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The Impact of Wine Style and Sugar Addition in *liqueur d'expedition* (*dosage*) Solutions on Traditional Method Sparkling Wine Composition

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Abstract: The purpose of this study was to investigate the effect of wine style and cane sugar addition in the *liqueur d'expedition* (*dosage*) solution on volatile aroma compounds (VOCs) in traditional method sparkling wine. There were 24 bottles of each treatment produced. Treatments were sparkling wine zero *dosage* (ZD); NV sparkling wine + sugar (BS); unoaked still Chardonnay wine + sugar (UC); Pinot noir 2009 sparkling wine + sugar (PN); Niagara produced Brandy + sugar (B) and Icewine (IW). The control treatment in the sensory analysis was an oaked still Chardonnay wine + sugar (OC) because the zero-*dosage* wine was not suitable for a difference test that compared wines with sugar to one without. Standard wine chemical parameters were analysed before disgorging and after *liqueur d'expedition* was added and included; pH, titratable acidity (TA g/L), alcohol (*v*/*v* %), residual sugar (RS g/L), free and total SO_2 and total phenolics (A.U.). Volatile aroma compounds (VOCs) analysed by Headspace Solid- Phase Micro-Extraction Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS) included two alcohols, and six ethyl esters. ZD wines had the highest foam height and highest dissolved oxygen level. Sugar affected VOC concentrations in all treatments at five weeks post-disgorging, but by 15 weeks after *liqueur d'expedition* addition, the wine with added sugar had similar VOC concentrations to the ZD wines. The type of wines used in the *dosage* solutions had more influence on VOC concentrations than sugar addition.

Keywords: sparkling wine; *liqueur d'expedition*; volatile aroma compounds; foam

1. Introduction

Four main methods are used to produce sparkling wine: the Charmat/Cuvé closed/tank method; transfer method; carbonation of the base wine; and *Methode Champenoise* (in Champagne only), though it is are referred to as the Traditional Method, Classic method, *Methode Traditionale* or bottle-fermented in other parts of the world. Although the term bottle-fermented can also refer to the transfer method, in this study it refers to the Traditional Method whereby the second fermentation occurs in the bottle that is ultimately sold to consumers. There are two main production phases to Traditional Method sparkling winemaking as reviewed in Kemp et al. [1]: the first stage is the first fermentation of grape juice to convert it into base wine; the second stage is when the base wines go through a second fermentation in the bottle after the addition of yeast and sugar (*liqueur de tirage*). Following a period of aging on the yeast lees (*sur lies*), bottles are riddled to move the sediment to the neck. Yeast biomass is then expunged by freezing the lower part of the bottle's neck and removing the sediment. Prior to closing the bottle with a cork, *dosage* (*liqueur d'expedition*) is inserted into each bottle and determines the

final sweetness of the wine as well as a consistency in winery style. The *liqueur d'expedition* is used to balance the wine and can also provide a wine with a unique flavour. Commonly referred to as *dosage*, it has been known to be produced using a range of sugar types (i.e., cane sugar, dextrose, beet sugar, liquid sugar or rectified concentrated grape must (RCGM)). Additionally, the wine used to make the *dosage* (+/− sugar) can include the same wine as that being disgorged, another sparkling wine from the cellar, oaked or unoaked still wine or wines aged in stainless steel or oak barrels depending on the required wine style of the winemaker [1,2]. Sparkling wines that do not have sugar added and are topped up with the same wine as that in the bottle are referred to in literature as *zero dosage*, *Brut Zero*, *Brut naturel*, *Nature* (<0.3%) or *Extra Brut* (<0.6%). Although produced for many years, zero *dosage* wines have recently increased in popularity [3]. Bottle-fermented sparkling wines range in sugar levels with *Brut* wines tending to be adjusted with up to 1.5 *w*/*v* % sugar, whereas *Extra-sec* generally contains 1.2%–2% sugar. *Sec* wines commonly have between 2% and 4% sugar, *Demi-sec* between 3% and 5% sugar and the now rare *Doux* wines contain more than 5% sugar [3]. Residual sugar levels (RS g/L) are known to impact sparkling wine flavour but can cause an "unbalanced" wine if levels are too high or too low. Additionally, it was reported that sucrose strongly suppressed sourness in sparkling wines while subtly enhancing flavour [4].

The length of time between disgorging and release of sparkling wines from the winery is decided by the wine producer, but the flavour of sparkling wine after disgorging and *dosage* addition is known to continuously change. The influence of grape variety, lees aging and bottle aging post-disgorging on volatile aroma compounds (VOCs) and foam has been studied [5], but sparkling wines were analysed 12 months after disgorging and did not have sugar added (zero *dosage* wines topped with the same wine but without sugar addition). The authors of the manuscript reported that Albariño, Verdejo, Godello and Prieto Picudo were richest in most of the VOCs analysed, but Verdejo and Prieto Picudo had the best foam physiognomies. These characteristics were maintained during lees aging and in bottle after disgorging. However, to the authors' knowledge, no studies have investigated the impact of wine style or sugar addition used in the *dosage* on VOCs during bottle aging post-disgorging.

The disparity in the chemical nature and the concentrations of compounds in sparkling wines makes their analysis difficult [6]. Therefore, in this study, the focus was on eight VOCs at 5, 10 and 15 weeks after disgorging and *dosage* addition as well as standard chemical parameters, dissolved oxygen and foam. With the short growing season in Ontario, this study will show that *dosage* can also be used to create a unique product with complexity and flavour.

2. Materials and Methods

2.1. Sparkling Wine Treatments

The non-vintage (NV) sparkling wine was donated by Trius Winery, Niagara on-the-Lake, Ontario, Canada and disgorged on 26 February 2014 and analysed at 5, 10 and 15 weeks post-disgorging on 2 April, 7 May and 18 June 2014 respectively. The NV wine consisted of 70% Chardonnay, 28% Pinot noir and 2% Pinot Meunier. Of these, 42% was from 2011, 25% from 2010, 16% from 2009, 13% from 2008 and 4% from 2007. The base wine was bottled for second fermentation in March 2012 and wines therefore had approximately 2 years of aging on yeast lees prior to disgorging. The oaked Chardonnay (OC) was from 2012 vintage and fermented and then aged in French oak barrels for 12 months, while the unoaked Chardonnay (UC) was from the 2013 vintage and fermented in stainless steel tanks. The brandy (B) was purchased from Forty Creek distillery in Niagara, Ontario, Canada and the Icewine (IW) was produced from Vidal grapes from the 2012 vintage and donated by Trius Winery. The oldest wine used in the study was the *Blanc de noir* 2009 produced from Pinot noir grapes and bottled in February 2010 with approximately 4 years in contact with yeast lees. All wines were analysed for chemical parameters according to Iland et al. [7] standard industry practices.

