,4-decadienoate, methyl (E)-geranoate, isoamyl acetate, ethyl dodecanoate, and ethyl decanoate can be clearly identified as pear-like aroma (>1/3 of positive answers). According to the study, an aroma is considered a potential pear-like aroma if the substance is perceived by at least two

consumers in each age group or by at least nine consumers in total (>10%). Ethyl octanoate, ethyl hexanoate, and isoamyl octanoate are thus also included in the substances described as pear aroma by the consumers. The literature statements [7–12] that butyl butyrate, ethyl pentanoate, and hexyl acetate are associated with pear aroma could not be certified in this consumer survey [18]. Otherwise, a proportion of qualified panelists have recognised the hitherto neglected compounds—ethyl decanoate, ethyl dodecanoate, and ethyl hexanoate—as pear-like aromas [18].

In the interpretation of GC-MS data, some authors [8, 19–21] only considered those aromatic substances, whose odour activity values (OAV) were over a value specified by the author (e.g., in Vilanova et al. [20] $\text{OAV} > 0.2$). In contrast, other researchers suggest that the significance of the synergies of similar smelling compounds should be considered [17, 22, 23]. This study attempts to capture the synergies of compounds which smell like pear. To our knowledge, there is no exemplary work on this topic. A valid stable isotope dilution assay headspace solid-phase microextraction gas chromatography mass spectroscopy (SIDA-HS-SPME-GC-MS) method should be developed to quantify the aromas largely responsible for the pear aroma of the wines. Applying this method, a selection of Austrian Pinot blanc wines of different vintages will be analysed, and the determined substances will be interpreted with respect to perception and wine style [24].

2. Material and Methods

2.1. Samples. A total of 102 Austrian samples of Pinot blanc were produced for the Austrian market and marketed as Austrian quality wine. All wines were described by the producers as varieties typical to the region. 101 samples are from the provinces of Burgenland, Lower Austria, Vienna, and Styria and 1 sample from Tirol. 60 wines from the vintage 2016, 34 wines from the vintage 2015, 5 wines from the vintage 2014, and 3 wines from the vintage 2013 were used for the gas chromatographic analysis. Following volatile aromatic substances were quantified: ethyl (E,Z)-2,4-decadienoate, methyl (E)-geranoate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, isoamyl octanoate, ethyl decanoate, and ethyl dodecanoate. These substances were selected for analysis because a previous sensory study showed that these compounds have potential pear-like flavors [18]. Two bottles of each wine were procured from the producers in an 8- to 13-month period after the harvest and stored at +4°C until analysis. Only samples from vintage 2015 and 2016 were used for the study of the influence of the cultivation region and the wine style.

2.2. Chemicals. Ethanol (99%) was procured from Aus-
trAlco (Österreichische Alkohol-handels-GmbH, Spillern, Austria), tartaric acid (L-(+)-tartaric acid, >99.5%), and caustic soda solution (sodium hydroxide solution 50–52%) from Sigma-Aldrich (St. Louis, USA). The anhydrous monopotassium phosphate, used for the analysis of aroma, came from Zeller (Hohenems, Austria). Synthetic wine was made from 12% ethanol, 4 g/L tartaric acid, and Milli-Q water

(Synergy UV, Ultrapure Type 1, Merck-Millipore, Billerica, USA). The pH was adjusted to pH 3.2 with sodium hydroxide.

The chemical standards were purchased from Altmann Analytics-Shop (Fluka products, Munich, Germany), Sigma-Aldrich (St. Louis, USA), and Merck Schuchardt (Hohenbrunn, Germany) and showed all the maximum available concentration (90%–99.5%). All noncommercially available standards were produced by on-site synthesis [25, 26].

2.3. Synthesis of Deuterated Standards. The (d_6)-ethanol necessary for the preparation of the deuterated standards (anhydrous, ≥ 99.5 atom % D) was obtained from Sigma-Aldrich (St. Louis, USA) as does the hexan-1-yl chloride (98%), octan-1-yl chloride ($\approx 98\%$), and the decan-1-yl chloride (98%). One milliliter methylene chloride (anhydrous, $\geq 99.8\%$, containing 40–150 ppm amylene as stabiliser, Sigma-Aldrich (St. Louis, USA)) and 3 mmol (d_6)-ethanol were placed in a 15 ml centrifuge tube, and 3.3 mmol of acid chloride was gradually added. This was followed by a gradual addition of 1.5 ml pentane (anhydrous, $\geq 99\%$, Sigma-Aldrich (St. Louis, USA)) and neutralisation by means of 10% hydrogen carbonate ($\geq 99.7\%$, Sigma-Aldrich (St. Louis, USA)). The organic phase was removed and the solution evaporated by means of a rotary evaporator (Büchi (Flawil, Switzerland), Vacuum controller V-800, and Rotavapor R-200, heating bath B-490) at 35°C. The finished product was dissolved in 50 ml 4-hydroxy-4-methyl-2-pentanone (98%, Merck, Darmstadt, Germany) and stored at +4°C. The stability, purity, and mass spectra of the standards were tested with GC-MS and assessed as satisfactory.

2.4. Quantitative Analysis Using HS-SPME-GC-MS

2.4.1. Sample Preparation. 3 ml of the sample was placed together with 1 g NaH_2PO_4 , 2 ml Milli-Q water, and 50 μl of an internal standard mixture ((d_5)-ethyl hexanoate, (d_5)-ethyl octanoate and (d_5)-ethyl decanoate, and 80 $\mu\text{g/L}$ end concentration) in a headspace vial (20 ml) with a magnetic stir bar in the autosampler (CombiPal, CTC Analytics (Zwingen, Switzerland)). The headspace solid-phase microextraction (HS-SPME) with a carbon wide range (WR)/polydimethylsiloxane (PDMS), bipolar 95 μm fibre (Sigma-Aldrich (St. Louis, USA)) was chosen as the sample application system. The pre-incubation time at 45°C was 5 min, the adsorption time at 45°C was 30 min, and the desorption time in the injector at 250°C was 30 min. Every sample was analysed in duplicate.

