Influence of cane and beet sugar for second fermentation on “fruity” aromas in Auxerrois sparkling wines.

Andrew Wilson[1], Hannah Charnock[1], Shufen Xu[2] and Belinda Kemp[1, 2]

[1]Department of Biological Sciences, Faculty of Mathematics and Science, Brock University, 1812 Sir Isaac Brock Way, St. Catharines, ON, Canada, L2S 3A1.

[2]Cool Climate Oenology and Viticulture Institute (CCOVI), Brock University, 1812 Sir Isaac Brock Way, St. Catharines, ON, Canada, L2S 3A1.

*Corresponding author: Bkemp@brocku.ca

Abstract

Traditional Method sparkling wine production requires a sugar addition to the base wine to initiate the second alcoholic fermentation in bottles. This study aimed to identify differences in “fruity” volatile aroma compounds (VOCs) in Traditional Method sparkling wines produced from the addition of either cane sugar or beet sugar to Auxerrois base wines. Wines underwent a second fermentation in bottles inoculated with IOC 18-2007 yeast and fermented at 15 °C. Standard chemical analysis was carried out on base wines and sparkling wines. The concentrations of fourteen “fruity” volatile aroma compounds representing five classes of compounds were analysed by Headspace-Solid-Phase Micro-Extraction-Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS). Cane and beet sugars were analysed in de-aromatised wine and distilled water to establish the concentrations of VOCs present in the sugar products prior to addition to wine. Wines were analysed on the day of inoculation and bottling and again after the second fermentation. Beet sugar significantly ($Pt < 0.05$) increased the concentration of linear fatty acid-derived ethyl esters (ethyl octanoate, ethyl hexanoate, and ethyl butyrate) compared to cane sugar in sparkling wine. These results are attributed to higher concentrations of medium-chain fatty acids found in beet sugar due to the duration of sugar beet storage prior to processing. Recommended future research includes

monitoring aroma compounds during ageing on lees, sensory analysis, and an investigation of a wider range of sugar products permitted for use in sparkling wine production.

Keywords Sparkling wine, sugar, volatile aroma compounds, medium-chain fatty acids, ethyl esters.

Introduction

Auxerrois is a *Vitis vinifera* grape variety that is believed to have been spontaneously crossed between two cultivars of *V. vinifera* in Northern France (Bowers *et al.,* 2000; Bowers *et al.,* 1999) following analysis of microsatellite loci and comparison of the microsatellite genotypes within sets of cultivars. Auxerrois is often referred to as the offspring of Pinot noir and Gouais blanc variety (Bowers *et al.,* 2000), although these are the proposed parents and not definitively proven (Bowers *et al.,* 2000). Gouais blanc is an ancient cultivar that has effectively disappeared from the wine world after several unsuccessful attempts at banning the variety (Bowers *et al.,* 1999). Despite this, Gouais Blanc is responsible for creating many cultivars today due to its widespread planting in Medieval France (Bowers *et al.,* 1999). Auxerrois is a primary component in the production of Crémant d'Alsace and has been shown to have cold tolerance and disease resistance. Few studies exist that have focused on Auxerrois grapes and wines, and there is an absence of studies concerning Auxerrois wine aroma compounds.

Sparkling wines are produced using four principal methods: forced carbonation, pressurised Charmat tank, Transfer method, and Traditional Method/Méthode Champenoise. The Traditional Method of sparkling wine production involves the second alcoholic fermentation taking place in a bottle—the same bottle that will eventually be sold to the customer (Kemp *et al.,* 2015). To achieve this, it is necessary to acclimatise the yeast to the high acid, high alcohol, and low pH environment. This involves the addition of a highly concentrated syrup made with base wine, yeast and sugar *(Liqueur de Tirage)* to the base wine. This process occurs in several stages: rehydration, acclimation, and proliferation (Kemp *et al.,* 2020). The sugar required to carry out the secondary fermentation is 22 - 23 g/L of sucrose, which will achieve approximately six atmospheres of pressure in the finished wine (Kemp *et al.,* 2015, 2020).

Sugar source is a topic that has been gathering interest in producing consumer food products (Urbanus *et al.,* 2014a). Sensory studies by Urbanus *et al*. (2014a & b) found that cane and beet sugars are discernible sensorially. However, the type of sugar used in the production of sparkling wine has rarely been considered in published studies. Sugar can be added at three stages of sparkling wine production; 1) To increase potential alcohol in the juice before fermentation (where

permitted), 2) To base wine for the second alcoholic fermentation *(Liqueur de tirage)*, and to sparkling wine to achieve the desired balance and flavour *(Liqueur de dosage)*.

