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From the Editor

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Samara Kuehne is professional editor of Food Quality & Safety. *Reach her at skuehne@wiley.com.*

Effect of dissolved carbon dioxide on the sensory properties of still white and red wines

R. GAWEL[1,2], A. SCHULKIN[1], P.A. SMITH[1,2], D. ESPINASE[1] and J.M. MCRAE[1]

[1] The Australian Wine Research Institute, Glen Osmond, SA, 5064, Australia; [2] Wine Australia, Kent Town, SA, 5071, Australia

Corresponding author: Dr Richard Gawel, email richard.gawel@awri.com.au

Abstract

Background and Aims: Still wine contains a significant but sub-saturated concentration of carbon dioxide (CO_2) that remains following alcoholic fermentation. The concentration of CO_2 can be adjusted by winemaking practices and may influence wine sensory properties. This study set out to define for the first time the effect of a sub-saturated level of dissolved carbon dioxide (DCO_2) on the taste, overall aroma, flavour and mouthfeel attributes of still white and red wine.

Methods and Results: The concentration of DCO_2 of two white and two red wines was adjusted to represent the range encountered in their respective wine types. Ethanol concentration of both wine types, the pH of the white wines and red wine tannin concentration were varied to assess the sensory implications of wine matrix interaction with DCO_2. Differences in 'spritz' intensity were differentiable within the range of DCO_2 concentration found in still white and red wines. A higher DCO_2 concentration generally increased perceived sweetness and reduced bitterness and astringency perception. The DCO_2 did not influence fruit aroma or flavour intensity and few consistent interactions between DCO_2 and the wine matrix were observed.

Conclusions: At still wine concentration DCO_2 directly influences the taste and astringency of wine, but in a manner that is different from other beverage systems with a saturated level of CO_2.

Significance of the Study: The study is the first to explore the effect of a sub-saturated (non-sparkling) concentration of DCO_2 on the aroma, taste, flavour and mouthfeel of wine and provides practical guidance as to how to modulate DCO_2 in table wines to achieve a desired taste and mouthfeel.

Keywords: *carbonation, flavour, mouthfeel, spritz, taste, wine*

Introduction

The bottling specification of still white and red table wine typically includes a target concentration of dissolved carbon dioxide (DCO_2), as when low, wines can taste 'flat' and give the impression that they lack freshness; but when DCO_2 concentration is excessive, wines can elicit a 'spritz' sensation that is incongruent with consumer expectations of still wine.

A still wine is defined in the USA as one containing less than 3.92 g/L DCO_2 (US Code of Federal Regulations 2012), while in Australia 5 g/of DCO_2 is used to legally differentiate sparkling from still and semi-still wine (Food Standards Australia New Zealand 2004). Within these broad limits, however, winemakers routinely adjust DCO_2 concentration to a level that is consistent with a desired wine style by direct gas exchange with N_2 (sparging) or by using membrane contactors (Nordestgaard 2018). The range of DCO_2 concentration typically applied by winemakers in commercial practice is 0.5–1.8 g/L for white wines and 0.5–1.0 g/L for red wines (Müller-Späth 1982, Peynaud 1984).

The oral sensation elicited by DCO_2 has a detection threshold of 0.26 g/L in water (Le Calvé et al. 2010) and has been described as 'tingling', 'prickling', 'burning', 'fizzy' and 'spritzy' (Green 1996, Hewson et al. 2009, Clark et al. 2011). The dominant sensory mechanism that underlies the oral perception varies depending on DCO_2 concentration. When DCO_2 is saturated at atmospheric pressure, as is the case in beer and sparkling wine, the release of CO_2 bubbles in the mouth is thought to activate oral mechanoreceptors that indicate the percept of foaming. At a lower DCO_2 concentration typical of still wine, chemoreception is the dominant mechanism, whereby carbonic anhydrase production of carbonic acid activates oral nociceptors attuned to perceiving noxious substances which include DCO_2 (Dessirier et al. 2000, Carstens et al. 2002). Specifically, perception of DCO_2 involves the excitation of the intracellular proton gated transient receptor potential channel A1 nociceptors that are embedded within the oral mucosa (Wang et al. 2010). This channel modulates the transduction of intraneuronal acidification by weak acids in general and by DCO_2 in particular (Wang et al. 2011), and is co-expressed on the same receptor with the channel transient receptor potential channel V1 which conveys information about a broad range of percepts that include capsaicin heat, astringency and palate warmth elicited by ethanol (Trevisani et al. 2002, Kurogi et al. 2015).

The effect of DCO_2 on the taste qualities and spritz of model systems involving a saturated level of DCO_2 at atmospheric pressure (>5 g/L) (Dalmolin et al. 2006) representing beer (Clark et al. 2011), apple cider (Symoneaux et al. 2015) and aqueous systems likened to 'soft drinks' (Yau and McDaniel 1992, Cowart 1998, Hewson et al. 2009) has been reported. In these saturated systems increasing DCO_2 has mostly been found to suppress sweetness and enhance sourness (Cowart 1998, Hewson et al. 2009, Clark et al. 2011, Symoneaux et al. 2015). Others have reported,

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however, that sweetness is unaffected by DCO_2 (Yau and McDaniel 1992) and that the effect of carbonation on the perception of sourness is dependent on the concentration of the acidulant (Yau and McDaniel 1992, Hewson et al. 2009). The bitterness of hop acids in model beer was shown to be suppressed by carbonation (Clark et al. 2011). Conversely, the bitterness of apple tannins in a model apple cider system was unaffected by carbonation (Symoneaux et al. 2015). The effect of carbonation on mouthfeel has been little studied compared with taste properties. Symoneaux et al. (2015) found that carbonation increased astringency perception of apple tannins, while Clark et al. (2011) observed that carbonation contributed to the warm mouthfeel elicited by ethanol at low concentration but suppressed the perception of warmth at higher concentration.

Still wines differ from other alcoholic beverages in that: (i) their DCO_2 concentration is well below saturation level at consumption; (ii) they are higher in alcohol and acidity; and (iii) in the case of red wine they are higher in monomeric and polymeric flavan-3-ols that contribute to their bitterness and astringency (Peleg et al. 1999). Given that the perception of DCO_2 is both somatosensory and chemosensory in origin, it is feasible that DCO_2 in still wine could also affect the perception of the mouthfeel attributes of perceived viscosity, astringency and orally perceived warmth from ethanol. This study investigates the direct and interactive effect of DCO_2 with ethanol, pH and tannin concentration on the perceived intensity of the tastes, overall fruit aroma and flavour and mouthfeel attributes of still white and red wines.

Materials and methods

Commercial bottled white (Chardonnay, Viognier) and red (Shiraz and Cabernet Sauvignon) wines, each from a single bottling run and sealed under screw cap were sourced from the winery (2016 Yalumba 'Y Series', S. Smith & Sons, Angaston, SA, Australia) (Table 1).

Four nominal levels of DCO_2 were obtained for the white wines ('high', 'medium-high', 'low' and untreated Control) and three nominal levels of DCO_2 were obtained for red wines ('high', 'low' and untreated Control). The treatments were obtained by blending the original wine with the same wine that had been saturated with CO_2 (for the high and medium-high concentration), or that had been de-carbonated (for the low concentration). The saturated component of the blend was obtained by releasing pressurised CO_2 into wine (500 mL) contained in polycarbonate containers. The de-carbonated component of the blend was obtained by extensively sparging with N_2 (BOC, North Ryde, NSW, Australia). Specifically, 'high' and 'medium-high' levels DCO_2 were achieved by blending 20 and 10% carbonated wine with the Control wine, respectively, while a 'low' level of DCO_2 comprised 100% sparged wine.

Full factorial designs were constructed to assess the interactions of wine matrix components and DCO_2 on wine sensory characters. White wine factorial design included 16 treatments reflecting four (DCO_2) × two (ethanol) × two (pH) levels, and the red wine factorial design included 12 treatments covering three (DCO_2) × two (ethanol) × two (tannin) concentration levels. The levels were selected to reflect ranges typical of the respective wine type. The white wines were pH adjusted using L-(+)-tartaric acid (Sigma Aldrich, St Louis, MO, USA) to produce two pH levels each (pH 3.2 and 3.4). Ethanol (96% v/v ethanol, Tarac Technologies, Nuriootpa, SA, Australia) was added to increase alcohol by 1% v/v to give a concentration of 13.2 and 14.2% v/v in Chardonnay and 13.5 and 14.5% v/v in Viognier. The matrix composition of the red wines was also varied by increasing the ethanol concentration by 1% to give 13.8 and 14.8% alcohol in the Shiraz wines and 14.7 and 15.7% alcohol in the Cabernet Sauvignon wines. The tannin concentration was increased in the red wines by adding two times the recommended rate (200 mg/L) of commercial wine tannin (Oenotannin Mixte MG, Oenofrance, Bordeaux, France). Wines were bottled (3 × 750 mL) following DCO_2 variation and wine matrix modifications and were resealed with screw caps, with the seal wrapped in Parafilm Wines were stored at 4°C for up to 48 h prior to sensory assessment.

Sensory assessment

Eight and nine tasters were used for the white and red wine component of the study, respectively (mean age 53, SD 7). Each taster had over 5 years of extensive experience in rating the intensity of overall aroma, flavour and mouthfeel attributes of still white and red wines including spritz.

Training and practice protocols were similar for the white and red wine assessments. Tasters were trained over two, 2 h sessions where they selected and defined appropriate descriptors that applied to a representative set of wines from the study. Standards for the selected aroma and palate attributes were presented and refined following discussion (Table S1). The accepted reference standards were made available to the tasters for referral thereafter. Training also involved the ranking of two or three samples with a varying level of DCO_2, pH, viscosity, ethanol hotness, bitterness and astringency presented in water and in wine. Following training, tasters practised rating the intensity of the selected attributes with the same scale and under the same conditions used during the formal assessment using 12 randomly selected treatment combinations described earlier. Discussion after the practice session also served to finalise the attribute list (Table S2). Tasters were found to be performing to an acceptable standard as determined by their ability to discriminate between samples, and by agreement with the panel means (SensomineR, sensominer.free.fr/; FactomineR, factominer.free/fr).

A preliminary trial showed that a significant loss in DCO_2 occurred when the wines were poured from the bottle into the tasting glass, and that the DCO_2 concentration in the glass further reduced in the interim between pouring

Table 1. Composition of wines used in the study.

Wine	pH	TA (pH 7)	Ethanol (% v/v)	Glucose + fructose (g/L)
Chardonnay	3.41	4.4	13.2	3.3
Viognier	3.41	5.0	13.5	4.0
Shiraz	3.59	4.9	13.8	0.8
Cabernet Sauvignon	3.64	5.2	14.7	1.2

and tasting (Figure S1). Consequently, measurement of DCO_2 (n = 8 for each DCO_2 concentration) was simultaneously taken from the wine glass at the time that the wines were being assessed during the formal sensory sessions using an Orbisphere CO_2 analyser with a sensitivity of 0.025 g/L (Hachultra Model 3658, Geneva, Switzerland) that had been adapted for 'in-glass' sampling.

The formal sensory assessments of the white and red wines were conducted separately. In each study, aliquots of wine were poured directly from bottles into three-digit-coded, polycarbonate stemless wine glasses of dimensions typical of restaurant and domestic wine consumption to a 150 mL pouring mark (Vino stemless 400 mL, 100 mm height, 80 mm bowl, 60 mm opening, Model PS-46, Polysafe, St Peters, NSW, Australia). The temperature of the wines in glass when tasted was 10 ± 0.4°C for white wines and 23 ± 0.2°C for red wines. Tastings were conducted in isolated booths under sodium lighting. To balance carry over effects the wines were presented two times, in a modified Williams Latin Square incomplete random block design generated by Fizz sensory acquisition software version 2.51 (Biosystèmes, Couternon, France). Different bottles of the wines prepared in the same way were used as presentation replicates. Attribute intensity was rated using an unstructured 15 cm line scale with anchor points representing 'low' and 'high' intensity placed at 10 and 90%, respectively. Tasters were given eight sets of two wines during white wine assessment and six sets of two wines during red wine assessment. The tasting of the first of the two samples commenced less than 60 s after pouring followed by a 60 s rest before assessment of the second sample. New sets of two samples were assessed after 10 min rest periods. Formal evaluation was completed in two sessions conducted on consecutive days, with each presentation replicate presented in a single session. Data were acquired using Fizz sensory software version 2.51.

Data analysis

Attribute ratings were analysed using a fully crossed ANOVA blocked on assessors considered as random factors, with compositional variables (DCO_2, pH and ethanol concentration for white wines, and DCO_2, ethanol and tannin concentration for red wines) considered as fixed factors. Analyses were conducted using MINITAB 14.13 (Minitab, State College, PA, USA).

Results and discussion

White wines were adjusted to give high, medium-high, Control (unchanged), and low (N_2 sparged) concentration of DCO_2. Mean DCO_2 concentration in the wine glass when tasted by the panellists were 0.47, 1.06, 1.79 and 2.56 g/L for Chardonnay wines and 0.42, 0.92, 1.90 and 2.87 g/L for Viognier wines (Figure 1). The DCO_2 concentration was adjusted in red wines to give high, Control (unchanged) and low (N_2 sparged). The DCO_2 concentration in red wines was lower than in white wines to reflect commercial practice (Peynaud 1984) with 0.13, 0.62 and 0.90 g/L in Shiraz wines and 0.11, 0.44 and 0.78 g/L in Cabernet Sauvignon wines when sensory assessment was made. Figure 1 shows that pouring wine from the bottle into wine glasses prior to sensory assessment can result in substantial 'in-glass' variation in DCO_2 concentration at the point of tasting. The variation was most likely caused by differences in the amount of agitation resulting from pouring as variation because of passive losses of DCO_2 from the wine while standing in the glass prior to tasting would have been minimal as the wines were tasted within 2 min of pouring (Figure S1). Strategies to standardise the amount of agitation that occurs during pouring of wines need to be considered in future sensory research involving a narrower range of DCO_2 concentration than was being considered in this study.