2.4.2. Device Parameters. For the analysis, a gas chromatograph from Agilent Technologies (6890 N series II (Santa Clara, USA)) was used. The injector temperature was 250°C. The column installed was a ZB-WAXplus (60 m $\times$ 0.25 mm ID $\times$ 0.25 μm d_f) from Phenomenex (Torrance, USA). Helium (grade 5.6 (Linde GmbH (Stadl-Paura, Austria))) was used as the carrier gas at a constant flow of 1.2 ml/min. The total flow was set at 19.04 psi 26.4 ml/min. The unit was operated in the splitless mode. The temperature program began with a holding time of 8 minutes at 40°C injection temperature and was then heated up to

TABLE 1: Results of the expert survey/perception threshold as per the BET process [18].

Aromatic substance	Average typicity (%) ^{*1}					Total	Detection threshold µg/L
	T1	T2	T3	T4	T5		
Ethyl (E,Z)-2,4-decadienoate	28	36	0	0	16	80	2
Methyl (E)-geranoate	0	0	48	10	0	58	8
Isoamyl acetate	0	0	0	52	0	52	30
Ethyl hexanoate	10	0	0	4	38	52	14
Ethyl octanoate	0	42	0	16	0	58	80
Isoamyl octanoate	2	22	6	0	0	30	28
Ethyl dodecanoate	0	0	26	0	14	40	70
Ethyl decanoate	0	18	2	0	16	36	88

T1: overripe pear; T2: fresh pear; T3: cooked, processed pear; T4: exotic pear candy; T5: oily waxy pear-like.*10 = not typical; 100 = very typical.

a temperature of 168°C by an increase of 3°C/min. Then, the oven was heated by 20°C/min up to 250°C and kept constant at this temperature for another 5 minutes. The total running time was 75 minutes. A mass-selective detector from Agilent Technologies (Type 5975 (Santa Clara, USA)) was used as the detector. The temperature of the transfer line was 250°C. The electron multiplier voltage was 1400 V. Samples were measured in single-ion monitoring (SIM) mode. The evaluation was made using the relative ratio of the peak area of the sample to the peak area of the internal standard.

2.4.3. Calibration and Validation of the Method. An external calibration was carried out in a synthetic wine by means of six calibration points and the use of an internal standard. For the validation, the limit of detection (LOD) and the limit of quantitation (LOQ) were determined in accordance with the German Standard DIN 32645 [27, 28]. To attain the reproducibility, the coefficient of variation was calculated from ten replicate analyses. The average recovery was identified as quintuple determination for a level of concentration.

2.5. Calculations of the Results and Statistics. In order to describe the pear aroma in wines, the concept of pear-specific odour activity value has been introduced as a mathematical merging of the analytical and sensory aroma analyses. It was calculated according to (1) taking Table 1 into account. The data in Table 1 were collected by two different expert panels using descriptive tests and as per the “best estimated threshold” procedure (threshold value tests) [18, 29]. The 18 experts for determination of the detection threshold were members of the official sensory wine control. However, the aromas were classified by six other experts into five defined pear groups on a scale of 0–100 mm, analogous to their typicity (overripe pear (T1), fresh pear (T2), cooked, processed pear (T3), exotic pear candy (T4), and oily waxy pear-like (T5)). These six experts were experienced in the field of descriptive studies and were trained on the different sensations. The terms (T1 to T5) arose as consensus of these experts [18]:

$$\text{OAV}_{\text{T1-T5}} = \sum_{i=1}^{8 \text{ esters}} \text{OAV}_i \cdot \text{typicity}_i. \quad (1)$$

Odour activity value (OAV) is defined as the ratio of each compound to its detection threshold concentration

[30]. The different pear-specific odour activity values ($\text{OAV}_{\text{overripe pear}}$, $\text{OAV}_{\text{fresh pear}}$, $\text{OAV}_{\text{cooked, processed pear}}$, $\text{OAV}_{\text{exotic pear candy}}$, and $\text{OAV}_{\text{oily waxy pear-like}}$) result from the sum of the products of the defined typicity and the odour activity value of each compound. The overall pear-specific odour activity value (OAV_{pear}) is the sum of the five OAV-groups [18].

The statistical analysis of the data was performed using SPSS Statistics 22.0 (IBM) regarding differences from the vintage (2013–2016), the origin (Burgenland, Lower Austria, Styria, and Vienna for the vintages 2015 and 2016), and wine style (Leithaberg DAC, wines (>13.0% vol) and wines (≤13.0% vol) for the vintages 2015 and 2016). For the categorisation of the alcohol, the information on the bottle label was used.

First, the data set was tested for normal distribution using an exploratory data analysis. No outliers were eliminated. In the case of a normal distribution, the mean values of the independent samples were compared using a one-factorial analysis of variance and tested for variance homogeneity. If variance homogeneity was proved, an evaluation was done using the Tukey B test at the significance level of 0.05. If there was no variance homogeneity, the Dunnett-T3 test was also used at the significance level of 0.05. If the respective data set was not distributed normally, a Kruskal–Wallis test was conducted at the significance level of 0.05 and evaluated in the form of pairwise comparisons. The influence of the vintage, the origin, and the wine style on the concentration of the individual measured aromatic substances and the calculated pear-specific OAV could be examined using this test [31–33].

3. Results and Discussion

3.1. Results of the HS-SPME-GC-MS-Method Development. In addition to the relevant aroma compounds—isoamyl acetate, ethyl hexanoate, ethyl octanoate, isoamyl octanoate, ethyl decanoate, methyl (E)-geranoate, ethyl dodecanoate, and ethyl (E,Z)-2,4-decadienoate—other methyl and ethyl esters of cis-trans isomers of decadienoic acid were also determined according to Brandes et al. [25, 26]. On the one hand, all of these analytes belong to the same group of chemicals, and on the other hand, they have different polarities which makes the analysis difficult [9]. The expected concentration ranges of the different analytes span from

FIGURE 1: The response progression at different adsorption temperatures—compounds with (d₅)-ethyl-hexanoate and (d₅)-ethyl octanoate as internal standard.