In Canada, raw cane sugar is imported, then processed in sugar refineries and recrystallised (Canadian Sugar Institute, 2021). Unlike cane sugar, beet sugar does not require milling; rather, sugar beets are sliced into noodle-like “cossettes”, which soak in water to attain the sugar. After pressing, the cossettes are separated from the sugar-water mixture and the water is removed by evaporation so sugar crystallisation can occur (Food and Agriculture Organisation (FAO), 1999). Sugar beets are typically stored before processing, resulting in elevated levels of medium-chain fatty acids (MCFAs) in the final sugar (Batista *et al.*, 2002). MCFAs are intermediates in the biosynthesis of long-chain fatty acids and fatty acid toxicity in wine increases as pH decreases (Malherbe *et al.*, 2007; Restrepo *et al.*, 2019). During fermentation, MCFAs have been shown to have negative effects on fermentation, with decanoic acid exhibiting greater inhibitory effects on yeast growth compared to octanoic acid (Bauer & Pretorius, 2000; Malherbe *et al.*, 2007; Restrepo *et al.*, 2019). Therefore, the use of beet sugar for increasing potential alcohol in primary fermentation could be undesirable. Based on stable carbon isotope analysis, it was stated that many European countries, as well as Chile, use beet sugar for the second alcoholic fermentation while Brazilian and Argentinian wineries use cane sugar (Martinelli *et al.*, 2003).

Wine aroma is a complex matrix of many diverse aroma compounds and is influenced by grape variety, winemaking practices/decisions (including yeast strain, nutrient type) and duration of ageing (Guth, 1997; Torrens *et al.*, 2008; González-Álvarez *et al.*, 2012; Kelly *et al.*, 2020). Aroma compounds are classified according to their chemical structure, e.g., higher alcohols/fusel alcohols are compounds with more than two carbon atoms (Cordente *et al.*, 2021). These higher alcohols are derived from yeast amino acid metabolism via the Ehrlich pathway, which consists of three steps: the initial transamination of the amino acid to the corresponding α-keto acid analogue, decarboxylation to an aldehyde, and reduction to the corresponding higher alcohol by the enzyme alcohol dehydrogenase (Hazelwoood *et al.*, 2008; Cordente *et al.*, 2021). Higher alcohols are also substrates for acetate ester production, a reaction initiated by yeast alcohol acetyltransferases (Cordente *et al.*, 2021). Acetate esters impart ‘fruity’ or solvent flavours to wine (Lyons *et al.*, 2021). Esters are the most abundant VOCs in wine, are synthesised from the condensation of an alcohol and a carboxylic acid, and in wine are either ethyl esters (ethanol condensing with a fatty acid), or acetate esters (an alcohol condensing with acetic acid) (Rice *et al.*, 2019; Longo *et al.*, 2021). Ethyl esters also impart “fruity” characteristics to wine and are produced by yeast

metabolism of MCFA precursors (Lyons *et al.*, 2021). Aroma compounds that contribute fruity characteristics to wine and their respective detection thresholds can be found in Table 1. This preliminary study aimed to identify differences in "fruity" VOCs in sparkling wines produced from the use of either cane sugar or beet sugar added to the base wine for the second fermentation of Auxerrois wines.

Materials and methods

1. Experimental design

The sparkling base wine (20 L), produced from Auxerrois grapes, was donated by Oxley Estate Winery, Ontario, Canada. Six 750 mL bottles were fermented with the addition of 24 g/L of cane sugar to the base wine (ACS) and six with 24 g/L of beet sugar addition (ABS). Both sugars were processed and supplied by Lantic Rogers (Taber, Alberta, Canada). The cane sugar was produced from imported sugar cane and beet sugar from beets grown in Alberta, Canada. The base wines were chemically analysed before yeast acclimation (base wine), on the first day of the second fermentation (immediately after bottling (Day 0)), and at the end of the second fermentation (eight weeks post-bottling/second fermentation). Analysis of VOCs occurred on the day of *Liqueur de tirage* addition (Day 0) and post-fermentation once wines had reached dryness. Dryness was determined by residual sugar analysis and pressure level. To establish differences between the "fruity" VOCs of the cane and beet sugar products themselves, 8 g/L of each sugar was prepared in de-aromatised wine (10 % v/v ethanol) and deionised water in triplicate and transferred to 20 mL amber glass vials (MicroLiter, Millville, NJ, USA), according to the sample preparation technique used by Botezatu *et al.*, (2016) for analysis by HS-SPME-GC-MS. The decision to use 8 g/L of sugar to establish VOC concentrations in each sucrose product prevented the overloading of peaks and interference from sugars during the HS-SPME-GC-MS analysis.