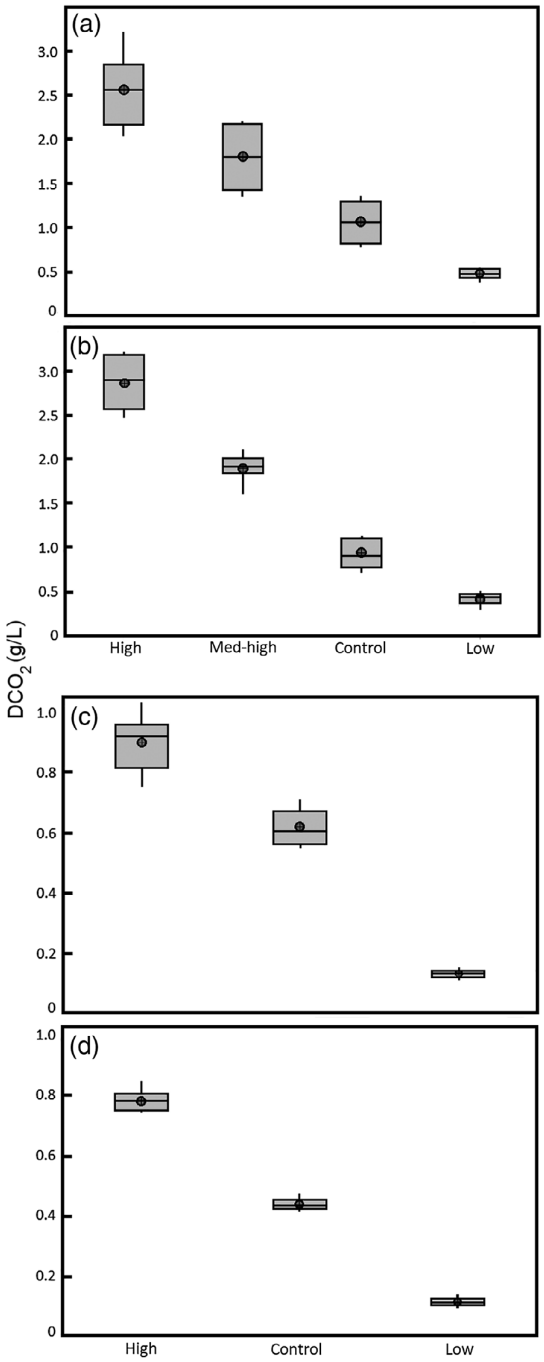

Figure 1. Box and whisker plots of dissolved carbon dioxide (DCO_2) concentration in the white, (a) Chardonnay and (b) Viognier, and red, (c) Shiraz and (d) Cabernet Sauvignon, wines measured in the wine glass at tasting. Circles represent mean concentration.

The significance (P values) of the main effects and interactions involving DCO_2 in the white and red wines is shown in Tables 2 and 3, respectively. The significance of effects not involving DCO_2 is shown in Tables S2 and S3.

Table 2. Statistical significance of dissolved carbon dioxide concentration and interactions with pH and ethanol concentration on sensory properties of Chardonnay and Viognier wines.

	Chardonnay				Viognier			
	DCO_2	**DCO_2 × pH**	**DCO_2 × EtOH**	**DCO_2 × pH*EtOH**	**DCO_2**	**DCO_2 × pH**	**DCO_2 × EtOH**	**DCO_2 × pH*EtOH**
Mouthfeel								
Spritz	**0.001**	*0.083*	0.817	0.808	**0.001**	**0.001**	0.154	0.221
Astringency	**0.031**	*0.090*	0.735	0.955	0.398	0.403	0.703	0.594
Hotness	0.590	0.793	0.539	0.215	0.928	0.546	0.438	0.951
Viscosity	0.995	0.669	0.878	0.294	0.304	0.235	0.799	0.837
Taste								
Sweetness	**0.006**	0.912	0.605	0.233	0.701	0.962	0.796	0.974
Acidity	0.310	0.217	0.938	0.372	*0.097*	0.713	**0.006**	0.749
Bitterness	**0.001**	0.201	0.790	0.314	0.157	0.447	0.763	0.351
Overall fruit flavour	**0.010**	0.564	0.962	0.599	0.710	0.343	0.865	0.932
Aroma								
Overall fruit aroma	0.444	0.289	**0.047**	0.150	0.476	0.294	0.693	0.681

$P < 0.1$ shown in italics; $P < 0.05$ shown in bold. DCO_2, dissolved carbon dioxide; EtOH, ethanol.

Table 3. Statistical significance of dissolved carbon dioxide concentration and interactions with tannin and ethanol concentration on sensory properties of Shiraz and Cabernet Sauvignon wines.

	Shiraz				Cabernet Sauvignon			
	DCO_2	**DCO_2 × Tannin**	**DCO_2 × EtOH**	**DCO_2 × Tannin × EtOH**	**DCO_2**	**DCO_2 × Tannin**	**DCO_2 × EtOH**	**DCO_2 × Tannin × EtOH**
Mouthfeel								
Spritz	**0.001**	0.472	0.549	0.119	**0.001**	0.619	*0.099*	0.813
Astringency	0.175	0.831	0.273	0.158	**0.016**	0.109	0.921	0.435
Hotness	0.417	0.917	0.320	*0.089*	0.463	0.260	**0.040**	0.253
Viscosity	0.117	0.459	0.490	0.896	0.797	0.196	0.982	0.715
Taste								
Sweetness	**0.001**	0.819	0.130	0.465	**0.003**	0.736	0.663	0.269
Acidity	*0.070*	0.675	0.996	0.775	0.679	0.840	0.346	0.526
Bitterness	*0.078*	0.817	0.484	0.601	0.658	0.928	0.219	0.619
Overall fruit flavour	**0.005**	0.594	0.248	0.298	*0.099*	0.622	0.733	0.380
Nasal perception								
Overall fruit aroma	0.502	0.120	0.913	0.317	**0.038**	0.573	0.618	0.227
Pungency	0.804	0.367	0.858	0.254	**0.001**	0.418	0.667	0.391

$P < 0.1$ shown in italics; $P < 0.05$ shown in bold. DCO_2, dissolved carbon dioxide; EtOH, ethanol.

Effects of DCO_2 on mouthfeel attributes

'Spritz' intensity significantly increased with higher DCO_2 concentration in both white and red wines ($P < 0.001$, Figure 2; $P < 0.001$, Figure 3) within the ranges typically applied by winemakers in commercial practice (Müller-Späth 1982). In the red wine study DCO_2 effects were independent of ethanol or tannin concentration. In the white wine study, a $DCO_2 \times$ pH interaction on spritz perception was observed; however, this may have been because of loss of DCO_2 incurred during the pH adjustment process due to stirring.

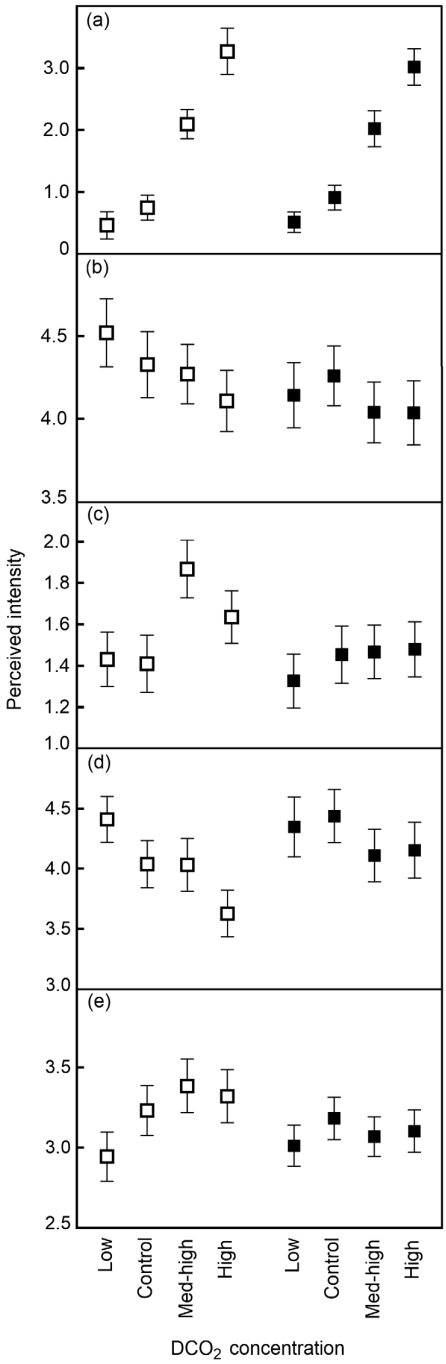

Figure 2. Effect of DCO_2 concentration on the mean intensity rating of (a) spritz, (b) astringency, (c) sweet, (d) bitter and (e) overall fruit flavour for Chardonnay (□) and Viognier (■) wines. Error bars represent ±2 SE.

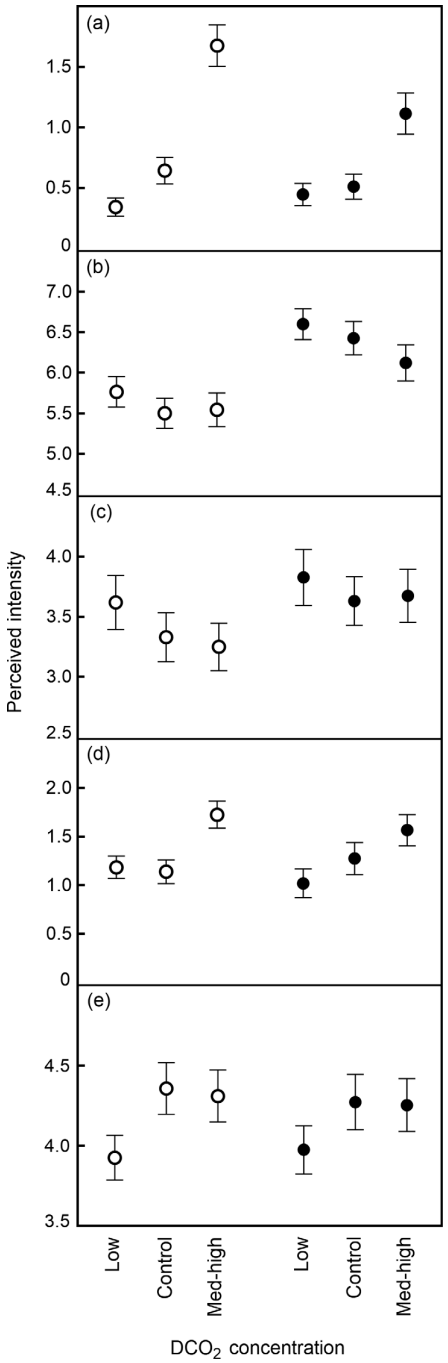

Figure 3. Effect of DCO_2 concentration on the mean intensity rating of (a) spritz, (b) astringency, (c) sweet, (d) bitter and (e) overall fruit flavour for Shiraz (○) and Cabernet Sauvignon (●) wines. Error bars represent ±2 SE.

'Astringency' defined as a drying sensation perceived in the mouth (Table S1) was perceived in both the white and red wines. With red wines, it is widely recognised that their astringency is caused by polyphenol–salivary protein binding activity. With white wines, however, which contain a significantly lower concentration of polyphenols, astringency is mostly elicited by organic acids. pH has been shown to be a strong predictor of the astringent intensity of aqueous systems (Lawless et al. 1996) and white wine (Gawel et al. 2014) which is consistent with the result that the Chardonnay and Viognier wines at the lower pH (3.2) were

significantly more astringent than those at pH 3.4 ($P = 0.004$, $P < 0.001$).

The astringency of the Chardonnay wines significantly decreased with increasing DCO_2 concentration ($P = 0.031$, Figure 2). Higher DCO_2 concentration also significantly decreased the astringency of the Cabernet Sauvignon wines ($P = 0.016$), with a similar trend in the Shiraz wines (Figure 3). Symoneaux et al. (2015) found that a saturated level of DCO_2 increased astringency perception in model apple cider that included apple polyphenols. Their result contradicts those of this red wine study but can be explained by the higher level of DCO_2 used (5 g/L) which may have resulted in more efficient binding of salivary proteins with polyphenols because of lower pH in a saturated system (Fontoin et al. 2008). Another possibility for increased astringency in saturated DCO_2 systems is that in-mouth 'foaming' could potentially mechanically displace the salivary film thereby positively influencing astringency perception by increasing oral friction or allowing greater access to polyphenols to oral surfaces causing epithelial constriction (Payne et al. 2009), or by allowing greater access to astringency receptors (Schobel et al. 2014).

The possible reasons for the observed decrease in astringency perception by lowering the concentration of DCO_2 when tasted at equivalent pH are speculative. The suppression of astringency by DCO_2 may simply be an attentional effect because of distraction by the perception of 'spritz' character. This is possible as Clark et al. (2011) noted that the astringency of hop acids in model beer decreased with carbonation, but this was accompanied by differences in the foaming properties and increased carbonation perception. Another explanation involves mucin unfolding (Racz et al. 2018), whereby the significantly higher salivary bicarbonate concentration in higher flow rate saliva (Thaysen et al. 1954) binds more salivary Ca^{++} enabling mucins to unfold and hydrate, increasing oral lubrication and thereby decreasing the perception of astringency. This explanation is contingent upon DCO_2 stimulating salivary flow having not been established but is plausible given that capsaicin (another trigeminal stimulant) strongly stimulates salivary flow (Kono et al. 2018). Last, the suppression of astringency by DCO_2 might be the result of competition for the same receptor sites embedded within the oral nociceptors (Kurogi et al. 2015).

