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ORIGINAL RESEARCH ARTICLE

The impact of *dosage* sugar-type and aging on Maillard reaction-associated products in traditional method sparkling wines

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ABSTRACT

Liqueur d'expedition (dosage) is a final sugar addition made to sparkling wine which determines the sweetness and balance of the finished product. In the present study, the influence of *dosage* sugar composition on Maillard reaction-associated products and precursors in traditional method (bottle-fermented) sparkling wines was evaluated over 18-months of storage in climate-controlled cellar conditions (14 °C, 70 % relative humidity). Evaluated *dosage* sugar-types included glucose, fructose, cane-derived sucrose, beet-derived sucrose, maltose, and Must Concentrate Rectified (MCR) Sucraisin®, which were compared to a zero *dosage* (no sugar added) control. Maillard reaction-associated products were quantified by headspace solid-phase microextraction coupled to gas-chromatography-mass spectrometry (HS-SPME-GC/MS), and precursors including sugars and amino acids, were measured by enzymatic assay and proton (1H) nuclear magnetic resonance (NMR) spectroscopy, respectively. Partial least squares discriminant analysis (PLS-DA) was used to effectively discriminate between wines based on aging duration but did not adequately separate wines treated with different *dosage* sugar-types. Decreases in alanine and glycine were observed after 18-months of cellar aging, suggesting that Maillard reaction product formation may be partially related to their depletion. Benzaldehyde and ethyl-3-mercaptopropionate were identified as discriminatory Maillard reaction-associated compounds when comparing 0- and 18-month aged wines, with benzaldehyde increasing and ethyl-3-mercaptopropionate decreasing over the aging period. This research contributes to an increased understanding of Maillard reaction pathways in the unique low-temperature and low pH sparkling wine matrix and establishes the relatively greater effect of aging duration compared to *dosage* sugar-type on the formation of Maillard reaction-associated products. The combined application of HS-SPME-GC/MS and 1H NMR based metabolomics presents new insights into the chemical composition of sparkling wines during aging.

KEYWORDS: sparkling wine, cellar aging, HS-SPME-GC/MS, 1H NMR, *dosage*, Maillard reaction

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INTRODUCTION

Sparkling wine is a rapidly growing sector of the global wine industry, with over 20 million hectolitres produced each year (OIV, 2020). The technology used to produce sparkling wine is a critical factor in determining wine style and quality. Although several methods exist, traditional method or *Méthode Champenoise* wines have been widely studied for their aging potential and complexity (Culbert *et al*., 2017).

Traditional method sparkling wines undergo a second fermentation of the base wine which takes place in the same bottle that is later purchased by the consumer (Figure 1). The second fermentation is initiated by the addition of *liqueur de tirage* (*tirage*), a combination of yeast, sugar, nutrients, and an adjuvant/riddling aid. Subsequently, the wine is aged on the yeast lees (*sur lies*) at cellar temperature (15 ± 3 °C) with the duration ranging from 9 months to several years depending on the regulations associated with the wine's region of production. At the end of the aging period, lees are riddled and disgorged from the bottle. The displaced volume is accounted for by the addition of *dosage*, which determines the sweetness of the finished wine (Kemp *et al*., 2017). *Dosage* can be a mixture of wine (aged or non-aged), grape must, or a blend of wine and grape must, which can include the addition of cane or beet sugar (sucrose), liquid sugar (dextrose), rectified concentrated grape must, oxidized wine, SO_2, citric acid, tannin, and occasionally brandy, Icewine, or other spirits (Kemp et al., 2015, 2017). The level of residual sugar in *dosage* is categorized as: zero *dosage*/*brut nature* (0 - 3 g L^{-1}), *brut* (< 12 g L^{-1}), extra *brut* (12 - 17 g L^{-1}), *sec* (17 – 32 g L^{-1}), *demi sec* (32 – 50 g L^{-1}), and *doux* (50 + g L^{-1}) (Di Gianvito *et al*., 2019; International Organisation of Vine and Wine (OIV), 2021; Jackson, 2014). While sparkling wines with no sugar added in *dosage* (zero *dosage* or *brut nature*) are commonly produced, sugar additions in *dosage* can impart unique chemical and sensory attributes to the finished wine (Kemp *et al*., 2017; McMahon *et al*., 2017; Wilson *et al*., 2022), thereby influencing finished wine quality.

Kemp *et al.* (2017) demonstrated that the wine type in *dosage* impacts volatile aroma compounds during aging. The authors compared sparkling wines with zero *dosage* to those with *dosage* prepared in Chardonnay still wines, Pinot noir sparkling wines, the same non-vintage sparkling wine as the treatment wine, or Brandy, each dosed with *brut* (8 g L^{-1}) levels of cane sugar. The type of wine used in *dosage* had a significant influence ($p < .05$) on the composition of assessed volatile aroma compounds (ethyl esters: ethyl octanoate, ethyl hexanoate, ethyl butanoate, ethyl isobutyrate, ethyl isovalerate, ethyl-2-methylbutyrate; and alcohols: 2-phenylethanol, 1-hexanol) in the finished sparkling wines after 15-weeks of aging, although the treatment with zero *dosage* was not different from the *dosage* treatment with sugar added to the same sparkling wine. McMahon *et al.* (2017) studied the influence of different sugar-types (glucose, fructose, and sucrose) in *dosage* at *brut* or *demi sec* residual sugar levels on consumer preference, aroma, and taste attributes. Although the wines were not aged following *dosage* addition, fructose and sucrose showed higher ratings ($p < .05$) for caramelized, vanilla, and honey aromas compared to glucose. Additionally, findings revealed a consumer preference for wines sweetened with sucrose ($p < .05$) compared to glucose or fructose. Further, sparkling wines with increasing sucrose levels in *dosage* (from 0 – 31 g L^{-1}) have been associated with improved foam formation but reduced stability, possibly due to modifications of the wine's viscosity (Crumpton *et al*., 2018a).

It is of note that many studies related to wine chemistry do not specify the source of sucrose, whether they are sugar beet or sugar cane derived, despite sensory differences reported between the two products (Urbanus *et al.*, 2014; Wilson *et al*., 2022). Beet sugar is characterized by off-dairy, oxidized, earthy, and barnyard orthonasal aromas, compared to cane sugar with dominant fruity retronasal (aroma by mouth) qualities (Urbanus *et al*., 2014). Wilson *et al.* (2022) compared the use of cane and beet sugar in *tirage* for sparkling wines made from Auxerrios (*Vitis vinifera*) and found that wines made with beet sugar had a higher concentration of linear fatty-acid derived ethyl esters, leading to fruity, tropical, and apple aromas. This was attributed to differences in sugar production, and specifically to higher concentrations of medium-chain fatty acids created in sugar beets during extended storage prior to processing (Wilson *et al*., 2022). Stable carbon isotope analysis of sugars in sparkling wines by Martinelli *et al.* (2003) revealed that beet sugar is commonly used in Europe and South America, while cane sugar is typically used in Brazil, Australia, and America.

Charnock *et al.* (2022a) suggested that *dosage* sugar may be involved in the formation of compounds related to the

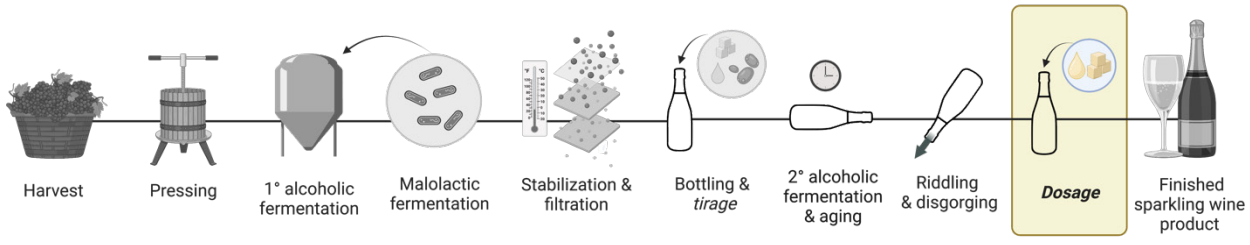


FIGURE 1. Simplified overview of the traditional method sparkling wine production process, with an emphasis on *dosage*, reproduced and adapted from Charnock *et al.* (2022a) with permission of the copyright owner.

Maillard reaction during prolonged storage of finished sparkling wines. The Maillard reaction is a non-enzymatic reaction between the carbonyl group of a reducing sugar and the amine group of amino acids, peptides, or proteins which has been typically studied in high-heat conditions, although it also occurs at low-temperatures over a longer period of time (Ames, 1990; Charnock *et al*., 2022a; Nursten, 2005). Previous studies on Maillard reaction-associated products in model wine conditions have shown that carbonyl compounds react with amino acids under low temperature and low pH (pH 3.5) conditions similar to sparkling wine (Pripis-Nicolau *et al*., 2000). Thus, the Maillard reaction may be an important contributor to sensory changes during sparkling wine production and aging under low pH (pH 3 - 4) and low temperature (15 ± 3 °C) conditions. Several Maillard reaction-associated compounds have been identified in aged sparkling wines, and exhibit roasted, bready, nutty and caramel aromas (Cutzach *et al*., 1999; Jeandet *et al*., 2015; Keim *et al*., 2002; Le Menn *et al*., 2017; S. Marchand *et al*., 2000; Pripis-Nicolau *et al*., 2000; Silva Ferreira *et al*., 2003; Tominaga *et al*., 2003b). However, limited research has evaluated the relationship between Maillard reaction-associated products and sugar or amino acid levels in sparkling wines during post-disgorgement aging (Le Menn *et al*., 2017). The formation of Maillard reaction-associated compounds in sparkling wine may be, at least in part, linked to interactions between *dosage* sugars and amine-containing compounds, (Charnock *et al*., 2022a) liberated from autolyzed yeast cells and assimilated into the wine matrix prior to disgorgement (Alexandre and Guilloux-Benatier, 2006; Riera, 2016; Tudela *et al*., 2012). Alternatively, sugars may undergo acid-catalyzed degradation during the aging period, in turn producing compounds which may participate in the Maillard reaction cascade (Charnock *et al*., 2022a). Thus, it is anticipated that utilizing different sugar-types in *dosage* may alter the composition of the finished wine due to differences in sugar structures.

Various methods for the quantification of heterocyclic Maillard reaction-associated compounds have been proposed, most recently with an optimized headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC/MS) method developed by Burin *et al.* (2013), however, the authors only evaluated red and white still wines. This method has been applied to aged base wines for Champagne production (Le Menn *et al*., 2017) and more recently has been adapted for the measurement of furan-derivatives in base wines subject to accelerated aging (Medeiros *et al*., 2022). In addition to measuring the formation of Maillard reaction-associated products, simultaneously monitoring the relative composition of reactant compounds (i.e., amino acids, sugars) during wine aging is necessary to understand their potential role in these reaction pathways. However, previous studies in model wine have shown that amino acid levels decrease (mg L^{-1} range) without a proportional increase in the formation of Maillard-associated aroma compounds (μg L^{-1} range) (Grant-Preece *et al*., 2013). This is attributed to the participation of amino acids in side reactions, leading to a suite of alternative compounds than those being measured with a targeted approach. Although revealing the direct role of reactant compounds is therefore challenging in Maillard-associated pathways, relative concentration changes in precursor compounds are nevertheless important to understand these interactions in complex environments. Recently, ^{1}H NMR-based metabolomics have emerged as a valuable tool for the characterization of chemical components in a complex matrix (Wishart *et al*., 2022). NMR is a rapid, non-destructive, targeted technique that can simultaneously identify and quantify different chemical families of metabolites in a single sample. It has previously been applied to wine for authenticity purposes and regional discrimination (Gougeon *et al*., 2018; Gougeon *et al*., 2019a; Gougeon *et al*., 2019b; Le Mao *et al*., 2021; Le Mao *et al*., 2023; Son *et al*., 2009).