2. Base wine chemical analysis

Sparkling base wines from the 2020 vintage were cold stabilised at –2 °C for four weeks with an addition of 1 g/L cream of tartar, then filtered to 0.45 µm using a SUPRAdisk lenticular filter (Scottlabs, Niagara-on-the-Lake, Ontario, Canada) before 20 L was transferred in a glass carboy from Oxley Estate Winery to the Cool Climate Oenology and Viticulture Institute (CCOVI), Brock University, Ontario, Canada. The free SO_2 was maintained at 20 mg/L and wines were stored at 10 °C until bottling. YAN (mg N/L) was adjusted to ensure a level of 60 mg N/L using Fermoplus Intergrateur (AEB, Lodi, California, USA). Yeast acclimation for the second alcoholic fermentation

was carried out according to Kemp *et al*., (2020) with IOC 18-2007 yeast and 23 g/L of sugar added to the base wine the day prior to bottling. 730 mL of base wine was added to 750 mL sparkling wine bottles, and then 20 mL of the yeast, wine and sugar solution (*Liqueur de tirage)* was added. A pneumatic crown capper (Air-Matic-1 2016 Zambelli Enotech, (Camsiano, Italy) attached to a Mastercraft® 3 gallon air compressor (Canadian Tire® St. Catharines, Ontario, Canada) was used to seal the bottles with crown caps. Scelnox crown caps (29 mm) and had an SM802 closure (Nuance Winery Supplies, St Catharines, Ontario, Canada). The plastic closure (bidule) conformed to AFNOR NF H 35-022 and NF H 35-029 standards. Bottles were stored horizontally in the dark at a temperature of 15 °C to complete the second fermentation.

3. Wine chemical analysis

Free and total sulphur dioxide (SO_2) levels were carried out using the aspiration method according to Iland *et al*., (2017). Titratable acidity (TA g/L) and pH was analysed by Hanna Instruments Autoitrator (HI 84502 Woonsocket, USA) using pH buffers 4.0, 7.0 and 8.2. Analysis of residual sugar (g/L fructose and glucose), primary amino nitrogen (mg N/L), ammonia (mg N/L), L-malic acid (g/L), and acetic acid (g/L) were carried out using Megazyme® assay kits (K-FRUGL, K-PANOPA, K-AMIAR, K-LMAL, and K-ACET {Bray, Ireland}), and an Ultrospec 3100 Pro Spectrophotometer (Cambridge, England). Alcohol (% v/v) was analysed by Gas Chromatography-Flame Ionization Detection (GC-FID) according to the method of Nurgel *et al*., (2004).

4. Head Space-Solid Phase Micro-Extraction Gas-Chromatography-Mass Spectrometry (HS-SPME-GC-MS)

4.1 Reagents, chemicals, and standards

All aroma standards, sparkling wine samples and sugar standards (cane and beet sugar) were prepared according to the method by Botezatu *et al*. (2016). 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 a 0.22 µM filter (Millipore, Canada). Individual standard stock solutions at 1000 ppm were prepared in anhydrous ethanol. From individual stock solutions, a composite standard solution was prepared with deionised water with optimised concentration levels for each analyte. The composite was subsequently diluted (Standard 6') and prepared in de-aromatised wine as a working standard to prepare Standards 1-6. Additional working standards were prepared at higher concentrations (Standard 7', Standard 8') to encompass higher volatile levels in several samples and were used to prepare their respective standards (Standard 7,

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Standard 8). Each reference compound was identified by its 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 3). The purchased deuterated internal standards were analysed by EI-MS and matched to the GC-MS EI spectrum (Botezatu *et al.*, 2016).

4.2 Sample preparation of wines for volatile aroma compound (VOC) analyses

Samples were prepared according to Botezatu *et al.*, (2016). The wine was degassed by filtering it through Fisher Scientific® P8 filter paper using a Sentino® pump (New York, USA). To a 20 mL amber glass vial, 3 g NaCl, followed by 8.51 mL of Milli-Q and 0.45 mL of wine. Finally, 40 µL of the deuterated internal standard ethyl hexanoate-d11 standard was added and the vial was closed with a screw cap immediately. The final dilution for the wine for VOC analysis was 20-fold.

4.3 Preparation of volatile aroma compound (VOC) standards

Volatile aroma standards were prepared according to Botezatu *et al.* (2016) and Kemp *et al*. (2017). Standards were prepared by first creating a composite standard of target VOCs and diluting it with Milli-Q water, then preparing standards 6', 7' and 8' from the composite to prepare the respective standards as outlined in Table 2. To each 20 mL amber glass vial (MicroLiter, Millville, NJ, USA), 3 g of reagent grade NaCl (Bioshop, Burlington, ON, Canada), 8.51 mL of Milli-Q water, de-aromatised sparkling wine matrix, and standard 6', 7' or 8' were added. The matrix was made to ensure the different standards remained consistent and was prepared by adding 0.2 mL of 15.3 % alcohol in 8 mL of de-aromatised wine. De-aromatised wine samples were obtained by evaporating 750 mL of sparkling wine using a rotary evaporator. The residue was dissolved in deionised water and anhydrous reagent grade ethanol and prepared to 10 % v/v ethanol. Using a rotary evaporator to de-aromatise wine is an established academic practice (Geffroy *et al.*, 2020). A volume of 40 µL of ethyl hexanoate-d11 solution C was added and the vial was capped with a screw/thread headspace cap PTFE/silicone (MicroLiter, Millville, NJ, USA) immediately.

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179 Table 1. The fourteen aroma descriptors, odour threshold (µg/L), stock solution purity, chemical
180 CAS no., and supplier details of the standards.

