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Analysis of Volatile Components of Varietal English Wines Using Stir Bar Sorptive Extraction/Gas Chromatography-Mass Spectrometry

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Abstract: Aroma is an important property of wine and it can be influenced significantly by enological practices. The aim of this work was, by use of stir bar sorptive extraction/gas chromatography-mass spectrometry (SBSE/GC-MS), to compare semi-quantitative concentrations of the volatile constituents of stainless steel tank-fermented/matured Huxelrebe, Ortega, Schönburger and Siegerrebe varietal wines from a commercial English vineyard, with corresponding wines produced by oak cask ('barrel') fermentation/maturation. Aroma profiles of tank and barrel wines were different, with more volatiles detected and net concentrations being higher in barrel wines. Long chain ethyl carboxylate esters were generally more abundant in barrel wines, whereas acetate esters were generally more prominent in tank wines. By conducting a short (~7 month) maturation period in secondhand (third or fourth fill) casks, it was possible to make wines with more complex aromas, but without obvious oak aroma.

Keywords: stir bar sorptive extraction with thermal desorption; gas chromatography-mass spectrometry; volatile organic compounds; wine

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1. Introduction

Aroma is a very important property of wine, contributing much to its overall quality and commercial value. The aroma profile of a wine is the result of the combined contributions of hundreds of organic compounds, some of which are present in concentrations lower than μg/L (parts per billion) [1]. Some of these low concentration components have exceptionally low odour threshold values (OTVs; OTV is the lowest concentration of a specific odour compound that is perceivable by the human sense of smell), sometimes in the ng/L (parts per trillion) range [2], and hence make a significant individual contribution to the overall wine aroma. Many others, present in concentrations lower than their OTV, may make a valuable background contribution.

The low concentration of most wine volatile compounds makes the use of an extractive enrichment or focusing technique essential. Such a technique is stir bar sorptive extraction (SBSE) [3]. SBSE has been used to extract volatile compounds from grape juice [4–10] and wine [9–17], including wines that have had prolonged contact with oak wood, such as maturation in oak casks [12,13], and also including liquid (solvent) desorption in one case [15], as opposed to the standard thermal desorption. Similarly, SBSE has been used to extract volatile compounds from grape juice and wines of grapes to which French oak extract was applied at the ripening stage (veraison) [9,10]. In the case of wine, all SBSE studies have been carried out on wines made from warm climate varieties, such as Malvasia [12], Merlot [14], Pinotage [16], Verdejo [9] and Petit Verdot [10], all growing in warm regions. Of two SBSE investigations on the influence of oak maturation on wine aroma, one was on Madeira wines [12] and the other on red wine [13], and both studies involved wines that mostly had prolonged maturation periods (>1 year) in oak casks.

The aim of this work was to determine for the first time the aroma profiles of English varietal wines made from Huxelrebe, Ortega, Schönburger and Siegerrebe grapes from a commercial vineyard. Samples of these wines were derived from the same musts as those used in previous studies [4–6]. In particular, we report here a comparison of aroma profiles of varietal wines (Huxelrebe, Ortega, Schönburger) fermented and aged in stainless steel tanks, and wines from the same must that were fermented and matured in secondhand oak casks. By using a short (~7 month) residence in secondhand (third or fourth fill) Burgundian Nevers oak casks, the winemaker's aim was to make wines with greater aroma complexity and earlier maturity than the standard tank wines, but without obvious oak aroma character, as it was felt that this would clash with the 'aromatic' aromas (Muscat or Gewürztraminer type) of the base wines. All the varieties here have either Muscat or Gewürztraminer as part of their ancestry [5].

2. Materials and Methods

2.1. Materials

Acetone and water ('super pure' quality) were acquired from Romil (Cambridge, UK). *n*-Tetradecane 99+% was obtained from Aldrich (Gillingham, UK). Standard Twister^{TM} stir bars coated with 24 µL of PDMS (length: 10 mm, film thickness: 0.5 mm) were obtained from Gerstel (Mülheim an der Ruhr, Germany).

2.2. Wine Samples

Huxelrebe, Ortega and Schönburger and Siegerrebe wines of the 2004 vintage were obtained as gifts from the commercial 22-acre Chilford Hundred vineyard (Cambridgeshire, UK).

2.3. Winemaking and Ageing

Processing of the Chilford grapes was conducted at the vineyard and the adjacent winery. Each variety was harvested, destalked, crushed and pressed separately, using a horizontal pneumatic press. The varietal musts were pumped into separate stainless steel tanks, sulphited (30–50 mg/L) and allowed to settle for 24 h. The clarified juices were then pumped to separate clean stainless steel tanks, whence samples (1 L) were taken of each and analysed by SBSE/gas chromatography-mass spectrometry (GC-MS) as described by Caven-Quantrill et al. [5] (see Figure 1). To ensure the start of fermentation, yeast (Lallemand EC1118, Montréal, QC, Canada) was added to each tank and fermentation was allowed to proceed for 24 h, after which the fermenting musts were transferred to either fresh tanks or barrels to finish fermentation at ca. 12–15 °C and to begin the ageing process (a total of 7 months for each wine, with no further racking). The bulk of the wines spent the time in stainless steel tanks, but small amounts were fermented and aged in secondhand Nevers oak casks (228 L) from Burgundy. Fermentation proceeded to dryness, all the wines having 1.5–3.8 g/L of reducing sugars, and subsequent maturation took place under airlock at 10–15 °C. The free SO_2 level was maintained at 30–50 mg/L (ppm) and temperature was kept low in all cases in order to discourage malolactic fermentation (MLF). After 7 months, our sample wines (1 L of each) were taken from tank and cask and bottled. The bottles, stoppered with sterile conventional corks, were stored horizontally in a cool (ca. 10–15 °C), dark place until opened for analysis nearly 2 years after the vintage.