To the best of our knowledge, no prior literature has investigated the role of *dosage* sugar-type in the formation of Maillard reaction-associated products in sparkling wine. Through a time-course analysis, the present study evaluates the composition of reaction products and precursors over 18-months of bottle aging by analytical techniques including HS-SPME-GC/MS and ^{1}H NMR. Various *dosage* treatments including fructose, glucose, sucrose (both cane and beet derived), maltose, Must Concentrate Rectified (MCR) Sucraisin®, and a zero *dosage* control were evaluated.

MATERIALS AND METHODS

1. Chemicals and reagents

D-Glucose monohydrate (> 99.5 %), D-Fructose (> 99.5 %), and maltose monohydrate (> 95.0 %) were reagent grade and purchased from BioShop Canada Inc. (Burlington, ON, Canada). MCR Sucraisin® rectified grape must concentrate was purchased from L'Institut Œnologique de Champagne (IOC, Épernay, France). Commercial cane and beet-derived sucrose products were processed and supplied by Lantic/Rogers (Taber, AB, Canada). Potassium metabisulfite (KMS) was purchased from Vines to Vintages (Jordan, ON, Canada). HPLC-grade water was purchased from Fisher Chemical (Fair Lawn, USA), 750 mM phosphate buffer, 5 mM 3-(Trimethylsilyl)-1-propanesulfonic acid-d_6 sodium salt (DSS-d_6), 5.84 mM 2-chloro pyrimidine-5-carboxylic acid, and 99.9 % D_2O was purchased from Sigma Aldrich (St. Louis, USA). Chemical standards for HS-SPME-GC/MS were obtained from the following suppliers according to Table 1: 1-2, 4-5, 8-15, 17-19, 21-22 from Sigma Aldrich (St. Louis, USA); 3, 16, and 20 from Fisher Scientific (Mississauga, ON); 6 and 7 from Tokyo Chemical Industry (Tokyo, Japan), and internal standards a-c from CDN Isotopes (Pointe-Claire, QC, Canada). Milli-Q water was obtained from Biocel water purifying system (Millipore, Etobicoke, ON, Canada) and filtered through a 0.22 μm filter (Millipore). Sodium chloride (NaCl; ≥ 99 %) was purchased from Fisher Chemical (Fair Lawn, USA). Absolute anhydrous ethanol was purchased from Greenfield Global (Mississauga, ON,

Canada). GC-MS grade methanol (≥ 99.8 %) was purchased from Millipore Sigma (Burlington, USA).

2. Experimental design

2.1 Sparkling winemaking

Disgorged sparkling wine was produced and supplied by the Niagara College Teaching Winery in Niagara-on-the-Lake, ON, Canada. The base wine was a 2015 vintage and comprised of 59 % Chardonnay and 41 % Pinot noir. Grapes were harvested at 18.8 (± 0.5) °Brix. Following primary fermentation, the base wine was bottled for secondary alcoholic fermentation in November 2016 and aged on yeast lees for three years prior to disgorging in November 2019. For both alcoholic fermentations, the oenological *Saccharomyces cerevisiae* strain EC1118 (Lallemand Inc., Montreal, QC, Canada) was used.

Dosage solutions were prepared in filtered and degassed sparkling wine from the same production lot as the bottles used for treatments. The *dosage* composition was modified by the introduction of six sugar treatments (D-glucose (GLU); D-fructose (FRU); sucrose derived from sugar beets (BET) and sugar cane (CAN); maltose (MAL); and the commercial product MCR Sucraisin® (MCR). The control treatment had no sugar added in the *dosage* solution (CTR) (Figure 2). *Dosage* solutions were prepared to target 6.5 ± 1 g L^{-1} sugar per 750 mL bottle, as per the *brut* style (< 12 g L^{-1} residual sugar). Sulfur dioxide (SO_2) was added to each *dosage* solution (as potassium metabisulfite) to ensure 900 mg L^{-1} free SO_2 prior to addition. *Dosage* treatments were added to individual wine bottles on a commercial bottling line at Millesime Sparkling Wine Processing Inc. (St. Catharines, ON). Wines were bottled at 6 atmospheres (atm) with 20 mL of *dosage* solution added to each bottle. A total of 12 bottles per treatment were prepared. Samples were bottled with cork closures (Diam technical cork, Céret, France) and wire cages, and wines were subsequently cellared at the Cool Climate Oenology & Viticulture Institute (CCOVI) at Brock University (St. Catharines, ON) with environmental controls (14 °C and 70 % relative humidity).

2.2 Wine chemical analysis

At intervals of 0, 9, and 18-months post-*dosage* addition, triplicate bottles of each treatment and control wines were collected from the cellar and analysed in triplicate for each parameter. Standard wine chemical analysis including pH, titratable acidity (TA, g L^{-1} tartaric acid equivalent), alcohol (*v/v* %), free and total SO_2 (ppm), degree of browning (A_{420}), sugar composition (g L^{-1}) and organic acids (g L^{-1}) were performed immediately. Additionally, basic chemical analysis was carried out on the un-treated sparkling wine used to prepare the *dosage* solutions [12.3 % (*v/v*) alcohol, pH 3.05, 8.9 g L^{-1} total acidity, 3.20 g L^{-1} malic acid, 0.12 g L^{-1} acetic acid, 0.75 g L^{-1} residual sugar].

Prior to analysis, wine samples were degassed at room temperature (20 °C) by vacuum filtration through P8 (20 µm) filters (Fisher Scientific, Mississauga, ON, Canada) using a Sentio® microbiology pump (Pall® Life Sciences, New York, NY, USA). pH and TA were measured using a Hanna Instruments HI 84502 auto-titrator (Woonsocket, RI, USA) calibrated with standard solutions of pH 4.0, 7.2, and 10.0 and a pump calibration standard to ensure auto titration accuracy (HI 84502-55, Hanna Instruments, Woonsocket, RI, USA). Ethanol content (*v/v* %) was analysed by gas chromatography-flame ionization detection (GC-FID) according to a modified method by Nurgel *et al.* (2004), with modifications of an Agilent 6890 GC-FID (Agilent, Santa Clara, CA, USA) equipped with a DB wax column (30 m x 0.25 mm x 0.25 µm), an Agilent 7638B automated split/spitless injector (Agilent, Santa Clara, CA, USA) and an internal standard of 0.1 % butanol. Free and total SO_2 levels were analysed by the aspiration method outlined by Iland *et al.* (2015). Degree of browning was measured as the absorbance at λ_{420nm} (A_{420}) in a 1 cm spectrophotometric cell using a Cary 60 UV-Vis spectrophotometer (Agilent, Santa Clara, CA, USA) multiplied by 1000 (mAU). Sugar and organic acid composition (g L^{-1}) was analysed by Megazyme® enzymatic assay kits (K-FRUGL, K-MASUG, K-LMAL, K-LACET; Bray, Ireland).

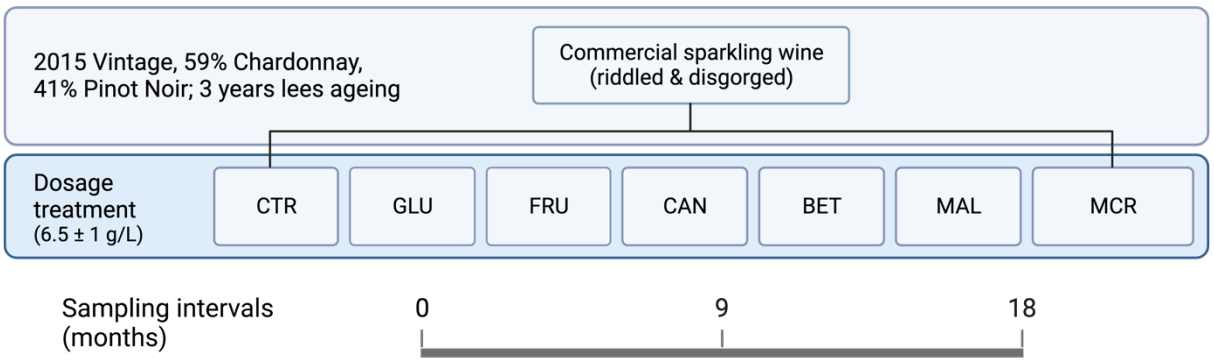


FIGURE 2. Experimental design for dosage sugar-type treatments in disgorged commercial sparkling wine [CTR, control (zero dosage); GLU, glucose; FRU, fructose; CAN cane-derived sucrose; BET, beet-derived sucrose; MAL, maltose; MCR, rectified concentrated grape must (1:1 glucose: fructose)].

The analysis of metal ions in the un-aged sparkling wines following *dosage* addition was carried out by inductively coupled plasma–mass spectrometry (ICP-MS) and inductively coupled plasma–optical emission spectrometry (ICP-OES) techniques following U.S. Environmental Protection Agency (USEPA) Methods 200.8 and 6010D, respectively (USEPA, 1994; USEPA, 2018). This method has been previously applied to sparkling wine by Charnock *et al*. (2022b). Sample replicates showed mean relative standard deviations of 5.4 ± 7.4 %. Arsenic content was below the limit of detection (< 10 μg L^{-1}**)** for all wine samples except for wines treated with cane sugar (sucrose; CAN) in *dosage*, and as such, no statistical evaluation was carried out due to the high proportion of < LOD data (Wood *et al*., 2011).

Following basic chemical analysis, 45 mL from each wine bottle were transferred directly into two 50 mL conical tubes (Eppendorf, Mississauga, ON, CA) and sealed with screw caps and parafilm wrap. Samples were immediately transferred to a -40 °C freezer for storage until ^{1}H NMR and HS-SPME/GC-MS analyses (Sections 2.4 and 2.5, respectively).

2.3 Sugar purity analysis by high performance liquid chromatography (HPLC)

Sugar purity analysis by high-performance liquid chromatography (HPLC) was carried out by Eurofins Experchem Laboratories (Toronto, ON) according to Association of Official Analytical Chemists (AOAC) (1982).

2.4 1H NMR spectroscopic analysis of amino acids

2.4.1. Sample preparation

^{1}H NMR sample preparation and analysis was carried out at The Metabolomics Innovation Centre (TMIC), Wishart Node (University of Alberta, Edmonton, AB). Wine samples from the 0-month and 18-month aging intervals were assessed. Sparkling wine samples were shipped frozen to TMIC and stored at -40 °C until analysis, at which time they were thawed on ice for 8 hours prior to sample preparation. Thawed samples were sonicated in an ice water bath to remove dissolved carbon dioxide and were subsequently centrifuged at 11,000 rpm for 20 minutes at 4 °C (Eppendorf 5810R benchtop centrifuge, Eppendorf, Hamburg, Germany). Samples were then filtered to remove unwanted macromolecules, primarily proteins, in order to ensure spectral clarity (Mercier et al., 2011). Centrifugal filter units (Amicon Ultra-0.5 mL 3 kDa MWCO, Merck Millipore, Burlington, USA) were rinsed five times prior to sample filtration to ensure the removal of residual glycerol from filter membranes. During each rinse, approximately 0.5 mL of HPLC grade water was centrifuged at 10,000 rpm for 10 minutes at 4 °C. The exterior tube was subsequently replaced, and 450 uL aliquots of wine were transferred to each pre-washed filter for to centrifugation at 11,000 rpm for 20 minutes.

To 200 uL of wine filtrate, 50 uL of a standard buffer solution was added (750 mM phosphate buffer, 5 mM 3-(Trimethylsilyl)-1-propanesulfonic acid-d_6 sodium salt (DSS-d_6), 5.84 mM 2-chloro pyrimidine-5-carboxylic acid and 54 % D_2O (pH 7.0)). The pH was maintained at 7.0 for analysis to accommodate the global fitting routine for processing software and metabolite identification with a pH-sensitive compound library (Mercier et al., 2011). The inclusion of 2-chloro pyrimidine-5-carboxylic acid allows for improved spectral phasing due to a down field signal which does not interfere with known biological metabolites (Trimigno *et al*., 2018). Deuterated water provided a field frequency lock and DSS-d_6 was employed as a chemical shift reference (^{1}H, δ 0.00 ppm). Solutions were vortexed thoroughly and centrifuged at 10,000 rpm for 5 minutes at 4 °C to eliminate sample precipitates in the sample. The supernatant (250 uL) was then transferred to a 3 mm NMR tube (Bruker, Milton, ON) for analysis.