Sparkling wine *dosage* treatments were: NV sparkling wine without sugar addition but with addition of the same wines zero *dosage* (ZD); NV sparkling wine + sugar (BS); unoaked still Chardonnay

wine + sugar (UC); Pinot noir 2009 sparkling wine + sugar (PN); Niagara produced Brandy + sugar (B); Icewine (IW). The control treatment in the sensory analysis was an oaked still Chardonnay wine + sugar (OC). Granulated cane sugar (Redpath Sugar Ltd., Toronto, ON, Canada) additions were; ZD = 0 g/L, BS = 299.6 g/L, UC = 299 g/L, OC = 273.1 g/L, PN = 299.6 g/L, IW = 76.8 g/L and B = 295.8 g/L. The control for chemical and volatile aroma analysis was ZD (without sugar) but the control for the sensory analysis was the OC because the composition of ZD without sugar addition made it unsuitable to be included in a difference test. After sugar addition, samples of each *dosage* were taken for standard wine chemical, and VOC analysis. Sulfur dioxide (SO_2) additions were made after analytical samples were taken to ensure each *dosage* had a free SO_2 level of 900 mg/L. Wines were analysed prior to, and after sugar addition to ensure a residual sugar level of 300 g/L in the *dosage* solutions to reach a residual sugar (RS) level of 8 g/L ($\pm$ 2 g/L) in each 750 mL bottle. Wines were bottled at the same pressure 6 atmospheres (atm) and the *dosage* solution addition in each bottle was 20 mL. Wines were disgorged by trained staff on a commercial disgorging line at Trius Winery, Niagara-on-the-Lake, Ontario and sealed with a cork closure and *muselet* (wire cage) before being transported immediately to the Cool Climate and Oenology Institute (CCOVI) at Brock University, Ontario. Prior to VOC analysis, wines were stored in a horizontal position in a specialised wine cellar at a temperature of 14 °C with a relative humidity of 70%.

2.2. Chemical Analyses

The wine used to make the individual *dosage* solutions, final *dosage* solutions and the sparkling treatment wines after disgorging/*dosage* addition were analysed at 5, 10 and 15 weeks for standard wine chemical parameters. Three bottles of the control and treatment wines were analysed in triplicate for pH, titratable acidity (TA g/L), alcohol (v/v %), residual sugar (RS g/L), free and total SO_2 and total phenolics (A.U.) [7]. All wines were degassed at room temperature (20 °C) prior to chemical and sensory analysis. Degassing was carried out using a vacuum filtration system that included an EMD Millipore 90 mm Glass Vacuum Filter Holder with a borosilicate glass funnel, base, tubulated cap, a PTFE-coated stainless steel screen and an anodised aluminum spring clamp (Millipore Inc., Etobicoke, ON, Canada). For alcohol levels (v/v %), three bottles, each one measured in duplicate, were analysed by a modified-method from Nurgel et al. [8] using a Gas Chromatographer coupled to a Flame Ionisation Detector (GC-FID). Method modifications included the GC-FID (Agilent 6890, Agilent Inc., Santa Clara, CA, USA) equipped with a DB wax column, a 7683B injector and the internal standard was 0.1% butanol. Residual sugar (g/L) was analysed using an enzymatic kit (Megazyme, Chicago, IL, USA) and total phenolics was measured according to Iland et al. [7] by absorbance reading (A.U.) at 280 nm. Dissolved oxygen (mg/L) levels in each treatment wine were analysed using a dissolved oxygen meter (DOM) from Hanna Instruments (Model 9146, Woonsocket, RI, USA).

2.3. Reagents, Chemicals and Standards

All volatile aroma compound standards and sparkling wine samples were prepared according to the method by Botezatu et al. [9]. Reference compounds and their suppliers can be found in Table 1. Milli-Q water was obtained from Biocel (Millipore Inc., Etobicoke, ON, Canada) and filtered through 0.22 µM filter (Millipore, Canada). All stock standard solutions (standard A) were prepared using ethanol (Chromasolv® HPLC grade, Sigma-Aldrich, Oakville, ON, Canada). From standard A, a composite standard solution was made and labelled as standard C which was used to prepare Standard 6′ as a working standard. Each reference compound was identified by their EI spectrum according to Enhanced ChemStation MSD (E.02.00.493)/Wiley spectral databases (NIST 08) and published literature. These compounds were also confirmed using qualifying and quantifying ions (Table 1). The purchased deuterated internal standards were analysed by EI-MS and matched to the GC-MS EI spectrum [9].

Table 1. List of volatile aroma compounds analysed, their aroma descriptors and odour thresholds.

Aroma Compound	Aroma Descriptors	Odor Threshold (µg/L)	Purity (%)	CAS No.	Supplier
d11 Ethyl hexanoate ISTD	N/A	N/A	98.7	2159-19-5	CDN Isotopes, Pointe-Claire, QC, Canada
Ethyl ester: Linear fatty acid derivatives					
Ethyl octanoate	Fruity, apricot, pineapple	580 [a]	>99	106-32-1	Sigma Aldrich
Ethyl hexanoate	Apple, blackberry	62 [d]	99	123-66-0	Sigma Aldrich
Ethyl butanoate	Acid fruit, candy, strawberry	20 [b] and 125 [d]	99	105-54-4	Sigma Aldrich
Ethyl esters: Branched acid derivatives					
Ethyl isobutyrate	Apple, citrus, tropical fruit	15 [c]	99	97-62-1	Sigma Aldrich
Ethyl isovalerate	Mint, fruit	3 [c]	98	108-64-5	Sigma Aldrich
Ethyl-2-methylbutyrate	Sweet fruit	18 [c]	99	7452-79-1	Sigma Aldrich
Alcohols					
2-Phenylethanol	Roses	14,000 [c]	99	60-12-8	Sigma Aldrich
1-Hexanol	Herbal, green, grass	8000 [b]	99.5	111-27-3	Sigma Aldrich

[a] Etiévant [10], odour thresholds determined in wine; [b] Guth et al. [11], odour thresholds determined in 10% ethanol/water solution; [c] Ferreira et al. [12], odour thresholds determined in 10% ethanol/water solution with 7 g/L glycerol at pH 3.2; [d] San Juan et al.[13] odour threshold determined in 10% ethanol/water solution at pH 3.2.

2.4. Sample Preparation of Wines for Volatile Aroma Compound (VOC) Analyses

Samples were prepared according to Botezatu et al. [9]. To a 20 mL amber round-bottomed glass vial, 3 g NaCl and a stir bar were added, followed by 8.06 mL of Milli-Q and 0.90 mL of wine for a 10-fold dilution. Finally, 40 µL of the deuterated internal standard ethyl hexanoate-d11 standard C was added and the vial was closed with magnetic screw cap immediately. The final dilution for the wine for VOC analysis was 10-fold. Samples were incubated at 40 °C and stirred at 600 rpm for 1 min before being exposed to the fiber for 30 min at 40 °C with stirring at 600 rpm.

2.5. Preparation of Volatile Aroma Compound (VOC) Standards

Volatile aroma standards were prepared according to Botezatu et al. [9]. To a 20 mL round-bottomed amber glass vials (MicroLiter, Millville, NJ, USA), 3 g of reagent grade NaCl (Bioshop, Burlington, ON, Canada) and a stir bar were added, then 8.06 mL of Milli-Q water and the matrix (14% hydro-ethanolic solution) and composite standards as indicated in Table 1. A volume of 40 µL of ethyl hexanoate-d_{11} solution C was added and the vial was capped with a magnetic screw/thread headspace cap PTFE/silicone (MicroLiter, Millville, NJ, USA) immediately.

2.6. Headspace Solid- Phase Micro-Extraction Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS)

The HS-SPME-GC-MS method from Botezatu et al. [9] was used to analyse VOCs. A 2 cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco Inc., Bellefonte, PA, USA), 23 gauge SPME fiber was used for sampling. Samples were incubated at 40 °C with a conditioned stir bar before exposing the fiber for 30 min at 40 °C at 600 rpm. The samples were analysed using an Agilent (Mississauga, ON, Canada) 7890A gas chromatograph coupled to a 5975C mass selective detector (MSD) equipped with a Gerstal MPS2 XL autosampler (Linthicum Heights, MD, USA). The GC was equipped with a Deans Switch and two columns: a HP-5MS 5% phenyl methyl siloxane column (30 m, 0.25 mm i.d., 0.25 µm film thickness) coupled with a secondary DB-Wax capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) (Agilent Technologies Inc., Santa Clara, CA, USA) . The liner was a SPME inlet liner (0.7 mm i.d.; Supelco). Helium was used as the carrier gas with a flow rate of 0.5 mL/min in the first column, and 1.5 mL/min in the second column. Oven temperature programming began at 35 °C for 3 min, and then increased 3 °C/min up to 105 °C where it was held for 10 min. Temperature was then increased by 2 °C/min up to 140 °C, before holding for 10 min.