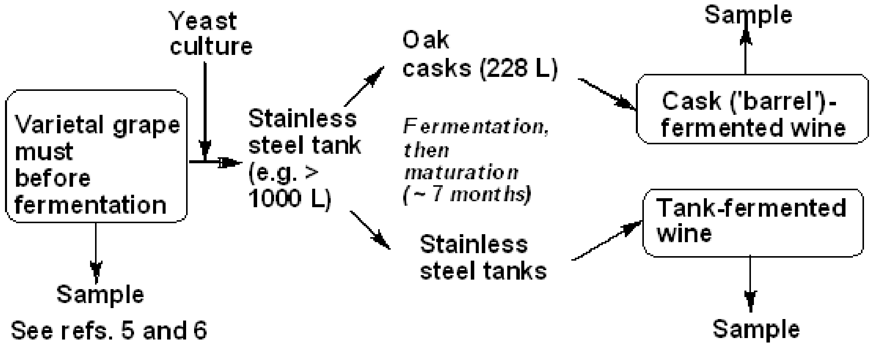

Figure 1. Sample origin and relationships. The full scheme applies to the musts and wines of the varietals Huxelrebe, Ortega and Schönburger. Tank wine only was produced from the must of Siegerrebe.

2.4. Determination of Routine Viticultural and Oenological Parameters

Specific gravities of musts were determined using a wine hydrometer, with adjustment for temperature. Malic and tartaric acids in musts and wine were determined by reversed-phase high-performance liquid chromatography (HPLC), as described previously [18]. D- and L-lactic acids in wine were determined by a previously reported chiral ligand-exchange HPLC method [18]. Free SO_2 levels were determined by the Ripper method [19]. Determination of ethanol content (% v/v) was carried out using a standard gas chromatography-flame ionisation detection GC-FID procedure [20]. Residual reducing sugar in the wines was assayed by the Lane and Eynon method [19].

2.5. Sampling Conditions for SBSE

Subsequent SBSE and thermal desorption gas chromatography/mass spectrometry TD-GC/MS analyses (triplicate analyses for each sample) were conducted as previously optimized [4] using a 20 mL sample volume and a 5:1 injection split ratio [17].

Prior to use, the stir bars were conditioned at 300 °C in a helium stream (100 mL/min) for 1 h using a TC-1 tube conditioner (Gerstel). A preconditioned Twister^{TM} stir bar was added to each of the sample vials before being capped and placed onto a Gerstel Twister™ stirrer plate (TS-1). Samples were stirred under the previously optimised ambient temperature juice conditions (1000 rpm for 2 h [4]. On completion, stir bars were removed from the vials, washed with 'super pure' water (5 mL) and blotted dry on a lint-free tissue. The stir bars were finally spiked directly with internal standard solution (*n*-tetradecane 0.02% w/v in acetone, 1 µL) then transferred to a clean preconditioned thermal desorption tube and placed onto a TDS-A autosampler for analysis. Addition of *n*-tetradecane directly to the stir-bar allowed it to be used for semi-quantifying the extracted volatile compounds, that is, it acts as both an extraction internal standard and a GC internal standard, since its recovery into the stir bar polydimethylsiloxane (PDMS) phase is 100% [4].

2.6. Instrumentation and Conditions

For an account of the method development and optimisation of the TD-GC/MS procedure, see references 4 and 17. The SBSE analyses were performed using an automated TDS-2/TDS-A thermal desorption unit (Gerstel) mounted on an Agilent 6890 gas chromatograph system coupled to a quadrupole Agilent 5973 electron ionisation (70 eV) mass spectrometric detector (Agilent Technologies, Palo Alto, CA, USA), equipped with an Agilent Innowax (crossed linked polyethylene glycol) capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The carrier gas was helium with a constant column flow rate of 1 mL/min (mean velocity 36 cm/s).

The analytes were cryofocused in a programmed temperature vaporising injector (PTV) (CIS-4, Gerstel) held at −50 °C with liquid nitrogen prior to injection. A packed liner containing 20 mg of

Tenax TA was used in the PTV. Stir bars were thermally desorbed in a stream of helium carrier gas at a flow rate of 70 mL/min and by programming the TDS 2 from 20 °C to 300 °C (5 min) at a rate of 60 °C/min. After desorption and cryofocusing, the CIS-4 was programmed from −50 °C to 260 °C (20 min) at 12 °C/s to transfer the trapped aroma volatiles onto the analytical column. The TDS-2 was operated in the split mode to provide a 5:1 split ratio, as previously optimized [17]. The GC oven temperature was programmed from 40 °C (5 min) to 240 °C (20 min) by increasing the temperature at 3 °C/min, and the MS was operated in scan mode (35–300 amu). The temperature of the mass selective detector transfer line was retained at 250 °C throughout.

2.7. Identification of Volatile Components and Determination of Semi-Quantitative Concentrations

Agilent MS Chemstation software, equipped with the Wiley 275L, Nist 98 and Frutarom's proprietary mass spectral libraries, was employed for identification of the volatile compounds, along with ethyl ester retention indices (as secondary confirmation), which were determined, whenever possible, by co-injection of authentic aroma standards under identical GC-MS conditions and calculated by linear interpolation relative to the retention times of C_2–C_{20} ethyl esters [21]. Confirmation of the presence of oak lactones in barrel wines (and of their absence in tank wines) was achieved by selected ion chromatography (SIC) using the $m/z = 99$ ion.

Semi-quantitative concentrations were calculated using the expression (peak area/IS peak area) × IS concentration (IS = internal standard), and hence are only approximate values. However, our major interest in this work is with regard to differences in wine volatile component levels of varietal tank and corresponding barrel wines of a single vintage.