2.4.2. NMR analysis

^{1}H NMR spectra were acquired at 25 °C with a 700 MHz Bruker NMR with a 5 mm cryoprobe and z-axis pulsed field gradient (PFG) following a standard one-dimensional pre-saturation NOESY pulse sequence (noseypr1d) to supress the water signal. This included a 2 s recycling delay with pre-saturation (80 Hz pulse power), proton 90 ° pulse width approx. 8 μs, 50 ms mixing time, 4 s acquisition time, 12 ppm sweep width, and 128 scans. The singlet produced by the DSS methyl groups was used as an internal standard for chemical shift referencing (set to 0 ppm, concentration 5.0 mM). All spectra were shimmed so that the DSS linewidth was below or equal to 1 Hz. Shimming adjusted DSS signal peak to be without shoulders or other artefacts. ^{1}H NMR spectra were processed and analysed for quantification by the MagMet fully automated analysis software package with a custom metabolite library for beer and wine (http://magmet.ca, Accessed August 11, 2022). MagMet software has been previously shown to perform with absolute concentration accuracy of 90 % or greater and is freely available through web servers (Ravanbakhsh *et al*., 2015; Wishart *et al*., 2022). Amino acids in the MagMet metabolite library for beer and wine analysis included: alanine, arginine, choline, glutamine, glycine, histidine, isoleucine, proline, leucine, phenylalanine, threonine, tyrosine, uracil, uridine, and valine. Chemical shifts and coupling constants for amino acid determination are shown in Supplementary Table S1. Analytes with all concentrations below the limit of quantification (< 40 μM) were removed from data analysis. ^{1}H NMR analyses were run on triplicate bottles with analytical duplicates, and the mean relative standard deviation was 8.6 ± 6.3 % for repeated measurements on the same bottle.

2.5 Headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC/MS) analysis of Maillard reaction-associated products

The HS-SPME-GC/MS method for the determination of Maillard reaction-associated compounds was adapted from Burin *et al*. (2013). Additional compounds relevant to the Maillard reaction in sparkling wine were incorporated into the method. Standard compounds numbered 1-3, 8, 12, 14-17, and internal standards a-b (according to Table 1) were

TABLE 1. Monitored ions, corresponding internal standard, retention time, linearity, range, recovery, variance, limit of detection and quantification, odour description, sensory threshold, and reported range in wine literature for each Maillard reaction-associated compound.

	#	Compound	ISTD	CAS	RT / sec	**Ions (*m/z*)**[b]	R^2	Range (µg/L)	% Recovery	LOD [c](µg/L)	LOQ [d](µg/L)	Odour descriptor	Sensory threshold	Range reported in wine literature
Internal Standard	a	Benzaldehyde-d_6		17901-93-8	33.83	82, 94, **112**	-	-	-	-	-	-	-	-
	b	Furfural-d_4		1219803-80-1	23.99	70, 99, **100**	-	-	-	-	-	-	-	-
	c	2-Methylpyrazine-d_6		1219804-84-8	20.80	100	-	-	-	-	-	-	-	-
Aromatic	1	Benzaldehyde	a	100-52-7	33.84	*77*, **106**	0.999	1.16 –100	101.3	0.38	1.16	Bitter almond, sweet, buttery [1,2]	3.0–3.5 mg/L [*,3]	*7* mg/L[1]; 4 mg/L[4]
Aromatic/Thiol	2	Benzenemethanethiol	a	100-53-8	43.38	**91**, 124	0.998	1.92–100	96.3	0.63	1.92	Gun flint, empyreumatic [5]	0.3 ng/L[†,5]	10–400 ng/L[6]
Thiol	3	Ethyl-3-mercaptopropionate	c	5466-06-08	36.24	**61**, 88, 134	0.998	8.35–100	100.1	2.76	8.35	Fruity, pleasant, Concord grape [7]	200 ng/L [‡,7]	40–12 000 ng/L [6]
N,S-heterocycle	4	Thiazole	c	288-47-1	14.93	**85**, 58	0.998	8.64–100	84.0	2.85	8.64	Popcorn, peanut [8]	38 µg/L [‡,9]	0–23 µg/L [9]; 4.6–8.2 µg/L [§,10]
	5	2-Ethylthiazole	c	15679-09-1	25.62	98, 112, **113**	0.997	9.92–100	103.1	3.37	9.92	Green, nutty [8]	n.a.	1.14–1.33 µg/L [§,10]
	6	2-Acetylthiazole	c	24295-03-2	38.18	112, **127**	0.992	5.63–100	95.9	1.86	5.63	Nutty, roasted hazelnut, popcorn [8,9]	3 µg/L [‡,9]	0–0.4 µg/L [11], 0–3 µg/L [9], 0.59–3.49 µg/L [§,10]
	7	2-Methylthiazole	c	3581-87-1	19.52	**58**, 70, 99	0.998	3.11–100	100.8	1.03	3.11	Green vegetable [8]	n.a.	n.d.
	8	2-Acetyl-2-thiazoline	c	29926-41-8	45.87	**43**, 60, 129	0.993	8.57–100	92.3	2.83	8.57	Roasted hazelnut [12]	<5 µg/L [‡,12]	0–0.2 µg/L [11]
O-heterocycle	9	3-Acetyl-2,5-dimethylfuran	b	10599-70-9	43.41	**123**, 138	0.993	2.48–100	98.8	0.82	2.48	Nutty, musty, cocoa, bready [13]	n.a.	n.d.
	10	2,3-Dihydrobenzofuran	b	496-16-2	42.59	65, **91**, 120	0.988	3.78–100	102.9	1.25	3.78	n.d.	n.a.	n.d.
	11	2-Acetylfuran	b	1192-62-7	30.19	95, **110**	0.997	2.49–100	98.5	0.82	2.49	Balsamic, burnt, sweet [8,10]	80 mg/L [**,14]	0.23–0.77 mg/L [15]; 1.47–14.70 µg/L [10]
	12	2-Furanmethanethiol	c	35828	28.37	**81**, 85	0.999	9.31–100	81.8	3.07	9.31	Roasted coffee, roasted sesame seeds, cooked wheat bread, burnt rubber, dried fruits, toasty [9,16,17]	0.4 ng/L [†,16], 1 ng/L [‡,9]	2–5500 ng/L [6]
	13	5-Methylfurfural	b	620-02-0	35.05	53, 81, **110**	0.999	2.48–100	97.7	0.82	2.48	Sweet, fruity, caramel, nutty, spicy [8,17]	>1 mg/L [18]	0.45–27.4 µg/L [§,10]; 11.3–40.1 µg/L [19]
	14	Furfural	b	27538-09-06	23.93	67, 95, **96**	0.997	3.46–300	97.8	1.14	3.46	Fruity, caramel, toasted [17]	14 mg/L [††,20]	0.279–0.871 mg/L [17]
	15	Homofuraneol	b	35796	23.94	**97**, 101	0.994	6.34–100	99.2	2.09	6.34	Strawberry, caramel, sweet [21,22]	10 µg/L [†††,23]	4390 µg/L [24]
	16	Furfuryl ethyl ether	b	6270-56-0	27.18	**81**, 98, 126	0.995	4.20–100	99.0	1.39	4.20	Stale beer, spicy, nutty, solvent, kerosine-like [18,25]	2.5 µg/L [**,25]	25–170 µg/L [§§,18]
	17	Ethyl-2-furoate	b	614-99-3	41.21	**95**, 112, 140	0.997	2.63–100	100.6	0.87	2.63	White flowers [19]	n.a.	n.d.
S-heterocycle	18	3-Acetylthiophene	c	1468-83-3	45.31	**111**, 126	0.996	9.81–100	87.2	3.24	9.81	n.d.	n.a.	0–2 µg/L [§§,8]
	19	2-Acetylthiophene	c	88-15-3	44.62	83, **111**	0.996	9.56–100	90.4	3.16	9.56	Mustard-like, onion, malty, roasted [8]	n.a.	1.65–2.32 µg/L [§,10]
	20	2,3-Dimethylthiophene	c	632-16-6	25.64	**97**, 111, 112	0.983	9.85–100	105.1	3.25	9.85	n.d.	n.a.	1.16–2.95 µg/L [§,10]
	21	2,5-Dimethylthiophene	c	638-02-8	23.84	**111**, 95	0.993	13.07–100	82.2	4.31	13.07	Green [8]	n.a.	1.30–1.31 µg/L [§,10]
N-heterocycle	22	2,3,5-Trimethylpyrazine	c	14667-55-1	35.40	42, **81**	0.997	2.56–100	86.7	0.84	2.56	Roasted hazelnut, peanut, cocoa, burnt [8,9]	n.a.	0–0.8 µg/L [§§,8]

[a]Internal standard used for quantification corresponds to deuterated compound identification letter.[b] Ions used for identification, with bolded ions used for quantification in SIM mode. [c] LOD: limit of detection in sparkling wine.[d]LOQ: limit of quantification in sparkling wine.*Sensory threshold reported in white wine (unspecified variety).**Sensory threshold reported in a neutral lager beer.†Sensory perception threshold reported in model wine hydroalcoholic solution (12% (v/v) ethanol, 5 g/L tartaric acid, pH 3.5).†† Sensory perception threshold reported in model wine hydroalcoholic solution (11% (v/v) ethanol, 5 g/L tartaric acid, pH 3.4, 7 g/L glycerin).†††Sensory perception threshold reported in model wine hydroalcoholic solution (11% (v/v) ethanol, 5 g/L tartaric acid, pH 3.5).§Concentrations reported in aged Champagne reserve wines (still wine). §§Concentrations reported in still white wine.‡Sensory threshold reported in water. [1](Delfini *et al.*, 1984) [2](de Souza Nascimento *et al.*, 2018) [3](Delfini, 1987) [4](Loyaux *et al.*, 1981) [5](Tominaga *et al.*, 2003a) [6](Tominaga *et al.*, 2003b) [7](Kolor, 1983) [8](Burin *et al.*, 2013) [9](S. Marchand et al., 2000) [10](Le Menn et al., 2017) [11](Keim et al., 2002) [12](Marchand et al., 2002) [13](Mosciano, 1992) [14](Vanderhaegen et al., 2003) [15](Martínez-García et al., 2021) [16](Tominaga *et al.*, 2000) [17](Torrens *et al.*, 2010) [18](Spillman *et al.*, 1998) [19](Ubeda *et al.*, 2019) [20](Ferreira *et al.*, 2000) [21](Escudero *et al.*, 2000) [22](Blank and Fay, 1996) [23](Kotseridis *et al.*, 2000). [24](Sawyer *et al.*, 2022) [25](Harayama *et al.*, 1995).

incorporated into the method while several compounds were removed as they failed to meet recovery and/or R^2 criteria with any internal standard.

2.5.1. Standard preparation

The method for standard preparation followed Kemp (2010). Concentrations of standards were prepared according to their anticipated concentrations in sparkling wine based on existing literature. Individual stock solutions of each analyte were prepared to 1000 mg L^{-1} in methanol. From individual stock solutions, a high concentration composite standard was prepared to 1 mg L^{-1} in 10 % *v/v* ethanol in Milli-Q water. This composite standard was subsequently diluted 10-fold to 100 μg L^{-1} as a working standard and diluted with sterile filtered de-aromatized wine. The de-aromatized wine served to maintain a comparable matrix and alcohol content across standards and samples, since SPME analysis is highly dependent on matrix composition (Rocha *et al*., 2001). De-aromatized wine was obtained by rotary evaporation of 500 mL of sparkling wine without *dosage* addition at 60 °C (Büchi Rotovapor R-200, Heating Bath B-490, New Castle, USA) to a final volume of 100 mL. The concentrated residue was dissolved in Milli-Q water and prepared to 500 mL in 10 % *v/v* ethanol. Internal standards were prepared separately to a 1 mg L^{-1} composite internal standard solution.