Aroma compound	Aroma descriptors	Odour threshold (µg/L)	Purity (%)	CAS no.	Supplier
Ethyl ester: Linear fatty acid derivatives					
Ethyl octanoate	Fruity, apricot, pineapple	2[a]	>99	106-32-1	Sigma Aldrich
Ethyl hexanoate	Apple, blackberry	62[d]	99	123-66-0	Sigma Aldrich
Ethyl butyrate	Candy, Strawberry	20[a] and 125[c]	99	105-54-4	Sigma Aldrich
Ethyl decanoate	Fruity, pleasant	200[b]	>99	110-38-3	Sigma Aldrich
Ethyl esters: Branched acid derivatives					
Ethyl-2-methylbutyrate	Sweet, Fruit	18[b]	99	7452-79-1	Sigma Aldrich
Ethyl isovalerate	Berry, Blackberry	3[a]	>98	108-64-5	Sigma Aldrich
Ethyl isobutyrate	Fruity, Banana	15[b]	99	97-62-1	Sigma Aldrich
Ethyl lactate	Fruity, Buttery	150000[d]	>98	97-64-3	Sigma Aldrich
Alcohols					
1-Hexanol	Herbal, green, grass	8000[a]	99.5	111-27-3	Sigma Aldrich
2-Phenylethyl ethanol	Roses	14000[b]	99	60-12-8	Sigma Aldrich
Acetates					
Ethyl acetate	Pineapple, Fruity, Balsamic	7500[a]	>99.5	141-78-6	Sigma Aldrich
Hexyl acetate	Apple, Cherry, Pear, Floral	670[d]	99	142-92-7	Sigma Aldrich

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Isoamyl acetate	Banana, Fruity	30[a]	>97	123-92-2	Sigma Aldrich
2-Phenylethyl acetate	Floral, Honey	250[a]	>98	103-45-7	Sigma Aldrich

181 *[a]Guth et al. odour thresholds determined in 10 % ethanol/water solution; [b]Ferreira et al., odour thresholds determined*
182 *in 10 % ethanol/water solution with 7 g/L glycerol at pH 3.2; [c] San Juan et al. odour threshold determined in 10 %*
183 *ethanol/water solution at pH 3.2, [d]Peinaldo et al. odour thresholds determined 10 % ethanol/water solution adjusted to*
184 *pH 3.5 with tartaric acid.*

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186 Table 2. A summary of the preparation of standards.

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Standards	Vol of Std 6' (mL)	Vol DI Water (mL)	Vol Matrix (mL)	Internal Std (mL)	Total volume (mL)
188 Std 1	0	8.51	0.45	0.04	9
Std 2	0.04	8.51	0.41	0.04	9
189 Std 3	0.08	8.51	0.38	0.04	9
Std 4	0.15	8.51	0.3	0.04	9
Std 5	0.30	8.51	0.15	0.04	9
190 Std 6	0.45	8.51	0	0.04	9
191 Std 7	0.45*	8.51	0	0.04	9
Std 8	0.45**	8.51	0	0.04	9

192 ** Prepared from Std 7'; ** Prepared from Std 8'.*

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194 **4.4 Headspace Solid-Phase-Micro-Extraction-Gas Chromatography-Mass Spectrometry (HS-**
195 **SPME-GC-MS) method**

196 The HS-SPME-GC-MS method for VOCs was modified from Hjelmeland *et al.* (2013)
197 and Botezatu *et al.* (2016). A 2 cm divinylbenzene/carboxen/polydimethylsiloxane
198 (DVB/CAR/PDMS) (Supelco Inc., Bellefonte, PA, USA), 23-gauge SPME fiber was used for
199 sampling. The samples were analysed using an Agilent (Mississauga, ON, Canada) 7890B gas
200 chromatograph coupled to a 5977B Inert Plus mass selective detector (MSD) equipped with a PAL
201 RSI 85 autosampler (Zwingen, Switzerland).

202 The GC was equipped with a 6 % cyanopropylphenyl and 94 % dimethylpolysiloxan DB-624Ultra
203 Inert column (30 m, 0.25 mm i.d., 1.4 µm film thickness) (Agilent Technologies Inc., Santa Clara,
204 CA, USA). The liner was a SPME inlet liner (0.7 mm i.d.; Supelco). Helium was used as the carrier
205 gas with a flow rate of 1.0 mL/min. Oven temperature programming began at 40 ºC for 5 minutes
206 and then increased 3 ºC /min up to 180 ºC. Temperature was then increased by 30 ºC /min up to 240
207 ºC before holding for 1 minute. The run time for this method was 54.667 minutes. The inlet

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208 temperature was at 250 ºC and the SPME fiber was desorbed in split mode (20:1 ratio) with a flow
209 rate of 20 mL/min. The solvent delay was 3.5 minutes. The fiber was prebaked for 15 minutes and
210 post baked for 5 minutes at 270 ºC. Samples were warmed at 40 ºC and agitation at 600 rpm for 1
211 minute before being exposed to the fiber for 30 minutes at 40 ºC with agitation at 600 rpm,
212 followed by desorption in the inlet for 10 min. An electron ionisation source was used, with a
213 source temperature of 230 ºC and electron energy of −70 eV. The MSD transfer line was set at 240
214 ºC. The samples were measured using synchronous scan and selected ion monitoring (SIM mode).
215 The scan parameters ran from 40 m/z to 300 m/z; both scan and SIM acquisitions were performed
216 with an EMV Gain Factor of 2. All analyses were carried out in duplicate. Target and confirmation
217 ions for each compound are listed in Table 3.