<0.1 to >2000.0 µg/L. This fact also represents a large challenge to the method [33].

For the method development, three different fibres (100 µm PDMS, 95 µm carbon wide range (WR)/PDMS-bipolar, and 85 µm polyacrylate) were tested. A satisfying response could only be achieved with the carbon wide range bipolar fibre in all compounds. The polar compound isoamyl acetate produced no response with respect to PDMS fibre as well as ethyl (E,Z)-2,4-decadienoate with respect to polyacrylate. The response of ethyl decanoate and ethyl dodecanoate was also extremely low with polyacrylate. This is consistent with the results of Antalick et al. [9].

As described by various authors [9, 34–37], there is a correlation between the ionic strength of the solution and the adsorption of the analytes on the fibre. The concentration of the salt as well as the choice of the salt is important. Trials were conducted with three different salts and different amounts (NaCl, NaH₂PO₄, and MgSO₄). Ethyl (E,Z)-2,4-decadienoate in particular showed the highest response with NaH₂PO₄. The salt amount for this salt is increased above 1 g/5 ml; however, there were adverse effects on the adsorption of isoamyl acetate. Therefore, 1 g/5 ml NaH₂PO₄ was chosen for these experiments.

The adsorption temperature is also crucial for the SPME process. In theory, a higher temperature leads to an increase in the partial vapour pressure of the analytes, but at the same time to a reduction of the adsorption onto the fibre [9]. As shown in Figures 1 and 2, the responses of ethyl (E,Z)-2,4-decadienoate and ethyl dodecanoate were poor at 30°C and reached the optimum at 50°C. On the other hand, the response of isoamyl acetate decreased already between 30°C and 40°C but was still satisfactory at 45°C. An adsorption temperature of 45°C and an adsorption time of 30 min were chosen for the method. Several studies [9, 24] have shown that wine esters have different SPME extraction profiles. Quantification problems can only be avoided if the internal standard is structurally as similar as the analyte [9, 24, 38]. Therefore, three deuterated standards (d₅)-ethyl hexanoate (used for isoamyl acetate, ethyl hexanoate), (d₅)-ethyl octanoate (used for ethyl octanoate), and (d₅)-ethyl decanoate (used for ethyl decanoate, isoamyl octanoate, methyl (E)-geranoate, ethyl dodecanoate, and decadienoic esters)

FIGURE 2: The response progression at different adsorption temperatures—compounds with (d₅)-ethyl decanoate as internal standard.

were synthesised and used. They behaved similarly to the analytes, and therefore, their choice for the quantification of the different analytes was useful (Figures 1 and 2).

3.2. Stability of the Deuterated Standards. (d₅)-Ethyl hexanoate, (d₅)-ethyl octanoate, and (d₅)-ethyl decanoate were synthesised from (d₆)-ethanol and the corresponding acid chloride. Since these internal standards show more than one deuterium, their retention times and mass spectra differ slightly from their protonated counterparts. The stability of the deuterated esters should be checked, since transesterification reactions can take place in an acidic solution such as wine in the presence of ethanol and other alcohols [9]. Stability was measured over 18 hours in wine and in synthetic wine. It has been found that (d₅)-ethyl hexanoate, (d₅)-ethyl octanoate, and (d₅)-ethyl decanoate were sufficiently stable over a period of at least 8 hours. The sample series were adapted to these conditions, and all samples were analysed in less than 8 hours. These statements are consistent with the findings of Antalick et al. [9].

3.3. Quantification of Decadienoic Acid Esters. For the qualitative detection of the different methyl and ethyl ester of the decadienoic acid, a self-synthesised mixed standard was available [25, 26]. The quantification of the ethyl (E,Z)-2,4-decadienoate equivalents was done using a procured pure standard. For the quantification of the methyl esters and ethyl esters, the quantifier mass of 182 and 196, respectively, was used to get a good separation of the peaks (Figure 3). The usually used quantifier mass of 151 was not suitable because ethyl dodecanoate showed a response with a similar retention time (Table 2) [25, 26].

The quantification of decadienoic acid esters was done in accordance with the sum parameter (µg/L ethyl (E,Z)-2,4-decadienoate equivalents). There are four cis-trans isomers for both methyl and ethyl esters, which is due to the structural peculiarity (two double bonds with different substitutions) of the decadienoic acid. From the viewpoint that cis compounds have higher energy and thus lower boiling points, the isomers elute in the following order: (a) (Z,Z)-2,4-, (b) (E,Z)-2,4-, (c) (Z,E)-2,4-, and (d) (E,E)-2,4-decadienoate [25, 39].

FIGURE 3: Separation of (a) methyl (Z,Z)-2,4-decadienoate and (b) ethyl (E,Z)-2,4-decadienoate.

TABLE 2: Calibration table.

Substance	<i>m/z</i> quant	<i>m/z</i> qual	RT min	<i>R</i> ²	Calibration range (μg/L)
Isoamyl acetate	70	55; 43	17.667	0.996	14.56–2912.00
Ethyl hexanoate	88	99; 60	23.580	0.999	13.92–2784.00
Ethyl octanoate	88	101; 127	33.213	0.996	14.56–2912.00
Ethyl decanoate	88	101	41.769	0.999	14.50–2899.20
Isoamyl octanoate	127	70	42.589	0.998	0.25–50.85
Methyl (E)-geranoate	114	69	43.789	0.996	0.05–10.45
Ethyl dodecanoate	88	101	49.398	0.989	0.24–48.90
Decadienoic acid ester ^{*1}	196 (182 ^{*2})	151	49.267	0.996	0.08–15.91

^{*1}Calculated as ethyl (E,Z)-2,4-decadienoate equivalents; ^{*2}for methyl esters.