3. Results and Discussion

3.1. General

The SBSE/GC-MS method used in this work was optimised, as described previously, using a mixture of 46 pure volatile compounds of known concentrations in a synthetic grape must medium [4], and in a corresponding synthetic wine medium containing 12.0% ethanol by volume [17]. By changing the GC injector split ratio from 20:1 for the synthetic must volatiles to 5:1 for the synthetic wine volatiles, it was possible to compensate for the decreased SBSE sensitivity caused by the presence of ethanol in the wine medium. In this way, the GC peak areas of the extracted volatiles were generally much more similar for the two media. Hence, it was possible to monitor changes in volatile component composition caused by the alcoholic fermentation of real grape must to wine by direct comparison of data for wines in this work with data for the corresponding musts, as reported in our previous reports [5,6], and as outlined in Figure 1. Briefly, compared with the original musts, the wines were characterized by much higher overall levels of volatiles, especially with regard to ethyl alkanoate and acetate esters, fatty acids, 2-phenylethanol and β-damascenone, whereas certain alcohols, carbonyl compounds and some terpenoids were more abundant in the musts.

The casks used for barrel fermentation/aging at Chilford Vineyard were secondhand Nevers Oak 228 litre 'pièces', that previously held up to three vintages worth of Burgundy Chardonnay wine. By using secondhand casks and a short maturation period (ca. seven months), the winemaker at Chilford Vineyard intended to produce more complex and more intense wines, but without pronounced oak aromas and flavours, because it was thought that these would clash with the natural strongly aromatic character of the wines. At the same time, the barrel wines would still benefit from typical accelerated maturation compared with the tank wines.

The level of free SO_2 (30–50 mg/L) in all the wines after primary fermentation, combined with the relatively low fermentation temperatures (12–18 °C) and maturation temperatures (10–15 °C), completely discouraged malolactic fermentation (MLF) [22] (Table 1), so the difference in aroma profiles of corresponding tank and barrel wines is due entirely to ageing for seven months in different containers. MLF is a secondary fermentation caused by resident or added bacteria (e.g., *Lactobacillus*, *Oenococcus* and/or *Pediococcus* spp.); it converts L-malic acid to the 'softer' L-lactic acid, but also produces changes to the aroma profile. The occurrence of MLF in some of the wines would have invalidated the comparisons made here. Typically, very low levels of the major MLF product L-lactic acid (25–44 mg/L) [23] (Table 1) confirm that none of the wines underwent even partial MLF. This is supported by the observation of (at best) only trace levels of diacetyl in the wines. The small depletions of malic acid observed (Table 1) are typical of those caused by ordinary yeast metabolism during alcoholic fermentation [23]. MLF causes a significant reduction in the total acidity of wine and, hence, would be undesirable here, since the juice acidities (except Huxelrebe) were low-moderate, and in any case they were lowered naturally as a result of alcoholic fermentation (Table 1).

Analysis of all the wines was performed nearly two years after the vintage (consumers typically begin to drink English wines of about this age) over a short period of time.

Table 1. Summary of routine enological parameters [a].

Parameter	**Huxelrebe 2004 must**	**Ortega 2004 must**	**Schönburger 2004 must**	**Siegerrebe 2004 must**
°Brix (±0.2°) [b]	19.4	21.6	19.6	21.6
Tartaric acid concentration. (%*w/w*, %Relative standard deviation (RSD) = 2.7) (n = 3)	0.57	0.41	0.50	0.30
Malic acid concentration. (%*w/w*, %RSD = 1.4) (n = 3)	0.38	0.16	0.33	0.13

[a] All juices were 'fermented to dryness', having residual reducing sugar content of 1.5–3.8 (±0.2) g/L. For all the wines made from these juices, malic acid levels dropped by 8–12% (±1%), which is normal for alcoholic fermentations using the *S. cerevisiae* EC1118 strain at 15–20 °C [23]. Tartaric acid levels dropped by ca. 10% (presumably as a result of precipitation of potassium hydrogen tartrate during and after alcoholic fermentation). L-Lactic acid levels were only 25–44 (±2) mg/L and D-lactic acid levels were 195–326 (±6) mg/L, thus indicating the absence of malolactic fermentation (MLF). The % ethanol by volume range was 11.7–12.5 (±0.2); [b] °Brix = sugar content in g/100 g juice.

3.2. Wine Volatiles

Since the semi-quantitative concentrations of volatiles quoted in Table 2 are only approximate, a comparison of these with their corresponding literature odour threshold values (OTV) will not give a definitive indication of extent of contribution to global wine aroma, especially as literature OTVs vary according to the medium. However, it is possible to make a tentative suggestion on the contribution of a particular wine volatile, especially if its semi-quantitative concentration is much higher than its OTV. Certainly, the suggestions here are in broad agreement with the informal organoleptic assessments made by a number of independent qualified or experienced tasters, as well as by the authors. The major wine volatiles are summarized next.

Table 2. Volatile compounds [a] identified in stainless steel tank- and barrel-fermented/aged 2004 Huxelrebe, Ortega and Schönburger wines, and stainless steel tank-fermented/aged 2004 Siegerrebe wine using stir bar sorptive extraction/gas chromatography-mass spectrometry (SBSE/GC-MS).