A significant suppressive effect of DCO_2 on perceived 'hotness' was confined to the Cabernet Sauvignon wine with the highest ethanol concentration applied in the study (15.7% v/v) ($P = 0.04$). In all other wines which contained a lower concentration of ethanol, DCO_2 did not influence palate hotness (Tables 2, 3), which is consistent with Clark et al. (2011) who found that the warming aspect of ethanol in model beer did not significantly change after carbonation. Dissolved CO_2 did not affect perceived 'viscosity' in any wine either directly or interactively with the matrix components (Tables 2, 3).

Effects of DCO_2 on taste attributes

'Sweetness' was influenced by DCO_2 in the Chardonnay ($P = 0.006$) wine with the two highest concentration values of DCO_2 resulting in wines that were rated significantly higher in sweetness than the Control and sparged wines with a similar trend observed in the Viognier wines (Figure 2). Higher DCO_2 concentration significantly reduced 'bitterness' intensity of the Chardonnay wine ($P < 0.001$, Figure 2) with a similar effect on the bitterness of the Viognier wine. Dissolved CO_2 similarly affected the perceived sweetness and bitterness of the red wines. The perceived sweetness of both the Cabernet Sauvignon and Shiraz wines with the highest DCO_2 concentration was significantly ($P = 0.003$, $P < 0.001$) greater than the same wines with the lowest DCO_2 concentration (Figure 3). The perceived bitterness in both red wines tended to be less intense when DCO_2 concentration was high, although this trend was not significant (Figure 3).

Dissolved CO_2 concentration did not significantly affect 'acidity' perception in either white wine where pH was equalised after modifying the DCO_2 concentration. This approach was taken as pH changes resulting from the formation of the weak carbonic acid by CO_2 can be ameliorated by standard acid adjustment processes used throughout the winemaking process. The range of DCO_2 concentration in the red wine study was significantly lower reflecting commercial practice (Figure 1), so wine pH was unaltered following carbonation or de-carbonation. As per the white wines, DCO_2 did significantly affect perceived acidity; however, there was some evidence that DCO_2 had a suppressive effect on the acidity of the Shiraz wine ($P = 0.070$).

Many studies have investigated the effect of saturated or near-saturated levels of DCO_2 on the sweet, sour and bitter tastes in model systems often with conflicting results. Dissolved CO_2 at saturated levels decreased the sweetness of aqueous systems containing sugars (Cowart 1998, Hewson et al. 2009, Clark et al. 2011); however, as observed in this study, a sub-saturation level of DCO_2 has also been found to increase sweetness perception in model aqueous systems (Cometto-Muñiz et al. 1987). Others have found complex effects between DCO_2 concentration and bitterness in model systems representing various beverages. Suppression of the bitterness of quinine sulfate (QS) by DCO_2 was found to occur in model solutions representing Champagne wine (Thuillier 2007) and carbonated soft drinks (Cowart 1998). Cometto-Muñiz et al. (1987) used a range of concentration values of DCO_2 and QS and found a concentration-related response whereby DCO_2 suppressed QS bitterness but only at higher QS concentration. The perceived acidity of water was increased following CO_2 addition (Hewson et al. 2009) which is most likely because of the formation of carbonic acid species which under normal atmospheric conditions produce a slightly acidic solution (pH = 5.7) (Chaix et al. 2014). When presented, however, in aqueous solutions containing organic acids, a system more analogous to wine, DCO_2 either had no effect (Hewson et al. 2009) or suppressed perceived acidity (Cometto-Muñiz et al. 1987, Symoneaux et al. 2015). This effect may be explained by the predominance (greater than 98%) of CO_2 over both the undissociated and dissociated forms of carbonic acid that are present at wine pH (Chaix et al. 2014).

The increase in perceived sweetness by DCO_2 is consistent with the reduction in bitterness as different tastes mutually suppress each other (Keast and Breslin 2003), but the physiological mechanisms underlying DCO_2 induced reduction in bitterness and increases in sweetness are unclear and warrant further investigation.

Effect of DCO_2 on flavour and aroma attributes

'Overall fruit flavour' intensity was influenced by DCO_2 concentration in the Chardonnay, and in the Shiraz and Cabernet Sauvignon wines. The effect, however, was confined to a lower perceived intensity at the lowest DCO_2 concentration achieved by N_2 sparging, a process known to inadvertently reduce the concentration of volatile

compounds that influence flavour intensity (Figures 2, 3). There was no significant change in overall fruit flavour intensity as a result of increasing DCO_2 concentration over that of the Control wines. Contrary to this result, carbonation increased flavour intensity elicited by volatile compounds both with and without a trigeminal component when presented in model aqueous systems. Corresponding increases in the concentration of volatiles in in vivo nasal and in vitro throat effluent suggest the possibility of enhanced delivery of odorants to the olfactory epithelium (Pozo-Bayón et al. 2009, Saint-Eve et al. 2009, 2010). The difference in results between this and previous studies may be attributed to differences in the concentration of DCO_2 used. The previous studies used a saturated level of DCO_2 which may have caused volatile stripping and subsequent convection throughout the nasal cavity by the action of gas bubbles. This was unlikely to have occurred in this study because of the concentration of DCO_2 being well below saturation (Dalmolin et al. 2006).

The concentration of DCO_2 also did not significantly affect 'overall fruit aroma' in the Chardonnay, Viognier and Shiraz wines, and its effects were inconsistent in the Cabernet Sauvignon wines. When presented orthonasally CO_2 has been also been shown to suppress the fruity aromas elicited by *n*-amyl acetate (Cain and Murphy 1980). Suppression was perceived even when CO_2 and the volatile compounds were presented individually to each nostril, therefore precluding competition at the receptor level. This showed that the perceptual interaction between CO_2 and volatiles is at least partially cognitive. Electrophysical studies support this hypothesis—whereby a direct multimodal convergence of CO_2 trigeminal and odorant information onto neurons in the olfactory piriform cortex has been shown (Albrecht et al. 2010, Carlson et al. 2013). While the concentration of CO_2 in the headspace above the wine in the glass was not measured, it is likely to have been below the reported orthonasal (irritation) detection threshold of CO_2 in air (5.2% v/v) (Melzner et al. 2011) which may explain why 'overall fruit aroma' intensity was unaffected by headspace CO_2.

A nasal attribute labelled as 'pungency' attributed to ethanol (Table S1) was identified as relevant in the red wine study and presumably was the result of high ethanol concentration particularly in the Cabernet Sauvignon wines. The intensity of pungency in the Cabernet Sauvignon wines was significantly suppressed by DCO_2 ($P < 0.001$) with its effect being independent of ethanol or tannin concentration. In contrast to overall fruit aroma, the perception of nasal pungency elicited by ethanol in the Cabernet Sauvignon wine was suppressed by CO_2. Frasnelli et al. (2011) showed that chemesthetic stimuli that share a common receptor suppress each other when presented orthonasally supporting that the suppressive nature of CO_2 on ethanol induced pungency involves competition for receptor sites in the oral mucosa.

Conclusions

Still wine is unique in that it is a non-distilled alcoholic beverage that contains a sub-saturated level of DCO_2 whereby CO_2 gas formation/foaming does not occur in-mouth during tasting. This study set out to define for the first time the effect of a sub-saturated level of DCO_2 on the taste, overall aroma, flavour and mouthfeel attributes of still white and red wine. The pH, ethanol and tannin concentration were co-varied with DCO_2 to assess possible interactive effects with DCO_2 on sensory properties. First, it was demonstrated that the spritz sensation elicited by DCO_2 could be clearly differentiated both within the legally defined and the commercially accepted concentration ranges for still white and red wines. In contrast to results from model studies involving saturated levels of DCO_2, we found that DCO_2 at still wine concentration increased perceived sweetness and decreased bitterness in both white and red wines. Furthermore, DCO_2 did not influence fruit aroma or flavour intensity and suppressed the astringent/drying sensation in both white and red wines even though the compositional cause of the sensation differed between the two wine types. While some of the variations between studies could be attributed to methodological differences, particularly in the white wine study where the effect of pH changes because of DCO_2 were nullified, it was notable that the same sensory trends were observed in the red wines where pH was unaltered following DCO_2 addition. There was little evidence for the expected interaction of DCO_2 with ethanol concentration on either perceived viscosity or palate hotness, suggesting that DCO_2 and ethanol at still wine concentration act independently despite sharing related sensory receptors and pathways associated with oral irritation. These results demonstrate that DCO_2 at still wine concentration directly influences the taste and astringency in wine but in a manner that is different from other beverage systems involving a saturated level of CO_2.

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Figure S1. Evolution of CO_2 in glass.

Table S1. Attribute definitions and composition of reference standards.

Table S2. Statistical significance of interactions between pH and ethanol concentration on sensory properties of Chardonnay and Viognier wines.

Table S3. Statistical significance of interactions between tannin and ethanol concentration on sensory properties of Shiraz and Cabernet Sauvignon wines.

Sources of volatile sulfur compounds in wine

M.E. SMITH[1], M.Z. BEKKER[1], P.A. SMITH[1,2] and E.N. WILKES[1]

[1] The Australian Wine Research Institute, Urrbrae, SA 5064, Australia; [2] Flinders Centre for Marine Bioproducts Development (CMBD), and Department of Medical Biotechnology, School of Medicine, Flinders University, Bedford Park, SA 5042, Australia

Corresponding author: Dr Mark Smith, email mark.smith@awri.com.au

Abstract

Undesirable volatile sulfur compounds with aromas, such as boiled or rotten egg, sewage and rubber, can impact negatively on wine sensory attributes. The identity of these molecules is known but knowledge gaps exist about their source and ways to manage them in winemaking. This review focuses on the chemistry of the three main compounds: hydrogen sulfide, methanethiol and dimethyl-sulfide. Discussion centres on their possible origins and the efficacy of methods currently used to control them during wine production. The role of metals, both in the vineyard and in the winery, in the formation and release of these three volatile sulfur compounds is described. Oxygen management during fermentation and bulk ageing is discussed along with the impact of the bottle closure.

Keywords: *copper, iron, oxygen, sulfide, volatile sulfur compound*

Introduction

Volatile sulfur compounds (VSCs) have a significant impact on the flavour and aroma of wine. Reductive winemaking techniques, particularly in new world wine regions, have seen an increase in the contribution of VSCs to the aroma of some wine styles. Sulfur chemistry is, however, a double-edged sword, with some VSCs associated with positive aroma attributes and others responsible for far less desirable aromas. The same winemaking techniques that favour formation of the positive VSCs are likely also to favour the negative ones (often referred to as reductive aromas). Studies have shown that, in general, consumers react negatively to wine with reductive aromas (Lattey et al. 2007, Lockshin et al. 2009).

Some VSCs which can impart positive characters to wine include 3-mercaptohexan-1-ol (3MH), 3-mercaptohexyl acetate (3MHA), 4-mercapto-4-methylpentan-2-one (4MMP) and 4-mercapto-4-methylpentan-2-ol (4MMPOH). Box tree, grapefruit, citrus zest and passionfruit have been used to describe the aromatic quality of these five compounds which have an odour detection threshold (ODT) in the range 0.8–60 ng/L (Darriet et al. 1995, Bouchilloux et al. 1998, Tominaga et al. 1998, 2000, 2003b).

Negative aromas, such as boiled or rotten egg, cabbage, sewage, faecal, burnt rubber, garlic, onion and struck flint, are often referred to as 'reduced' or reductive aromas. The VSCs responsible for these include hydrogen sulfide (H_2S), methanethiol (MeSH), dimethyl sulfide (DMS) and benzenemethanethiol, also known as benzylmercaptan. Benzenemethanethiol has been identified as having a struck flint/burnt match aroma often found in Chardonnay and Sauvignon Blanc wines and can be considered part of the style rather than a defect (Tominaga et al. 2003a) when found at an appropriate concentration. As such, benzenemethanethiol will not be discussed here. Small amounts of the reductive VSCs have been shown to be beneficial to aromatic complexity (de Mora et al. 1993, Siebert et al. 2009), possibly due to changes in their sensory perception with changing concentration (Lopes et al. 2009) or synergistic effects. Hydrogen sulfide and MeSH have an ODT of 1–3 µg/L in wine (Siebert et al. 2009, Solomon et al. 2010). Dimethyl sulfide is also termed a reductive aroma, although in low concentration it is described as having blackcurrant, red fruit and truffle aromas and is considered to enhance the bouquet in some wine styles (Spedding and Raut 1982, Segurel et al. 2004, Escudero et al. 2007, Vidal and Aagaard 2008). At high concentration, DMS can impart canned corn, asparagus or vegetal aromas (Mestres et al. 2000). Table 1 summarises several of the common VSCs in wine. This review will focus on the chemistry of H_2S, MeSH and DMS with respect to their formation, potential precursors and methods to manage them during wine production. A brief discussion of the biosynthesis of these VSCs is included.

Possible precursors of H_2S, MeSH and DMS in wine

Grapes. Inorganic sulfate is the major source of sulfur in grapes. Sulfate is naturally occurring and the concentration in grapes is largely dependent on the mineral composition of the soil in which the grapevine is grown as well as the water used for irrigation (Leske et al. 1997). Wine grapes in Australia contain an average of 260 mg/L (+/− 121 mg/L) of sulfate ions (measured as K_2SO_4) (Leske et al. 1997). In the USA, Canada and Europe, the sulfate content of must ranges between 30 and 2200 mg/L (Zee et al. 1983). In addition to inorganic sulfate, a small amount of sulfur is present in the form of the vitamins, thiamine and biotin, as well as the sulfur-containing amino acids, peptides and the tripeptide, glutathione (Ribéreau-Gayon et al. 2006).