TABLE 3: Validation table.

Substance	LOD (μg/L)	LOQ (μg/L)	Srt (%)	Recovery (%)
Isoamyl acetate	24.378	80.580	4.55	100.71
Ethyl hexanoate	23.001	76.030	3.40	94.38
Ethyl octanoate	9.23	30.530	2.34	104.5
Ethyl decanoate	17.495	57.828	2.38	108.6
Isoamyl octanoate	0.051	0.168	2.80	103.1
Methyl (E)-geranoate	0.047	0.154	3.16	107.3
Ethyl dodecanoate	0.117	0.388	9.67	92.6
Decadienoic acid ester ^{*1}	0.036	0.119	3.64	102.4

^{*1}Calculated as ethyl (E,Z)-2,4-decadienoate equivalents.

3.4. Calibration and Validation Results of the Analytical Method. An external calibration was carried out in the synthetic wine by means of six calibration points and the use of an internal standard mixture. The functions were linear with regard to the concentration range of the analytes common in wine (correlation coefficient of all analytes ≥ 0.989) (Table 2).

The limit of detection (LOD) and limit of quantification (LOQ) were determined in accordance with the German Standard DIN 32645 [27, 28] and can be found in Table 3. This is particularly necessary because the LOD and LOQ may be outside the linearity range at high calibration ranges (isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate) if a signal-noise validation procedure would be performed [40].

The detection limits varied depending on the compound, especially in the concentration range between 0.119 μg/L and

80.580 μg/L and were below the expected concentrations in wine. For the determination of the repeatability (Srt), a wine was measured 10 times. The calculated repeatability (2.34%–9.67%; coefficient of variation, Table 3) was similar to that reported in the literature [9, 33, 36]. The recovery varied from 92.6% (ethyl dodecanoate) to 108.6% (ethyl decanoate). These very good recovery values were obtained mainly due to the use of deuterated internal standards [9, 33, 41].

3.5. Influence of Vintage and Origin on the Pear Aroma of Austrian Pinot Blanc Wines. 102 Austrian Pinot blanc wines of different vintages and origins were measured for the pear aroma's primary substances using the SIDA-HS-SPME-GC-MS. The concentrations of isoamyl acetate (Table 4) fluctuated from 211.1 to 5816.7 μg/L. According to the literature [6], this compound ranges in wine from 30 and 8100 μg/L.

TABLE 4: Concentrations of esters regarding vintage and wine-growing region.

Parameter ($\mu\text{g/L}$)	Vintage 2013 ($n = 3$)	Vintage 2014 ($n = 5$)	Vintage 2015 ($n = 34$)	Vintage 2016 ($n = 60$)	Burgenland ($n_{2015} = 7$)	Lower Austria ($n_{2015} = 14$)	Styria ($n_{2015} = 7$)	Vienna ($n_{2015} = 6$)	Burgenland ($n_{2016} = 9$)	Lower Austria ($n_{2016} = 35$)	Styria ($n_{2016} = 7$)	Vienna ($n_{2016} = 8$)
Isoamyl acetate	Mean	444.4 ^a	563.9 ^a	1535.1 ^{ab}	2139.9 ^b	1261.0 ^{ab}	1538.9 ^{ab}	1045.9 ^a	2416.6 ^{ab}	1358.5 ^{ab}	2106.0 ^{ab}	2898.1 ^b
	SD	114.0	191.7	881.1	1074.0	724.5	785.25	414.7	1002.0	812.4	970.1	1149.5
	Lowest	294.7	211.1	226.4	332.2	419.0	226.4	499.9	1149.5	332.2	387.1	600.4
	Highest	571.3	758.2	3994.2	5816.7	2291.7	2908.5	1743.0	3994.2	2996.2	4861.1	4331.3
Ethyl hexanoate	Mean	927.3 ^a	772.5 ^a	1034.9 ^a	867.9 ^a	1037.6 ^{ab}	1056.7 ^{ab}	883.6 ^{ab}	1157.1 ^b	834.5 ^a	864.8 ^{ab}	872.3 ^{ab}
	SD	240.8	115.6	254.4	174.3	281.3	222.1	140.7	305.6	219.9	156.7	234.6
	Lowest	655.7	639.1	546.3	473.0	640.2	546.3	661.2	742.7	546.8	587.1	473.0
	Highest	885.1	978.4	1635.7	1252.4	1512.6	1445.8	1100.7	1635.7	1174.9	1252.4	1085.9
Ethyl octanoate	Mean	1235.7 ^a	1092.7 ^a	1389.4 ^a	1275.4 ^a	1358.7 ^a	1422.7 ^a	1271.7 ^a	1484.7 ^a	1084.3 ^a	1288.1 ^a	1429.1 ^a
	SD	179.2	130.4	278.5	276.0	268.9	278.0	192.5	320.4	298.0	256.1	212.0
	Lowest	1002.2	972.5	743.7	760.5	934.8	743.6	955.5	1070.8	760.5	880.6	1128.3
	Highest	1267.3	1324.9	2029.4	1824.2	1858.4	1831.8	1476.7	2029.4	1556.3	1824.2	1793.6
Ethyl decanoate	Mean	490.6 ^a	576.2 ^a	774.5 ^a	723.6 ^a	729.8 ^{ab}	786.5 ^{ab}	763.0 ^{ab}	812.2 ^{ab}	546.9 ^a	703.1 ^{ab}	1054.7 ^b
	SD	54.1	82.0	225.0	299.1	193.7	240.4	216.2	222.4	185.0	281.0	232.3
	Lowest	523.9	511.7	408.1	280.5	570.2	466.2	454.2	408.1	310.5	282.0	671.2
	Highest	414.4	713.2	1210.8	1578.8	1127.0	1210.8	986.2	1128.5	850.5	1588.0	1580.3
Isomayl octanoate	Mean	3.06 ^a	3.60 ^a	5.30 ^a	5.07 ^a	5.4 ^a	5.0 ^a	5.49 ^a	5.71 ^a	3.98 ^a	4.86 ^a	7.79 ^a
	SD	0.26	0.30	1.85	2.25	0.239	1.05	2.26	1.93	1.16	2.15	2.13
	Lowest	2.85	3.25	1.89	2.23	1.89	3.34	2.88	3.00	2.23	0.230	4.63
	Highest	3.43	4.11	9.25	11.20	9.11	6.87	8.93	9.25	5.72	11.21	11.05
Methyl geranoate	Mean	0.19 ^a	0.12 ^a	0.18 ^a	0.28 ^a	0.17 ^a	0.18 ^a	>0.047 ^a	0.24 ^a	0.19 ^a	0.35 ^a	0.18 ^a
	SD	0.07	0.02	0.09	0.62	0.06	0.07	0.02	0.14	0.11	0.79	0.08
	Lowest	>0.047	>0.047	>0.047	n.d.	>0.047	>0.047	>0.047	>0.047	>0.047	n.d.	>0.047
	Highest	0.29	0.16	0.47	4.73	0.28	0.32	0.18	0.47	0.46	4.73	0.35
Ethyl dodecanoate	Mean	25.97 ^a	22.87 ^a	30.11 ^a	33.94 ^a	26.46 ^a	32.17 ^a	28.30 ^a	31.66 ^a	25.57 ^a	33.60 ^a	44.58 ^a
	SD	16.80	7.22	17.6	18.57	14.92	21.07	9.60	15.31	11.85	18.91	21.34
	Lowest	2.23	10.12	3.29	3.74	5.19	3.29	13.93	3.76	8.6	3.73	25.20
	Highest	36.75	32.48	65.23	94.28	58.73	65.23	42.75	47.17	44.9	80.9	94.30
Decadienoic acid ester	Mean	0.34 ^a	0.59 ^a	0.56 ^a	0.54 ^a	0.56 ^a	0.56 ^a	0.54 ^a	0.56 ^a	0.48 ^a	0.57 ^a	0.53 ^a
	SD	0.04	0.07	0.15	0.28	0.18	0.14	0.13	0.12	0.15	0.33	0.18
	Lowest	0.29	0.50	0.28	0.15	0.37	0.28	0.32	0.39	0.23	>0.036	0.30
	Highest	0.38	0.63	0.94	1.83	0.94	0.83	0.72	0.73	0.70	1.83	0.86