Retention Times (min)	RI [b]	Component [c]	Huxelrebe		Ortega		Schönburger		Siegerrebe	%RSD (CV) [d]
			2004		2004		2004		2004	
			Tank	Barrel	Tank	Barrel	Tank	Barrel	(Tank)	n = 3
2.24	92	Acetaldehyde	37	58	45	47	33	37	19	5.9
3.48	200	Ethyl acetate	820	380	1900	1300	12,000	1500	700	7.4
3.51	203	1,1-Diethoxyethane (Acetal)	-	240	-	170	-	220	990	6.8
3.80	230	2-Methylbutanal	tr.	-	-	-	-	-	tr.	-
3.86	235	3-Methylbutanal (Isovaleraldehyde)	tr.	tr.	tr.	tr.	-	tr.	11	7.3
4.28	273	2,4,5-Trimethyl-1,3-dioxolane (Acetaldehyde-2,3-butanediol acetal)	-	181	-	121	-	241	720	3.1
4.58	300	Ethyl propionate	41	33	43	30	119	83	101	4.9
4.75	308	Ethyl isobutyrate	92	88	150	118	330	350	123	6.2
5.06	323	Diacetyl	tr.	tr.	tr.	tr.	tr.	tr.	-	-
5.91	364/365	α-Pinene/Isobutyl acetate	410	129	300	230	-	190	870	7.0
5.91	365	Isobutyl acetate	-	-	-	-	520	-	-	7.6
6.59	400	Ethyl butyrate	1420	1270	1470	1470	2100	1060	3800	5.0
7.06	413	Ethyl-2-methyl butyrate	87	87	74	65	113	280	55	6.0
7.12	415	Camphene	tr.	tr.	tr.	tr.	-	-	25	5.3
7.60	430	Ethyl isovalerate	150	190	125	116	160	370	69	6.8
7.74	434	n-Butyl acetate	65	33	29	26	26	17	50	6.3
7.88	437	1,1-Diethoxy-3-methylbutane (Isovaleraldehyde diethyl acetal)	-	50	-	26	-	41	67	4.2
8.00	440	Hexanal	tr.	tr.	-	tr.	tr.	tr.	-	-
8.30	449	2-Methyl-1-propanol (Isobutyl alcohol)	68	26	110	71	710	170	190	7.4
8.43	452	β-Pinene	11	tr.	tr.	tr.	-	tr.	tr.	9.0
8.81	465	2,2,6-Trimethyl-6-vinyltetrahydropyran	14	35	tr.	24	21	30	190	3.8
9.06	470	1-Ethoxy-1-pentoxyethane (Acetaldehyde ethyl amyl acetal)	-	167	-	48	-	130	290	4.2
9.75	484/485	2-Methylbutyl acetate/3-Methylbutyl acetate (Isoamyl acetate)	60,000	19,000	48,000	27,000	44,000	18,000	19,000	6.6
10.11	500	Ethyl pentanoate (Ethyl valerate)	-	-	tr.	tr.	-	-	39	3.1
10.29	504	δ-3-Carene	tr.	14	tr.	tr.	tr.	11	tr.	8.3
10.56	510	1-Butanol	tr.	tr.	15	12	26	14	25	5.1
11.12	523	Isobutyl butyrate	12	tr.	tr.	tr.	tr.	tr.	tr.	7.3
11.25	525	Ethyl-2-butenoate (Ethyl crotonate)	-	-	37	29	37	17	-	6.9
11.25	525/527	Ethyl-2-butenoate (Ethyl crotonate)/β-Myrcene	45	42	-	-	-	-	83	5.1
11.62	535	α-Terpinene	tr.	21	tr.	16	tr.	tr.	12	5.8
11.73	538	n-Amyl acetate	38	16	36	28	35	16	31	1.4
11.97	543	2-Heptanone	11	17	13	30	15	tr.	41	2.3
12.10	546	Heptanal	-	-	-	-	-	-	tr.	-
12.27	550	Methyl hexanoate	45	53	18	23	45	25	33	8.6
12.42	554	Limonene	200	100	55	50	56	110	230	8.8

Table 2. *Cont.*

Retention Times (min)	RI [b]	Component [c]	Huxelrebe		Ortega		Schönburger		Siegerrebe	%RSD (CV) [d]
			2004		2004		2004		2004	
			Tank	Barrel	Tank	Barrel	Tank	Barrel	(Tank)	n = 3
12.64	559	Isoamyl isobutyrate/1,8-Cineol	35	19	-	-	-	-	35	7.9
12.64	559	Isoamyl isobutyrate	-	-	17	16	27	87	-	7.6
13.26	573	2-Methyl-1-butanol/3-Methyl-1-butanol (Isoamyl alcohol)	10,700	8600	10,200	7800	18,000	21,000	22,000	5.5
14.53	600	Ethyl hexanoate	86,000	91,000	59,000	68,000	67,000	43,000	97,000	5.5
14.69	604	γ-Terpinene	54	48	31	37	29	32	154	3.3
15.09	616	(*E*)-β-Ocimene	tr.	16	tr.	12	tr.	tr.	81	1.6
15.35	622	1-(1-Ethoxyethoxy) hexane (Acetaldehyde ethyl hexyl acetal)	-	11	-	-	-	-	20	3.3
15.69	630/631	p-Cymene/Isoamyl butyrate	156	150	79	111	88	116	238	3.1
16.18	641	Hexyl acetate	39,000	13,700	22,000	12,900	18,200	3500	6400	5.0
16.22	642	α-Terpinolene	13	39	tr.	13	12	12	71	4.8
16.26	643	3-Methylbutyl-2-methyl butyrate (Isoamyl-2-methyl butyrate)	-	-	-	-	tr.	15	-	5.7
16.67	653	Octanal	tr.	tr.	-	-	-	-	tr.	-
17.36	669	Ethyl-(*Z*)-3-hexenoate	38	36	-	19	-	108	-	5.0
17.56	673	Ethyl-(*E*)-3-hexenoate	17	16	14	22	tr.	tr.	20	7.2
17.67	-	(*E*)-3-Hexenyl acetate*	200	80	148	156	44	18	133	5.0
18.01	682/685	(*Z*)-3-Hexenyl acetate/Propyl hexanoate	400	227	176	149	760	198	157	3.7
18.71	700	Ethyl heptanoate	138	122	51	59	68	99	310	5.0
18.78	702	6-Methyl-5-hepten-2-one	tr.	tr.	-	-	21	tr.	21	7.2
18.78	702	6-Methyl-5-hepten-2-one/(*E*)-2-Hexenyl acetate	-	-	21	16	-	-	-	6.7
19.13	710	Ethyl-(*E*)-2-hexenoate	42	103	330	228	168	225	360	3.5
19.17	711	Ethyl lactate	122	126	28	28	76	105	52	4.2
19.23	712	(*Z*)-Rose oxide	tr.	tr.	30	28	75	100	260	7.8
19.52	719	Isobutyl hexanoate	19	tr.	34	37	36	42	-	8.6
19.59	721	1-Hexanol	2400	2100	1320	1350	1700	1140	3400	6.4
20.04	731	(*E*)-3-Hexenol	26	23	17	24	13	14	36	8.3
20.45	741	Heptyl acetate	70	71	54	28	50	tr.	-	3.0
20.89	752	(*Z*)-3-Hexenol	34	32	-	48	138	123	82	2.0
20.96	753	2-Nonanone (Methyl heptyl ketone)	70	112	172	96	87	40	230	4.1
21.10	757	Methyl octanoate	600	630	240	250	540	230	330	5.1
21.39	763	3-Octanol	-	-	-	-	-	-	tr.	-
23.64	800	Ethyl octanoate	370,000	370,000	274,000	310,000	310,000	206,000	360,000	3.4
23.88	822	1-Octen-3-ol	tr.	tr.	-	-	tr.	tr.	tr.	-
23.94	824	Acetic acid	450	118	278	360	244	273	350	2.9
24.01	825	1-Heptanol	11	-	13	tr.	15	11	-	6.2
24.12	828	Isoamyl hexanoate	710	910	570	610	610	630	900	3.4
24.26	831	Furfural	tr.	tr.	tr.	14	13	tr.	12	5.9