Six calibration standards were prepared over a range of 1–100 μg L^{-1} for all analytes except for furfural, which included a seventh standard at 300 μg L^{-1}. Acceptable regression coefficients over the range of analysis were determined for all analytes ($R^2 > 0.9$, Table 1). To each 10 mL amber glass vial (La-Pha-Pack®, Langerwehe, Germany), 1.5 g of NaCl, standard composite solution, and a modified matrix of de-aromatized wine were added to a total volume of 4.9 mL. The de-aromatized wine was modified to match the matrix ratio of deionized water and de-aromatized wine for all standards, according to the added volume of working standard solution. This required 1 part of 10 % *v/v* ethanol in deionized water to 9 parts of 10 % *v/v* ethanol in sterile filtered de-aromatized wine. Finally, 0.1 mL of the 1 mg L^{-1} internal standard composite solution was added for a final concentration of 20 μg L^{-1}. Vials were immediately sealed with a PFTE/silicone screw cap (VWR, Radnor, USA) and stored at 4 °C for < 24 hr until analysis.

2.5.2. Sample preparation

Wine samples from 0-month and 18-month aging intervals were assessed. Samples at 9-months could not be analytically evaluated due to COVID-19 impacts on equipment access. Samples were degassed prior to analysis by vacuum filtration as previously described. Samples were prepared in 10 mL amber glass vials containing 1.5 g NaCl and contained 4.9 mL wine and 0.1 mL composite internal standard solution. Vials were immediately sealed by screw cap and stored at 4 °C for < 24 hr until analysis.

2.5.3. GC-MS analysis

Analysis was carried out using an Agilent 7890B GC and 5977B quadrupole MSD (Santa Clara, CA, USA) equipped with a DB-624UI capillary column (30 m x 0.25 mm, 1.4 μm film thickness, Agilent Technologies, Santa Clara, CA, USA) and PAL RSI 85 autosampler (CTC Analytics, Zwingen, Switzerland). The autosampler was outfitted with a Peltier stack tray cooler which held samples at 4 °C until analysis (model G4565A, CTC Analytics, Zwingen, Switzerland). An 85 μm Carboxen/ polydimethylsiloxane (CAR/PDMS) coated 23-guage metal alloy SPME fibre (Supelco®, Bellefonte, USA) was utilized for headspace analysis, as outlined by Burin *et al.* (2013). Instrumental parameters followed Burin *et al.* (2013) and required vial agitation 250 rpm for 5 min at 40 °C before inserting the fibre into the headspace for 55 min at 40 °C throughout continued agitation (250 rpm). The sample was desorbed from the fiber to the inlet at 250 °C for 5 min. The carrier gas was Helium (Ultra-high purity 5.0, Linde Canada Inc., Mississauga, ON, Canada) at a flow rate of 1.0 mL min^{-1}, and the oven program was as follows: initial temperature 40 °C for 4 min, ramped 2 °C min^{-1} to 160 °C, held 1 min, ramped 5 °C min^{-1} to 230 °C, held 5 min. All analysis was carried out in selective-ion monitoring (SIM) mode, with ions used for identification and quantification shown in Table 1. The ionization mode was electron impact (70 eV), and the interface was held at 280 °C. All integration data was processed with Agilent OpenLab software (2.4.5.9, Agilent Technologies).

2.5.4 Analytical performance

Compound identification was carried out by a comparison of retention times and mass spectra to pure compounds by the NIST spectral database. Standard concentrations were quantified based on the ratio of peak area of compound to the corresponding deuterated internal standard. Analyses were run on triplicate bottles with analytical duplicates, and the mean relative standard deviation was 3.7 ± 0.1 % for repeated measurements on the same bottle. The limits of detection (LOD) and limits of quantification (LOQ) for each compound were determined as the ratio of the standard error of the y-intercept over the slope multiplied by 3.3 or 10, respectively, and are shown in Table 1. Accuracy (percent recovery) was measured by spiking control wine in triplicate with all compounds at 20 μg L^{-1}.

3. Statistical analysis

Statistical analysis was carried out using XLSTAT Version 2021.2.1 (Addinsoft Inc., New York, NY, USA). Standard wine chemical data, amino acids, and Maillard reaction-associated products were analysed by two-way Analysis of Variance (ANOVA) with Tukey's Honest Significant Difference (HSD) tests to evaluate the individual effect of *dosage* sugar-type (treatment), aging duration (time), and their interactions. Significance was established at $p < .05$. To assess the relative effect size of treatment and time on each variable, Eta-squared (η^2) values were derived from a two-way ANOVA with all interactions included in the model. Effect size is considered small with Eta-squared values > 0.01, medium when levels > 0.06, and large for values > 0.140 (Lakens, 2013).

Multivariate statistical analysis was first carried out by principal component analysis (PCA) as an unsupervised method to observe patterns within amino acid and Maillard reaction-associated product composition (Supplementary Figures S1 – S4). Subsequently, a supervised analysis by partial least squares discriminant analysis (PLS-DA) was carried out using MetaboAnalyst 5.0 web-software to separately assess amino acid and Maillard reaction-associated product data. Prior to multivariate models, each dataset was mean centred and $\log_{10}$-transformed. Log_{10}-transformation reduces relative concentration distributions to better fit a normal distribution, which was assessed by a visual comparison of histograms (data not shown). To validate the explained variation and predictive ability (R^2 and Q^2, respectively) of the models, a cross-validation using five components was carried out for each analysis. PLS-DA maximizes the separation between features and visualizes the distribution of samples in a two-dimensional space, which is supported by loading plots and Variable Importance in Projection (VIP) scores to identify key discriminating analytes in the model. VIP scores > 1 indicate potentially discriminating compounds (Cocchi *et al*., 2018). PLS-DA revealed significantly predictive models when assessing the aging interval for both amino acids ($Q^2 = 0.59$; $R^2 = 0.66$) and Maillard reaction-associated products ($Q^2 = 0.98$; $R^2 = 0.98$) (Figure 3) (Bevilacqua and Bro, 2020; Szymańska *et al*., 2012). However, predictive PLS-DA models were not generated when comparing the *dosage* sugar treatments in the wines aged for 18-months for amino acids ($Q^2 = -0.14$; $R^2 = 0.17$) nor Maillard reaction-associated products ($Q^2 = 0.21$; $R^2 = 0.50$) (data not shown). Negative values for Q^2 show that the model has no predictive relevance (Szymańska *et al*., 2012), and $R^2 < 0.50$ explain less than 50 % of the variation within the model.

RESULTS AND DISCUSSION

1. Wine chemical composition

The chemical composition of the sparkling wines during aging is shown in Table 2. Basic chemical parameters including ethanol, pH, TA, malic acid, acetic acid, SO_2, and the degree of browning were significantly different between aging intervals as well as between *dosage* treatments. The interaction between treatment and time was also significant for all basic chemical parameters. Ethanol content varied between sampling intervals and *dosage* treatments at the 9- and 18-month periods. The interaction between treatment and time showed no trends consistent with sugar treatment nor aging duration (data not shown). We therefore speculate this variation is the result of individual second alcoholic fermentations taking place per bottle following the traditional method of sparkling wine production (ethanol range of 1.5 % *v/v* across all samples) and is therefore not related to treatment nor time effects. Malic and acetic acids had a 0.22 and 0.03 g L^{-1} range in concentration, respectively, and differences are also attributed to the second alcoholic fermentation where variation in yeast growth under stress conditions (e.g., CO_2 pressure, high alcohol, low pH, low temperature) can influence organic acid composition (Berbegal *et al*., 2022). Both free and total SO_2 levels decreased during the aging period, which we expect is due to mild oxidation during storage and is consistent with an increase in the degree of browning observed over 18-months of cellar aging. Additionally, pH slightly increased by 3.2 % during aging which may be related to the simultaneous decrease in TA (11.7 % decrease), likely as a result of tartaric acid precipitation during aging (Crumpton *et al*., 2018b). No consistent relationships were observed to relate sugar type to any basic wine chemical parameter during aging.

As expected, sugar concentrations were different between *dosage* treatments. The control sparkling wine contained the lowest concentration of residual sugar, although high variability of residual sugar levels in control wines was observed (range of 1.10 g L^{-1}). Both *dosage* treatments with sucrose additions (6.5 ± 1 g L^{-1}; cane and beet-derived sugar) contained < 1.4 g L^{-1} sucrose and higher concentrations of glucose and fructose at each sampling interval, suggesting the separation of glucose and fructose monomers by incomplete acidic hydrolysis. The complete acidic hydrolysis of sucrose can occur rapidly at low pH (pH 3), similar to that of sparkling wine (Jakob *et al*., 2020; Pinhero Torres *et al*., 1994). The glucose and fructose composition of wines treated with MCR Sucraisin® in *dosage* agrees with the anticipated sugar composition of concentrated and dehydrated grape must, as discussed later in Section 2.

For *dosage* treatments containing maltose, sugar cane- and sugar beet-derived sucrose, the corresponding sugar decreased over time (Supplementary Table S2). For example, the concentration of maltose decreased over 18-months of aging by approximately 0.7 g L^{-1}. Maltose is an exogenous sugar to grapes and has been widely investigated for its role in the Maillard reaction due to its abundance in seeds and plant materials and therefore various food products (Ajandouz and Puigserver, 1999; Kwak and Lim, 2004; Omari *et al*., 2021; Shen *et al*., 2018). It has not been previously reported in the context of winemaking. Sucrose levels decreased by approximately 0.3 g L^{-1} in wines treated with cane-derived sugar (sucrose) in *dosage*, and 0.1 g L^{-1} for treatments of beet-derived sugar. Wines treated with fructose in *dosage* showed the opposite effect, whereby the concentration of fructose increased between 0-, 9-, and 18-month intervals with an approximate 2.8 g L^{-1} overall increase. In these same wines, glucose levels decreased during aging by 1.6 g L^{-1}, suggesting the gradual isomerization of glucose to fructose which can be facilitated by metal ions in solution (Lara-Serrano et al., 2021). Bottle variation in residual glucose and fructose concentrations also contributed to this variability. For wines with glucose in *dosage*, glucose levels did not differ between the final 18-month interval and either the 0- or 9-month concentrations, however differences between the 0-and 9-month aging periods were found, with the lowest concentration at 9-months. No increase in fructose was observed in the glucose *dosage* wines. The isomerization of glucose to fructose is reported to be independent of the initial glucose concentration (Carraher *et al*., 2015), indicating that the suggested isomerization may be dependent

TABLE 2. Sparkling wine chemical composition evaluated over 18-months of cellar aging with different *dosage* sugar treatments [zero sugar control (CTR); D-glucose (GLU); D-fructose (FRU); sucrose derived from sugar beets (BET) and sugar cane (CAN); maltose (MAL); and MCR Sucraisin® rectified grape must concentrate (MCR)].