All the parameters are given with their mean, standard deviation (SD), lowest amount, and highest amount. For each parameter, different letters in the same row indicate significant differences between variants ($p \leq 0.05$).

It is known from the literature that there is a reduction in the isoamyl acetate concentration during wine storage due to transesterification and hydrolysis reactions [42], which can be confirmed by this study. The wines of the 2016 vintage show significantly higher levels than the wines of the 2013 and 2014 vintages. Within the 2015 and 2016 vintages, a significant difference could be observed between the samples from Styria. Wines of Styria are highly regarded by the consumer for their fruitiness which is partly based on high levels of fermentative aromas like isoamyl acetate which, however, have low stability [42].

The concentrations of ethyl hexanoate (473.0 to 1635.7 $\mu\text{g/L}$), ethyl octanoate (743.7 to 2029.4 $\mu\text{g/L}$), ethyl decanoate (280.5 to 1578.8 $\mu\text{g/L}$), and ethyl dodecanoate (2.23–94.28 $\mu\text{g/L}$) (Table 4) also corresponded to the average data from the literature [9, 33, 43]. These aromas are counted among the classical fermentation aromas (tertiary aromas) and are due to the amino acid or fatty acid metabolism of the yeast [6]. As expected, there was no significant difference between the vintages (Table 4 left side) of the wines and the contents of these four aromatic substances. Interestingly, there were determined significant differences between the wines from Vienna (2015 vintage) and Burgenland (2016 vintage) regarding the ethyl hexanoate and between the wines from Burgenland (2016 vintage) and from Styria (2016 vintage) regarding the ethyl decanoate (Table 4 right side). These differences are likely the result of the choice of wines (sample size and age). Rather, it can be assumed that the largest influence on the content of isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, and ethyl dodecanoate derived from the fermentation conditions, in particular, the choice of yeast, fermentation temperature, yeast-available nitrogen, pH value, concentrations of unsaturated fatty acids, sterol, and the oxygen content [6, 44, 45].

Concentrations of 1.89–11.20 $\mu\text{g/L}$ were measured from the isoamyl octanoate compound (Table 4). In the literature, concentrations for red wine are specified in the range from 1.2 to 4.0 $\mu\text{g/L}$. Antalík et al. [9] assumed that the concentration of this ester is higher in rose wine, white wine, and red wine vinified by carbonic maceration than in classical red wines. 41 of the 102 wines, however, showed concentrations below 4.00 $\mu\text{g/L}$. The average of all 102 wines was 5.01 $\mu\text{g/L}$, which was slightly higher than the above mentioned range for red wines. There was no significant impact from the vintage and the origin of wines with this aroma compound.

In addition, the ethyl and methyl esters of the decadienoic acid were detected in the wines. Quantifiable concentrations only resulted from ethyl (E,Z)-2,4-decadienoate and ethyl (Z,Z)-2,4-decadienoate (quantified as ethyl (E,Z)-2,4-decadienoate equivalent). Ethyl (Z,E)-2,4-decadienoate and methyl (Z,Z)-2,4-decadienoate were also detectable, but below LOQ. All other cis-trans-isomers were not detected. The Austrian Pinot blanc wines had calculated concentrations from 0.15 to 1.83 $\mu\text{g/L}$, calculated as ethyl (E,Z)-2,4-decadienoate (Table 4). Because these compounds were already verified in grapes of the vine genus *Vitis labrusca* and their hybrids, it would be possible to assign them to the

primary aromas. On the other hand, the results showed that there were no significant differences in vintage and origin.