Temperature went through one more ramp up of 4 °C/min up to 250 °C, before holding for a final 10 min. The run time for this method was 101 min. The MSD interface was held at 250 °C. The inlet temperature was 250 °C and the SPME fiber was desorbed in splitless mode. The solvent delay was 5 min. The fiber was prebaked for 10 min and post baked for 20 min. Samples were warmed at 40 °C and stirred at 600 rpm for 1 min before being exposed to the fiber for 30 min at 40 °C with stirring at 600 rpm, followed by desorption in the inlet for 10 min. Electron ionisation source was used, with a source temperature of 230 °C and electron energy of −70 eV. The samples were measured using synchronous scan and selected ion monitoring (SIM mode). The scan parameters ran from 35 m/z to 400 m/z, and both scan and SIM acquisitions were performed with an EMV Gain Factor of 7. All analyses were carried out in duplicate.

2.7. Data Processing of Volatile Aroma Compounds (VOCs)

VOCs were identified according to Botezatu et al. [9] using the ChemStation MSD (E.02.00.493) by Agilent, in addition to their authentic standards and concentrations found in current literature. The quantifying ions (Table 2) were extracted, and the ratio of the standard over the internal standard was plotted against the concentration of the VOC to fit a linear equation where the intercept was set to zero. Spiked samples were prepared to calculate the percent of recovery.

Table 2. Summary of parameters for volatile aroma compound quantification.

Volatile Aroma Compound	Retention Time (mins)	Target Ions (m/z)	Confirming Ions (m/z)	Standard Curve (R^2)
Ethyl hexanoate-d11—IS	26	91	50, 110	-
Ethyl esters: Linear fatty acid derivatives				
Ethyl butanoate	15.77	88	101, 60	0.9774
Ethyl hexanoate	26.8	88	115, 60	0.9746
Ethyl octanoate	41.31	88	101, 129	0.9936
Ethyl esters: Branched acid derivatives				
Ethyl Isobutyrate	13.57	11,243	71, 116	0.9954
Ethyl isovalerate	18.39	881.3	85, 130	0.9934
Ethyl 2-methylbutyrate	18.82	57,229	102, 130	0.9832
Alcohols				
1-Hexanol	22.87	56	55, 84	0.9678
2-Phenylethanol	50.62	92	88, 122	0.9294

2.8. Foam Analyses

Foam analysis was carried out using a video technique by Lynch and Bamford [14] with beer and Curioni et al. [15] with sparkling wine. Along with a timer, a high quality digital SLR camera with video capability (Canon EOS 70D, Canon Canada Inc., Mississauga, ON, Canada,) was used with a 250 mL glass volumetric cylinder free of scratches, faults, or marks. Each wine was opened and a waiting period of five minutes prior to pouring was imposed to reduce variability between samples. Once filming commenced, the bottle was held at a 45° angle and wine was poured into the top of the 250 mL volumetric cylinder and monitored until all foam diffused. The resultant film was analysed using Windows Media Player recording the time it took for the dissipation of foam (FDsec), the foam height between top of the foam and the underlying wine immediately upon pouring (FHcm), and the volume of the underlying wine after the foam had dissipated (mL). The room temperature was 20 °C and wines ranged in temperature from 18.5 °C to 19 °C to be close to the room temperature to avoid loss through gushing.

2.9. Sensory Analysis

Except for ZD wine, all wines were analysed using the A-Not A difference test [16]. The ZD wine was deemed unsuitable to include in a difference test because of a lack of sugar addition that would skew results. The A-Not A difference test can be superior to the triangle test, duo-trio

test, and same-difference test in terms of statistical power [17]. It is used to measure the overall sensory difference of one or more products from a reference sample A (OC—control) using a sureness rating [17,18]. Participants were asked to state on ballot papers whether the sample poured was A or, not A. The sureness rating was included to minimise the response bias of assessors. In this study, participants were asked to indicate how sure they were about their decision using a simple scale of very sure, sure, unsure and very unsure [19]. The sureness rating for "A" and for "Not A" responses were analysed using the *R*-index which represents the probability of participants distinguishing between the wines tasted [19,20].

The sensory panel consisted of 16 participants that included 13 Niagara winemakers and 3 oenology postgraduate students (10 males and 6 females), following ethics approval from the Research Ethics Board at Brock University (file #14-03) with clearance granted from 9/5/2014 to 9/30/2015. The sensory session began when wines (50 mL) were poured into clear ISO wine glasses and participants were asked to familiarise themselves with the wines retronasally. To improve the panelists' overall sensory perception of samples, a familiarisation session took place [17]. After 20 min of familiarising themselves with the wines, panelists had a 20-minute break and cleansed their palates with distilled water and unsalted crackers. All wine glasses were assigned a 3-digit number specific to each wine treatment and to each participant. Participants were also assigned 3-digit numbers to ensure anonymity. Following the familiarisation session, the A-Not A test was carried out according to Kemp et al. [19]. The wines were served in a randomised order per participant and no two assessors had the same order of wines. Each participant consumed unsalted crackers and distilled water between sample pairs. All treatment wines were tasted in duplicate to allow for sparkling wine bottle variation. Compusense 5.2 (Compusense, Guelph, ON, Canada) was used for responses. The room temperature was 20 °C with continuous airflow to prevent air contamination.

2.10. Statistical Analysis

Analysis of Variance (ANOVA), Tukey's Post-hoc test, Principal Component Analysis (PCA), Chi-squared test (χ^2), and standard deviations were calculated using XLSTAT Version 2014 software (Addinsoft, Paris, France). The *R*-index was calculated according to Kemp et al. [19].

3. Results and Discussion

3.1. Chemical Analyses

3.1.1. Chemical Parameters of the Wines Used as *Dosage* Bases before Sugar Addition

There was a statistically significant difference (p = 0.001) for all chemical parameters analysed between the wines that were used for making the *dosage* solutions (Table 3). B had the highest pH, alcohol and lowest acidity while PN had the highest acidity and the IW had the lowest alcohol level due to their respective methods of production. Residual sugar (RS g/L) levels were found to be highest in IW due to the fact that yeast only consume approximately half the juice sugar during fermentation with a legal requirement for Icewines to have a minimum residual sugar of 125 g/L. The lowest RS was found in the NV and Pinot noir wines likely due to completion of secondary fermentation in bottle. The highest free and total SO_2 was found in OC because SO_2 had already been added by the winery as part of the preparation for the *dosage* solution. The lowest free and total SO_2 was observed in the brandy. Total phenolic compounds were highest in OC wine (12 A.U.) and lowest in the NV sparkling wine (4.4 A.U.). No differences in total phenolic compounds in Charmat (secondary fermentation in tank) wines (Pinot noir (48%), Chardonnay (10%) and Riesling (42%)) when sugar was added at rates of 10 g/L, 20 g/L and 30 g/L was reported by Stefenon et al. [21]. These contradictory results could be attributed to the higher levels of phenolic compounds in the wines used as the base for *dosages* in our study, different analytical methods in the two studies and/or different sparkling wine production techniques used in each study.

Table 3. Chemical analyses of the wines used for *dosage* prior to sugar addition.