Exogenous sources of sulfur in the vineyard. Sulfur may be applied to vines in the elemental form to protect against fungal growth such as downy and powdery mildew. It has been shown that H_2S can be produced from elemental sulfur with reducing compounds produced by yeast (Rankine 1963, Rauhut 1993). A large number of pesticides and fungicides registered for use in the vineyard (Essling and Lord 2015) contain sulfur moieties, but little is known about the potential for these to break down either enzymatically or non-enzymatically to H_2S, thiols or other sulfides. At the time of grape harvesting, sulfur, in the form of gaseous SO_2, or more commonly, an aqueous solution of potassium metabisulfite (PMS), is often added to the grapes to protect from microbial spoilage and oxidation.

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Table 1. Aroma descriptors and odour detection threshold for some volatile sulfur compounds found in wine.

Compound	Odour descriptor	Odour detection threshold (µg/L)
Hydrogen sulfide	Boiled or rotten egg	1.1–1.6† (Siebert et al. 2009)
Methanethiol	Burnt rubber, sewage, cabbage	1.8–3.1† (Solomon et al. 2010)
Ethanethiol	Onion, faecal	1.1† (Goniak and Noble 1987)
Benzenemethanethiol	Struck flint,	0.3 ng/L‡ (Tominaga et al. 2003a)
Dimethylsulfide	Boiled cabbage, asparagus, canned corn, blackcurrant, truffle	25† (Goniak and Noble 1987)
Diethylsulfide	Garlic, rubber	0.9† (Goniak and Noble 1987)
Carbon disulfide	Rubber, sulfidy	>38† (Spedding and Raut 1982)
Dimethyl disulfide	Cabbage, intense onion	29† (Goniak and Noble 1987)
Diethyl disulfide	Onion, garlic, burnt rubber	4.3† (Goniak and Noble 1987)
Methylthioacetate	Cheese, egg, sulfurous	50§ (Baxter and Hughes 2001)
Ethylthioacetate	Sulfurous, onion	10§ (Baxter and Hughes 2001)
Methionol	Cauliflower, cabbage	500§ (Mestres et al. 2000)
Benzothiazole	Rubber	50‡ (Mestres et al. 2000)
4-Mercapto-4-methyl-pentan-2-one	Box tree, cat urine	3.3 ng/L† (Darriet et al. 1995)
3-Mercaptohexan-1-ol	Passionfruit, grapefruit	60 ng/L‡ (Tominaga et al. 1998)
3-Mercaptohexylacetate	Passionfruit, box tree	4 ng/L‡ (Tominaga et al. 1995)
4-Mercapto-4-methylpentan-2-ol	Citrus zest	55 ng/L‡ (Tominaga et al. 1998)

†Determined in wine. ‡Determined in model hydroalcoholic solution. §determined in beer.

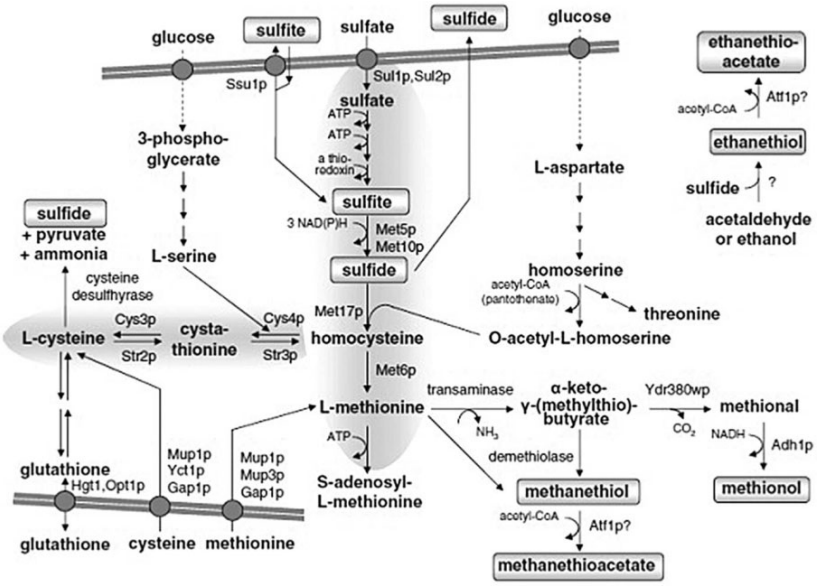

Figure 1. Sulfate and sulfate metabolism in *Saccharomyces cerevisiae* yeast (Ugliano and Henschke 2009), reproduced with permission from Springer Science + Business Media B.V.

Biosynthesis of H_2S, MeSH and DMS. Significant research has been undertaken to explain the evolution of H_2S during the fermentation of grape juice [see for example Rauhut (1993, 2009), Swiegers and Pretorius (2007), Ugliano and Henschke (2009), Cordente et al. (2012)]; a brief overview follows.

It is well established that sulfate (SO_4^{2-}) and sulfite (SO_3^{2-}) are converted to sulfide (S^{2-}) by *Saccharomyces cerevisiae* yeast via the sulfate reduction sequence as shown in Figure 1 (Rankine 1963, Eschenbruch 1974, Vos and Gray 1979, Stratford and Rose 1985, Giudici and Kunkee 1994, Jiranek et al. 1995). If the amount of yeast assimilable nitrogen (YAN) is sufficient, H_2S produced by yeast is converted to the sulfur-containing amino acids, cysteine and methionine. (Figure 1) (Park et al. 1994, Jiranek et al. 1995, Moreira et al. 2002, Bell and Henschke 2005, Swiegers and Pretorius 2007, Ugliano and Henschke 2009). One of the mechanisms of release of H_2S during fermentation occurs in a low YAN environment and has been shown to be greatly increased when sulfite is present (Stratford and Rose 1985, Jiranek et al. 1995). Sulfite may be added as an antioxidant and an anti-microbial to must and juice at a rate of up to 200 mg/L, and its uptake by yeast is largely unregulated (Ugliano and Henschke 2009). While sulfate reduction is tightly regulated by yeast, sulfite reduction is uncontrolled; the capacity for H_2S production during times of low YAN would therefore appear to be substantial (Jiranek et al. 1995). The role of the sulfite permease, which effluxes sulfite from the cell, in regulating this reaction is unknown (Park and Bakalinsky 2000, Nardi et al. 2010).

During fermentation, H_2S is being produced, and varying quantities are released into the must. The chemistry that occurs between H_2S and other molecules present in the must is not well understood and is the subject of ongoing research. It is therefore possible that during fermentation, a pool of sulfur-containing precursors to H_2S, MeSH and DMS are being produced, and not all are being released in a gaseous form. Currently, several VSCs can be measured by GC headspace methodology, and the results obtained are only that of volatile species rather than a total content including soluble precursors and soluble VSCs (Siebert et al. 2010). Recent evidence suggests that there are additional

sources of H_2S and other VSCs produced by yeast, such as glutathione and S-amino acids, but their pathways and regulation are still poorly understood (Winter et al. 2011).

Cysteine, methionine and glutathione. As can be seen in the sulfate reduction pathway in yeast (Figure 1), H_2S is converted to cysteine, methionine and glutathione via a series of enzyme-catalysed reactions. During the growth phase of yeast, the sulfur amino acids are used in protein synthesis, while later in fermentation all three molecules can be excreted from the cell and appear in the finished wine. Glutathione can be degraded to its constituent amino acids including cysteine when cellular nitrogen levels are deficient (Elskens et al. 1991, Hallinan et al. 1999) and cysteine desulfhydrase is known to release H_2S from cysteine under nitrogen-deficient conditions (Tokuyama et al. 1973). It has been reported recently that methionine can produce MeSH via transamination to form the α-keto acid, followed by a demethiolase activity (Perpète et al. 2006). In addition, MeSH can be esterified to MeSAc (Rauhut et al. 1996), possibly by yeast alcohol acetyltransferases (Ugliano and Henschke 2009). In the presence of metal ions, cysteine and S-methyl cysteine can release H_2S by desulfhydration while methionine can release MeSH under similar conditions (Gruenwedel and Patnaik 1971).

Thioesters. The thioacetates of MeSH and ethanethiol (EtSH) have been observed in wine in the range 0–180 µg/L (Leppanen et al. 1980, Mestres et al. 2000, Fedrizzi et al. 2007, Moreira et al. 2010) and their odour descriptors and ODT are listed in Table 1. They have a higher ODT than that of their corresponding thiols, and hence their presence may go unnoticed during a sensory evaluation. Thioesters can be hydrolysed to the corresponding thiols at low pH (Leppanen et al. 1980), potentially providing another source of VSCs in finished wine.

Other organic compounds including disulfides and polysulfides. A disulfide (RS-SR) is the oxidised dimer of the corresponding thiol (RSH) and could be a source of volatile free thiols in wine. Mestres et al. (2000) reviewed the content of organic sulfur compounds in wine and reported that the concentration of disulfide ranged from 0 to 85 µg/L. Bobet et al. (1990) showed that in the presence of 30 mg/L free SO_2, diethyldisulfide (DEDS) can be reduced in a model wine system to EtSH and an organic thiosulfate. The oxidation of thiols to disulfides is a reversible process and is likely mediated by the redox state of the wine at any given point in time. Nedjma and Hoffmann (1996) showed that H_2S can react with thiols in the presence of Cu^{2+} at wine pH to form symmetrical and asymmetrical dialkyl disulfides and trisulfides. Peroxide radicals, produced from the interaction of Fe^{2+} ions and oxygen, have been shown to oxidise thiols to disulfides; the reaction being faster in the presence of Cu^{2+} ions (Jocelyn 1972).

A study by Smith and Reed (1994) showed Cu^{2+} oxidised glutathione and cysteine to their corresponding disulfides with the rate of reaction slower for the larger thiol, glutathione, due to the stability of the proposed intermediate Cu(II)-glutathione complex. This process is shown in Figure 2 and produces thiol radicals which can then react to form a disulfide. Given the large numbers of reactive molecules present in wine, it is possible that thiol radicals combine with other compounds with an unknown potential of these thiol sinks to release the original thiol. For example, Nikolantonaki and Waterhouse (2012) showed that quinones react with thiols, and this will be discussed later in this review.

The oxidation of thiols to disulfides by arylglyoxals, which are similar to quinones, in acidic conditions has been demonstrated (Mosslemin et al. 2011). The presence of chelating ligands, such as EDTA, has been shown to decrease the oxidation of MeSH to DMDS (Vasserot et al. 2003) while enhancing the reactivity of iron towards oxygen (Buettner and Jurkiewicz 1996). This redox reaction, shown in Figure 3, can be mediated by several transition metals and also increases the possibility for the formation of asymmetrical disulfides which have not been well studied in wine and which may have an ODT significantly different to that of the symmetrical disulfides and the component thiols listed in Table 1.

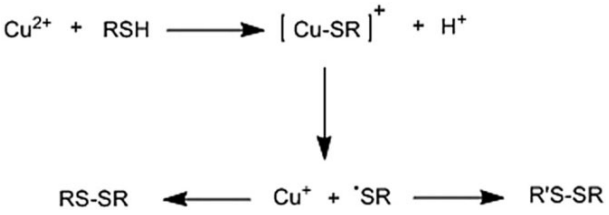

Figure 2. Oxidation of thiols by Cu^{2+}.

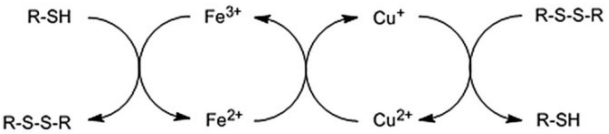

Figure 3. Possible mechanism for the oxidation/reduction cycle of thiols/disulfides.

Sulfite added during winemaking. Sulfite is a common additive used during the winemaking process either in the gaseous form (SO_2) or more commonly as PMS solution (Jackson 2014). While it is added to prevent microbial spoilage, the primary role of SO_2 is that of an antioxidant via reaction with hydrogen peroxide (H_2O_2) generated from O_2 via a series of redox steps (Danilewicz 2003, Waterhouse and Laurie 2006, Elias and Waterhouse 2010, Oliveira et al. 2011). As mentioned previously, *S. cerevisiae* uses this inorganic source of sulfur in the formation of sulfur-containing amino acids. In a low YAN environment, sulfite enhances H_2S production by yeast (Jiranek et al. 1995). In juice and wine, the amount of sulfite present is expressed in terms of free (unbound) and total (bound + unbound) SO_2. The concentration found in must and wine is in the range 50–200 mg/L total SO_2 (Rauhut 1993). It has been shown that the added sulfite can be converted to H_2S by yeast (Eschenbruch 1974, Stratford and Rose 1985, Monk 1986, Jiranek et al. 1995). During storage and at bottling, further additions of PMS can be made to protect the wine from oxidation during maturation (both in tank and in bottle) and to limit the risk of microbial spoilage.

Throughout the progression from grapes to wine, enzymatic and non-enzymatic reduction of sulfite to sulfide occurs, with possible further reactions to form other VSCs and non-volatile sulfur-containing compounds. To date, the focus of much research has been on the yeast-driven reduction of sulfite, however, the role of metals in reducing SO_2 in industrial processes has been known for decades. Metals such as tin (Muneera et al. 1983) have been shown to oxidise SO_2 in the presence of O_2 to sulfate but when the partial pressure of SO_2 is greater than that of O_2, the metal reduces SO_2 to sulfide. In bottled wine, the available oxygen from migration through the closure is highly dependent on the closure type (Godden et al. 2005), but at least initially, the concentration of SO_2 is much greater than that of O_2. The reduction of SO_2 to H_2S by low valent metal ions (Fe^{2+}, Cu^{+}), as described by Muneera et al. (1983), then becomes possible.