218 For the purpose of this study, base wine VOC analysis refers to wines that were analysed after
219 sugar, yeast, and nutrient addition *(Liqueur de tirage)* on the day of inoculation and bottling.
220 Sparkling wines refer to bottles that underwent VOC analysis eight weeks after bottling when the
221 second fermentation was complete.

222 Table 3. Retention times (minutes), target ions (m/z), confirming ions (m/z), and standard curve
223 (R^2) of the volatile aroma compounds analysed.

Aroma compound	Retention Time (mins)	Target Ion (m/z)	Confirming Ion (m/z)	Standard Curve (R^2)
Ethyl hexanoate-d11 - Internal standard	26	91	50, 110	-
Ethyl ester: Linear fatty acid derivatives				
Ethyl octanoate	41.30	88	101, 129	0.9417
Ethyl hexanoate	26.8	88	115, 60	0.9882
Ethyl butyrate	15.77	88	101, 60	0.9785
Ethyl decanoate	45.60	88	101, 60	0.9164
Ethyl esters: Branched acid derivatives				
Ethyl-2-methylbutyrate	18.82	57	102, 130	0.9898
Ethyl Isovalerate	20.29	88	85	0.9960
Ethyl Isobutyrate	14.54	41	60, 88, 101	0.9267
Ethyl Lactate	19.21	45	43, 75	0.9219
Alcohols				
1-Hexanol	22.87	56	55, 84	0.9921
2-Phenylethyl alcohol	50.62	92	88, 122	0.9831
Acetates				

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Ethyl acetate	6.92	88	70, 61, 43	0.9958
Hexyl acetate	29.79	43	56, 73, 84	0.9827
Isoamyl acetate	21.86	43	87, 73	0.9962
2-Phenylethyl acetate	43.48	104	91, 43	0.9908

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225 **4.5 Statistical Analysis**

226 VOCs were identified by matching their spectrum to the NIST library (Botezatu *et al.,* 2016) using
227 the OpenLAB CDS (Version 2.4.5.9 by Agilent), in addition to their authentic standards. The
228 quantifying ions (Table 3) were extracted, and the ratio of the standard over the internal standard
229 was plotted against the concentration of the VOC to fit a linear equation where the intercept was set
230 to zero. Statistical analysis of data included Student T-Tests and standard deviations carried out
231 using XLSTAT (Version 3. 2017, Addinsoft, Paris, France). Odour activity values (OAV) of each
232 compound analysed in the wines after the second fermentation were calculated by dividing the
233 concentration of each compound by its odour threshold, detailed in Table 1 (Duan *et al.,* 2015).

234 ## Results

235 Base wines used for sugar additions did not differ in chemical composition because they were from
236 the same base wine that was divided into two equal volumes. The base wine chemical parameters
237 were as follows; 9 g/L titratable acidity (TA), pH 3.3, 2 g/L of residual sugar, YAN 13 mg N/L, 9.8
238 % v/v alcohol, 4.5 g/L of malic acid and 0.2 g/L acetic acid. The cane and beet sugars were also
239 analysed for the same volatile aroma compounds as the wines (Table 4). Significant differences
240 were found in the de-aromatised wine for beet sugar compared to cane sugar for two linear chain
241 fatty acid derivative ethyl esters: ethyl hexanoate *(*Pt < 0.05) and ethyl butyrate (Pt < 0.05); two-
242 branched acid derivative ethyl esters: ethyl isovalerate (Pt < 0.05) and ethyl isobutyrate (Pt < 0.05);
243 three acetates: ethyl acetate (Pt < 0.05), hexyl acetate (Pt < 0.05), and isoamyl acetate (Pt < 0.05). 1-
244 Hexanol was not detected in the de-aromatised wine or distilled water. In distilled water, significant
245 differences were found between the two sugars for one linear chain fatty acid derivative ethyl ester:
246 ethyl butyrate (Pt < 0.05), two-branched acid derivative ethyl esters: ethyl-2-methylbutyrate (Pt <
247 0.05) and ethyl isobutyrate (Pt < 0.05), and two acetates: ethyl acetate (Pt < 0.05) and isoamyl
248 acetate (Pt < 0.05).