Chemical Analysis	NV Sparkling	Oaked Chardonnay	Unoaked Chardonnay	Pinot Noir 2009	Vidal Icewine	Brandy
pH	3.10 ± 0.01 ^{e}	3.32 ± 0.01 ^{d}	3.4 ± 0.01 ^{c}	3.1±0.01 ^{e}	3.6 ± 0.01 ^{b}	3.9 ± 0.02 ^{a}
TA (g/L)	8.40 ± 0.1 ^{b}	4.50 ± 0.1 ^{d}	6.5 ± 0.1 ^{c}	9.2 ± 0.1 ^{a}	8.2 ± 0.1 ^{b}	0.2 ± 0.20 ^{e}
Residual sugar (g/L)	0.40 ± 0.1 ^{b}	26.9 ± 2.6 ^{b}	1.0 ± 0.1 ^{b}	0.4 ± 0.1 ^{b}	223 ± 15.4 ^{a}	4.2 ± 0.10 ^{b}
Alcohol (% *v*/*v*)	12.30 ± 0.1 ^{d}	12.8 ± 0.1 ^{c}	13.1 ± 0.1 ^{b}	12.3 ± 0.1 ^{d}	10.5 ± 0.1 ^{e}	38.8 ± 0.10 ^{a}
Free SO_2 (ppm)	8.00 ± 1.0 ^{b}	864 ± 42 ^{a}	41 ± 3 ^{b}	5 ± 1 ^{b}	60 ±1 ^{b}	6 ± 1.00 ^{b}
Total SO_2 (ppm)	64.00 ± 2.1 ^{d}	959 ± 14 ^{a}	139 ± 6 ^{c}	55 ± 2 ^{d}	477 ± 25 ^{b}	7 ± 1.00 ^{e}
Total phenolics (A.U.)	4.40 ± 0.7 ^{d}	12 ± 0.89 ^{a}	5.3 ± 0.09 ^{d}	7.4 ± 0.10 ^{c}	8.4 ± 0.10 ^{c}	10.5 ± 0.39 ^{b}

All values are representative means of triplicate measurements (± = standard deviation of the means). ND = not detected and ± represents the standard deviation. Means identified by different letters were significantly different as determined by the post-hoc Tukey's test at $p < 0.05$.

3.1.2. Chemical Parameters of Wines at 15 Weeks after *Dosage* Addition

Treatment wines, after *dosage* addition, were analysed for their standard chemical parameters at 5, 10 and 15 weeks after *dosage* addition (Table 4 and supplementary data). Only chemical data at 15 weeks is presented here (Table 4). Wines that had *dosage* solution made from sparkling wines all had higher pH levels than those *dosage* solutions made from still wine additions. There was no difference in acidity (TA g/L) between treatment wines and, as expected, the ZD wine had low levels of residual sugar compared to the other wines. Alcohol level was highest in B due to the higher alcohol level in the brandy compared to the other treatments. The chemical composition of the wines that were used to make the *dosage* solutions before sugar addition were statistically significant ($p < 0.05$) probably due to their diverse production methods. However, the wines after *dosage* addition at 5, 10 and 15 weeks showed no difference in titratable acidity (TA g/L).

Table 4. Chemical analyses of treatment wines 15 weeks after disgorging with *dosage* addition.

Chemical Analysis	BS	ZD	OC	UC	PN	IW	B
pH	3.20 ± 0.02 ^{a}	3.35 ± 0.02 ^{b}	3.09 ± 0.01 ^{c}	3.09 ± 0.01 ^{c}	3.31 ± 0.01 ^{a}	3.08 ± 0.01 ^{c}	3.09 ± 0.01 ^{c}
TA (g/L)	8.2 ± 0.1 ^{a}	8.2 ± 0.1 ^{a}	8.0 ± 0.1 ^{a}	7.9 ± 0.1 ^{a}	8.0 ± 0.1 ^{a}	8.2 ± 0.2 ^{a}	7.9 ± 0.1 ^{a}
Residual sugar (g/L)	7.6 ± 0.1 ^{a}	1.1 ± 0.1 ^{d}	7.5 ± 0.2 ^{a,b}	7.7 ± 0.3 ^{a}	7.1 ± 0.1 ^{a,b}	6.3 ± 0.1 ^{c}	7.0 ± 0.3 ^{b}
Alcohol (% *v*/*v*)	12.4 ± 0.1 ^{b}	12.3 ± 0.1 ^{b}	12.4 ± 0.1^{b}	12.3 ± 0.1 ^{b}	12.3 ± 0.1 ^{b}	12.3 ± 0.1 ^{b}	12.9 ± 0.1 ^{a}
Free SO_2 (ppm)	5 ± 1 ^{a,b}	4 ± 1 ^{b}	5 ± 1 ^{a,b}	3 ± 1 ^{a}	4 ± 1 ^{b}	4 ± 1 ^{b}	4 ± 1 ^{b}
Total SO_2 (ppm)	54 ± 12 ^{a,b}	49 ± 6 ^{a,b}	59 ± 7 ^{a}	52 ± 1 ^{a,b}	48 ± 2 ^{b}	46 ± 1 ^{b}	53 ± 2 ^{a,b}
Total phenolics (A.U.)	1.4 ± 0.2 ^{a,b}	1.5 ± 0.2 ^{b}	1.8 ± 0.1 ^{a,b}	1.3 ± 0.1 ^{b}	1.8 ± 0.3 ^{a,b}	1.6 ± 0.1 ^{a,b}	2.1 ± 0.1 ^{a}

All values are representative means of triplicate measurements (± = standard deviation of the means). ND = not detected and ± represents the standard deviation. Means identified by different letters were significantly different as determined by the post-hoc Tukey's test at $p < 0.05$. NV sparkling wine + sugar (BS); NV sparkling wine zero *dosage* (ZD); oaked still Chardonnay wine + sugar (OC); unoaked still Chardonnay wine + sugar (UC); Pinot noir 2009 sparkling wine + sugar (PN); Icewine (IW) and Brandy + sugar (B).

3.1.3. Dissolved Oxygen

The two highest levels of dissolved oxygen (DO) were found in the both NV wines (ZD and BS), which suggests that these wines were more susceptible to oxidation than the other treatment wines. The lowest level was found in IW while the highest was in ZD (6.6 mg/L). ZD might have had less protection against oxidation than wines with sugar addition. The next highest DO was found in the BS at 5.0 mg/L, then UC (4.6 mg/L), OC (4.4 mg/L), B (3.6 mg/L), PN (3.5 mg/L), and the lowest level was in IW (3.1 mg/L).

3.2. Volatile Aroma Compounds (VOCs) in the Wines

Unsurprisingly, there were differences in the concentrations of VOCs across all wines used for the *dosage* solutions before sugar was added to them. B had the highest levels of ethyl ester linear fatty acid derivatives; ethyl hexanoate and ethyl octanoate, as well as an alcohol, 1-hexanol, but it also had the lowest concentration of 1-phenylethanol. Of the ethyl ester branched acid derivatives, ethyl

isobutyrate was found to be highest in PN, and ethyl isovalerate highest in the NV sparkling wine that was the wine all *dosage* solutions were added to but also used in two treatments (ZD and BS). The OC wine had the highest ethyl butanoate and 1-phenylethanol while the UC wine had the lowest concentration of 1-hexanol. Vidal Icewine had the lowest ethyl butyrate and Pinot noir 2009 had the lowest ethyl hexanoate and ethyl octanoate, possibly due to longer lees aging than the other wines, but the highest ethyl-2-methylbutyrate.