Time	Txmt	Ethanol (% v/v)	pH	TA [a] (g L^{-1})	Malic acid (g L^{-1})	Acetic acid (g L^{-1})	Free SO_2 (mg L^{-1})	Total SO_2 (mg L^{-1})	Browning A_{420} (mAU)	Glucose (g L^{-1})	Fructose (g L^{-1})	Residual sugar (g L^{-1})	Maltose [b] (g L^{-1})	Sucrose (g L^{-1})
0 months	CTR	12.4 ± 0.1	3.06 ± 0.01 c	9.7 ± 0.2 b	3.15 ± 0.03 a	0.12 ± 0.00	2 ± 1 ab	51 ± 1 a	85.1 ± 4.5	0.28 ± 0.00 a	0.50 ± 0.02 a	0.78 ± 0.02 a	n.d.	n.d.
	GLU	12.4 ± 0.1	3.04 ± 0.01 ab	9.6 ± 0.2 ab	3.14 ± 0.02 a	0.12 ± 0.00	2 ± 1 b	56 ± 1 c	88.9 ± 2.2	6.23 ± 0.21 e	0.14 ± 0.01 a	6.36 ± 0.21 b	n.d.	n.d.
	FRU	12.3 ± 0.1	3.03 ± 0.01 a	9.5 ± 0.2 ab	3.17 ± 0.05 ab	0.12 ± 0.00	1 ± 1 b	54 ± 1 b	88.0 ± 3.5	1.77 ± 0.11 b	4.42 ± 0.58 d	6.19 ± 0.47 b	n.d.	n.d.
	CAN	12.3 ± 0.1	3.05 ± 0.01 abc	9.1 ± 0.3 ab	3.18 ± 0.03 ab	0.12 ± 0.00	1 ± 1 a	62 ± 2 de	82.7 ± 3.2	2.72 ± 0.44 c	4.52 ± 0.39 d	7.24 ± 0.15 c	n.d.	1.31 ± 0.06 b
	BET	12.2 ± 0.3	3.05 ± 0.01 abc	9.1 ± 0.3 a	3.23 ± 0.04 b	0.12 ± 0.00	2 ± 1 b	58 ± 1 c	87.3 ± 2.7	3.68 ± 0.09 d	3.84 ± 0.10 c	7.52 ± 0.19 c	n.d.	1.12 ± 0.09 a
	MAL	12.1 ± 0.3	3.05 ± 0.01 bc	9.0 ± 0.5 a	3.21 ± 0.04 ab	0.12 ± 0.00	3 ± 1 b	61 ± 0 d	83.3 ± 2.7	0.17 ± 0.01 a	0.53 ± 0.03 a	0.70 ± 0.02 a	6.23 ± 0.12	n.d.
	MCR	12.2 ± 0.3	3.04 ± 0.01 ab	9.1 ± 0.4 a	3.23 ± 0.04 b	0.12 ± 0.00	4 ± 1 c	64 ± 1 e	87.5 ± 5.2	3.76 ± 0.11 d	2.84 ± 0.13 b	6.59 ± 0.21 b	n.d.	n.d.
		B	A	B	AB	A	C	C	A		A	A	C	C
9 months	CTR	12.4 ± 0.1 c	3.05 ± 0.00 a	9.7 ± 0.1 d	3.21 ± 0.03 bc	0.13 ± 0.00	1 ± 1	54 ± 4 ab	91.7 ± 2.1 c	0.89 ± 0.18 b	1.00 ± 0.16 b	1.87 ± 0.22 b	n.d.	n.d.
	GLU	12.3 ± 0.1 bc	3.06 ± 0.02 ab	9.5 ± 0.1 c	3.12 ± 0.01 a	0.12 ± 0.01	1 ± 1	57 ± 5 ab	89.7 ± 4.6 bc	5.67 ± 0.22 d	0.12 ± 0.03 a	5.79 ± 0.22 c	n.d.	n.d.
	FRU	12.0 ± 0.3 a	3.08 ± 0.01 bc	9.4 ± 0.1 bc	3.23 ± 0.03 c	0.13 ± 0.00	1 ± 1	55 ± 5 ab	87.7 ± 4.4 abc	0.40 ± 0.33 a	5.94 ± 0.55 e	6.35 ± 0.32 d	n.d.	n.d.
	CAN	12.4 ± 0.1 bc	3.06 ± 0.01 ab	9.3 ± 0.0 ab	3.22 ± 0.02 bc	0.13 ± 0.00	1 ± 1	51 ± 4 a	87.5 ± 2.1 abc	3.60 ± 0.04 c	3.84 ± 0.04 d	7.44 ± 0.07 e	n.d.	1.18 ± 0.03 b
	BET	12.4 ± 0.1 c	3.07 ± 0.02 bc	9.3 ± 0.1 ab	3.19 ± 0.04 bc	0.13 ± 0.00	2 ± 1	56 ± 7 ab	84.3 ± 4.3 ab	3.64 ± 0.12 c	3.60 ± 0.32 d	7.24 ± 0.36 e	n.d.	1.05 ± 0.09 a
	MAL	12.0 ± 0.2 a	3.08 ± 0.01 bc	9.3 ± 0.0 a	3.17 ± 0.03 ab	0.13 ± 0.00	1 ± 1	61 ± 5 b	83.2 ± 3.0 a	0.32 ± 0.01 a	0.35 ± 0.02 a	0.67 ± 0.02 a	5.95 ± 0.12	n.d.
	MCR	12.1 ± 0.1 ab	3.09 ± 0.01 c	9.3 ± 0.1 a	3.19 ± 0.03 bc	0.13 ± 0.00	1 ± 0	50 ± 2 a	87.7 ± 0.5 abc	3.76 ± 0.07 c	2.85 ± 0.04 c	6.61 ± 0.11 d	n.d.	n.d.
		AB	B	B	B	C	B	B	A		B	A	B	B
18 months	CTR	12.5 ± 0.2 b	3.24 ± 0.07 a	7.8 ± 0.4 a	3.18 ± 0.02 a	0.12 ± 0.00	1 ± 0	49 ± 3	91.7 ± 3.7 ab	0.34 ± 0.06 a	0.57 ± 0.06 a	0.91 ± 0.11 a	n.d.	n.d.
	GLU	12.0 ± 0.4 a	3.19 ± 0.01 c	8.3 ± 0.4 b	3.17 ± 0.04 a	0.12 ± 0.00	1 ± 0	50 ± 4	89.5 ± 4.9 a	6.00 ± 0.44 c	0.20 ± 0.13 a	6.20 ± 0.40 b	n.d.	n.d.
	FRU	12.3 ± 0.1 ab	3.14 ± 0.03 ab	8.1 ± 0.3 ab	3.16 ± 0.05 a	0.12 ± 0.00	1 ± 0	47 ± 5	94.3 ± 4.9 ab	0.15 ± 0.02 a	7.21 ± 0.34 d	7.36 ± 0.35 cd	n.d.	n.d.
	CAN	12.1 ± 0.1 ab	3.14 ± 0.01 ab	8.2 ± 0.1 ab	3.15 ± 0.05 a	0.12 ± 0.00	1 ± 0	49 ± 8	89.5 ± 7.5 a	3.72 ± 0.08 b	4.00 ± 0.07 c	7.72 ± 0.11 d	n.d.	0.98 ± 0.08
	BET	12.1 ± 0.1 ab	3.17 ± 0.02 bc	8.3 ± 0.2 b	3.18 ± 0.05 a	0.12 ± 0.00	1 ± 0	43 ± 0	96.2 ± 1.6 ab	3.56 ± 0.30 b	3.66 ± 0.39 c	7.22 ± 0.69 cd	n.d.	1.02 ± 0.05
	MAL	12.0 ± 0.3 a	3.15 ± 0.01 ab	8.4 ± 0.0 b	3.17 ± 0.06 a	0.12 ± 0.00	1 ± 0	50 ± 3	95.2 ± 5.2 ab	0.45 ± 0.01 a	0.36 ± 0.02 a	0.80 ± 0.03 a	5.52 ± 0.09	n.d.
	MCR	12.0 ± 0.4 a	3.12 ± 0.01 ab	8.3 ± 0.1 b	3.18 ± 0.04 b	0.13 ± 0.01	1 ± 0	44 ± 5	100.0 ± 7.2 b	3.91 ± 0.12 b	3.03 ± 0.16 b	6.94 ± 0.10 c	n.d.	n.d.
		A	C	A	A	B	A	A	B		C	B	A	A
***p*-value**	Txmt	***	***	*	***	***	***	***	**	***	***	***	–	***
	Time	*	***	***	*	***	***	***	***	n.s.	***	***	***	***
	Tx x Time	**	***	***	***	***	***	***	***	***	***	***	–	***
F-statistic	Txmt	6.09	3.96	2.75	4.72	4.24	4.48	5.20	3.06	2201.63	1411.62	2126.41	–	17.09
	Time	3.76	388.09	337.45	3.32	18.20	36.04	84.49	40.45	1.41	17.67	9.40	61.38	30.86
	Tx x Time	2.85	7.53	6.16	3.73	3.11	4.51	6.06	3.44	33.98	32.98	11.95	–	9.24
Eta-squared (η^2)	Txmt	0.199	0.024	0.019	0.153	0.125	0.104	0.083	0.075	0.962	0.940	0.979	–	0.134
	Time	0.041	0.780	0.776	0.036	0.177	0.279	0.447	0.330	0.000	0.004	0.001	–	0.485
	Tx x Time	0.187	0.091	0.085	0.242	0.183	0.210	0.192	0.168	0.030	0.044	0.011	–	0.145

Mean value ± standard deviation of triplicate bottles analysed in duplicate (n=3) at each time interval. Comparisons of treatment means was carried out via two-way ANOVA followed by Tukey's post-hoc means separation tests. Significance: n.s. = $p > .05$; * = $p < .05$;** = $p < .01$; *** = $p < .001$. Means followed by different lowercase letters in each column (each time interval assessed separately) are significantly different; different capital letters in the same column indicate that means for each time interval are significantly different. n.d. = value below the limit of detection.[a] TA expressed as g L^{-1} tartaric acid equivalents.[b] Maltose was evaluated via one-way ANOVA followed by Tukey's post-hoc means separation tests for sparkling wines treated with maltose in *dosage* (MAL).

TABLE 3. Sugar purity and composition.

Evaluated sugar product	Composition (%)					
	Fructose	Glucose	Lactose	Maltose	Sucrose	Total Sugar
D-Glucose monohydrate	< 0.1	90.7	< 0.1	< 0.1	< 0.1	90.7
D-Fructose	99.4	< 0.1	< 0.1	< 0.1	< 0.1	99.4
Sucrose (cane sugar)	< 0.1	< 0.1	< 0.1	< 0.1	99.9	99.9
Sucrose (beet sugar)	< 0.1	< 0.1	< 0.1	< 0.1	99.9	99.9
Maltose monohydrate	< 0.1	0.5	< 0.1	87.3	< 0.1	87.8
MCR Sucraisin®	40.0	51.8	< 0.1	< 0.1	< 0.1	91.7

on fructose levels or other matrix components. Additionally, sugar levels during aging are confounded by bottle variation in residual glucose levels following second fermentation, as previously discussed. As expected, changes in glucose levels for wines treated with glucose in *dosage* are consistent with residual sugar levels (sum of glucose and fructose). Minor changes may also be due to the transformation of sugars in the Maillard reaction or via isomerization during aging.

The analysis of metal ions content in sparkling wines following *dosage* sugar treatments is shown in Supplementary Table S5. Metal ions (e.g., Fe^{2+}, Cu^{2+}, Al^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}) have been shown to accelerate the Maillard reaction in model conditions (Hayase *et al*., 1996; Kwak and Lim, 2004; Omari *et al*., 2021; Rizzi, 2008), although limited research exists pertaining to the metal composition of sparkling wines (Charnock *et al*., 2022b; Focea *et al*., 2017; Jos *et al*., 2004a; Jos *et al*., 2004b; Rapa *et al*., 2023). Further, the impact of metals on the formation of Maillard reaction-associated products in sparkling wines has not yet been reported. The present analysis of metals in sparkling wines serves to contribute to our understanding of wine metal composition and screen for variation attributable to *dosage* sugar. All metals measured in wines were below the internationally regulated maximum allowable limits established by the OIV (International Organisation of Vine and Wine (OIV), 2015). No differences between *dosage* treatments were observed for metals apart from boron and barium. Boron levels were highest among control, glucose, fructose, and MCR Sucraisin® wines, with the lowest levels in cane and beet-derived sucrose treatments. Barium levels were also highest in the control wine, with the lowest levels in fructose, MCR Sucraisin®, and maltose treatments. Small concentration ranges for boron and barium across all *dosage* treatments of 0.6 mg L^{-1} and 3 μg L^{-1}, respectively, indicates that bottle variation is the driving factor for differences between wines, and thus metal content does not appear to be associated with sugar treatment.