Table 2. *Cont.*

Retention Times (min)	RI ^{b}	Component ^{c}	Huxelrebe		Ortega		Schönburger		Siegerrebe	%RSD (CV) ^{d}
			2004		2004		2004		2004	
			Tank	Barrel	Tank	Barrel	Tank	Barrel	(Tank)	n = 3
24.41	835	(*E*)-Linalool oxide (Furanoid)/Nerol oxide	-	-	-	-	-	-	592	0.6
24.42	-	Nerol oxide *	168	289	216	214	167	164	-	0.7
24.72	842	Octyl acetate	76	166	83	27	92	67	-	3.0
25.29	856	(*E*)-Theaspirane	-	-	-	-	-	39	-	5.3
25.39	858	2-Ethyl-1-hexanol	-	-	-	-	17	-	-	5.3
25.59	863	Decanal	11		-		tr.		tr.	1.1
25.75	867	Camphor	16	tr.	-	-	-	-	tr.	8.7
26.16	-	Geranyl ethyl ether *	183	310	142	185	90	100	450	3.5
26.32	880	Benzaldehyde/Vitispirane (Unknown isomer) *	118	320	-	-	53	115	-	4.9
26.32	880	Benzaldehyde	-	-	90	129	-	-	2100	4.9
26.33	-	Vitispirane (Unknown isomer) *	-	-	-	-	-	70	-	4.6
26.49	884	Propyl octanoate	410	660	269	255	305	70	283	2.8
26.59	886	2-Nonanol	14	25	22	66	36	33	19	3.1
26.87	893	(*Z*)-Theaspirane	24	41	-	-	17	26	-	7.8
27.16	900	Ethyl nonanoate	330	280	204	280	200	250	390	4.0
27.28	903	Propionic acid	tr.	tr.	tr.	tr.	tr.	tr.	-	-
27.49	-	Ethyl-2-hydroxyhexanoate *	29	51	20	18	26	60	11	6.4
27.76	913/917	Linalool/Isobutyl octanoate	690	690	446	535	580	446	5050	1.6
28.05	922	1-Octanol	226	121	62	83	108	293	75	2.6
29.27	948	Isobutyric acid	-	-	17	24	26	25	-	5.6
29.44	958	Methyl decanoate/2-Undecanone	435	570	-	-	-	-	-	2.8
29.44	958	Methyl decanoate	-	-	264	344	470	241	340	2.7
29.48	956	4-Terpineol	-	-	13	-	-	-	-	5.2
29.97	967/973	β-Cyclocitral/Hexyl hexanoate	37	40	37	22	41	38	-	4.4
30.15	973	Hotrienol	40	246	226	268	83	123	383	0.4
30.29	976	γ-Butyrolactone	37	29	36	40	27	30	17	6.5
30.50	981	Ethyl-2-furoate	18	57	12	19	16	37	23	1.0
31.57	1000	Ethyl decanoate	260,000	310,000	230,000	290,000	260,000	176,000	310,000	4.7
31.96	1024	Isoamyl octanoate	2700	3500	2000	2300	2300	2300	3300	6.3
31.98	1024	1-Nonanol	-	-	-	43	-	-	-	0.2
32.06	1026	Citronellyl acetate	194	40	217	40	190	81	50	3.1
32.23	1031	(*E*)-β-Farnesene	80	91	123	83	102	73	121	5.3
32.62	1039	Diethyl succinate	990	1670	551	1020	549	3420	704	1.0
33.10	1053	Ethyl-9-decenoate	240	2050	6800	7600	20,200	9400	410	4.3
33.18	1055	α-Terpineol	97	130	80	105	16	58	274	2.0
33.91	1074	3-(Methylthio)-1-propanol (Methionol)	14	tr.	-	-	tr.	15	15	11.1