3.3. Volatile Aroma Compounds (VOCs) in the Dosage Solutions

No discernible trend could be observed within families of VOCs in the BS compared to the ZD although some differences in several VOC concentrations were observed between them (Figure 1). ZD *dosage* solution had higher levels of ethyl isobutyrate, ethyl-2-methylbutyrate, ethyl isovalerate, ethyl hexanoate and 2-phenylethanol compared to BS. Although the BS *dosage* had higher ethyl butanoate, ethyl octanoate and 1-hexanol compared to the ZD so the addition of sugar increased some compounds but decreased others.

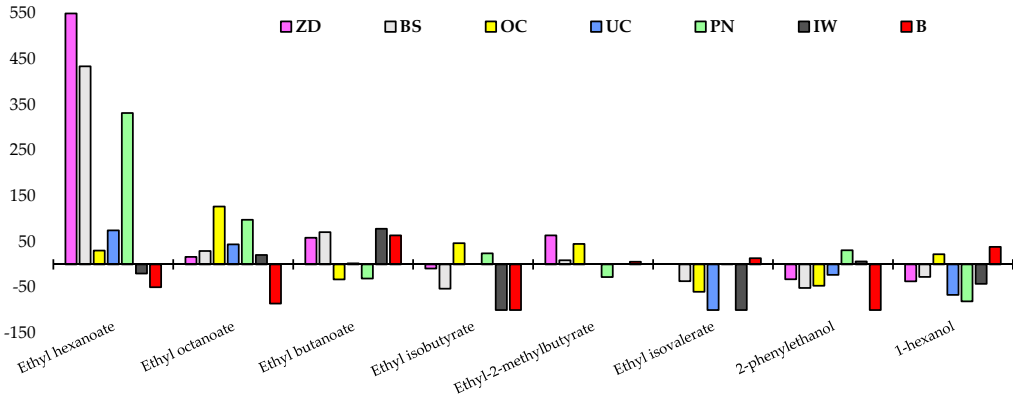

Figure 1. The increases and decreases of the mean concentrations (%) of each volatile aroma compound (VOC) between wines used for the *dosage* solutions. NV sparkling wine without sugar addition = zero *dosage* (ZD), NV sparkling wine + sugar (BS), unoaked still Chardonnay wine + sugar (UC), Pinot noir 2009 sparkling wine + sugar (PN), Brandy + sugar (B), Icewine (IW) + sugar and oaked still Chardonnay wine + sugar (OC).

Ethyl hexanoate increased in all *dosage* solutions except in B and IW, with the biggest increase found in ZD which did not have sugar added, although this change might have been due to bottle variation or oxygen ingress at disgorging. There was no 2-Phenylethanol detected in the B *dosage* solution even though prior to sugar addition it had a concentration of 5842 µg/L which is likely due to sugar addition at 300 g/L added to the high alcohol medium. Ethyl octanoate also increased in all *dosage* solutions except for B, whereas Ethyl butanoate decreased in PN and OC but not the other *dosages*. Ethyl isobutyrate only increased in PN and OC and decreased in the other *dosages*. Ethyl-2-methylbutyrate decreased in PN and increased in the other wines, but was not detected in UC. Ethyl isobutyrate, and ethyl isovalerate were not detected in UC and IW *dosage* solutions and ethyl isobutyrate was also not detected in B *dosage* solution. These results suggest that at levels of 300 g/L residual sugar in the actual *liqueur d'expedition* before its addition to each individual wine bottle, ethyl ester branched acid derivatives were negatively impacted in some wines. For 1-hexanol, it decreased in all *dosage* solutions except for B and OC, whereas 2-phenylethanol decreased in all *dosages* except for PN, and a very small (7%) increase in IW.

Upon comparison of BS and ZD, ethyl hexanoate increased in both *dosage* solutions along with ethyl octanoate, ethyl butanoate and ethyl 2-methylbutyrate and a decrease was observed in both treatments for ethyl isobutyrate, ethyl isovalerate, 2-phenylethanol and 1-hexanol. VOCs were affected similarly for both NV *dosage* solutions except that BS had higher percent of increase, and decrease of

compounds, except for ethyl hexanoate and ethyl-2-methyl butyrate which had higher percent increase in ZD than BS. The most likely explanation for the lack of specific trends in the decline and escalation of VOC concentrations in the *dosage* solutions is the physio-chemical composition of the wines, diverse grape varieties, and the range of wine styles, aging time and differences in production techniques of the wines used in the *dosage* solutions [22].

3.4. Volatile Aroma Compounds (VOCs) in Wines at 5, 10 and 15 Weeks Post-Disgorging

All wines were analysed for 8 VOCs (6 ethyl esters and 2 alcohols) over 15 weeks after disgorging, sugar addition and closure with cork. This section explains VOC differences in treatment wines over 15 weeks.

3.5. Five Weeks Post-Disgorging

At 5 weeks post-disgorging, BS had lower levels of alcohols than ZD. It was also higher in ethyl hexanoate and ethyl butanoate but lower in ethyl octanoate and ethyl isobutyrate than ZD (Table 5). All VOC concentrations in the treatment wines, except ethyl butanoate, were found to be significantly different statistically ($p < 0.05$) at 5 weeks post-disgorging. Ethyl-2-methylbutyrate was not detected in BS but was reported in Chardonnay sparkling wine enriched with glycosylated precursors at 3.3 µg/L even though in the German study sugar was not added after disgorging [23]. Differences in VOCs found in ZD compared to BS were detected in small concentrations at 5 weeks' post-disgorging which suggests that at levels of 8 g/L (±2) residual sugar, concentrations of alcohols and some ethyl ester branched acid derivatives were impacted. At this stage, ethyl isovalerate and ethyl hexanoate were found to be lower in ZD than BS. However, the introduction the *dosage* solution (+sugar) into the bottles increased ethyl isobutyrate, ethyl isovalerate, and ethyl hexanoate, whereas BS had the lowest ethyl octanoate. The high concentration of 2-phenyethanol (fermentation/yeast-derived VOC) in OC was likely due to extraction from oak during the production process of the wine used in the *dosage* solution [24]. UC had the lowest 1-hexanol and B had the lowest 2-phenylethanol. The leaf aldehyde, 1-hexanol, was found in the highest concentration in B, likely due to high concentrations in the brandy from the production method, and/or due to the ripeness level of the base material. Additionally, B had the lowest concentration of 2-phenylethanol.

PN wines had the highest ethyl isobutyrate, ethyl-2-methylbutyrate, and ethyl isovalerate, though the lowest ethyl hexanoate concentration. IW was found to have the lowest concentrations of ethyl isobutyrate, ethyl butanoate and ethyl-2-methylbutyrate. BS had the lowest ethyl octanoate and UC the lowest ethyl isovalerate. IW was found to have the lowest concentrations of ethyl isobutyrate, ethyl butanoate, ethyl-2-methylbutyrate and 1-hexanol.

Interestingly, B had the highest concentration of 4 of the 8 VOCs analysed: ethyl butanoate, ethyl hexanoate, ethyl octanoate and 1-hexanol. Of the monocarboxylic acid esters, the most important in brandy are those based on ethanol and saturated carboxylic acids, such as hexanoic (caproic), octanoic (caprylic) and decanoic (capric) acids [25]. In our study, the ranges of ethyl hexanoate and ethyl octanoate were 132–1687 µg/L and 349–23,429 µg/L, respectively, with B having the highest concentration of both compounds. Our results for these compounds are higher than those reported in Cava wines (produced from Chardonnay grapes), which is most likely due to the inclusion of brandy in one of our treatment wines [26]. However, it is unclear in the study as to whether a sugar *dosage* solution was added to the Cava wines. It is important to state that previous studies of sparkling wine VOCs have focused on base wines, aged wines, vineyard soil types, and grape maturity [26–29]. Few peer-reviewed studies on sparkling wines have stated the ingredients of the *dosage* solution, the time after disgorging that the VOC analysis took place or have examined sparkling wine aroma development post-*dosage* addition. Although Pérez-Magariño et al. [5] analysed VOCs in sparkling wines at 12 months post-disgorging, the wines did not have a sugar addition after disgorging (zero *dosage*), and were also produced from varieties not included in our study.