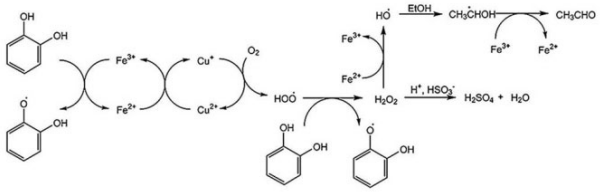

Figure 4. Wine redox chemistry showing the interaction of iron, copper, phenolic substances and sulfur dioxide (HSO_3^-). Adapted from Elias and Waterhouse (2010).

DMS precursors. Dimethyl sulfide is found in wine in a wide range of concentration, up to 946 µg/L in some wines (Loubser and Du Plessis 1976, de Mora et al. 1987, 1993, Park et al. 1994, Anocibar Beloqui et al. 1996), and it has been shown that DMS increases with wine ageing (Siebert et al. 2010, Ugliano 2013).

The production of DMS in beer is well known in the brewing industry with S-methyl methionine (SMM) being shown to be the main precursor (White and Wainwright 1976, Anness and Bamforth 1982, Dickenson 1983). Recent work has shown that grapes contain SMM at a concentration ranging from a few µg/L up to 5 mg/L (Segurel et al. 2004, 2005, Loscos et al. 2008), suggesting SMM could be the precursor to DMS in wine. In addition, methylation of methionine to SMM is catalysed by an S-methyltransferase enzyme (Figure 4) (Bourgis et al. 1999). The quantity of SMM in finished wine has not been reported, and therefore the potential for a given wine to produce DMS is unknown.

It has been shown that cysteine can produce DMS during fermentation of grape must (de Mora et al. 1986), while the reduction of dimethylsulfoxide (DMSO) to DMS has been observed in the brewing industry (Anness and Bamforth 1982) and more recently in wine (de Mora et al. 1993); with both processes being influenced by yeast. Nitrogen supplementation of fermenting grape must has also been observed to stimulate DMS formation post-fermentation by an unknown mechanism (Ugliano et al. 2009).

Release mechanisms—conversion of precursors to H_2S, MeSH and DMS

Wine redox chemistry, iron and copper. A key series of redox reactions in wine are illustrated in Figure 4 which describes how oxygen interacts with transition metals to produce the superoxide radical which can then react with organic compounds.

Oxygen cannot directly react with organic compounds due to its ground state electronic configuration as a triplet diradical, however, transition metals such as Fe and Cu catalyse the reaction by producing the superoxide radical which can in turn react with organic compounds (Haber and Weiss 1934): the role of Fe and Cu in this cycle has been reviewed (Danilewicz 2003, 2007, 2011, 2013, Waterhouse and Laurie 2006, Danilewicz et al. 2008, Elias et al. 2009, Elias and Waterhouse 2010). In the absence of Fe and Cu, the rate of reaction of O_2 with wine antioxidants (e.g. SO_2) is almost zero, demonstrating the importance of these two metal ions (Danilewicz 2007). Iron alone can interact with O_2, but the rate of reaction is slow as the resulting Fe^{3+} must be first reduced back to Fe^{2+} before another O_2 molecule can be reduced (Figure 4). It is the ability of the two metal ions to redox cycle that rapidly speeds up this reaction; indeed, only a catalytic amount of Cu (~0.05 mg/L) is needed to greatly accelerate the reduction of O_2 (Danilewicz 2007).

The concentration of iron found in wine ranges from 0 to 5 mg/L, and Cu is routinely added to treat sulfidic wine prior to bottling at a concentration between 0 and 1 mg/L (Viviers et al. 2013). Copper salts are used as a fungicide treatment in the vineyard, and recent work has shown this to have the potential to significantly increase the Cu concentration in the resulting wine (La Pera et al. 2008). The Organisation Internationale de la Vigne et du Vin has set the maximum allowable limit of Cu in wine at 1 mg/L, but many countries have their own legislation for Cu concentration in wine (US/China 0.5 mg/L, Europe 1.0 mg/L) (Tariba 2011, Wine Australia 2014). Australia has no limit on the concentration of Cu in wine (Australian Government 2015). Although the relative concentration of Cu and Fe is low in most wines, given the catalytic nature of Fe and Cu as shown in Figure 4, their impact on reactions in wine is likely to be significant. The chemistry of Cu in white wine has been reviewed recently (Clark et al. 2015a).

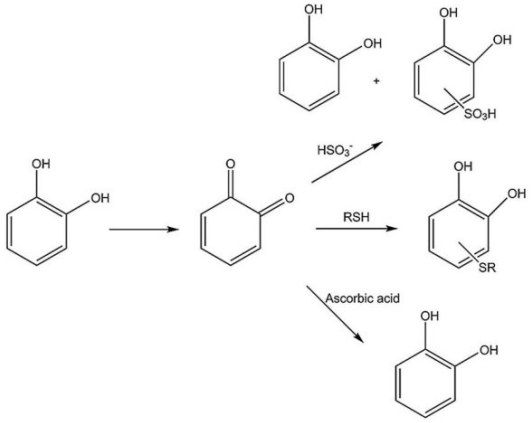

Figure 5. Reactions between quinones and relevant wine compounds.

It is not only the redox state of the metals that determines their reactivity; the coordination state of the metal is equally important. Indeed, the molecules coordinated to the metal determine the redox potential of the metal complex. Pohl and Sergiel (2009) developed a method to determine the species present in wine and beer using a two-column solid phase extraction. Copper was found to exist mostly as hydrophobic species, and the authors hypothesise that these were Cu complexes containing anthocyanins, flavonoids and other phenolic substances such as tannins.

As shown in Figure 4, phenolic substances play a critical role in wine chemistry by enabling Fe and Cu to redox cycle, ensuring these two metals are available in the required oxidation state to react with oxygen. Phenolic substances are in significant molar excess relative to Fe and Cu in wine with a concentration of 100–850 mg/L in white wine, 730–4170 mg/L in red wine and 340–1300 mg/L in rosé wine (Neveu et al. 2010). They can be oxidised to quinones, via the redox cycle shown in Figure 4, and can then react with a range of wine components (Figure 5), including thiols such as the tripeptide glutathione, via a Michael addition to form, for example, 'grape reaction product' (S-glutathionyl caftaric acid) (Singleton et al. 1985, Cheynier et al. 1986, Nikolantonaki et al. 2014). Loss of varietal aroma due to the reaction of 3MH and 4MMP with quinones has been demonstrated (Nikolantonaki et al. 2014). Nikolantonaki and Waterhouse (2012) have shown H_2S to be the most reactive of the major wine thiols towards 4-methyl-1,2-benzoquinone in a model wine system. In the presence of sulfite, the quinones are reduced back to phenolic substances via a 1,2-addition producing a sulfate ion (Danilewicz 2003). It is possible that thiol adducts of quinones are reversible and as such, these molecules could act as precursors to VSC production.

Other naturally occurring metal ion levels – do they play a role? While discussion so far has focused on the role of Fe and Cu on wine chemistry, there are many more transition metals present naturally in wine, some of which are capable of interacting with sulfur-containing compounds. Viviers et al. (2013) demonstrated the effect of five metals (Cu, Fe, Zn, Al, Mn) on the production of H_2S, MeSH and DMS in Chardonnay and Shiraz, showing that metals can act individually or in combination to influence the concentration of H_2S, MeSH and DMS in wine (Viviers et al. 2013). In a survey of 80 French wines, Cabrera-Vique et al. (2000) found that the concentration of Mn ranged from ~0.3 to 7.8 mg/L which is similar to the Fe concentration (~0.9–9.2 mg/L) found by Ferreira et al. (2008). Cacho et al. (1995) showed that Mn, along with Fe, affected the rate of non-enzymatic oxidation in white wine, indicating the ability of Mn to catalyse Fenton-like reactions in wine. Recently, it was reported that Mn was responsible for the oxidative degradation of MeSH (Ferreira et al. 2014) although the authors did not see a similar role for Mn in their recent study (Viviers et al. 2013). More research is needed in this area to determine the role other metals may play in the modulation of H_2S, MeSH and DMS.

Current management strategies

DAP additives during fermentation. Diammonium phosphate (DAP) and other nitrogen-based yeast nutrients can be added during a ferment to increase the amount of YAN present, which enables the yeast to convert the H_2S produced via the sulfate reduction pathway into the sulfur-containing amino acids, cysteine and methionine (Ugliano et al. 2009). Addition of DAP generally occurs before or early on in fermentation and can be combined with a pump-over in the case of red wine to increase the oxygen concentration of the ferment. It has traditionally been thought that the H_2S present is volatilised during this process while the increase in YAN from the DAP ceases the production of H_2S (Jackson 2014). Viviers et al. (2013) recently showed that the aerative winemaking practices commonly used to remove reductive aromas did not result in a physical displacement of VSCs, but that the O_2 was involved in a reactive manner for H_2S, as well as other VSCs. The O_2 treatment created a favourable environment for VSC removal, either through increased yeast activity or potentially by formation of larger molecular mass sulfur compounds. This supports the earlier conclusions about the role of oxygen in the removal of sulfides in ferments (Waterhouse and Laurie 2006).

Copper fining. Tanner in 1969 suggested that the addition of Cu or Ag ions to a wine containing a high concentration of H_2S resulted in the formation of copper sulfide (CuS) or silver sulfide (AgS) precipitates (Tanner 1969). Winemakers sometimes add $CuSO_4$ to wine to prevent or remove reductive odours post-fermentation. Often $CuSO_4$ is added just prior to bottling as a standard addition; anecdotally both for protection against VSC evolution post-bottling and to lift fruit characters. A bench sensory trial is normally conducted to ascertain how much Cu is needed to remove the VSCs (Iland et al. 2004). Calculations done by Clark and co-workers have shown that Cu^{2+} is added in ~25-fold excess stoichiometrically to treat VSCs (Clark et al. 2015b). There is scant evidence of the formation of any CuS precipitate, indicating that either the particle size is smaller than the pore size of industry filtration units or that CuS is not formed, or alternatively, yet unknown, mechanisms are occurring. This is an ongoing area of research. There have been several studies that have demonstrated that the presence of residual Cu in bottled wines actually results in higher VSC concentration post-bottling (Ugliano et al. 2011, Viviers et al. 2013).

Aeration. Winemakers who choose not to add $CuSO_4$ to treat reductive aromas pre-bottling often use an aerative racking step to physically displace the VSCs (Jackson 2014). The addition of oxygen during this process is likely to induce the redox processes shown in Figure 4 involving quinone formation and possible thiol trapping. Another possible consequence of aeration is the oxidation of thiols to sulfides or polysulfides which have a higher ODT, and thus the wine appears free of VSCs. The result of this intervention is a wine that may contain a hidden pool of precursors which can potentially be converted back to the VSCs at a later stage. Bekker et al. (2015) showed that although O_2 treatment during fermentation did result in a significant decrease in MeSH and EtSH concentration, it did not result in an increase in that of the corresponding disulfides, dimethyldisulfide and diethyldisulfide. This suggests that the chemical trapping of thiols as a consequence of increased quinone formation is a more likely mechanism.

The role of lees in controlling VSCs. There is evidence that yeast lees are able to remove volatile thiols via a disulfide linkage with the cysteine residues of mannoproteins in the cell wall (Lavigne and Dubourdieu 1996, Palacios et al. 1997, Vasserot et al. 2003). The role of Cu in this process was studied, and it has been proposed that Cu bridges the free SH moieties of the cysteine residues and free thiols before disulfide formation (Vasserot et al. 2003).Wine is regularly stored in oak barrels in the presence of yeast lees. Gentle stirring of the lees in addition to controlled aeration through the barrel staves is thought to limit the production of H_2S and MeSH (Lavigne-Cruège and Dubourdieu 2001). In the case of white wine, it has been demonstrated that racking the wine off the lees and aerating followed by returning the lees back to the wine after 48 h completely removes MeSH and EtSH (Lavigne-Cruège and Dubourdieu 2001). What is lacking in these studies is assessment of the concentration of polysulfides (e.g. DMDS, DEDS and ethylmethyl disulfide) present in the wine which may explain why despite these winemaking methodologies being employed, VSCs can reappear.

Oxygen exposure during fermentation or maturation. Addition of O_2 during the cell growth phase of yeast in alcoholic fermentation has been shown to be beneficial to yeast health resulting in less H_2S produced during fermentation (du Toit et al. 2006). A study on the impact of O_2 dosage and timing during fermentation on the concentration of VSCs in wine has shown the beneficial effects of O_2 (Bekker et al. 2015). Wines treated with air or 40% O_2/60% N_2 during fermentation showed a significant decrease in VSCs after 12 months in bottle compared with wine treated with N_2 or no gas. The impact on VSCs of O_2 introduced during 6 months' bottle storage, was studied in a series of Spanish red wines (Ferreira et al. 2014). Oxygen was transferred into the wine in known amounts over a 6-month period, and the wines were analysed for VSCs. The more oxygen introduced to the wine, the greater the reduction in H_2S concentration, while a low concentration of oxygen resulted in an increase in MeSH and DMS.

Micro-oxygenation. Micro-oxygenation was originally developed as a way of simulating, in stainless steel tanks, the ageing effects of wine in oak barrels (Gómez-Plaza and Cano-López 2011). The introduction of a continual O_2 supply during maturation can result in quinone production which, as discussed earlier, may result in thiol trapping and a reduction in the overall VSC concentration.

Oxygen-related effects of closures. The amount of O_2 present in wine at bottling will play a significant role in the rate at which H_2S, MeSH and DMS are formed and consumed (Brajkovich et al. 2005, Godden et al. 2005, Skouroumounis et al. 2005, Kwiatkowski et al. 2007, Lopes et al. 2009, Caillé et al. 2010, Dimkou et al. 2011, Silva et al. 2011, Ugliano et al. 2011). A recent review of the effect of oxygen on wine aroma evolution during bottling summarises this area (Ugliano 2013). A study that investigated the impact of closures on a Semillon wine showed that 63 months after bottling wine stored under screwcap retained a higher SO_2 concentration, higher varietal aromas but also a higher concentration of reductive aromas (Godden et al. 2005). The same wine stored under natural and synthetic corks showed lower varietal character and a lower level of reductive aromas. The change in VSC concentration post-bottling due to the impact of dissolved O_2 as well as closure-derived O_2 was reported in 2011 (Ugliano et al. 2011). This demonstrated that low O_2 exposure at bottling and during bottle ageing can have a significant impact on H_2S, MeSH and DMS during the life of a wine.