2. Sugar Purity Analysis by HPLC

Each sugar product was evaluated as received by the supplier. Table 3 summarizes the composition and purity of sugars prior to their use in the preparation of *dosage* solutions. Reagent grade sugars showed lower purity than reported by the supplier (> 99.5 % for D-Glucose monohydrate and D-Fructose; > 95.0 % for maltose monohydrate), although D-Fructose composition was comparable at 99.4 %. Commercially available sucrose products derived from either sugar cane or sugar beets showed very high purity (99.9 % sucrose). Therefore, differences in the chemical composition of sparkling wines treated with cane or beet sugar in *dosage* are therefore attributable to 0.1 % of impurities in each refined sugar product, or bottle-to-bottle variation. MCR Sucraisin® is obtained from concentrating and dehydrating grape must and is approximately 1:1 glucose and fructose with trace amounts of minor sugars from the grape juice (Kliewer, 1967). Our analysis of MCR Sucraisin® shows a 1.3 glucose-fructose ratio, accounting for 91.7 % of the sugar composition. Glucose predominates in unripe grapes, although the ratio gradually decreases during ripening, reaching approximately 1 by maturity, after which point fructose becomes the primary sugar component in overripe grapes (Kliewer, 1967). The high glucose-fructose ratio of 1.3 in MCR Sucraisin® is therefore consistent with a sparkling wine must source, where glucose dominates the composition in early grape harvests.

3. ^{1}H NMR analysis of amino acids

Seven amino acids and nutrients including alanine, choline, glycine, proline, leucine, phenylalanine, and tyrosine were identified and quantified by ^{1}H NMR in sparkling wines aged for 0- and 18-months post-*dosage* and are shown in Table 4. Arginine, glutamine, isoleucine, threonine, uracil, and valine were below the limit of quantification. Aging time was a significant contributor to alanine, glycine, leucine, and tyrosine levels. Mean concentrations of alanine and glycine decreased over the 18-month aging period (by 12.3 and 11.2 %, respectively), indicating that they may degrade or participate in reactions including the Maillard reaction (Charnock *et al*., 2022a; Pripis-Nicolau *et al*., 2000; Zhang *et al*., 2018). Due to the varied fate of amino acids, decreasing amino acid levels do not necessarily equate to a proportional increase in Maillard reaction-associated products (Grant-Preece *et al*., 2013). A decrease in amino acids during bottle aging of red and white table wines has previously been reported (Cassino *et al*., 2019; Zhang *et al*., 2018). Zhang *et al.* (2018) observed a decrease in all evaluated amino acids

TABLE 4. Sparkling wine amino acid and nutrient composition (mg L^{-1}) evaluated at 0-and 18 months of cellar aging with different *dosage* sugar treatments [zero sugar control (CTR); D-glucose (GLU); D-fructose (FRU); sucrose derived from sugar beets (BET) and sugar cane (CAN); maltose (MAL); and MCR Sucraisin® rectified grape must concentrate (MCR)].

Time	Txmt	Alanine	Choline	Glycine	Proline	Leucine	Phenylalanine	Tyrosine
0 months	CTR	71.32 ± 4.69 abc	6.92 ± 0.25	180.65 ± 17.25 ab	253.38 ± 31.50	16.46 ± 2.77 b	10.24 ± 1.61 a	7.74 ± 0.33 a
	GLU	69.37 ± 1.56 ab	6.84 ± 0.16	166.83 ± 12.21 a	270.74 ± 9.60	14.91 ± 0.20 ab	11.88 ± 1.20 ab	7.93 ± 0.14 ab
	FRU	67.72 ± 0.89 a	6.75 ± 0.18	256.06 ± 32.08 d	268.85 ± 8.39	15.43 ± 3.46 ab	12.34 ± 0.31 b	7.93 ± 0.17 ab
	CAN	75.39 ± 1.27 c	6.96 ± 0.13	263.04 ± 20.99 d	274.62 ± 8.58	15.74 ± 2.99 ab	12.05 ± 0.77 ab	8.22 ± 0.18 b
	BET	69.45 ± 2.23 ab	6.92 ± 0.08	225.40 ± 12.87 cd	271.30 ± 9.11	15.47 ± 3.27 ab	12.07 ± 0.52 b	8.01 ± 0.21 ab
	MAL	73.05 ± 2.90 bc	6.89 ± 0.14	189.32 ± 20.4 abc	265.86 ± 5.80	15.40 ± 2.57 ab	11.13 ± 0.66 ab	7.77 ± 0.17 a
	MCR	71.08 ± 2.55 abc	6.88 ± 0.11	211.92 ± 36.76 bc	273.38 ± 9.06	11.54 ± 2.04 a	12.15 ± 1.32 b	7.94 ± 0.17 ab
		B		B		A		A
18 months	CTR	63.22 ± 4.89 ab	7.01 ± 0.28	136.22 ± 34.83 a	268.06 ± 8.32	19.12 ± 2.69	11.53 ± 1.26	8.36 ± 0.25
	GLU	68.00 ± 4.16 b	7.11 ± 0.19	175.12 ± 14.34 ab	277.54 ± 8.63	16.24 ± 1.93	11.03 ± 2.04	8.56 ± 0.27
	FRU	57.89 ± 5.50 a	6.58 ± 0.57	218.56 ± 20.37 b	244.50 ± 29.73	16.40 ± 3.38	10.88 ± 1.44	7.90 ± 0.61
	CAN	68.30 ± 7.96 b	7.15 ± 0.11	211.48 ± 30.89 b	274.33 ± 7.78	14.29 ± 2.79	11.57 ± 1.35	8.42 ± 0.26
	BET	56.12 ± 2.71 a	6.68 ± 0.53	214.52 ± 40.34 b	264.42 ± 26.67	16.99 ± 1.74	12.14 ± 0.43	8.32 ± 0.43
	MAL	65.00 ± 7.17 ab	6.62 ± 0.86	155.58 ± 10.77 a	256.05 ± 30.53	17.49 ± 4.05	12.40 ± 1.07	8.09 ± 0.93
	MCR	57.80 ± 3.49 a	6.51 ± 0.42	214.27 ± 35.57 b	251.11 ± 12.51	17.42 ± 1.61	10.11 ± 1.59	8.10 ± 0.32
		A		A		B		B
p-value	Txmt	***	n.s.	***	n.s.	n.s.	n.s.	***
	Time	***	n.s.	***	n.s.	**	n.s.	***
	Tx x Time	*	n.s.	*	n.s.	n.s.	n.s.	*
F-statistic	Txmt	7.96	2.15	19.64	1.90	1.88	1.43	2.02
	Time	88.62	0.77	17.70	2.47	9.92	1.43	14.43
	Tx x Time	2.80	1.46	2.48	2.01	1.98	3.33	1.24
Eta-squared (η^2)	Txmt	0.214	0.139	0.535	0.119	0.110	0.086	0.117
	Time	0.397	0.008	0.080	0.026	0.096	0.014	0.139
	Tx x Time	0.075	0.095	0.067	0.126	0.115	0.200	0.071

Mean value ± standard deviation of triplicate bottles analysed in duplicate (n=3) at each time interval. Comparisons of treatment means was carried out via two-way ANOVA followed by Tukey's post-hoc means separation tests. Significance: n.s. = $p > .05$; * = $p < .05$; ** = $p < .01$; *** = $p < .001$. Means followed by different lowercase letters in each column (each time interval assessed separately) are significantly different; different capital letters in the same column indicate that means for each time interval are significantly different

apart from asparagine over 12-months of bottle aging for Cabernet-Sauvignon. This included a decrease in alanine, leucine, proline, and phenylalanine; however, Zhang *et al.* did not include glycine, choline, and tyrosine. Similarly, Cassino *et al.* (2019) identified a slow but progressive decrease in the concentration of amino acids over a 24-month period for red and white table wines stored at 12 °C. This contradicts our findings, where leucine and tyrosine levels increased by an average of 12.4 and 4.0 %, respectfully over 18-months of bottle aging. Amino acids have been shown to increase during sparkling wine aging in contact with yeast lees, but not in their absence (Condé *et al*., 2017; Culbert *et al*., 2017; Martínez-Rodríguez *et al.*, 2002; Prokes *et al.*, 2022). The reason for the increase in leucine and tyrosine is unclear, but may be linked to trace amounts of residual yeast solids remaining in each bottle post-disgorgement, allowing levels to rise as cell membranes release amino acids into the wine matrix during their breakdown (Alexandre and Guilloux-Benatier, 2006). The concentrations of choline and proline were not influenced by time, treatment, nor their interactions. Interactive effects of aging duration and *dosage* sugar treatment on amino acid and nutrient composition were observed (Supplementary Table S3). Specifically, alanine decreased between 0- and 18-months of aging for wines treated with MCR Sucraisin®, beet-derived sucrose, and fructose by 13.3, 13.3 and 9.8 mg L^{-1}, respectively. Despite time and treatment interactions for glycine and tyrosine, there was no evidence for further systematic trends when comparing the amino acid composition of the same *dosage* treatment during aging.

Multivariate analysis by PLS-DA revealed a sufficiently predictive model ($Q^2 = 0.59$; $R^2 = 0.66$) with results illustrated in Figure 3A-C. PLS-DA is an effective discrimination technique to maximize and visualize the separation between features. Ellipses at the 95 % confidence interval are grouped by aging duration of 0 and 18 months (T0 and T18, respectively). PC1 (36.6 %) and PC2 (16.6 %) accounted for 53.2 % of the total variability in the amino acid data

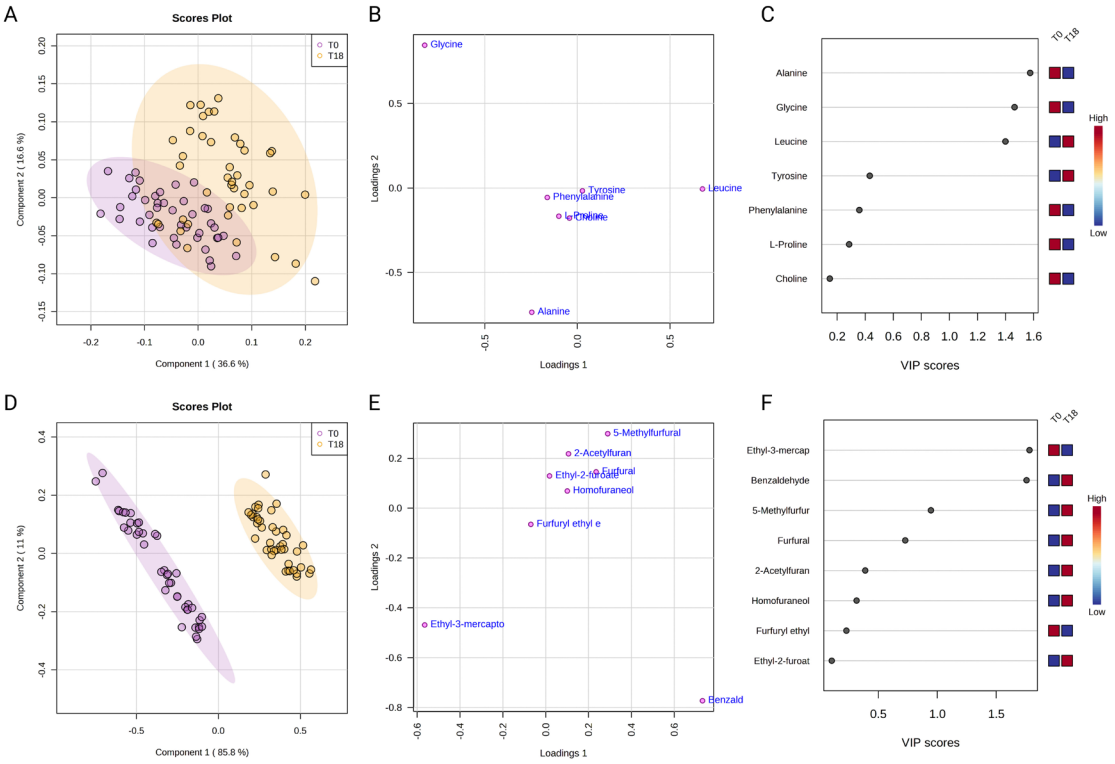


FIGURE 3. Partial least squares discriminant analysis (PLS-DA) of amino acids (A-C) and Maillard reaction-associated products (D-F) in sparkling wines aged with various *dosage* sugary-types aged for 0 (T0) and 18 months (T18). Scores plot of sparkling wine samples (A,D), loading plots show key differentiating variables (B,E), and Variable Importance in Projection (VIP) scores (C,F). VIP > 1 indicates variables with significant ($p < .05$) fold changes, whereby blue denotes a significant fold change decrease and red indicates a significant fold change increase.