Table 2. *Cont.*

Retention Times (min)	RI [b]	Component [c]	Huxelrebe		Ortega		Schönburger		Siegerrebe	%RSD (CV) [d]
			2004		2004		2004		2004	
			Tank	Barrel	Tank	Barrel	Tank	Barrel	(Tank)	n = 3
34.26	1083	Propyl decanoate	280	450	206	230	210	129	260	4.2
34.36	1086	Neryl acetate	39	14	44	26	53	30	-	1.9
34.60	-	1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) *	48	101	34	23	93	164	74	1.6
34.83	1098	Geranyl nitrile	-	-	-	-	-	-	-	5.9
34.91	1100	Ethyl undecanoate	61	74	56	78	23	33	190	6.8
35.00	1102	Ethyl geranate	-	-	-	-	78	63	-	3.2
35.16	1107	(*E*,*E*)-α-Farnesene	33	12	36	13	23	24	78	8.3
35.41	1113	Isobutyl decanoate	190	160	114	140	160	180	270	6.7
35.47	1115	Geranyl acetate	76	-	118	14	63	-	-	5.4
35.55	1117	Ethyl-(*E*)-2-decenoate	36	46	tr.	11	63	26	16	2.3
35.73	1122	1-Decanol	541	491	962	2220	656	818	672	0.6
35.88	1126	β-Citronellol	108	86	133	169	158	220	660	5.5
36.33	1138	Diethyl pentanedioate (Diethyl glutarate)	18	46	36	41	20	34	-	4.3
36.40	1140	Ethyl phenylacetate	93	94	21	48	33	172	34	4.1
37.06	1158	Nerol	44	33	25	-	-	-	-	4.0
37.06	1158	Nerol/Methyl dodecanoate (Methyl laurate)	-	-	-	-	76	50	274	2.0
37.14	1160	Ethyl nicotinate	-	-	-	-	36	14	-	4.7
37.14	1160/1161	Ethyl nicotinate/β-Damascone	-	-	37	-	-	44	-	5.2
37.41	1167	2-Phenylethyl acetate	17,000	-	15,800	6800	19,300	6000	-	3.3
37.41	1167/1169	2-Phenylethyl acetate/(*E*)-β-Damascenone	-	4470	-	-	-	-	3080	1.2
38.56	1197	Hexanoic acid	1660	2880	1910	2450	2140	1200	910	3.5
38.63	1200	Ethyl dodecanoate (Ethyl laurate)	32,000	43,000	21,000	42,000	25,000	20,000	112,000	7.3
38.71	1202	Geraniol	123	140	134	163	149	79	-	1.4
38.81	1205	Geranyl acetone	86	87	16	39	79	94	161	4.1
39.17	1216	Isoamyl decanoate	1800	2300	1100	1500	1100	1800	5000	8.7
39.53	1226	Benzyl alcohol	190	200	27	37	35	50	113	5.1
39.59	1227	(*Z*)-β-Methyl-γ-octalactone (cis-Oak lactone)	-	39	-	46	-	108	-	3.0
39.73	1232	Ethyl-3-phenylpropionate	26	48	27	33	22	58	68	3.0
40.09	-	Ethyl-3-hydroxyoctanoate *	39	92	63	66	58	54	45	1.8
40.52	-	Ethyl-3-methylbutyl butanedioate *	730	960	220	290	330	2100	420	5.4
40.68	1260	2-Phenylethanol	12,400	6580	6800	5030	10,800	17,400	9400	1.2
41.32	1278	β-Ionone	34	41	tr.	14	36	60	34	2.5
41.43	1282	Propyl dodecanoate	-	-	-	-	-	-	53	6.8
41.73	1291	Benzothiazole	13	13	13	23	-	-	21	6.9
41.90	1293	(*E*)-β-Methyl-γ-octalactone (trans-Oak lactone)	-	115	-	153	-	152	-	
42.22	-	3,7-Dimethyl-1,5-octadiene-3,7-diol (Terpendiol) *	tr.	-	24	34	-	-	-	5.3

Table 2. *Cont.*

Retention Times (min)	RI [b]	Component [c]	Huxelrebe		Ortega		Schönburger		Siegerrebe	%RSD (CV) [d]
			2004		2004		2004		2004	
			Tank	Barrel	Tank	Barrel	Tank	Barrel	(Tank)	n = 3
42.35	1309	2-Phenylethyl butyrate	130	31	87	73	100	107	35	7.9
42.77	1321	1-Dodecanol	200	170	290	910	170	280	910	5.6
43.76	1351	Diphenyl oxide	24	tr.	12	18	14	42	20	11.0
43.90	1354	(*Z*)-Nerolidol	109	46	45	-	36	36	12	5.6
44.24	1365	γ-Nonalactone	80	32	-	-	-	-	85	5.8
45.13	1391	Diethyl malate	98	214	-	-	-	-	-	4.0
45.20	1393	(*E*)-Nerolidol	2000	2200	1800	2100	1600	2500	2300	9.0
45.43	1400	Ethyl tetradecanoate (Ethyl myristate)	900	1700	410	1200	450	800	4500	13.3
45.81	1412	(*E*)-Methyl cinnamate	40	51	17	27	-	-	56	10.2
46.07	1421	Isoamyl laurate	12	-	36	46	39	55	100	10.6
46.08	1421	1-Tridecanol	-	-	-	-	-	-	-	6.3
46.10	1422	Octanoic acid	59,000	66,000	37,000	43,000	49,000	28,000	52,000	6.2
46.95	-	Ethyl-3-hydroxydecanoate *	1200	1900	620	450	610	380	310	9.0
47.09	1454	Diethyl suberate	100	90	12	30	15	60	460	15.1
47.58	1469	(*E*)-Ethyl cinnamate	220	360	250	360	140	170	560	8.4
48.06	1485	2-Phenoxyethanol (Rose ether)	12	tr.	11	21	tr.	12	-	7.9
48.53	1500	Ethyl pentadecanoate	24	31	25	24	16	12	131	5.6
48.82	1509	2-Phenylethyl hexanoate	390	270	143	200	150	290	240	6.2
49.18	1520	Nonanoic acid	82	123	58	78	61	38	126	6.2
49.23	1522	1-Tetradecanol	-	-	-	-	-	-	1800	7.8
49.24	1522	1-Tetradecanol/δ-Decalactone	127	150	118	400	130	320	-	7.5
49.74	1538	2-Methoxy-4-vinylphenol	300	250	380	390	450	110	83	5.0
51.71	1600	Ethyl hexadecanoate (Ethyl palmitate)	1300	7700	2000	3700	1600	1500	8100	8.3
51.95	-	Ethyl-2-hydroxy-3-phenyl propionate *	240	163	-	-	137	400	225	2.4
52.12	-	2,3-Dihydrofarnesol *	400	300	760	1050	500	1600	1600	5.5
52.45	-	Ethyl-9-hexadecenoate	-	-	450	570	-	920	-	2.8
52.60	1632	Decanoic acid	87,000	113,000	54,000	68,000	72,000	41,000	101,000	5.8
53.25	-	Ethyl-3-hydroxydodecanoate *	1300	1500	410	340	190	108	190	8.0
53.71	-	(*Z*,*E*)-Farnesol *	113	31	30		27		-	2.5
54.26	1690	α-Hexylcinnamic aldehyde	tr.	21	tr.	tr.-	-	-	-	9.4
54.31	1692	Geranic acid	-	-	-	-	-	-	1120	4.8
54.45	1697	(*E*,*E*)-Farnesol	2700	1600	2200	3200	3700	3100	8300	5.4
54.62	1703	γ-Dodecalactone	200	300	450	270	380	182	450	4.6
54.95	1715	2-Phenylethyl octanoate	530	310	212	207	253	266	398	2.3
55.15	1722	1-Hexadecanol	81	64	135	135	60	20	370	4.6
55.57	1737	para-Vinylphenol	82	112	550	510	250	250	142	4.7