Conclusions

There are many possible sulfur-containing compounds occurring naturally in grapes or added in the vineyard and winery, which could lead to subsequent production of H_2S, MeSH and DMS in wine. From the mineral composition of the soil in which wines are grown, to sulfur-containing pesticides and fungicides, sulfur is present at the beginning of the journey from grape to glass. The addition of sulfite to the grape bin, through to just prior to bottling is a standard practice in the wine industry. Yeasts that convert sulfate and sulfite to H_2S through to the sulfur-containing amino acids and glutathione also play a significant role in H_2S, MeSH and DMS production.

The role of O_2 has been shown to be influential in the redox cycles discussed and thus management of the introduction of O_2 at varying stages of the winemaking process is a key part to understanding the evolution of VSCs and is an active area of research. Transition metals including Fe and Cu play a central role in these redox cycles and together with O_2, their concentration, redox state and coordination state need to be monitored to understand better why wines, seemingly unaffected by VSCs, can change significantly during maturation and post-bottling.

Current management strategies employed in the wine industry need further investigation to understand better any untoward negative effects that may become obvious over a longer time frame. It is now clear that addition of Cu to treat VSCs is a double-edged sword affecting both unwanted and desirable sulfur compounds.

The origin and fate of many VSCs remains unclear, and although several leads have been discussed, many of the sources of VSCs and the triggers for their release into wine from precursors remain to be established. The chemistry of the wine matrix is complex, and the solutions currently employed to deal with VSCs may not be achieving the desired result. A better understanding of both the role of yeast in VSC production and the role of transition metals and O_2 in their release is required.

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The effect of wine closures on volatile sulfur and other compounds during post-bottle ageing†

Juan He,[a] Qin Zhou,[a] Jim Peck,[b] Rollin Soles[c] and Michael C. Qian[a]*

ABSTRACT: The effect of wine closures on volatile composition during post-bottle ageing was investigated for Pinot Noir and Chardonnay wines. Natural cork, synthetic cork and screw caps with Saran–tin, Saranex and low density polyethylene (LDPE) liners were investigated over 3 years of storage. Dissolved O_2 and SO_2 as well as colour were monitored. Volatile sulfur compounds, esters, terpene alcohols, and C_{13}-norisoprenoids were quantified every 6 months over 3 years. The results showed that the LDPE screw cap gave the highest dissolved oxygen, lowest free SO_2 and total SO_2 in both wines, while the Saran–tin screw cap gave the lowest dissolved oxygen and the highest free SO_2 and total SO_2. A decrease of H_2S, methanethiol, and thioacetates were observed during the 3 year ageing process for both wines, and their concentrations decreased most in wines sealed with LDPE screw caps and synthetic closures. The decrease of thioacetates was independent of closure type. The accumulation of dimethyl disulfide during storage was not obvious in these two wines. For both wines, no elevated sulfur compounds under any closure were detected; neither dimethyl disulfide nor dimethyl trisulfide were found in any of the experimental wines. Other volatiles also changed with in bottle ageing. Ethyl esters decreased during ageing, whereas ethyl 2-methylbutanoate and ethyl 3-methylbutanoate increased during ageing. Terpene alcohols showed a decreasing trend with ageing, as was β-damascenone, but β-ionone remained unchanged in Pinot Noir wine during ageing. Both wines under LDPE closure had the highest acetaldehyde at 36 months of ageing. Wine closure affected the concentration of terpene alcohols and C_{13}-norisoprenoids. Closure had no impact on nerolidol and citronellol, but LDPE screw-capped wine had lower concentrations of linalool at 36 months of storage. Both wines with LPDE screw cap closures had higher β-damascenone. Closure had no effect on β-ionone and other compounds. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: screw cap; volatile sulfur compound; wine; bottle ageing; oxidation; β-damascenone; sulfur dioxide

Introduction

Although natural cork has dominated the wine industry as the wine stopper of choice for over 300 years, it is considered to be a less than perfect seal. The major dissatisfactions in the wine industry with it have derived from oxidation and cork taint. Poor quality corks can spoil the wine with a 'cork taint', an off-flavour associated with musty, dull characteristics caused by the presence of 2,4,6-trichloroanisole. Wine makers are eagerly exploring other alternatives for wine closures. Plastic corks were tested by wine makers in the past, and many issues were found after the wines were bottled and marketed. Screw caps now arouse new interest as an alternative wine closure.

Depending on the type of wines and storage conditions, wine closure could have a dramatic impact on wine post-bottling behaviour. It was found that wine developed differently during in-bottle storage based on the types of closures. Free SO_2, used as an antioxidant for wine, was highly affected by the type of closure. Loss of SO_2 in wine is often associated with a high rate of oxygen ingress (oxygen transmission rate, OTR) or high initial dissolved O_2. Loss of SO_2 is often correlated with an increase in wine browning (OD_{420}). Wine closure has been reported to affect the aroma of the wine. It has been reported that screw-cap closure-bottled Semillon wines tended to have a higher fruity, citrus sensory score and were low in oxidized aroma.[1] It was also found that screw cap wines contained higher concentrations of 3-mecaptohexanol and 3-mecaptohexyl acetate than wines sealed with cork,[2] which contributed positively to Sauvignon Blanc wines. However, in a long-term storage study, a 'reductive' sulfur off-aroma was discernible in Riesling and Chardonnay wines under tin-saranex screw cap and in glass ampoules where the oxygen transmission was minimal.[3]

Accumulation of volatile sulfur compounds has been blamed for the perceived 'reductive' off-aromas observed in screw cap closured wines. It was proposed that the reductive off-flavour taint was due to the increase of thiols (methanethiol and ethanethiol) in the screw-capped wines.[4] Methanethiol and ethanethiol emit a smell like rotten cabbage and have much lower sensory thresholds (sensory threshold 1.1 μg/l for methanethiol) than dimethyl disulfide (sensory threshold 20–45 μg/l) and diethyl disulfide (sensory threshold 4.3–40 μg/l).[5] Under

* Correspondence to: Michael C. Qian, 100 Wiegand Hall, Corvallis, OR 97331, USA. E-mail: Michael.qian@oregonstate.edu

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[a] Department of Food Science & Technology, Oregon State University, Corvallis, OR 97331, USA

[b] G-3 Enterprises, Modesto, CA 95354, USA

[c] Argyle winery, Dundee, OR 97115, USA

screw cap closure, it is assumed that the low oxygen ingress through the closure cannot efficiently oxidize thiols to the corresponding disulfides, resulting in the accumulation of thiols producing the 'reductive' off-aroma.[4]

Wine in bottle ageing is a slow and complex process. Short-chain fatty acid esters can be hydrolysed to their corresponding acids to impart a less fruity (apple, banana and pineapple-like) character.[6,7] Terpene, terpene alcohol and C_{13}-norisoprenoid compounds and precursors can be hydrolysed or converted to other aroma-active compounds to alter the aroma profile of the wine.[7] Oxidation of some wine components can induce the formation of some off-flavour compounds such as acetaldehydes. Many compounds, including 1,1,6-trimethyl-1,2-dihydronaphthalene, 2-furfural, ethyl 2-furancarboxylate and dimethyl sulfide, have been reported to increase with wine ageing.[8–10] The objective of this study was to investigate the impact of wine closures on volatile sulfur composition and other volatile compounds during storage.

Materials and Methods

Chemicals

Sodium sulfide, methanethiol (MeSH), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and isopropyl disulfide (Iso-ProDS) were purchased from Sigma-Aldrich (St Louis, MO, USA). Methyl thioacetate (MeSOAc), ethyl thioacetate (EtSOAc) were obtained from Johnson Mattey Catalog Company Inc. (Ward Hill, MA, USA). Ethyl methyl sulfide (EMS), dimethyl sulfide (DMS), diethyl disulfide (DEDS) were supplied by TCI America (Portland, OR, USA). Methanol was obtained from J.T. Baker (Phillipsburg, NJ, USA), and the ethanol was from AAPER Alcohol and Chemical Co. (Shelbyville, KY, USA). Other volatile standards were from Sigma-Aldrich unless specified.

Selection of the Wine Closures

Three types of commercial screw cap closures with low, medium and high oxygen permeability were provided by G-3 Enterprises (Modesto, CA, USA). The liner types within the caps were a tin foil (Saran–tin), a Saranex and a polyethylene (low-density polyethylene, LDPE). The Saranex liners contain layers of polyethylene, polyvinylidene chloride and expanded polyethylene foam, whereas the Saran–tin has a layer of tin foil laminated between Saran and LDPE foam, which greatly reduces the oxygen ingress into the bottle. Commercial natural cork (standard grade A, 45 × 24 mm; Amorim Cork America, Napa, CA, USA) was carefully selected for adherence to grade, and the bottles sealed by these were stored both upright and inverted. One common extruded synthetic cork was obtained commercially from a local supplier in USA. The co-extruded synthetic corks consist of a closed-cell polymer foam inner core with a solid, polymer, flexible outer 'skin'. The dimensions were 24 mm diameter by 38 mm length.

Wine Bottling

One red (Pinot Noir) and one white wine (Chardonnay) from the 2006 vintage were made using commercial winemaking practices at Argyle Winery in Oregon. The wines were bottled in August 2007. Empty bottles were sparged with nitrogen before they entered the filling machine. A gravity flow filler with 12 filling heads was running at 32 bottles per minute. About 15 ml of headspace volume was left for screw cap closures and about 10 ml of headspace was left for cork and synthetic closures. Before the capper and corker, the bottle headspace was purged with nitrogen gas. Corks were inserted using a vacuum corker. One hundred and fifty bottles of each wine were sealed with each of the closures (300 bottles for the natural corks). All of the samples were stored at the winery under controlled conditions (12–13°C) until analysis. An ACR Smart Reader Datalogger Plus was placed in one of the wine cases to record the temperatures to which the wines were exposed for the duration of the test. Samples were taken at 6-month intervals for 3 years. Six bottles of wine from each treatment were analysed for dissolved oxygen, SO_2 and colour, three bottles of wine were used for volatile sulfur analysis.

Measurement of the Oxygen Transmission Rate

Oxygen transmission rate (OTR) for each closure was tested using Mocon Oxtran models 2/61 and 2/21 Mocon, (Minneapolis, MN, USA). The wine bottles were cut off using a DeWalt 25.4 cm (10 inch) tile cutter with diamond blade. The glass edges were then smoothed off using a Leco GP-6 polisher and 240 grit wet polishing disks. After wiping down with a cloth towel and brief drying, the bottle necks were glued to OTR testing platforms with Devcon 2-Ton epoxy. These testing platforms were then affixed to a nitrogen manifold for equilibration, where a constant flow of nitrogen is used to simulate the anoxic conditions of the headspace of a bottle of wine. Prior to testing, the Mocon instruments are calibrated using the two-point method. The lower point is a zero obtained by testing a stainless steel tube. A NIST certified Mocon calibration film is used to set the upper point. Equilibration in OTR testing can be defined as the point where the OTR reading is constant. Equilibration times vary significantly depending upon the materials in the closure and their thickness. Very thick closures such as synthetic stoppers may require 4 months to reach equilibrium. A polyethylene lined screw cap may only require five days. Screw caps were therefore placed on the equilibration system for at least a week, moved to a Mocon, and then tested twice to confirm equilibrium. Additional testing is performed as many times as needed to ensure that equilibrium has been achieved. Stoppers such as natural and synthetic cork are tested monthly until equilibrium is confirmed. OTR units of measurement are expressed as ml of oxygen per closure per day in air.

Measurement of Dissolved Oxygen, Wine Colour and SO_2

The dissolved oxygen was measured using an Orbisphere 3650 dissolved-oxygen meter (Hach Company, Loveland, Colorado, USA). Bottles were inverted with a small amount of water added to the punt. A 0.356 cm (0.14 inch) diameter diamond drill bit in a Dremmel tool was used to bore a hole in the bottom of the bottle. After the hole was completed, the bottle was tipped to drain off the water and a 0.318 cm (0.125 inch) diameter stainless steel siphon tube was inserted into the hole. At the top of the siphon tube was a 2.54 cm (1 inch) diameter rubber stopper with a nitrogen gas supply which when held down into the punt, created a seal, generating pressure that forced the wine out through the siphon and through the Orbisphere at approximately 100 ml/min. The reading on the Orbisphere was monitored during siphoning until reaching to a stabile level and recorded. During

siphoning, the wine exiting the Orbisphere was directed into two 175-ml bottles for further analysis of SO_2 and absorbance. Siphoning was continued until nearly all wine was expelled and nitrogen gas flowed through the apparatus. Nitrogen purging of the empty bottle and apparatus was continued until the Orbisphere readout decreased to zero. Wine colour were monitored by measuring the absorbance at 420/520 nm in red wine and 420 nm (Lamda 35; Perkin-Elmer, Waltham, MA, USA). Free and total SO_2 were measured in a Hitachi 911automatic analyser (Tokyo, Japan) using in-house protocols based on colorimetric methods described in test kits 'free sulfur dioxide colorimetric method for the manual determination in wine' and 'total sulfur dioxide colorimetric method for the manual determination in wine' (ANPEOS Pty Ltd, Boroniam, Victoria, Australia). Free SO_2 in wine were reacted with *p*-fuschine and formaldehyde under acidic conditions, and the absorbance was measured at 570 nm. Total SO_2 was reacted with 5,5′-dithio-bis(2-nitrobenzoic) acid at pH 7 and the absorbance was measured at 415 nm. Interference of polyphenols and the colour of the wine were eliminated by subtracting the absorbance of sample blank. SO_2, absorbance and OTR measurements were all performed in duplicate. Six samples were analysed for each closure and each wine.