249 Table 4. Volatile aroma analysis of cane and beet sugars in de-aromatised wine and distilled water.

	De-aromatised Wine	**Distilled Water**

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Compound	CANE	BEET	CANE	BEET
Ethyl esters: Linear fatty acid derivatives (µg/L)				
Ethyl octanoate	137 ± 0.01	137 ± 0.01	137 ± 0.006	137 ± 0.0005
Ethyl hexanoate	258 ± 0.001	259* ± 0.02	260 ± 0.01	260 ± 0.01
Ethyl butyrate	23* ± 0.60	21 ± 0.04	14* ± 0.10	9 ± 0.01
Ethyl decanoate	86 ± 0.02	86 ± 0.20	86 ± 0.10	86 ± 0.10
Ethyl esters: Branched acid derivatives (µg/L)				
Ethyl-2-methylbutryate	4 ± 0.20	4 ± 0.20	4* ± 0.20	3 ± 0.10
Ethyl isovalerate	34 ± 0.10	35* ± 0.10	35 ± 0.001	35 ± 0.2
Ethyl isobutyrate	36 ± 0.20	40* ± 0.20	33 ± 0.40	34* ± 0.10
Ethyl lactate	2681 ± 89	2695 ± 8	2529 ± 70	2565 ± 11
Alcohols (µg/L)				
2-Phenylethyl alcohol	9201 ± 0.01	9201 ± 0.003	9200 ± 0.01	9200 ± 0.01
Acetates (µg/L)				
Ethyl acetate	1847 ± 35	1984* ± 22	1902* ± 20	1840 ± 40
Hexyl acetate	37 ± 0.02	38* ± 0.01	37 ± 0.05	37 ± 0.04
Isoamyl acetate	74 ± 0.04	75* ± 0.1	74 ± 0.2	75* ± 0.02
2-Phenylethyl acetate	13 ± 0.006	13 ± 0.003	13 ± 0.01	13 ± 0.01

250 *± = standard deviation of the means, ND refers to quantities not detectable. Comparison of treatment means was*
251 *carried out using the Student T-test at Pt < 0.05. Significance: NS = Pt > 0.05, * = Pt < 0.05, ** = Pt < 0.01 and *** =*
252 *Pt < 0.001 and **** = Pt < 0.0001. (N = 6, 3 samples being measured in replicate).*
253

254 Post-fermentation YAN (ammonia and amino acids (mg N/L)) and malic acid (g/L) were found to
255 be statistically different between sugar treatments. However, these differences were so small that
256 they would not be considered important from the perspective of winemaking (Table 5). Malolactic
257 fermentation was not carried out on the base wines hence the high malic acid levels (4.2–4.7 g/L).

Table 5. Sparkling wine chemical composition.

Parameter	ABS	ACS	*Significance*
Titratable acidity (g/L)	9.6 ± 0.3	9.5 ± 0.2	*NS*
pH	3.3 ± 0.0	3.3 ± 0.0	*NS*
Residual sugar (g/L)	0.4 ± 0.0	0.3 ± 0.0	*NS*
Ammonia (mg N/L)	4.2 ± 0.4	0.5 ± 0.6	***
Amino acids (mg N/L)	13.3 ± 0.5	9.2 ± 0.4	***
YAN (mg N/L)	12.7 ± 0.5	9.8 ± 0.7	***
Malic acid (g/L)	4.7 ± 0.1	4.2 ± 0.1	***
Acetic acid (g/L)	0.2 ± 0.0	0.2 ± 0.0	*NS*
Alcohol (% v/v)	11.0 ± 0.1	11.0 ± 0.1	*NS*
Pressure (ATM)	6.4 ± 0.1	6.3 ± 0.1	*NS*

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*Treatment codes: ABS = Auxerrois made with beet sugar, ACS = Auxerrois made with cane sugar. ± = standard deviation of the means and comparison of treatment means was carried out using the Student T-test at Pt < 0.05. Significance: NS = Pt > 0.05, * = Pt < 0.05, ** = Pt < 0.01 and *** = Pt < 0.001. (N = 6 (3 bottles of each treatment was measured in duplicate)).*

Base wines were analysed after sugar, yeast and nutrient addition *(Liqueur de tirage)* on the day of inoculation and bottling. In the base wines, significant differences were observed for all linear fatty acid derivative esters: ethyl octanoate, ethyl hexanoate, ethyl butyrate, and ethyl decanoate, and branched acid derivative esters: ethyl isobutyrate, and ethyl lactate, and alcohols: 1-hexanol and 2-phenylethyl alcohol, and three acetates: ethyl acetate, hexyl acetate and isoamyl acetate (Table 6).

After the eight-week second fermentation, the finished sparkling wines were analysed (Table 6). In the sparkling wines, differences were also significant for several classes of VOCs; linear fatty acid derivative esters: ethyl octanoate, ethyl hexanoate, and ethyl butyrate, branched acid derivative ester: ethyl lactate, alcohols: 1-hexanol, and 2-phenylethyl alcohol, and acetates: hexyl acetate, and 2-phenylethyl acetate (Table 6).

Table 6. Chemical composition of Auxerrois base wines made from beet sugar (ABS) and cane sugar (ACS) on the day of inoculation and bottling and the corresponding sparkling wines after the second fermentation.