Table 2. *Cont.*

Retention Times (min)	RI [b]	Component [c]	Huxelrebe		Ortega		Schönburger		Siegerrebe	%RSD (CV) [d]
			2004		2004		2004		2004	
			Tank	Barrel	Tank	Barrel	Tank	Barrel	(Tank)	n = 3
55.91	1749	δ-Dodecalactone	260	440	188	191	220	175	230	4.7
57.15	-	3,4-Dihydro-8-hydroxy-3-methyl-1H-2-benzopyran-1-one (Ochracin) *	-	-	-	-	-	-	29	5.5
57.19	-	3,4-Dihydro-8-hydroxy-3-methyl-1H-2-benzopyran-1-one (Ochracin) */ 3a,4,5,7a-Tetrahydro-3,6-dimethylbenzofuran-2(3H)-one (Wine lactone) *	47	49	171	220	27	34	-	4.5
57.35	1800	Ethyl octadecanoate (Ethyl stearate)	125	530	150	200	210	200	1000	7.1
58.47	1841	Dodecanoic acid (Lauric acid)	12,000	12,000	14,000	11,000	19,000	13,000	31,000	8.3
59.07	1861/1863	Ethyl linoleate/Diethyl dodecanedioate	420	1100	800	1500	900	1300	-	11.3
59.10	1865	Ethyl linoleate	-	270	-	320	-	330	1700	8.2
60.80	-	Ethyl linoleolate *	113	270	230	320	160	330	500	7.3
61.30	1946	Benzyl benzoate	50	78	-	56	28	22	-	6.5
62.02	1973	δ-Tetradecalactone	270	380	320	370	280	270	330	6.9
63.94	2044	Tetradecanoic acid (Myristic acid)	1300	1040	254	420	277	188	3500	3.2
66.52	2140	Pentadecanoic acid	212	191	21	27	16	16	-	4.2
69.00	2230	Hexadecanoic acid (Palmitic acid)	4700	4200	670	2000	560	820	3900	5.5
71.41	2320	Heptadecanoic acid	450	250	-	-	-	-	22	4.5
73.96	2414	Octadecanoic acid (Stearic acid)	650	690	65	71	33	35	105	5.6
74.74	2443	9-Octadecenoic acid (Oleic acid)	1500	1700	-	145	-	-	-	6.2

[a] Semi-quantitative concentrations (ng/L) calculated from (peak area/IS peak area) × IS concentration; [b] Retention indices (RI) on a 30 m × 0.25 mm × 0.25 µm Innowax column, relative to C_2–C_{20} ethyl esters [21]; [c] Identification based on retention index, comparison of mass spectra and co-injection with authentic compounds, except for components marked *, which were based on comparison of mass spectra only; [d] Coefficient of variation (% CV) estimated from the SBSE-GC-MS analyses of a single wine sample (in triplicate; n = 3); - Indicates compound not detected; tr. Indicates concentration of <10 ng/L.

3.2.1. Alcohols

The 'fusel alcohols' 2-methyl-1-propanol (isobutyl alcohol) and 2- and 3-methyl-1-butanols, together with the normal straight chain volatiles such as 1-butanol and the benzenoid 2-phenylethanol, are present in all the wines and their combined concentrations are similar for tank and barrel wines. Individually, their concentrations are well below their literature OTVs, but their presence is likely to make a significant beneficial contribution to the background aroma [9,24,25].

3.2.2. Esters

All the wines, especially the barrel wines, had low levels of ethyl acetate and, since the OTV of this compound from wine is 1.60×10^8 ng/L, it is unlikely it played an individual role in organoleptic quality [26].

The C_5 and C_6 acetate esters are the most abundant acetate esters. The OTV of hexyl acetate is 2000–480,000 ng/L [27], so it is possible that this component contributed to the perceived pronounced fruity, green, apple/pear notes of the tank wines in particular.

From Table 2, certain ethyl esters were the most abundant of all the wine volatile compounds, the highest of which was ethyl octanoate, present in the concentration range 206,000 ng/L (Ortega barrel wine) to 370,000 ng/L (Huxelrebe wines). Since this ester's literature OTV is 5000–92,000 ng/L [27], it is likely to make a significant contribution to the overall organoleptic profile of all of the wines studied, providing a sweet odour reminiscent of apricot, banana and pineapple [28].

Another abundant ester is ethyl hexanoate, present in the concentration range 43,000 ng/L (Schönburger barrel wine) to 91,000 ng/L (Huxelrebe barrel wine). Again, because the literature OTV of this compound is reported to be 1000–36,000 ng/L [24] and 300–5000 ng/L [27], it is feasible that this ester contributed to the bouquet of all of the wines studied. The same could be said of ethyl decanoate (OTV 8000–12,000 ng/L [27]. The higher homologues (ethyl palmitate, etc.) are all present at low levels, and since the only available literature OTV for these oily/waxy esters was for ethyl palmitate (2×10^6 ng/L), their contribution is unknown.