Analysis of Volatile Sulfur by Headspace-SPME-GC-PFPD

Volatile sulfur compounds were analysed in all wines using HS-GC-PFPD described by Fang and Qian[11] with some modifications. An aliquot of 2 ml wine was diluted with 8 ml saturated NaCl solution in a 20 ml vial deactivated with dichlorosilane (Sylon CT™, 5% in toluene; Sigma-Aldrich Co., St Louis, MO, USA). An aliquot (100 μl) of internal standard solution (500 μg/l EMS and 2 μg/l isopropyl disulfide in methanol) and 100 μl 5% acetaldehyde (v/v dissolved in methanol), as described previously,[11] were added. An 85 μm Carboxen-PDMS StableFlex SPME fibre (Supelco, Bellefonte, PA, USA) was used for the extraction of sulfur compounds. Samples were pre-incubated for 5 min at 30°C with 500 rpm agitation, and then extracted for 20 min with 250 rpm agitation at the same temperature. The volatile compounds extracted by the SPME fibre were thermally desorbed at 300°C in the GC injector in splitless mode. Separation of the analytes was achieved using a DB-FFAP fused silica capillary column (30 m × 0.32 mm, 1.0 μm film thickness; Agilent, Palo Alto, CA, USA) with a constant nitrogen flow of 2.0 ml/min. The oven temperature program was as follows: 35°C held for 5 min, heated to 150°C at a rate of 10°C/min, held for 1 min, then heated to 220°C at a rate of 20°C/min with a final hold time of 5 min. The PFPD was held at 300°C and 500 V with the hydrogen flow rate at 14 ml/min, air 1 flow rate at 17 ml/min, and air 2 flow rate at 10 ml/min. Chromatographic identification of target sulfur compounds was performed by comparing retention times with those of authentic standards. Standard calibration curves were obtained by adding increasing amount of the target compounds mixture to the Chardonnay or Pinot Noir wine, respectively. Concentrations were calculated based on the square root of the peak area ratio of the compound to the internal standard. Three bottles of wine from each treatment were analysed and duplicate analyses were performed for each bottle.

Analysis of Acetaldehyde

Acetaldehyde was analysed using headspace GC with a flame ionization detector (FID). Wine sample (1 ml) and 20 μl of internal standard solution (2.5 mg/ml methyl propanoate in ethanol) were added to a 20 ml vial. Sample was equilibrated at 55°C for 15 min, and 1 ml of headspace was taken using a MPS multipurpose autosampler (Gerstel Inc., Baltimore, MD, USA) with a 2.5 ml headspace syringe maintained at 75°C. Sample was injected onto an Agilent 7890A GC (Agilent, Santa Clara, CA, USA) at a 1:10 split ratio. A DB-wax column (30 m × 250 μm × 0.5 μm, Agilent) was used for separation under a flow rate of 2 ml/min of helium. The GC oven temperature was maintained at 35°C for 4 min, and heated at a rate of 10°C/min to 150°C, and hold for 5 min. The FID temperature was set at 200°C. Standard acetaldehyde solution was prepared in aqueous ethanol solution. Chemstation E.02.01 was used to calculate the concentration of acetaldehyde.

Analysis of Other Volatiles by Stir Bar Sorptive Extraction-GC-MS

Esters, terpenoids, C_{13}-norisoprenoids and other volatiles in the wine were analysed using stir bar sorptive extraction (SBSE)-GC-MS.[12,13] A 10 ml aliquot of wine sample and 10 ml of water (saturated with NaCl) were added to a 20 ml vial. Volatile compounds in the wine were extracted with a stir bar coated with polydimethylsiloxane (PDMS) phase (1 cm length, 0.5 mm thickness, Gerstel Inc.). The analytes were thermally desorbed at the TDU in splitless mode, ramping from 35 to 300°C at a rate of 700°C/min, and held at the final temperature for 3 min. The CIS-4 was cooled to −80°C with liquid nitrogen during the sample injection, then heated at 10°C/s to 250°C for 10 min. Solvent vent mode was used for CIS-4 during the injection with a split vent purge flow of 50 ml/min beginning at 0.01 min. GC-MS analysis was performed on an Agilent 6890 gas chromatograph equipped with a 5973 mass selective detector (Agilent Technologies, Inc., Wilmington, DE, USA). The helium column flow was 2.5 ml/min. Separation was achieved using a HP-5 column (60 m × 0.32 mm ID, 0.25 μm film thickness; Agilent Technologies, Inc., Wilmington, DE). The oven temperature was programmed at 40°C for 2 min, then ramped to 220°C at a rate of 4°C/min, then increased to 250°C at a rate of 6°C/min and held at the final temperature for 6 min. MSD standard EI mode was used at 70 eV. The total mass ion chromatogram was obtained from 35 to 350 amu. Compounds were identified through a mass spectra library and retention indices of authentic standards. Selective mass ions were used to quantify the aroma-active compounds. Standard calibration curves were obtained by the standard addition technique mentioned above.

Results and Discussion

Oxygen Transmission Rate, Dissolved Oxygen, Free and Total SO_2, and Absorbance

The oxygen transmission of each type of closure is presented in Table 1. The wine type and storage time had little effect on the OTR value except for cork-up, for which a general decreasing trend of OTR was noticed. On average, LDPE caps had the highest oxygen transmission followed by synthetic cork, while Saran–tin showed the lowest OTR. It is normal for natural corks to show greater variability than other closures, particularly in the upright position. A more consistent oxygen transmission was observed in the inverted position than in the upright position.

Pinot Noir wines bottled with different closures had varied amounts of dissolved oxygen initially due to different ways of sealing right after bottling and bottling variations (Table 2).

Table 1. Oxygen transmission rate (± standard deviation) of different wine closures (ml O_2/day/cap)

Closure	Chardonnay			Pinot Noir		
	6 months	12 months	36 months	6 months	12 months	36 months
Synthetic	0.007 ± 0.0004	0.005 ± 0.001	0.007 ± 0.0001	0.006 ± 0.00055	0.006 ± 0.001	0.006 ± 0.0002
Cork-up	0.028 ± 0.03	0.011 ± 0.008	0.006 ± 0.002	0.009 ± 0.008	0.008 ± 0.007	0.004 ± 0.001
Cork-down	0.0006 ± 0.0006	0.0006 ± 0.0004	0.0006 ± 0.0002	0.0004 ± 0.0001	0.0005 ± 0.0003	0.0005 ± 0.0002
Saran	0.0009 ± 0.0002	0.0006 ± 0.0001	0.0006 ±0.0001	0.0006 ± 0.0002	0.0006 ± 0.0001	0.0006 ± 0.0001
LDPE	0.018 ± 0.003	0.018 ± 0.006	0.019 ± 0.002	0.017 ± 0.003	0.014 ± 0.002	0.015 ± 0.002
Saran–tin	0.00003 ± 0.000004	0.00002 ± 0.000002	0.00001 ± 0.00005	0.00006 ± 0.00003	0.00003 ± 0.00003	0.00004 ± 0.00001

LDPE, low-density polyethylene.

However, the wines consumed the oxygen after bottling, and the dissolved oxygen decreased dramatically in the first 6 months. The dissolved oxygen among the closures narrowed dramatically at 12 months of storage, ranging from 3 to 8 μg/l in both wines. After that, oxygen permeability of closure began to show its impact on dissolved oxygen content. At 36 months of storage, LDPE, with the highest oxygen permeability, showed the highest dissolved oxygen content, followed by synthetic cork; while foil, with the lowest OTR, showed the lowest dissolved oxygen content.

Free SO_2 serves as an antioxidant, protecting wine from oxidative reactions. It can be used as an indicator of oxygen exposure and oxidative status in wine.[14] The results demonstrated that both free and total SO_2 decreased with ageing; however, the rate of decrease was different among the closures. At 6 months of storage, free SO_2 was decreased substantially in Pinot Noir wine, but there was no difference among the closures. A closure effect started to show at 12 months of storage. For the three screw cap closures, Saran–tin had the highest free SO_2, LDPE had the lowest. The free SO_2 continued to decrease rapidly after 12 months for the LDPE closure in both Pinot Noir and Chardonnay wines, but the decreases were much slower for Saran–tin and Saran. Saran–tin retained the SO_2 slightly better than Saran although it has a much lower OTR. The cork closures had the same free and total SO_2 at 6 months of storage, regardless of the difference in OTRs, probably due to the initial dissolved oxygen at the bottling. The free SO_2 continued to decrease after 12 months of storage, while the synthetic cork decreased the most, in agreement with its high OTR.

Although the Saran closure had similar oxygen permeability to natural cork according to the Mocon data, the concentration of free SO_2 in Saran screw cap wine was higher than in the natural cork wine, which was in agreement with the dissolved oxygen data that the wine from Saran closure had slightly lower dissolved oxygen than the wine from inverted natural cork. Oxygen in wine is from ingress through the closure during storage and the initial dissolved oxygen at bottling. For high OTR, closures such as LDPE screw cap and synthetic cork, the dissolved oxygen and SO_2 correlated well with OTR, suggesting that oxygen diffused through the closure dominates. For wines with low OTR closures, the initial dissolved oxygen may play a more important role in wine behaviour.

The wine absorbance mirrored the data of both dissolved oxygen and free SO_2. The absorbance at 420 nm and 520 nm of Pinot Noir wine had an overall increasing trend during the storage period. The increase in both A_{420nm} and A_{520nm} was greatest with LDPE screw cap which had highest OTR, while the Saran–tin increased the least.

Volatile Sulfur Compounds

The volatile sulfur compounds commonly present in wine, including H_2S, methanethiol, ethanethiol, dimethyl sulfide, methyl thioacetate, ethyl thioacetate, dimethyl disulfide and dimethyl trisulfide, were monitored in this study. Overall, the concentrations of all volatile sulfur compounds were very low in both experimental wines (Table 3 and Table 4).

DMS concentration was not affected by wine in both wines indicating that oxidation status of the wine has no effect on DMS concentration. DMS concentration was reported to increase during ageing in many wines.[15–20] DMS can be formed from other non-volatile precursors during the ageing process. Dimethyl

Table 2. Dissolved O_2 and SO_2, and light absorbance (± standard deviation) of Pinot Noir and Chardonnay wines under different closures ($n = 6$)

Closure	0 months	6 months	12 months	18 months	24 months	30 months	36 months
Dissolved O_2 (mg/l)							
Pinot Noir							
Synthetic	141	10 ± 3	8 ± 1	7 ± 1	11 ± 1	11 ± 2	11 ± 4
Cork-up	75	5 ± 3	4 ± 1	7 ± 1	8 ± 1	6 ± 1	6 ± 1
Cork-down	178	4 ± 0	3 ± 1	5 ± 1	7 ± 1	3 ± 1	2 ± 1
Saran	161	8 ± 2	4 ± 1	4 ± 1	11 ± 1	7 ± 1	2 ± 1
LDPE	119	22 ± 5	7 ± 1	9 ± 1	18 ± 2	15 ± 2	31 ± 8
Saran–tin	70	14 ± 2	5 ± 1	2 ± 1	6 ± 1	5 ± 1	1 ± 1
Chardonnay							
Synthetic	67	7 ± 1	7 ± 1	8 ± 1	11 ± 1	12 ± 2	13 ± 1
Cork-up	86	5 ± 3	7 ± 1	7 ± 1	11 ± 3	6 ± 1	4 ± 3
Cork-down	74	3 ± 1	5 ± 1	7 ± 1	7 ± 1	4 ± 1	4 ± 1
Saran	116	9 ± 2	5 ± 0	6 ± 1	8 ± 1	7 ± 1	3 ± 1
LDPE	110	14 ± 2	7 ± 1	10 ± 1	19 ± 2	21 ± 3	21 ± 2
Saran–tin	104	11 ± 4	5 ± 1	4 ± 1	6 ± 1	4 ± 1	1 ± 1
Free SO_2 (mg/l)							
Pinot Noir							
Synthetic	33	25 ± 1	19 ± 0	14 ± 0	14 ± 1	11 ± 1	9 ± 1
Cork-up	33	22 ± 1	19 ± 1	14 ± 1	13 ± 2	15 ± 1	11 ± 1
Cork-down	33	21 ± 3	19 ± 1	16 ± 1	14 ± 1	12 ± 2	12 ± 2
Saran	33	27 ± 2	21 ± 0	16 ± 1	15 ± 1	15 ± 0	17 ± 1
LDPE	33	22 ± 2	13 ± 1	7 ± 1	8 ± 1	7 ± 1	4 ± 1
Saran–tin	33	25 ± 1	23 ± 1	19 ± 1	18 ± 1	16 ± 0	18 ± 1
Chardonnay							
Synthetic	25	24 ± 1	21 ± 1	14 ± 1	15 ± 1	11 ± 1	9 ± 1
Cork-up	25	22 ± 5	20 ± 1	17 ± 1	16 ± 2	16 ± 2	14 ± 3
Cork-down	25	26 ± 2	21 ± 1	18 ± 2	16 ± 2	15 ± 1	15 ± 2
Saran	25	23 ± 2	23 ± 1	20 ± 1	18 ± 1	17 ± 1	21 ± 1
LDPE	25	22 ± 2	15 ± 0	9 ± 2	6 ± 1	5 ± 1	2 ± 1
Saran–tin	25	23 ± 2	24 ± 2	20 ± 1	19 ± 1	22 ± 1	23 ± 1
Total SO_2 (mg/l)							
Pinot Noir							
Synthetic	68	52 ± 2	47 ± 0	38 ± 1	32 ± 1	30 ± 2	25 ± 0
Cork-up	68	50 ± 1	45 ± 2	40 ± 2	36 ± 5	38 ± 2	29 ± 3
Cork-down	68	50 ± 3	47 ± 2	45 ± 2	36 ± 2	35 ± 3	33 ± 3
Saran	68	58 ± 1	52 ± 1	42 ± 2	39 ± 1	40 ± 1	41 ± 1
LDPE	68	50 ± 1	39 ± 2	26 ± 2	23 ± 2	18 ± 4	10 ± 4
Saran–tin	68	58 ± 1	53 ± 1	50 ± 1	45 ± 1	43 ± 1	43 ± 1
Chardonnay							
Synthetic	97	90 ± 1	89 ± 1	79 ± 2	76 ± 1	73 ± 1	67 ± 1
Cork-up	97	90 ± 8	87 ± 2	82 ± 2	80 ± 4	83 ± 3	77 ± 6
Cork-down	97	93 ± 2	89 ± 1	85 ± 2	81 ± 3	80 ± 1	78 ± 4
Saran	97	90 ± 2	92 ± 1	88 ± 1	87 ± 2	85 ± 1	85 ± 2
LDPE	97	87 ± 3	78 ± 2	66 ± 3	62 ± 2	56 ± 2	44 ± 3
Saran–tin	97	92 ± 1	94 ± 3	88 ± 2	90 ± 1	90 ± 1	88 ± 2
Absorbance at 420 nm (au)							
Pinot Noir							
Synthetic	1.37	1.537 ± 0.005	1.631 ± 0.007	1.823 ± 0.018	1.758 ± 0.022	1.752 ± 0.02	1.786 ± 0.011
Cork-up	1.37	1.548 ± 0.012	1.583 ± 0.021	1.769 ± 0.037	1.617 ± 0.029	1.588 ± 0.026	1.718 ± 0.043
Cork-down	1.37	1.522 ± 0.012	1.566 ± 0.016	1.555 ± 0.036	1.470 ± 0.055	1.626 ± 0.059	1.638 ± 0.027
Saran	1.37	1.508 ± 0.008	1.523 ± 0.005	1.623 ± 0.028	1.651 ± 0.015	1.592 ± 0.008	1.57 ± 0.007
LDPE	1.37	1.563 ± 0.018	1.712 ± 0.015	1.836 ± 0.026	1.86 ± 0.022	1.904 ± 0.049	2.024 ± 0.094
Saran–tin	1.37	1.470 ± 0.019	1.636 ± 0.03	1.514 ± 0.011	1.530 ± 0.005	1.548 ± 0.009	1.559 ± 0.007
Absorbance at 520 nm (au)							
Pinot Noir							
Synthetic	1.48	1.583 ± 0.005	1.665 ± 0.008	1.833 ± 0.023	1.702 ± 0.011	1.698 ± 0.023	1.684 ± 0.009