	Base Wine		Sparkling Wine		Odour Activity Value (OAV)	
Compound (ppb)	**ABS**	**ACS**	**ABS**	**ACS**	**ABS**	**ACS**
Ethyl esters: Linear fatty acid derivatives (µg/L)						
Ethyl octanoate	389* ± 15	318 ± 11	437* ± 40	309 ± 15	2198.5	154.5
Ethyl hexanoate	784 ± 21	1065* ± 31	980** ±1 9	817 ± 10	15.8	13.2
Ethyl butyrate	487 ± 45	759* ± 70	695* ± 70	599 ± 52	5.6	4.8
Ethyl decanoate	755 ± 49	41547* ± 4078	2089 ± 1430	2457 ± 270	10.4	12.3
Ethyl esters: Branched acid derivatives (µg/L)						
Ethyl-2-methylbutryate	13 ± 0.5	15 ± 0.4	22 ±2	18 ± 1	1.2	1.0
Ethyl isovalerate	41 ± 0.2	44 ± 0.1	46 ±1	46 ± 1	15.3	15.3
Ethyl isobutyrate	89 ± 13	163* ± 7	179 ±48	206 ± 37	11.9	13.7
Ethyl lactate	7726* ± 146	5641 ± 25	9432* ±916	7620 ± 428	0.1	0.1
Alcohols (µg/L)						
1-Hexanol	616 ± 8	892* ± 47	696* ± 69	591 ± 30	0.1	0.1
2-Phenylethyl alcohol	41547 ± 4078	44479* ± 4778	49151* ± 1817	44274 ± 1957	3.5	3.2
Acetates (µg/L)						

Ethyl acetate	40445 ± 1360	59787* ± 1818	57841 ± 5009	58130 ± 3485	7.7	7.8
Hexyl acetate	80 ± 1	104** ± 1	83** ± 2	70 ± 1	0.1	0.1
Isoamyl acetate	800 ± 74	1238** ± 19	1015 ± 86	948 ± 105	33.8	31.6
2-Phenylethyl acetate	180 ± 16	200 ± 2	180** ± 10	160 ± 15	0.7	0.6

*†Treatment codes: Auxerrois beet sugar addition (ABS), Auxerrois sparkling wine made with cane sugar (ACS). ± = standard deviation of the means, ND refers to quantities not detectable. Comparison of treatment means was carried out using the Student T-test at Pt < 0.05. Significance: NS = Pt > 0.05, * = Pt < 0.05, ** = Pt < 0.01 and *** = Pt < 0.001. (N = 6, sugar in de-aromatised wine and distilled water were analysed in triplicate, 3 bottles of each sparkling wine treatment were analysed in triplicate).*

Discussion

1. Volatile aroma compounds in beet and cane sugars in de-aromatised wine and distilled water

Analysis of the VOCs present in cane and beet sugars before their addition to wines, in de-aromatised wine and distilled water, was carried out to establish the concentrations of "fruity" VOCs in the two sugar products. Other than ethyl acetate concentrations, VOC concentrations were similar in the two products. Additional differences might have been found if higher sugar concentrations were added to de-aromatised wine and more sugar products were tested. Urbanus *et al*. (2014a; 2014b) reported sensory differences between cane and beet sugars and attributed them to fatty acids present at high levels, which bestowed a "musty" or "earthy" aroma.

2. Volatile aroma compounds in base wines and sparkling wines

In the base wines, analysed on the day of inoculation for second fermentation, ethyl octanoate, ethyl hexanoate, ethyl butyrate and ethyl decanoate were both found to be significantly different (Pt < 0.05). Ethyl octanoate was the only one of these four esters with a higher concentration in beet sugar than in cane sugar wines (Table 6). The base wine had a lower concentration of ethyl octanoate than was reported in Cava sparkling base wines, and a comparable concentration of ethyl hexanoate (López de Lerma *et al.*, 2018; Martínez-García *et al.*, 2017). Ethyl lactate was also found at a higher concentration in the beet sugar wines. 1-Hexanol, 2-phenylethyl alcohol, ethyl acetate, hexyl acetate, isoamyl acetate and 2-phenylethyl acetate were all at higher concentrations in the cane sugar wine before fermentation was initiated.