Philipp et al. [18] found a sensorial detection threshold of 2 $\mu\text{g/L}$ in a 10% alcoholic solution; however, the specific odour threshold for perceived pear aroma was significantly higher. It was determined with an individual high dispersion and low reproducibility using the BET_{single} method and resulted in 20–400 $\mu\text{g/L}$. However, statistical calculation models have shown that these compounds are indeed relevant for the pear aroma in interaction with other aromatic substances (isoamyl acetate, ethyl dodecanoate, and methyl (E)-geranoate) determined with 24 volunteers [18].

Methyl (E)-geranoate was detected for the first time in Austrian wines and in general, firstly in Pinot blanc with a concentration up to 4.73 $\mu\text{g/L}$. This value corresponds to the data in the literature. Antalík et al. [9] have found 0.83 $\mu\text{g/L}$ in French red wines. However, this substance was found in high concentrations especially in Gewürztraminer and some sweet wines, for example, Coteaux du Layon from the Chenin blanc grape variety [46].

Table 5 shows the calculated pear-specific odour activity values in relation to the vintage. The total pear aromatic of the wines decreased with increasing age. This effect was primarily due to the decrease in isoamyl acetate concentrations (Table 4). With the decomposition of this aromatic substance, the sensorial perceived fruitiness of the wines decreased [42]. This is reflected in the significantly lower OAV of the “exotic pear candy” (OAV_{exotic pear candy}) in the 2013 and 2014 vintages. On the other hand, the OAV of the “overripe pear” (OAV_{overripe pear}) greatly depended on the vintage. 2014 and 2016 vintages have been described as rather cool vintages and thus have lower OAV_{overripe pear} than the very warm 2015 vintage.

Table 5 shows the different pear-specific-OAV associated to the origin of the wines. In Austria, well-known Pinot blancs are mainly produced in Styria (Südsteiermark, Vulkanland), Lower Austria (Thermenregion, Weinviertel, Klosterneuburg), Vienna, and Burgenland (Leithaberg DAC). However, the Pinot blanc is represented in all wine regions in Austria [1]. It is interesting to note that the wines of Styria (2015 vintage) and Burgenland (2016 vintage) showed lower total pear-specific odour activity value (OAV_{pear}) than the wines from Vienna (2015 vintage) and Styria (2016 vintage). This is mainly due to the already described lower isoamyl acetate content of wines from Styria (2015 vintage) and the very high isoamyl acetate content of wines from Styria (2016 vintage) and the resulting significantly lower OAV_{exotic pear candy}. Significant differences between the wines from Styria (2016 vintage) and Burgenland (2016 vintage) were found regarding the OAV_{fresh pear}. Since these differences have not been observed in the 2015 vintage, it could be due to the sampling (sample size and age). Similarly, a vintage or origin correlation with OAV_{cooked, processed pear} and OAV_{oily waxy pear-like} also is unlikely.

3.6. Influence of the Wine Style and the Alcohol Content on the Pear Aroma. Since there are no defined Pinot blanc styles in Austria, with the exception of the Leithaberg DAC

TABLE 5: Pear-specific odour activity value in relation to vintage and wine-growing region (sensorial-analytical-typograms).

Parameter (OAV _{pear})	Vintage 2013 (n = 3)	Vintage 2014 (n = 5)	Vintage 2015 (n = 34)	Vintage 2016 (n = 60)	Burgenland (n ₂₀₁₅ = 7)	Lower Austria (n ₂₀₁₅ = 14)	Styria (n ₂₀₁₅ = 7)	Vienna (n ₂₀₁₅ = 6)	Burgenland (n ₂₀₁₆ = 9)	Lower Austria (n ₂₀₁₆ = 35)	Styria (n ₂₀₁₆ = 7)	Vienna (n ₂₀₁₆ = 8)
Overripe pear	Mean SD	5.6 ^a 0.8	7.5 ^b 1.8	6.3 ^a 1.3	7.5 ^{ab} 2.0	7.6 ^{ab} 1.6	6.4 ^{ab} 1.0	8.3 ^b 2.2	6.0 ^a 1.6	6.3 ^{ab} 1.6	6.3 ^{ab} 0.9	6.4 ^{ab} 1.7
Fresh pear	Mean SD	7.1 ^a 0.8	9.0 ^a 1.7	8.3 ^a 1.9	8.8 ^{ab} 1.7	9.2 ^{ab} 1.6	8.4 ^{ab} 1.5	9.6 ^{ab} 2.0	6.9 ^a 1.8	8.3 ^{ab} 1.6	9.8 ^b 1.5	8.5 ^{ab} 1.9
Cooked, processed pear	Mean SD	2.4 ^a 0.3	3.3 ^a 0.8	2.8 ^a 0.5	3.3 ^{ab} 0.9	3.3 ^{ab} 0.6	2.8 ^{ab} 0.5	3.6 ^b 0.9	2.6 ^a 0.7	2.8 ^{ab} 0.6	2.9 ^{ab} 0.3	2.9 ^{ab} 0.7
Exotic pear candy	Mean SD	10.2 ^a 2.2	12.0 ^a 3.5	29.4 ^{ab} 15.4	39.6 ^b 18.7	29.5 ^{ab} 13.8	20.7 ^a 7.3	44.9 ^{ab} 17.3	25.7 ^a 14.4	39.1 ^{ab} 13.8	53.1 ^b 22.1	42.0 ^{ab} 20.1
Oily waxy pear-like	Mean SD	26.1 ^a 6.6	22.1 ^a 3.2	29.6 ^a 7.0	25.0 ^a 4.9	30.2 ^{ab} 6.0	25.5 ^{ab} 4.2	33.0 ^b 8.4	23.7 ^a 6.9	24.9 ^{ab} 6.0	25.7 ^{ab} 4.4	25.4 ^{ab} 6.2
Total	Mean SD	53.4 ^{ab} 2.1	49.2 ^a 1.3	78.8 ^b 5.5	82.0 ^b 6.8	79.9 ^{ab} 4.8	63.7 ^b 2.5	99.4 ^b 6.1	65.1 ^a 5.0	81.3 ^{ab} 5.8	97.9 7.8	85.1 ^{ab} 7.0

All the parameters are given with their mean and standard deviation (SD); for each parameter, different letters in the same row indicate significant differences between variants ($p \leq 0.05$).