Table 5. Concentration of volatile aroma compounds (VOCs) in sparkling wines at 5, 10 and 15 weeks post-disgorging.

Compound (μg/L)	ZD	BS	OC	UC	PN	IW	B
5 weeks							
Ethyl esters: Linear fatty acid derivatives							
Ethyl hexanoate	164 ± 7 ^{F}	1177 ± 20 ^{B}	522 ± 27 ^{D}	905 ± 26 ^{C}	132 ± 2 ^{F}	352 ± 17 ^{E}	1687 ± 89 ^{A}
Ethyl octanoate	651 ± 3 ^{B}	339 ± 10 ^{C}	381 ± 10 ^{B,C}	460 ± 42 ^{B,C}	349 ± 5 ^{B,C}	666 ± 125 ^{B,C}	23,429 ± 157 ^{A}
Ethyl butanoate	41 ± 3 ^{A}	53 ± 4 ^{A}	57 ± 7 ^{A}	52 ± 3 ^{A}	39 ± 1 ^{A}	30 ± 2 ^{A}	69 ± 15 ^{A}
Ethyl esters: Branched acid derivatives							
Ethyl isovalerate	33 ± 12 ^{D}	117 ± 1 ^{B}	76 ± 1 ^{C}	13 ± 6 ^{D}	154 ± 2 ^{A}	61 ± 1 ^{C}	59 ± 2 ^{C}
Ethyl isobutyrate	69 ± 11 ^{A,B}	56 ± 3 ^{A,B}	52 ± 10 ^{B}	41 ± 9 ^{B}	86 ± 1 ^{A}	38 ± 8 ^{B}	62 ± 7 ^{A,B}
Ethyl-2-methylbutyrate	12 ± 1 ^{C}	ND	7 ± 1 ^{D}	12 ± 1 ^{B,C}	25 ± 2 ^{A}	0.4 ± 1 ^{E}	18 ± 2 ^{B}
Alcohols							
2-Phenylethanol	24,527 ± 453 ^{B}	12,901 ± 1281 ^{C}	51,502 ± 6662 ^{A}	13,448 ± 165 ^{C}	14,263 ± 531 ^{C}	14,124 ± 895 ^{B,C}	5826 ± 23 ^{C}
1-Hexanol	736 ± 57 ^{B}	556 ± 54 ^{B,C}	433 ± 36 ^{BC}	577 ± 50 ^{B,C}	423 ± 6 ^{B,C}	209 ± 10 ^{C}	1458 ± 253 ^{A}
10 weeks							
Ethyl esters: Linear fatty acid derivatives							
Ethyl hexanoate	164 ± 6 ^{D}	164 ± 7 ^{D}	503 ± 28 ^{B}	457 ± 6 ^{B,C}	131 ± 6 ^{D}	339 ± 17 ^{C}	1750 ± 89 ^{A}
Ethyl octanoate	651 ± 3 ^{B}	650 ± 2 ^{B}	374 ± 11 ^{B}	619 ± 4 ^{B}	353 ± 5 ^{B}	577 ± 125 ^{B}	23,540 ± 157 ^{A}
Ethyl butanoate	41 ± ^{A,B}	41 ± 3 ^{A,B}	61 ± 6 ^{A}	42 ± 3 ^{A,B}	40 ± 2 ^{A,B}	32 ± 2 ^{B}	58 ± 15 ^{A}
Ethyl esters: Branched acid derivatives							
Ethyl isovalerate	117 ± 2 ^{AB}	118 ± 1 ^{A,B}	77 ± 1 ^{A,B}	56 ± 0 ^{B}	156 ± 2 ^{A}	62 ± 1 ^{A,B}	101 ± 63 ^{A,B}
Ethyl isobutyrate	68 ± 11 ^{AB}	68 ± 11 ^{A,B}	45 ± 10 ^{B}	ND	86 ± 1 ^{A}	31 ± 11 ^{C}	56 ± 7 ^{A,B}
Ethyl-2-methylbutyrate	11 ± 0 ^{C}	12 ± 1 ^{C}	7 ± 1 ^{D}	ND	27 ± 2 ^{A}	ND	17 ± 2 ^{B}
Alcohols							
2-Phenylethanol	23,321 ± 1251 ^{C}	23,527 ± 354 ^{C}	56,213 ± 6662 ^{A}	37,182 ± 2958 ^{B}	13,887 ± 532 ^{C,D}	14,757 ± 895 ^{C,D}	5842 ± 23 ^{D}
1-Hexanol	647 ± 68 ^{B}	648 ± 67 ^{B}	468 ± 50 ^{B}	213 ± 2 ^{B}	131 ± 2 ^{B}	289 ± 109 ^{B}	1278 ± 253 ^{A}
15 weeks							
Ethyl esters: Linear fatty acid derivatives							
Ethyl hexanoate	165 ± 6.5 ^{D}	164 ± 7, ^{D}	498 ± 27 ^{B}	456 ± 5.5 ^{B,C}	131 ± 1.6 ^{D}	339 ± 17 ^{C}	1750 ± 89 ^{A}
Ethyl octanoate	648 ± 1 ^{A}	651 ± 3 ^{A}	374 ± 10 ^{A}	619 ± 4 ^{A}	353 ± 5 ^{A}	577 ± 125 ^{A}	23,441 ± 157 ^{B}
Ethyl butanoate	41 ± 3 ^{A}	41 ± 3 ^{A}	51 ± 7 ^{A}	51 ± 11 ^{A}	39 ± 1 ^{A}	32 ± 2 ^{A}	58 ± 15 ^{A}
Ethyl esters: Branched acid derivatives							
Ethyl isovalerate	116 ± 2 ^{B}	117 ± 1 ^{B}	77 ± 1 ^{C}	56 ± 11 ^{B}	153 ± 2 ^{A}	61 ± 1 ^{D}	56 ± 0 ^{B}
Ethyl isobutyrate	68 ± 12 ^{A,B}	69 ± 11 ^{A,B}	47 ± 10 ^{B}	ND	87 ± 1 ^{A}	44 ± 8 ^{B}	56 ± 7 ^{A,B}
Ethyl-2-methylbutyrate	12 ± 0 ^{C}	12 ± 2 ^{C}	7 ± 0 ^{D}	ND	27 ± 1 ^{A}	ND	17 ± 2 ^{B}
Alcohols							
2-Phenylethanol	24,558 ± 453 ^{B,C}	24,527 ± 3314 ^{B,C}	56,213 ± 662 ^{A}	32,747 ± 6662 ^{B}	13,888 ± 895 ^{C,D}	14,758 ± 531 ^{C,D}	5859 ± 23 ^{D}
1-Hexanol	647 ± 68 ^{B}	648 ± 68 ^{B,C}	408 ± 35 ^{B,C}	212 ± 1.2 ^{C}	427 ± 5.7 ^{B,C}	286 ± 109 ^{B,C}	1278 ± 253 ^{A}

NV without sugar addition zero *dosage* (ZD), NV sparkling wine + sugar (BS), unoaked still Chardonnay wine + sugar (UC), Pinot noir 2009 sparkling wine + sugar (PN), Brandy + sugar (B), Icewine + sugar (IW) and oaked still Chardonnay wine + sugar (OC). ND = not detected or below LODs and LOQs and ± represents the standard deviation. Means identified by different letters were significantly different as determined the post-hoc Tukey's test at $p < 0.05$.