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302 **2.1 Esters in sparkling wines**

303 Ethyl octanoate (Pt < 0.05), ethyl hexanoate (Pt < 0.01), and ethyl butyrate (Pt < 0.05) were all
304 found to be at significantly higher concentrations in the wines treated with beet sugar compared to
305 cane sugar. Each were found at higher concentrations than their respective odour thresholds (2
306 µg/L, 62 µg/L and 25 µg/L, respectively) (Table 1). The OAV for each compound in both beet and
307 cane sugar treated wines was calculated to assess the relative importance of individual VOCs in
308 each sample (Francis and Newton 2005). The odour threshold of 125 µg/L was used to calculate the
309 OAV of ethyl butyrate (San Juan *et al.* 2011). Only the compounds with an OAV greater than 1
310 were accepted as potentially contributing to wine aroma in this study. All four ethyl esters (linear
311 fatty acid derivatives) had OAVs over 1 (Table 6). The elevated level of all three significant ethyl
312 esters could be due to ester formation during fermentation from fatty acids in the beet sugar. Fatty
313 acids have been identified as a source of spoilage in beet sugar and their levels require monitoring
314 during the process to keep within permissible limits (Marsili *et al.*, 1994). The condensation of
315 these esters requires ethanol to be present and the corresponding fatty acid (octanoic acid for ethyl
316 octanoate, hexanoic acid for ethyl hexanoate, and butyric acid for ethyl butyrate). All three of these
317 significant fatty acids have been found in beet sugar with higher concentrations resulting in
318 "unacceptable" sugar products (Moore *et al.*, 2003).

319

320 The only branched acid derivative that was found at statistically significant concentration
321 differences between treatments was ethyl lactate (fruity/buttery aroma) and it was at a higher
322 concentration in the beet sugar treatment (Pt < 0.05). However, it was below its odour threshold
323 level (150,000 µg/L) and had a low OAV in both beet and cane sugar wines (0.1) (Table 6). Ethyl
324 lactate is formed by condensation of lactic acid with ethanol and is typically found in higher
325 concentrations in beet sugar than cane sugar due to the microbial action during sugar beet
326 processing (Wojtczak *et al.*, 2013). Increases in all the linear fatty acid derivatives occurred in the

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beet sugar sparkling wines compared to their corresponding base wines. This was in contrast to VOC concentrations in the cane sugar sparkling wines, which were lower than their corresponding base wines, except for ethyl decanoate. Higher concentrations of esters were found in the sparkling wine made from beet sugars compared to its corresponding base wine, whereas ethyl-2-methylbutyrate and ethyl lactate increased in the sparkling cane sugar wines when compared to its corresponding base wine.

2.2 Higher alcohols in sparkling wines

1-Hexanol (herbal/green aroma) and 2-phenylethyl alcohol (rose/floral aroma) were both found to be higher in wines made from beet sugar compared to the cane sugar sparkling wines ($Pt < 0.05$). 2-Phenylethyl alcohol was above its odour threshold (14,000 µ/L) with an OAV of 3.5 in the beet sugar wine and an OAV of 3.2 in the cane sugar wines. However, 1-hexanol was below its odour threshold (8000 µg/L) with a low OAV (0.1) in both cane and beet sugar wines (Table 6). While it was found at a significant concentration in beet sugar wines, this compound was unlikely to impact the final wine aroma. The alcohols gained in the beet sugar fermentation are likely products of the fermentation (Lyons *et al.*, 2021).

2.3 Acetates in sparkling wines

Hexyl acetate (apple, cherry, pear, floral aroma) was found to be significantly higher ($P < 0.01$) in sparkling wines made from beet sugar but had a low OAV (0.1) and concentrations were not above its odour threshold (670 µg/L) (Table 6). 2-Phenylethyl acetate concentration was higher in the sparkling beet sugar wine ($Pt < 0.01$) but below its odour threshold of 250 µg/L, suggesting a negligible impact on the wine's aromatic profile with an OAV of 0.6 and 0.7 (Table 6). No clear pattern was able to be established for acetate compounds, but differences might be observed if a higher number of acetate compounds had been included in the analysis. In beet and cane sugar sparkling wines, the VOCs with low OAVs were ethyl-2-methylbutyrate, ethyl lactate, 1-hexanol, hexyl acetate and 2-phenylethyl acetate. Although this does not mean that those compounds with

high OAVs are important to the wine aroma, as Escudero *et al.* (2004) studied aroma compounds in Maccabeo wines using omission and addition experiments. The authors revealed that having a high OAV for a VOC is not necessary for, nor does it guarantee, an effect on the aroma of wine.

Conclusion

Auxerrois base wines underwent a second alcoholic fermentation in bottles with either cane sugar or beet sugar addition. Fourteen VOCs were chosen for this study due to their "fruity" attributes and were analysed in the sparkling wines after the eight-week long second fermentation. Beet sugar increased some "fruity" VOCs in the finished sparkling wines, likely due to the fatty acids present in the beet sugar that originate from its manufacturing process, which may have provided an elevated concentration of linear fatty acid-derived esters compared to cane sugar. Beet sugar increased 1-hexanol and 2-phenylethyl alcohol concentrations in wines, although only 2-phenylethyl alcohol was above its odour threshold. Our results confirm that the type of sugar used for the second fermentation of sparkling wines influences the concentration of the "fruity" VOCs after the second fermentation, but only a minor influence on the sparkling wine chemical composition. Future research should include VOC analysis of a higher number of compounds during cellar ageing when wines are ageing in contact with yeast lees, sensory analysis of the wines, and the inclusion of more sugar products (i.e., reconstituted Grape Must (RCGM)) and a wider range of VOCs.

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