TABLE 6: Concentrations of esters in relation to the wine stylistics and alcohol content.

Parameter ($\mu\text{g/L}$)		Pinot blanc 2015 ($\leq 13.0\%$ vol. alcohol); ($n = 18$)	Pinot blanc 2015 ($> 13.0\%$ vol. alcohol); ($n = 11$)	Leithaberg DAC 2015; ($n = 5$)	Pinot blanc 2016 ($\leq 13.0\%$ vol. alcohol); ($n = 35$)	Pinot blanc 2016 ($> 13.0\%$ vol. alcohol); ($n = 23$)	Leithaberg DAC 2016; ($n = 3$)
Isoamyl acetate	Mean	1455.1 ^{ab}	1967.6 ^{ab}	1392.5 ^{ab}	2095.3 ^{ab}	2298.2 ^b	909.1 ^a
	SD	789.3	1032.7	750.4	1249.3	706.6	413.1
	Lowest	226.4	419.0	446.8	387.1	1190.3	332.2
	Highest	3052	3994.2	2291.7	5816.7	3945.2	1277.1
	Mean	968.2 ^{ab}	1073.7 ^c	1048.7 ^{bc}	833.3 ^{ab}	928.9 ^{ab}	727.2 ^a
Ethyl hexanoate	SD	196.3	292.7	269.5	156.1	169.5	240.7
	Lowest	546.3	640.2	729.2	473.0	663.1	546.9
	Highest	1328.0	1635.7	1512.6	1174.9	1252.4	1067.4
	Mean	1337.4 ^{ab}	1416.6 ^b	1386.5 ^{ab}	1182.6 ^a	1426.0 ^{ab}	1122.7 ^a
Ethyl octanoate	SD	253.7	306.5	250.5	247.5	237.9	310.7
	Lowest	743.7	934.8	1172.6	760.5	880.6	844.3
	Highest	1625.3	2029.4	1856.4	1796.6	1824.2	1556.3
	Mean	741.1 ^a	845.5 ^a	799.1 ^a	686.2 ^a	797.1 ^a	527.1 ^a
Ethyl decanoate	SD	222.0	235.0	190.9	317.3	259.9	124.0
	Lowest	408.1	466.2	632.9	280.5	407.7	406.5
	Highest	1142.2	1210.8	1127.0	1580.3	1587.8	697.6
	Mean	5.11 ^a	5.31 ^a	6.32 ^a	5.16 ^a	5.10 ^a	3.21 ^a
Isoamyl octanoate	SD	1.77	1.73	02.05	2.33	2.13	0.72
	Lowest	2.88	1.89	3.78	2.2	2.88	2.24
	Highest	8.93	9.25	9.11	11.0	11.21	3.42
	Mean	0.17 ^a	0.23 ^a	0.15 ^a	0.37 ^a	0.16 ^a	0.19 ^a
Methyl geranoate	SD	0.08	0.10	0.03	0.80	0.07	0.06
	Lowest	>0.047	>0.047	>0.047	>0.047	n.d.	>0.047
	Highest	0.41	0.47	0.21	4.73	0.30	0.28
	Mean	30.31 ^a	34.31 ^a	28.71 ^a	60.67 ^a	39.51 ^a	26.13 ^a
Ethyl dodecanoate	SD	16.76	17.73	17.27	17.61	19.32	5.21
	Lowest	3.76	3.29	5.19	3.73	7.04	20.09
	Highest	59.10	65.23	58.73	94.28	80.94	32.80
	Mean	0.54 ^a	0.55 ^a	0.64 ^a	0.54 ^a	0.56 ^a	0.37 ^a
Decadienoic acid ester	SD	0.14	0.13	0.16	0.28	0.29	0.03
	Lowest	0.32	0.28	0.49	>0.036	0.25	0.35
	Highest	0.83	0.73	0.94	1.83	1.47	0.41
	Mean						

All the parameters are given with their mean, standard deviation (SD), lowest amount, and highest amount. For each parameter, different letters in the same row indicate significant differences between variants ($p \leq 0.05$).

(Districtus Austriae Controllatus), this classification was based on the alcohol content of the wines. For this reason, wines up to an alcohol content of 13% vol. and those above 13% vol. have been compiled in two groups for each vintage (2015 and 2016). The wines of the type “Leithaberg DAC” with a clearly defined development style have been kept separately. The typicity of DAC “Leithaberg” wines is rated in accordance with the national DAC Regulation (BGBl. (Federal Law Gazette) II Number 252/2009) [47] by a tasting commission and should be typical for the region: fruity, spicy, and have less fermentation aromas.

With regard to the aromatic substances (Table 6), significant differences in the concentrations of isoamyl acetate, ethyl hexanoate, and ethyl octanoate could be observed. The wines from the Leithaberg DAC (2016 vintage) showed the lowest concentrations, while wines with higher alcohol content tended to have higher values than the wines of the category up to 13% vol. To our knowledge, a direct correlation between higher alcohol content and higher ester content is not described in the literature yet. However, it has been shown that the perception of aroma compounds in fact

depends on the alcohol content [6]. The fact that Leithaberg DAC wines have lower concentrations of isoamyl acetate (2016 significant, 2015 tendency) than other Pinot blanc wines is understandable due to the defined wine style [47].