(Figure 3A-C). VIP variables > 1 included alanine, glycine, and leucine. The relative abundance of alanine and glycine decrease by 1.5-1.6-fold over the 18-month aging duration, while leucine shows an approximate 1.4-fold increase ($p < .05$).

4. GC-MS analysis of Maillard reaction-associated products

Maillard reaction-associated products in the aged sparkling wines at 0-months and 18-months post-*dosage* are shown in Table 5. Interaction results are shown in Supplementary Table S4. Eight compounds were measured > LOQ at both time intervals (benzaldehyde, ethyl-3-mercaptopropionate, 2-acetylfuran, 5-methylfurfural, furfural, homofuraneol, furfuryl ethyl ether, ethyl-2-furoate), and one compound was measured > LOQ only in wines aged for 18-months (2-methylthiazole). Benzenemethanethiol and 2-acetylthiazole were detected in all wines although < LOQ. Similarly, 2-ethylthiazole was detected but < LOQ in wines aged for 18-months, and < LOD at 0-months. 2-Methylthiazole was also < LOQ in wines at 0-months. Although many Maillard reaction-associated products included in this method have previously been reported in sparkling wine literature (Table 1), they were not detected in our wines. This may be due to method sensitivity or to differences in regional, varietal, and viticultural practices, which alter the chemical composition of grapes used for sparkling wine production (Jones *et al.*, 2014), thus modifying precursors available for age-related compound formation.

Aging duration was the primary driver for the development of Maillard reaction-associated compounds and had a stronger effect than *dosage* sugar type. Increases in concentration were observed for most compounds over the 18-month aging period, apart from ethyl-3-mercaptopropionate and furfuryl ethyl ether, which decreased. No systematic trends were observed in the formation of Maillard reaction-associated product families when comparing sugar treatments over the aging period.

The sensory relevance of odorants can be estimated by calculating their odour activity value (OAV), which is the ratio of the analyte's concentration to its sensory detection threshold. Generally, an OAV > 1 is considered important in influencing odour perception. No reported sensory threshold data is available for 2-methylthiazole or ethyl-2-furoate, and thus OAV values could not be calculated. Of the remaining analytes, ethyl-3-mercaptopropionate and furfuryl ethyl

ether are the only compounds present in our samples with OAV values > 1.

4.1 Benzaldehyde

Benzaldehyde is formed by the oxidation of benzylic alcohol or via Maillard reaction pathways with phenylalanine (Pripis-Nicolau *et al*., 2000), and has been reported to increase during Champagne aging in contact with yeast lees (Loyaux *et al*., 1981). It has been measured at levels up to 7 mg L^{-1} in aged Champagne (Delfini *et al.*, 1984). Benzaldehyde contributes a desirable bitter almond, sweet, and buttery odour to wine (de Souza Nascimento *et al*., 2018; Delfini *et al*., 1984), and has a sensory detection threshold of 3 – 3.5 mg L^{-1} (Delfini, 1987). Levels in the present study were below sensory detection thresholds, indicating that further aging is likely required for perceived sensory relevance. When comparing benzaldehyde levels in the wines prior to aging, differences were observed between treatments although they are attributed to bottle variation due to high variability between wines of the same sugar treatment. No differences were observed between the treatments after 18-months.

4.2 Ethyl-3-mercaptopropionate

Ethyl-3-mercaptopropionate has previously been identified in aged Champagne wines with concentrations ranging from 40 – 12 000 ng L^{-1} (Tominaga *et al*., 2003b). Tominaga *et al.* (2003b) reported the concentration of ethyl-3-mercaptopropionate to increase after 13-15 years of bottle aging, and subsequently decrease. Further, levels are also influenced by the aging time on lees prior to disgorging (Tominaga *et al*., 2003b). Our measured concentrations decreased during 18-months of bottle aging and ranged from 14.12 ± 1.18 to 50.90 ± 2.51 μg L^{-1} for both time intervals – levels surpassing those previously reported. Ethyl-3-mercaptopropionate levels decreased during aging for all sugar treatments, with an average decrease of 63.5 %. Cane-derived sucrose and MCR Sucraisin® treatments had the greatest decreases at 69.5 and 67.7 % during aging (corresponding to approximately 34.4 and 29.5 μg L^{-1}, respectively), while glucose *dosage* wines had the lowest overall decrease at 58.4 % after 18-months (24.0 μg L^{-1}). Ethyl-3-mercaptopropionate contributes to the empyreumatic aroma of aged Champagne wines (Tominaga et al., 2003b) although it has also been identified in Concord grapes, where it has fruity and "pleasant" qualities (Kolor, 1983). OAV values calculated in our samples ranged from 206 – 255 for wines at 0-months, and 71 – 96 in wines after 18-months aging. These results indicate that at both time points, ethyl-3-mercaptopropionate is an important contributor to sparkling wine aroma.

4.3 2-Methylthiazole

2-Methylthiazole is reported to have a green vegetable aroma (Kolor, 1983), and to the best of our knowledge, has not been previously reported in sparkling wine literature. It was found < LOQ in wines at 0-months and increased after 18-months to a maximum of 6.13 μg L^{-1} with no differences measured between treatments. To our knowledge, no reported sensory detection threshold is available for OAV determination.

4.4 2-Acetylfuran

2-Acetylfuran has balsamic, burnt, and sweet aroma qualities (Burin *et al*., 2013; Le Menn *et al*., 2017) with a relatively high sensory detection threshold of 80 mg L^{-1} (Vanderhaegen *et al*., 2003). It is reported to increase with base wine aging (studied up to 27 years) for several Champagne varieties (Chardonnay, Pinot noir, and Meunier), with a maximum reported concentration of 14.70 μg L^{-1} (Le Menn *et al*., 2017). Martínez-García *et al.* (2021) observed a similar trend for sparkling wines aged up to 15 months, with maximum levels reaching as high as 0.77 mg L^{-1}. While levels in our wines were much lower, ranging from 3.12 – 5.15 μg L^{-1}, significant variation due to *dosage* sugar treatment, aging duration, and their interaction was observed. 2-Acetylfuran concentrations varied between *dosage* sugar-types at both 0- and 18-month periods with the highest levels in the control, glucose, and cane sugar treatments after 18-months. 2-Acetylfuran levels unanimously increased during aging for all sugar treatments, although there was no systematic time by treatment interaction effect for any individual sugar type.

2-Acetylfuran is an intermediate Maillard reaction product with a complex formation pathway involving glucose or glucose and glycine. Glycine decreased during aging, suggesting its involvement in Maillard reaction-associated pathways, and may be particularly important for 2-acetylfuran levels. While mean glucose levels across all wines did not decrease over time, they decreased in glucose-*dosage* wines between the 0- and 9-month intervals, supporting its involvement in 2-acetylfuran formation. Future research on sparkling wines treated with glucose in *dosage* or potentially *tirage* is necessary to determine if its presence can generate 2-acetylfuran to levels with sensory relevance.

4.5 Furfural

Furfural has fruity, caramel, and toasted aroma qualities (Torrens *et al*., 2010), and is a precursor to 2-furanmethanethiol, a powerful aromatic thiol which forms in barrel-fermented white wines during aging (Blanchard *et al*., 2001). The formation of furfural via the Maillard reaction is well documented and is preferentially generated from reactions involving fructose under low pH (pH < 7) conditions (Nursten, 2005). During Champagne production, furfural increases with bottle aging, and has been reported to reach maximum levels of 3.8 mg L^{-1} after 25 years (Tominaga *et al*., 2003b). This is in agreement with Torrens et al. (2010) identifying furfural increases over 24 months of Cava aging. In our study, furfural levels were highest after 18-months (average of 214.9 μg L^{-1}) but did not vary with treatment nor the interaction between time and treatment. Despite the presence of additional fructose in wines with fructose *dosage*, no differences in furfural levels were observed between treatments after 18-months. It is possible that residual fructose levels in the wine following second fermentation are sufficient for Maillard activity and

furfural is therefore present in all wines, although below the sensory threshold of 14 mg L^{-1} (Ferreira *et al*., 2000).

4.6 5-Methylfurfural

5-Methylfurfural is a furan compound of interest in several wine aging studies (Bosch-Fusté *et al*., 2007; Burin *et al*., 2013; López de Lerma *et al*., 2010; Pereira *et al*., 2014; Spillman *et al*., 1998; Ubeda *et al*., 2019) and is described as sweet, fruity, caramel, nutty, and spicy (Burin *et al*., 2013; Torrens *et al*., 2010) with a sensory threshold > 1 mg L^{-1} (Spillman *et al*., 1998). It is formed via Maillard reaction pathways similar to that of furfural, whereby it originates from fructose reactions under acidic conditions. It is proposed that its formation is also related to residual fructose levels in the wines since no relationship was identified between the fructose *dosage* treatments and elevated 5-methylfurfural concentrations. Instead, 5-methylfurfural levels increased in all sparkling wines during aging, with no differences between treatment at either aging timepoint.

4.7 Homofuraneol

Homofuraneol is derived from Maillard reactions primarily with pentose sugars and is a complex furan derivative with strawberry, caramel, and sweet aroma qualities (Blank and Fay, 1996; Cutzach *et al*., 1999; Escudero *et al*., 2000). Homofuraneol levels were most influenced by aging duration (η^2 = 0.912), and to a lesser extent, sugar treatment (η^2 = 0.014). Contrary to our findings, Cutzach *et al*. (1998) found that homofuraneol levels diminished during the aging of young sweet red wines. This disparity may be attributed to higher residual sugar levels present in sweet wines than sparkling wines. Homofuraneol has been detected at concentrations with sensory relevance in Pinot noir and Chardonnay sparkling wines after 12 and 24 months of aging, respectively (Sawyer et al., 2022). Although homofuraneol levels in our study were below the sensory threshold (10 μg L^{-1}; Kotseridis *et al.*, 2000) in all wines in the present study, they approached an OAV of 1 after 18-months of aging. Differences between treatments were observed at 0-months, but not at 18-months. Since homofuraneol is primarily formed through interactions with pentose sugars, the sugar-type in *dosage* is unlikely to influence its formation during aging. Further research on the compositional changes of pentose sugars, endogenous to the grapes, during the aging process, is therefore warranted.

4.8 Furfuryl ethyl ether

Furfuryl ethyl ether has a spicy, nutty, and solvent-like aroma (Harayama *et al*., 1995; Spillman *et al*., 1998) and a low sensory threshold of 2.5 μg L^{-1} reported in beer (Harayama *et al*., 1995). Over 18-months, the concentration of furfuryl ethyl ether decreased by an average of 12 %, although all measured concentrations had an OAV > 1. OAV values were 3 for all 0-month wines except for the MCR Sucraisin® treatment with an OAV of 2. After 18-months, OAV values dropped to 2 for all wines aside from the control and glucose treatment which remained at 3. Treatment by time interaction data indicates that furfuryl ethyl ether concentration was unchanged in glucose and maltose *dosage* treatments over the aging period, while all other *dosage* treatments decreased. Cane-derived sucrose treatments had the greatest average decrease at 20.1 % (1.3 μg L^{-1}).