3.6. Ten Weeks Post-Disgorging

By 10 weeks post-disgorging, there was little difference between BS and ZD, thereby indicating that the sugar had little impact on VOCs by this time (Table 5). However, between 5 and 10 weeks, BS increased in 2-phenylethanol, 1-hexanol, ethyl isobutyrate, ethyl-2-methylbutyrate and ethyl octanoate but decreased in concentrations of ethyl hexanoate and ethyl butanoate. It is evident that 2-Phenylethanol was lower in BS wines compared to ZD at 5 weeks though by 10 weeks was slightly higher in BS compared to ZD. ZD showed an increase in ethyl isovalerate between 5 and 10 weeks which BS lacked.

Ethyl ester concentrations at 10 weeks post-disgorging were within ranges found in previous studies that investigated VOCs in sparkling wines made from Spanish grape varieties [5]. Unlike the results at 5 weeks, by 10 weeks post-disgorging, concentrations of ethyl butanoate in the treatment wines was statistically significant ($p < 0.05$). This was likely due to the high concentration found in B. The results of the other linear fatty acid derivatives were also statistically significant and at 10 weeks post-disgorging, B again, had the highest concentrations of ethyl hexanoate and ethyl octanoate. PN had the highest concentrations of branched acid derivatives by 10 weeks post-disgorging, but also the lowest concentrations of linear fatty acid derivatives. These results may be attributed to PN being the

oldest sparkling wine used in our study, with the longest lees aging time and ethyl ester concentrations are known to change with extended lees aging [27]. The lowest concentration of ethyl butanoate was found in IW, which also had the lowest concentration at 5 weeks post-disgorging. There was a significant statistical difference in all VOCs ($p < 0.05$) at 10 weeks.

Regarding alcohols, the highest concentration of 2-phenylethanol was found in OC wines and the lowest was in B, similar to 5 weeks post-disgorging. The 1-hexanol concentrations at 10 weeks post-disgorging were statistically significant ($p < 0.05$) due to the high amount found in B.

3.7. Post-Disgorging at 15 Weeks

At 15 weeks there was little difference between the ZD and BS for any compounds studied likely due to sugar hydrolysis (Table 5). It is interesting to note that by 15 weeks the concentrations of VOCs in ZD and BS were similar to the Brut wine prior to sugar addition.

Ethyl hexanoate, ethyl octanoate and ethyl octanoate was found to be at the highest concentration in B and lowest in PN. Although there was not a statistically significant difference between wines for ethyl butanoate, Brandy has high ethyl ester concentrations and ethyl esters decline during sparkling wine lees aging. The Pinot noir wine used in the *dosage* was from the 2009 vintage so had spent 5 years aging on yeast lees which may account for the different amounts of ethyl hexanoate in the final sparkling wines at this stage. In ZD, BS and PN, all *dosage* solutions made from sparkling wines were found to have statistically similar concentrations of ethyl hexanoate.

Branched acid derivatives were at their highest in PN and all 3 VOCs were statistically significant ($p < 0.05$). IW had the were lowest ethyl isobutyrate and the lowest ethyl-2-methylbutyrate was found in OC. UC had the lowest 1-hexanol and B was found to have the highest concentration just as it was at 5 and 10 weeks post-disgorging. Additionally, B had the lowest concentration of 2-phenylethanol while OC had the highest in 2-phenylethanol. There were greater changes in VOCs between 5 and 10 weeks than between 10 and 15 weeks post-disgorging.

The PCA (Figure 2) at 15 weeks' post-disgorging shows that the 3 treatment wines that had *dosage* solutions made from sparkling wines (second fermentation in bottle) are placed in the positive sector. The BS and ZD wines are extremely similar due to their close proximity. Likewise, OC and UC are also close together but near to IW, and these 3 wines used still wines in the *dosage* solutions. The ethyl esters that dominated B wine are responsible for its position on the peripheral of the PCA. These results suggest that by 15 weeks post-disgorging, the production methods used to produce the wines in the *dosage* treatments influenced the differences in the VOC results.

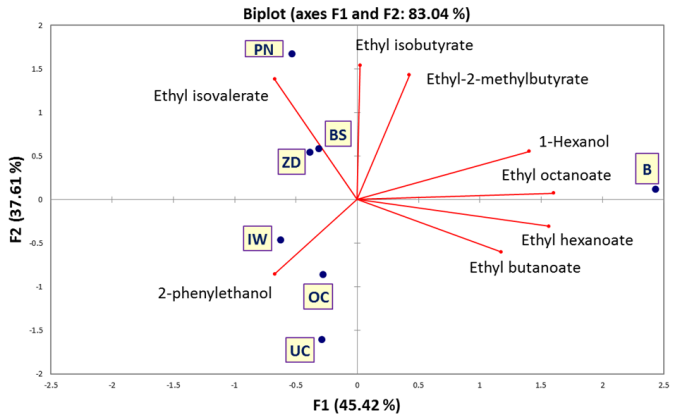

Figure 2. Principal Components Analysis (PCA) of the volatile aroma compounds (VOCs) in wines analysed at 15 weeks post-disgorging. NV sparkling wine without sugar addition = zero *dosage* (ZD), NV sparkling wine + sugar (BS), unoaked still Chardonnay wine + sugar (UC), Pinot noir 2009 sparkling wine + sugar (PN), Brandy + sugar (B), Icewine (IW) + sugar and oaked still Chardonnay wine + sugar (OC).

3.8. Odour Thresholds in Sparkling Wines

An 'absolute' or odour detection threshold is the concentration range below which the odour or taste of a substance is not detectable and above which individuals with a normal sense of smell or taste can readily detect the presence of the substance [22,30]. For populations, the detection threshold is defined as the concentration at which 50% of the group can detect the odorant [30]. The concentrations of ethyl isobutyrate, and ethyl isovalerate and ethyl hexanoate at 5, 10 and 15 weeks post-disgorging were above their respective odour thresholds (Tables 1 and 5). By 15 weeks, concentrations of 1-hexanol were below its odour threshold for all wines and ethyl-2-methylbutyrate only exceeded its odour threshold in PN. Ethyl octanoate was below its odour threshold in PN, OC and IW. By 15 weeks the 2-phenylethanol concentrations were above its sensory threshold except for the B and PN wines. Ethyl butanoate was above its odour threshold according to Guth [11], which was calculated in 10% water/ethanol. Conversely, San Juan et al. [13] used a 10% water/ethanol solution with a pH of 3.2 which resulted in its odour threshold increasing from 20 μg/L to 125 μg/L. Therefore, as wine is an acidic medium, we found that ethyl butanoate was below its odour threshold (125 μg/L) at all stages of analysis.

Another matrix effect within wine is referred to as an aroma buffer, and is caused by the numerous different VOCs found in wine. This aroma buffer effect describes how certain fusel alcohols, acids, esters and volatile phenols are not perceived separately within a wine [31]. This phenomenon is generally attributed to the presence of ethanol and a variety of fermentation-derived aromatic compounds. Since several compounds in alcoholic beverages are generally found coexisting as a result of fermentation, this causes the odour in question to be perceived as a mixture. Many aromatic compounds have similar odours and therefore, if one is lacking in concentration, the difference in the wine's profile would be negligible or undetectable by the average taster [31].