The sensorial-analytical-typograms of the pear aroma can be seen in Table 7, with regard to wine styles and alcohol content. The study revealed that wines belonging to the category “above 13.0 vol.% alcohol” tended to have higher total OAV_{pear}. This is due to various aspects. The alcohol-rich wines were characterised more by the overripe pear aromas than the wines with lower alcohol levels (2015 significant, 2016 tendency). Similar observations, some of them significant and some of which with tendency, could be made for the other categories: cooked pear, exotic pear candy, and oily waxy pear-like, while the impression exotic pear candy dominated the 2016 vintage. The wines of the 2015 vintage (alcohol content above 13% vol and Leithaberg DAC), however, showed significantly higher contents of overripe pears impression than the wines of the 2016 vintage. The sensorial-analytical-typograms depict what is well known about wines from these vintages: the 2015 vintage

TABLE 7: Pear-specific odour activity value in relation to wine stylistics and alcohol content (sensorial-analytical-typograms).

Parameter (OAV _{pear})		Pinot blanc 2015 (≤13.0% vol. alcohol); (n = 18)	Pinot blanc 2015 (>13.0% vol. alcohol); (n = 11)	Leithaberg DAC 2015; (n = 5)	Pinot blanc 2016 (≤13.0% vol. alcohol); (n = 35)	Pinot blanc 2016 (>13.0% vol. alcohol); (n = 23)	Leithaberg DAC 2016; (n = 3)
Overripe pear	Mean	7.0 ^{ab}	7.8 ^c	7.6 ^{bc}	6.0 ^{ab}	6.7 ^{ab}	5.2 ^a
	SD	1.4	2.1	1.9	1.1	0.3	1.7
Fresh pear	Mean	8.7 ^{ab}	9.3 ^b	9.1 ^{ab}	7.8 ^{ab}	9.3 ^{ab}	7.1 ^a
	SD	1.6	1.8	1.6	1.8	1.2	1.9
Cooked, processed pear	Mean	3.1 ^{ab}	3.4 ^b	3.3 ^{ab}	2.7 ^a	3.0 ^{ab}	2.3 ^{ab}
	SD	0.6	0.9	0.8	0.5	1.7	0.7
Exotic pear candy	Mean	27.9 ^a	36.9 ^{ab}	26.9 ^{ab}	38.7 ^{ab}	42.7 ^b	18.0 ^{ab}
	SD	13.8	18.1	13.2	21.7	0.5	7.6
Oily waxy pear-like	Mean	27.7 ^{ab}	30.8 ^b	30.0 ^{ab}	24.0 ^a	26.8 ^{ab}	20.8 ^{ab}
	SD	5.5	8.0	7.5	4.4	12.3	6.8
Total	Mean	74.4 ^{ab}	82.9 ^b	76.9 ^{ab}	79.1 ^{ab}	88.5 ^b	53.4 ^a
	SD	4.9	6.5	4.7	8.0	4.3	2.9

All the parameters are given with their mean, standard deviation (SD), lowest amount, and highest amount. For each parameter, different letters in the same row indicate significant differences between variants ($p \leq 0.05$).

was an overripe vintage, while 2016 was more fresh-fruity one. Wines with higher alcohol content were from more mature grape material than wines with lower alcohol content if not enriched or concentrated. The wines produced in this way showed more mature aromas than light-fruity wines. Therefore the division of the wine into two categories according to the alcohol content made sense either for the traditional wine description as well as for the discussion of the presented sensorial-analytical-typograms.

4. Conclusion

In the course of this present work, it is attempted to describe the synergies of eight short- and middle-chain esters as the main contribution to the pear aroma of Pinot blanc wines. The pear-specific odour activity values presented as sensorial-analytical-typograms were calculated from the analysed concentration of these esters in 102 Pinot blanc wines from Austria and the results of sensory studies [18]. The overall pear-specific odour activity value (OAV_{pear}) of the 102 wines ranged from 32.4 to 147.7. The largest contributors to the OAV_{pear} were the impressions for “exotic pear candy” (primarily characterised by isoamyl acetate) and “oily waxy pear-like” (characterised by ethyl hexanoate, ethyl decanoate). The sum of OAV “fresh pear” and “overripe pear” ranged from 8.6 to 24.5; however, they were important for the individual expression.

As cited by Philipp et al. [18], these sensorial-analytical-typograms serve the representation of subjective sensorial perceptions and highly specific chemical analyses of volatile substances. Since these are simplifications, it must be mentioned that the primary value of the sensorial-analytical-typograms lies in the consideration of the differences between the individual wines and not in an attempt to obtain a correlation between sensorial and chemical data.

In this paper, the influence of the vintage, the wine styles, and the origin on the pear aromatic of the Pinot blanc wines was examined. Differences in the concentration levels of

volatile substances were clear, easy, and understandable, presented in the proposed sensory-analytical-typograms. It was found that there was a vintage influence on the OAV_{overripe pear}. In mature years like 2015, the values are higher than in immature years like 2014 and 2016. In addition, the sensation of “exotic pear candy” drops with increasing age of the wines. This is accompanied by the reduction of isoamyl acetate [42]. On the other hand, an influence of the wine style on the pear aromatic wine was observed. It was determined that the Pinot blanc wines with higher alcohol content (category > 13.0 vol.%) tend to have a higher total pear-specific odour activity value than wines with a lower alcohol content. These results are in good accordance with the standard description of Austrian wines from these vintages and wine styles [48].

If it would be possible to measure a larger number of samples over several years, the question of regional disparities could be clarified better. In any case, further studies, especially on the technological and viticultural influence on the pear aromatic, are recommended. The pear-specific odour activity values model and the presentation in the form of sensorial-analytical-typograms seem well suited for the interpretation of such questions in the future.

Data Availability

All anonymized data that contributed to this research work can be requested from the corresponding author.

Conflicts of Interest

There are no relevant interests that could be perceived as conflicting.

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