Our measured levels of furfuryl ethyl ether were lower than those previously reported in table wine literature (25 – 170 μg L^{-1}; Spillman *et al*., 1998). Furfuryl ethyl ether has been shown to increase over 93 weeks of barrel aging for white wines (Spillman *et al*., 1998). However, the oxidative environment during barrel aging may account for differences in furfuryl ethyl ether formation and degradation when compared to sparkling wine bottle aging. In beer, furfuryl ethyl ether is formed from furfuryl acetate, a fermentation by-product (Harayama *et al*., 1995), although this compound has not yet been identified in wine. The degradation of furfuryl ethyl ether, derived during the fermentation process, likely explains its decrease in sparkling wines during aging (Spillman *et al*., 1998).

4.9 Ethyl-2-furoate

Ethyl-2-furoate is a Maillard intermediate compound produced by the esterification of ethanol and furoic acid, whereby furoic acid is generated via the Maillard reaction in the absence of oxygen (Cutzach *et al*., 1999; Vernin and Parkanyi, 1982). It has only recently been studied in sparkling wine (Medeiros *et al*., 2022; Ubeda *et al*., 2019) and is described as having an odour of white flowers (Ubeda *et al*., 2019). Sensory detection thresholds have not yet been reported. Medeiros *et al*. (2022) identified ethyl-2-furoate at 2 – 3 μg L^{-1} in sparkling base wines, with significantly higher levels observed for wines stored at 15 °C compared to 30 °C, presumably due to ethyl-2-furoate consumption in Maillard activity at higher temperatures. Ethyl-2-furoate concentrations increased during aging, supporting the suggested formation of ethyl-2-furoate via furfural (Medeiros *et al*., 2022). This agrees with our findings, as concentrations increased over 18-months of aging. Treatment effects were only present at the 0-month timepoint, with the lowest levels in glucose *dosage* wines (27.03 ± 1.98 μg L^{-1}) and the highest amounts in wines treated with cane-derived sucrose and MCR Sucraisin® (30.85 ± 1.27 and 30.81 ± 2.55 μg L^{-1}, respectfully). It is of note that in the present study, we have identified concentrations of ethyl-2-furoate substantially higher than those previously reported, with our measured levels ranging from 24.40 – 35.08 μg L^{-1}. Due to the absence of sensory threshold information, its impact on the aroma profile of sparkling wine remains to be elucidated.

4.10 Multivariate analysis of Maillard reaction-associated product composition during aging

PLS-DA results for Maillard reaction products at the two timepoints are shown in Figure 3D-F. PC1 (85.8 %) and PC2 (11.0 %) explain 96.8 % of the data variability, with benzaldehyde and ethyl-3-mercaptopropionate the most important analytes contributing to the discrimination between the two aging time points. Ethyl-3-mercaptopropionate decreased by 1.2-fold over 18-months of bottle aging, while benzaldehyde had a similar degree of increase over the same

TABLE 5. Maillard reaction-associated products (μg L^{-1}) in sparkling wines evaluated at 0- and 18-months of cellar aging with different *dosage* sugar treatments [zero sugar control (CTR); D-glucose (GLU); D-fructose (FRU); sucrose derived from sugar beets (BET) and sugar cane (CAN); maltose (MAL); and MCR Sucraisin® rectified grape must concentrate (MCR)].

Time		Txmt	Benzaldehyde	Ethyl-3-mercaptopropionate	2-Methylthiazole	2-Acetylfuran	5-Methylfurfural	Furfural	Homofuraneol	Furfuryl ethyl ether	Ethyl-2-furoate
		#	1	3	7	11	13	14	15	16	17
0 months		CTR	3.25 ± 0.50 a	46.38 ± 5.17 ab	n.q.	4.01 ± 0.36 b	5.11 ± 0.57	144.58 ± 13.07 ab	7.77 ± 0.25 ab	6.89 ± 0.48 b	27.77 ± 2.96 ab
		GLU	9.64 ± 1.26 c	41.18 ± 3.51 a	n.q.	3.46 ± 0.29 a	5.34 ± 0.44	140.77 ± 12.04 ab	7.68 ± 0.23 ab	6.63 ± 0.37 b	27.03 ± 1.98 a
		FRU	7.52 ± 2.56 bc	43.67 ± 2.83 ab	n.q.	3.53 ± 0.05 ab	5.56 ± 0.39	145.40 ± 1.33 ab	7.77 ± 0.05 ab	6.52 ± 0.35 ab	28.02 ± 1.31 ab
		CAN	2.71 ± 0.47 a	49.50 ± 3.01 b	n.q.	3.86 ± 0.30 ab	5.53 ± 0.43	133.32 ± 5.61 a	7.52 ± 0.12 ab	6.52 ± 0.35 ab	30.85 ± 1.27 b
		BET	8.00 ± 2.15 bc	44.37 ± 2.79 ab	n.q.	3.66 ± 0.23 ab	5.89 ± 0.36	148.20 ± 7.27 ab	7.81 ± 0.13 b	6.37 ± 0.41 ab	29.20 ± 1.12 ab
		MAL	3.26 ± 2.08 a	48.58 ± 6.90 ab	n.q.	3.66 ± 0.43 ab	5.63 ± 0.44	132.58 ± 10.46 a	7.48 ± 0.20 a	6.43 ± 0.32 ab	30.59 ± 2.22 ab
		MCR	5.88 ± 2.55 ab	50.90 ± 2.51 b	n.q.	3.81 ± 0.295 ab	5.92 ± 0.55	149.85 ± 7.75 b	7.73 ± 0.17 ab	5.89 ± 0.17 a	30.81 ± 2.55 b
			A	B	A	A	A	A	A	B	A
18 months		CTR	12.84 ± 2.49	17.03 ± 2.29 bc	5.75 ± 1.67	4.80 ± 0.14 b	9.38 ± 0.62	223.88 ± 14.72	9.40 ± 0.25	6.27 ± 0.23 d	30.58 ± 1.46
		GLU	16.27 ± 5.12	17.14 ± 0.64 bc	4.82 ± 1.19	4.75 ± 0.15 b	9.57 ± 0.21	217.95 ± 12.67	9.23 ± 0.25	6.36 ± 0.18 d	30.94 ± 1.84
		FRU	13.84 ± 4.38	14.12 ± 1.18 a	5.19 ± 1.59	4.61 ± 0.21 ab	9.37 ± 0.89	208.71 ± 12.49	9.07 ± 0.28	5.55 ± 0.26 bc	29.98 ± 1.74
		CAN	14.37 ± 6.88	15.10 ± 2.33 ab	4.74 ± 1.76	4.75 ± 0.27 b	9.31 ± 0.41	209.52 ± 17.00	9.04 ± 0.31	5.21 ± 0.17 ab	31.59 ± 1.96
		BET	17.18 ± 3.17	16.84 ± 1.69 abc	6.13 ± 0.57	4.31 ± 0.31 a	9.27 ± 0.54	212.66 ± 11.31	9.13 ± 0.23	5.43 ± 0.14 abc	30.04 ± 1.36
		MAL	10.43 ± 1.69	19.27 ± 1.06 c	5.21 ± 1.04	4.72 ± 0.20 ab	9.93 ± 0.33	216.55 ± 13.22	9.20 ± 0.26	5.83 ± 0.15 c	32.66 ± 1.31
		MCR	12.99 ± 2.40	18.53 ± 0.81 c	6.09 ± 0.78	4.41 ± 0.23 ab	9.83 ± 0.33	214.82 ± 8.93	9.27 ± 0.24	5.13 ± 0.37 a	30.58 ± 1.14
			B	A	B	B	B	B	B	A	B
p-value		Txmt	***	***	n.s.	**	*	n.s.	*	***	***
		Time	***	***	***	***	***	***	***	***	***
		Tx x Time	n.s.	**	n.s.	*	n.s.	n.s.	n.s.	**	n.s.
F-statistic		Txmt	6.49	6.20	1.81	3.61	2.37	1.89	2.54	18.25	4.56
		Time	141.20	1897.85	186.31	248.91	1362.56	876.68	964.63	140.66	19.19
		Tx x Time	1.13	3.43	0.77	2.62	1.43	1.65	1.36	3.68	1.79
Eta-squared (η^2)		Txmt	0.152	0.018	0.040	0.061	0.010	0.012	0.014	0.320	0.215
		Time	0.550	0.937	0.686	0.699	0.936	0.906	0.912	0.411	0.151
		Tx x Time	0.026	0.010	0.017	0.044	0.006	0.010	0.008	0.065	0.084

Mean value ± standard deviation of triplicate bottles analysed in duplicate (n=3) at each time interval. Bold values indicate OAV > 1. Comparisons of treatment means was carried out via two-way ANOVA followed by Tukey's post-hoc means separation tests. Significance: n.s. = $p > .05$; * = $p < .05$; ** = $p < .01$; *** = $p < .001$. Means followed by different lowercase letters in each column (each time interval assessed separately) are significantly different; different capital letters in the same column indicate that means for each time interval are significantly different. n.q. = value below the limit of quantification reported in Table 1.

time interval. 5-Methylfurfural has a VIP score nearing 1.0, suggesting that it is also influential to the model. PLS-DA was an effective technique for the separation of sparkling wines based on their aging duration (across *dosage* treatment) but did not discriminate well between *dosage* treatments. From this study, it is clear that the duration of wine aging has a greater effect on the composition of age-related compounds than *dosage* sugar-type. These results also suggest that the formation of Maillard reaction products may be partly related to the depletion of alanine and glycine precursors.

CONCLUSION

To our knowledge, this is the first study to evaluate *dosage* sugar-type in the context of Maillard reaction-associated products in sparkling wines. Maillard reaction-associated products were slowly formed and possibly also degraded in sparkling wines during 18-months of bottle aging. Our results clearly showed that the chemical composition of the wines, including sugar and amino acid precursors, is influenced to a greater extent by the aging duration of the wines rather than the *dosage* sugar composition. Amino acids alanine and glycine have been identified as key contributors to amino acid variability during aging, and both decrease during the aging period. Additionally, benzaldehyde and ethyl-3-mercaptopropionate were identified as key discriminating compounds when comparing wines aged for 0- and 18-months, with significant increases and decreases, respectively. No systematic trends were observed in the formation of Maillard reaction-associated product families when comparing sugar treatments over 18-months of aging. Further information pertaining to the Maillard reaction pathways of *dosage* sugars or sugars endogenous to wine is necessary to characterize these interactions in the unique low-temperature and low pH sparkling wine conditions. Specifically, model wine studies with controlled conditions are necessary for understanding reactions between single sugars and single amino acids, particularly those involving alanine and/or glycine. Since the duration of sparkling wine aging is a key limitation for this type of research, accelerated aging by the application of mild heating or metal ions may hasten the rate of chemical reactions to enable analysis on a shorter time scale than typical wine aging. An expanded investigation of additional Maillard reaction-associated compounds and organoleptic data that is specific to their detection thresholds in a sparkling wine matrix will also be highly beneficial. Future studies including the application of GC-Olfactometry and rapid sensory evaluation methods would provide valuable information regarding the identity of relevant aroma compounds and sensory differences in aged sparkling wines, respectively.

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AUTHOR CONTRIBUTIONS

Conceptualization, BK, HC, and GP; Investigation and Analysis, HC; Writing – Original Draft, HC; Data Visualization, HC; Review and Editing, BK and GP; Supervision, BK and GP; Funding acquisition, BK, HC. The authors declare no conflicts of interest in this research.

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