There are many factors that affect the concentrations of VOCs in sparkling wines including production techniques, grape variety, fining agents, yeast, ambient secondary fermentation temperature, type of sugar used during production, on-lees cellar storage temperature, *liqueur d'expedition* ingredients, oxygen ingress i.e., at disgorging, as well as closure type, SO_2 levels, carbon dioxide levels (CO_2) and ethanol levels. The impact of CO_2 on the perception of sugar and acidity in sparkling wine was confirmed by Thuillier [32]. Furthermore, increased CO_2 concentrations in sparkling wines resulted in increased intensity of mouthfeel attributes, with the attributes burn, bite, carbonation/bubble pain, and foamy showing the greatest differences in wines and after-numbing and tingly showing the least [33]. However, the impact of CO_2 and oxygen at disgorging on the concentrations of the VOCs studied is not known, yet both are likely to affect VOC concentrations as rising and collapsing bubbles act as continuous "portals" for volatile aromas in Champagne wine [34]. Additionally, carbonation has been reported to increase extremely volatile compounds i.e., ethyl butanoate, but not impact less volatile compounds, e.g., isoamyl acetate, due to a specific effect that is dependent on the physiochemical characteristics of each compound [35].

4. Foam Analyses

Protein concentrations, polysaccharides, alcohol levels, phenolic compounds and production processes all positively or negatively impact foam [2]. Due to different wine styles and production processes used to create the wines used as bases for the *dosage* solutions, we assessed the impact of the 20 mL *dosage* addition on the foam of the final sparkling wines. ZD had a foam height (FH) of 27.4 cm but the underlying wine volume in the glass, after the foam had dissipated, was 51 mL while BS foam height was 27.3 cm and underlying wine volume was 60 mL. Additionally, ZD took 168 seconds to dissipate (FD), whereas BS sugar was far quicker and dissipated in just 50 seconds indicating that without the 8 g/L of sugar addition ZD had better foam stability than BS. These dosages were NV wines, i.e., with the same wine in the *dosage* that was in the bottle, the only difference being sugar addition to BS. This difference in foam stability could be due to the increased viscosity of the wine with sugar addition that could have negatively impacted the foam.

The wine with the next highest foam stability was PN at 76 s, showing the benefit of using the oldest sparkling wine in the winery for *dosage* due to its positive impact on foam stability plus it had 27.1 cm of foam at pouring but 55 mL of underlying wine. OC wine took just 42 s for foam to dissipate and FH was 30 cm but it finished with the same volume of wine in the volumetric flask as PN. This may be due to a difference in compounds in Chardonnay wine compared to Pinot noir but were not analysed in this study, i.e., protein type and concentration. According to previous studies, Chardonnay sparkling wines were found to have a very small foam collar, a low foam height and short persistence, while Pinot noir sparkling wines exhibited higher height and longer persistence [36,37]. PN was the oldest sparkling wine and had extended time on yeast lees that could have released autolytic by-products such as yeast mannoproteins that are known to increase foamability [37]. Similar to the OC wines, B and IW had short foam dissipation times of 49 s and 43 s, but resulted in 54 mL and 57 mL of underlying wine but an FH of 28 cm and 28.3 cm respectively. B had the highest total phenolics after *dosage* addition and highest alcohol which might have contributed to these results because ethanol and phenolic compounds both influence foam [2]. Although the optimal concentration of total phenolic compounds in sparkling wine required to impact foam is unknown, the low level of 2.1 A.U. suggests that their impact on foam is negligible so ethanol level might have been responsible for the lower foam in B. The UC wine took 64 seconds to dissipate, had 28.3 cm of foam and resulted in 59 mL of underlying wine. Further in-depth foam analysis using a Mosalux system was not suitable for our trial as it is only used on base wines or degassed sparkling wines. A Computer Assisted Viewing Equipment (CAVE) system or the FIZZeyeRobot (robotic pourer) would have provided further insight into the foam but were not available for use in this study [38,39].

5. Sensory Difference Testing

The "A-Not A" results were analysed using the Chi-squared test (χ^2) at a significance level of 5% and resulted in a significant statistical difference between the wine samples ($p = 0.03$) [16]. An R-index score of 50% means that wines are deemed identical and a score of 100% means the wines were completely different and therefore not deemed suitable for a difference test [19]. The *R*-index of 73% in our study for the "A" samples demonstrates a clear difference between the sensory control (OC) and the other treatment wines. Quantitation of attributes using descriptive sensory analysis would provide further information as to how the wines differ with regards to their sensory flavour characteristics.

There were clear differences between wines produced using different wine styles in the *dosage* solutions with an addition of 20 mL of *dosage* solution for a target residual sugar level of 8 g/L. From 5 to 15 weeks, higher alcohols were more affected by sugar additions than ethyl esters except for ethyl hexanoate, although some initial differences in volatile aroma compounds (VOCs) were observed in the zero-*dosage* wine compared to the same wine without sugar addition (BS). However, these had vanished by 15 weeks post-disgorging and both had similar concentrations of VOCs. Further investigation into higher sugar concentrations in traditional method sparkling wines may result in greater differences than observed in our study. These results provide evidence to wineries of the need to withhold sparkling wines from release immediately after disgorging and until wines reach equilibrium. CO_2 impacts aroma detection in sparkling wines but as foam subsides in the glass, its presence or absence within sensory threshold ranges become more noticeable. Therefore, future research should involve additional families of VOCs, i.e., aldehydes, fatty acids, as well as descriptive sensory analysis every six months post-disgorging. A range of sugar levels in the same wines as well as blends of wine styles in the *dosage* solutions necessitates further investigation with regards to their influence on foam and sensory characteristics. Our study did not specifically separate the influence of CO_2 from sugar addition effects, thus further studies could focus on the combined influence of CO_2 and sugar on volatile aroma compounds. Future research could include in-depth analysis of residual enzymes from yeast cell lyses. Results could conceivably be used to characterise sparkling wines that have been produced using *dosage* solutions made using wines from a range of diverse production methods.

Supplementary Materials: The following are available online at http://www.mdpi.com/2306-5710/3/1/7/s1, Table S1: Chemical composition of ZD sparkling wines: 5 and 10 weeks post-disgorging, Table S2: Chemical composition of BS sparkling wines: 5 and 10 weeks post-disgorging, Table S3: Chemical composition of OC sparkling wines: 5 and 10 weeks post-disgorging, Table S4: Chemical composition of UC sparkling wines: 5 and 10 weeks post-disgorging, Table S5: Chemical composition of PN sparkling wines: 5 and 10 weeks post-disgorging, Table S6: Chemical composition of IW sparkling wines: 5 and10 weeks post-disgorging, Table S7: Chemical composition of B sparkling wines: 5 and 10 weeks post-disgorging.

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Author Contributions: Belinda Kemp conceived the idea for the study, designed the experiment, carried out the foam analysis, carried out the sensory analysis and sensory data analysis, and wrote the majority of this paper. Casey Hogan carried out all the standard chemical analyses and volatile aroma analyses. Shufen Xu assisted with the volatile aroma development method and wrote the HS-SPME-GC-MS methods section of the paper. Lisa Dowling assisted with the foam analysis, analysed the digital data and contributed to writing the paper. Debbie Inglis was the Principal Investigator, bringing all the researchers together with the industry partner, overseeing the research design and the main supervisor for Casey Hogan, contributed to writing the paper and is the Director of the Cool Climate Oenology and Viticulture Institute (CCOVI).

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

IS	Internal standard
NV	Non-vintage wine produced from a selection of base wines from a range of years
VOCs	Volatile aroma compounds

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