3.2.3. Fatty Acids

Other comparatively abundant common components of the wines were the long chain fatty acids, especially octanoic, decanoic, dodecanoic (lauric), hexadecanoic (palmitic), oleic and stearic acids. However, the levels of these compounds were all well below their reported odour threshold values, and so they will contribute only to background odour (and possibly to 'mouthfeel').

3.2.4. Terpenoids and Norisoprenoids

Forty eight volatile terpenoids and norisoprenoids were identified in the wines of this study and many are also common to grape juices [5,26]. The most abundant terpenoid in grape musts was linalool [5], but fermentation and other winemaking processes appear to have reduced its concentrations to well below its literature OTVs (1500–50,000 ng/L) in all the wines studied [24,26]. Likewise, all of the remaining terpenoids/norisoprenoids identified (apart from (*Z*)-rose oxide and (*E*)-β-damascenone) were also below their OTVs in the wines of this study, but it is likely that they provide an important synergistic background odour contribution [26].

3.2.5. Comparison of Volatile Compounds Identified in Tank- and Barrel-Fermented/Aged Wines

Over the years, it has been demonstrated by use of a variety of extraction/chromatographic techniques, that several viticultural/enological parameters can influence the aroma profile of wine. Recent examples include vineyard elevation [29], climatic or vintage variations [6,14,30], vine variety (cultivar) [5,15,30–32] and the use of oak or oak products [9,10,13,33–35]. To our knowledge, this is the first report of an SBSE/GC-MS based comparison of aroma profiles of 'unoaked' white wines with corresponding wines that have had a limited oak contact.

Overall, Table 2 indicates that tank wines and their barrel equivalents have a similar range of volatile components, but with different concentration profiles and with net concentrations of volatiles being rather greater in barrel wines. In fact, ca. 60% of the total concentration differences of volatile compounds observed in Table 2 are associated with higher levels in the barrel wines. Additionally, extra volatiles were detected in barrel wines (Table 2), including oak lactones.

Other than very low levels of oak lactones, typical oak volatile phenols (such as eugenol, guaiacol, 4-methylguaiacol and vanillin), as well as other oak volatiles (such as cyclotene, ethoxylactone and maltol) [22], were not detected in the barrel wines of this study and the low *p*-vinylphenol levels were similar in tank and barrel wines. This indicates that a short period of storage in used oak casks in the presence of lees fulfilled the winemaker's aim to produce more complex wines, but without obvious oak aroma and flavour. The typical oak aroma compounds mentioned above may largely have been removed from the wood in previous wines (in Burgundy) and/or may have been adsorbed as a result of prolonged contact with yeast: it is known that volatile phenols are selectively adsorbed by yeast lees [33]. The greater flavour complexity of the barrel wines, as perceived by experienced tasters at informal tastings, may be partly due to the presence of non-volatile oak compounds at low levels: such compounds are known to alter human perception of wine aroma [26].

Some major volatile compositional differences between corresponding tank and barrel wines seen in Table 2 are discussed in the next paragraphs.

Alcohols. The 'fusel alcohols' are present at similar levels in tank and barrel wines (see Section 3.2), but 1-hexanol, responsible for grassy, green fruit notes, is more abundant in tank wines, thus possibly making a contribution to the perceived more 'green' aroma of these wines.

Esters. Ethyl acetate and other acetate esters (such as hexyl acetate, 2-phenethyl acetate and the C_5 acetates) are generally more abundant in tank wines and could well contribute to the overall simple fruity aroma ('light acetate ester notes') of the tank wines in particular, as described informally by several independent experienced and qualified tasters. Conversely, Table 2 shows that ethyl carboxylate esters (ethyl octanoate, hexanoate and others) are collectively more abundant in barrel wines, possibly contributing to the organoleptically perceived softer, more exotic fruity notes of these wines, compared with the corresponding tank wines.

Acetals. Table 2 shows that, although acetals are not unique to the barrel wines, they are more abundant in these wines. Concentrations of acetals in oak-aged wines have been linked to the type of wood and the aging of the wine in oxidative conditions [34].

Lactones. Oak lactones (β-methyl-γ-octalactone diastereoisomers) occur in many wines that have matured in oak, but are not found in wine that has had no contact with oak. Already present in oak wood, and also formed during toasting of the cask during cooperage, these compounds are extracted slowly into the wine. Consequently, it may take from six months to one year in new oak casks for the coconut-like fragrance of oak lactones to become apparent. In most situations, the total content of these lactones rises during oak maturation, and there is also selective extraction of the more aromatic *cis*-isomer [32]. Continued use of casks (vintage after vintage) causes extensive leeching of volatile compounds from the woody material, so that later wines will have much lower levels of these compounds [35]. Indeed, in the barrel wines of this study, these two compounds are both well below ($\leq$108 ng/L) their OTVs—74,000 ng/L and 320,000 ng/L for the cis and trans isomers (respectively) as determined in red wine [13].

4. Conclusions

The SBSE/GC-MS method described here allowed the identification and semi-quantification of the volatile components (numbers in parentheses) of single vintage (2004), single varietal wines produced from *Vitis vinifera* cultivars Huxelrebe (tank wine: 158; barrel wine: 160), Schönburger (tank wine: 148; barrel wine: 158), Ortega (tank wine: 148; barrel wine: 155), and Siegerrebe wine (tank wine: 146). The results show that both greater numbers of volatiles, and also higher net levels of volatiles, were associated with the barrel wines. The major volatiles of wines were esters, with long chain fatty acid ethyl esters being generally more abundant in barrel wines and acetate esters being generally more abundant in tank wines. Long chain carboxylic acids, norisoprenoids and acetals were generally more abundant in barrel wines, and low levels of oak lactones—much below their odour threshold values (OTVs)—were found only in barrel wines. The use of a short (seven month) maturation time in used oak casks (third or fourth fill) gave more complex wines, but with no typical oak aroma, as perceived in informal tastings by experts.

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