(Continues)

Table 2. *(Continued)*

Closure	0 months	6 months	12 months	18 months	24 months	30 months	36 months
Cork-up	1.48	1.615 ± 0.008	1.628 ± 0.016	1.790 ± 0.037	1.625 ± 0.026	1.588 ± 0.019	1.656 ± 0.019
Cork-down	1.48	1.615 ± 0.008	1.638 ± 0.021	1.608 ± 0.028	1.468 ± 0.048	1.632 ± 0.065	1.606 ± 0.025
Saran	1.48	1.580 ± 0.015	1.567 ± 0.003	1.608 ± 0.028	1.620 ± 0.012	1.559 ± 0.009	1.515 ± 0.006
LDPE	1.48	1.630 ± 0.014	1.726 ± 0.020	1.825 ± 0.037	1.808 ± 0.035	1.848 ± 0.059	1.968 ± 0.077
Saran–tin	1.48	1.548 ± 0.012	1.695 ± 0.029	1.548 ± 0.011	1.510 ± 0.007	1.508 ± 0.006	1.490 ± 0.008
Absorbance at 420 nm (au)							
Chardonnay							
Synthetic	0.08	0.107 ± 0.007	0.117 ± 0.004	0.136 ± 0.002	0.151 ± 0.003	0.156 ± 0.003	0.183 ± 0.007
Cork-up	0.08	0.101 ± 0.004	0.129 ± 0.012	0.129 ± 0.004	0.153 ± 0.006	0.141 ± 0.006	0.164 ± 0.014
Cork-down	0.08	0.097 ± 0.001	0.117 ± 0.008	0.132 ± 0.003	0.142 ± 0.005	0.142 ± 0.004	0.156 ± 0.009
Saran	0.08	0.104 ± 0.006	0.110 ± 0.005	0.123 ± 0.001	0.140 ± 0.008	0.133 ± 0.003	0.154 ± 0.010
LDPE	0.08	0.108 ± 0.007	0.141 ± 0.025	0.150 ± 0.006	0.182 ± 0.007	0.181 ± 0.004	0.229 ± 0.009
Saran–tin	0.08	0.110 ± 0.005	0.111 ± 0.003	0.121 ± 0.002	0.131 ± 0.011	0.133 ± 0.003	0.149 ± 0.009

LDPE, low-density polyethylene.

Table 3. Volatile sulfur compounds in Chardonnay wine bottled with different closures ($n = 6$)

Closure	6 months	12 months	18 months	24 months	36 months
Dimethyl disulfide (μg/l)					
Synthetic	23.2 ± 8.3	22.3 ± 0.5	21.0 ± 0.8	20.8 ± 0.8	20.7 ± 1.0
Cork-up	27.8 ± 10.1	22.7 ± 0.5	24.8 ± 1.3	22.4 ± 1.1	22.0 ± 0.4
Cork-down	26.3 ± 8.6	23.5 ± 0.9	24.6 ± 2.0	21.6 ± 0.7	20.8 ± 0.6
Saran	24.4 ± 8.1	23.5 ± 0.8	25.3 ± 1.6	21.0 ± 0.4	21.7 ± 0.5
LDPE	24.8 ± 8.1	23.2 ± 0.6	23.6 ± 1.1	21.3 ± 1.1	21.4 ± 0.9
Saran–tin	25.2 ± 4.8	22.5 ± 1.7	28.2 ± 1.2	21.8 ± 1.3	22.4 ± 0.4
Ethyl thioacetate (μg/l)					
Synthetic	3.0 ± 0.4	1.7 ± 0.1	1.9 ± 0	0.8 ± 0.0	1.2 ± 0.1
Cork-up	2.9 ± 0.6	1.7 ± 0.1	1.3 ± 0	1.0 ± 0.1	1.2 ± 0
Cork-down	2.9 ± 0.5	1.8 ± 0.2	1.6 ± 0.1	0.9 ± 0.1	1.2 ± 0.1
Saran	3.0 ± 0.7	1.8 ± 0.1	1.5 ± 0.1	0.8 ± 0.0	1.3 ± 0
LDPE	2.9 ± 0.6	1.8 ± 0.2	1.6 ± 0.1	0.9 ± 0.1	1.2 ± 0.1
Saran–tin	2.9 ± 0.5	1.7 ± 0.2	1.5 ± 0	0.9 ± 0.1	1.3 ± 0.1
Methyl thioacetate (μg/l)					
Synthetic	10.9 ± 1.5	7.8 ± 0.4	6.4 ± 0.2	4.2 ± 0.2	3.6 ± 0.2
Cork-up	11.0 ± 1.6	7.9 ± 0.3	7.7 ± 0.4	4.6 ± 0.3	3.6 ± 0.1
Cork-down	10.8 ± 1.4	8.1 ± 0.5	7.6 ± 0.6	4.4 ± 0.2	3.5 ± 0.1
Saran	11.2 ± 1.6	8.2 ± 0.5	7.7 ± 0.6	4.3 ± 0.1	3.7 ± 0.1
LDPE	11.2 ± 1.7	8.3 ± 0.8	7.3 ± 0.3	4.6 ± 0.3	3.6 ± 0.2
Saran–tin	11.3 ± 1.1	7.9 ± 1.0	8.4 ± 0.3	4.8 ± 0.2	3.6 ± 0.2
Methanethiol (μg/l)					
Synthetic	6.5 ± 2.1	4.1 ± 0.1	3.7 ± 0.2	3.1 ± 0.5	4.2 ± 0.3
Cork-up	8.2 ± 1.6	4.3 ± 0.2	4.4 ± 0.3	3.7 ± 0.3	5.1 ± 0.3
Cork-down	5.8 ± 2.6	4.2 ± 0.3	4.5 ± 0.4	3.8 ± 0.6	5.6 ± 0.3
Saran	7.4 ± 2.5	6.7 ± 0.6	4.8 ± 0.2	4.2 ± 0.9	5.8 ± 0.2
LDPE	6.2 ± 1.7	5.3 ± 0.4	4.1 ± 0.2	3.2 ± 0.5	3.1 ± 0.2
Saran–tin	9 ± 2.6	5.5 ± 0.8	5.8 ± 0.3	4.0 ± 0.4	6.6 ± 0.5
Hydrogen sulfide (μg/l)					
Synthetic	NA	NA	15.9 ± 0.8	3.5 ± 0.8	2.3 ± 0.2
Cork-up	NA	NA	8.7 ± 0.2	3.8 ± 1.0	2.5 ± 0.2
Cork-down	NA	NA	8.8 ± 0.5	3.7 ± 0.9	2.3 ± 0.3
Saran	NA	NA	11.8 ± 1.0	3.9 ± 1.0	2.6 ± 0.2
LDPE	NA	NA	8.5 ± 0.3	3.8 ± 1.1	3.2 ± 0.9
Saran–tin	NA	NA	9.4 ± 0.9	3.8 ± 1.1	2.3 ± 0.3

LDPE, low-density polyethylene; NA, data were not obtained.

Table 4. Volatile sulfur compounds in Pinot Noir wine bottled with different closures ($n = 6$)

Closure	6 months	12 months	18 months	24 months	36 months
Dimethyl disulfide (μg/l)					
Synthetic	25.9 ± 7.9	24.9 ± 0.7	28.4 ± 3.3	19.9 ± 0.9	24.9 ± 0.8
Cork-up	24.4 ± 6.6	24.0 ± 1.0	26.9 ± 0.8	18.9 ± 0.6	26.2 ± 0.4
Cork-down	23.5 ± 6.5	24.9 ± 0.6	23.3 ± 2.0	20.0 ± 1.4	25.2 ± 0.6
Saran	22.6 ± 4.7	24.5 ± 0.8	27.0 ± 0.9	26.0 ± 1.0	25.0 ± 0.6
LDPE	24.7 ± 6.9	24.7 ± 0.9	25.4 ± 0.7	20.1 ± 0.8	25.0 ± 0.5
Saran–tin	23.6 ± 5.9	25.6 ± 1.4	28.1 ± 2.0	26.6 ± 1.2	26.3 ± 1.2
Methyl thioacetate (μg/l)					
Synthetic	5.0 ± 0.5	3.8 ± 0.1	2.6 ± 0.2	2.2 ± 0.2	1.7 ± 0.1
Cork-up	5.2 ± 0.7	3.7 ± 0.2	2.3 ± 0.1	2.0 ± 0.3	1.7 ± 0.1
Cork-down	5.1 ± 0.5	3.8 ± 0.1	2.4 ± 0.1	2.3 ± 0.2	1.8 ± 0.1
Saran	4.9 ± 0.5	3.7 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	1.7 ± 0.0
LDPE	4.9 ± 0.5	3.6 ± 0.2	2.2 ± 0.1	2.2 ± 0.1	1.6 ± 0.1
Saran–tin	4.9 ± 0.4	3.7 ± 0.2	2.6 ± 0.1	2.4 ± 0.1	1.7 ± 0.2
Methanethiol (μg/l)					
Synthetic	6.9 ± 1.4	ND	ND	ND	ND
Cork-up	4.5 ± 1.3	5 ± 0.3	2.6 ± 0.4	2.9 ± 0.3	ND
Cork-down	6 ± 3.5	5.4 ± 0.9	ND	3.2 ± 0.5	ND
Saran	8.8 ± 4.5	7.2 ± 1.8	2.7 ± 0.3	ND	2.8 ± 0.2
LDPE	7.4 ± 2.2	ND	ND	ND	ND
Saran–tin	13.2 ± 3.8	6 ± 0.8	ND	ND	3.4 ± 0.6
Hydrogen sulfide (μg/l)					
Synthetic	NA	NA	4.3 ± 0.5	1.9 ± 0.3	2.9 ± 0.5
Cork-up	NA	NA	4.0 ± 0.2	2.1 ± 0.5	3.6 ± 0.8
Cork-down	NA	NA	2.4 ± 1.5	2.8 ± 0.4	4.2 ± 0.7
Saran	NA	NA	4.4 ± 0.2	3.3 ± 0.5	3.9 ± 0.8
LDPE	NA	NA	3.1 ± 2.0	0.8 ± 0.1	0.7 ± 0.1
Saran–tin	NA	NA	5.0 ± 0.6	2.2 ± 0.1	3.5 ± 0.4

LDPE, low-density polyethylene; NA, data was not obtained; ND, not detected.

sulfoxide (DMSO), methionine sulfoxide (MSO), *S*-methylmethionine (SMM), dimethylsulfonium propanoic acid (DMSPA) and cysteine have been considered as possible precursors, although the study under basic hydrolysis condition suggests only *S*-methylmethionine can be converted to DMS.[21] A high initial concentration of those precursors could lead to the accumulation of DMS during in-bottle ageing. Storage temperature, dissolved oxygen, as well as nitrogen supplementation, could affect the conversion of the precursors and the accumulation of DMS in wine.[22–24] However, the accumulation of DMS during bottle ageing was not observed in the experimental Pinot Noir and Chardonnay wines. Instead, DMS maintained a constant level during storage. This discrepancy may be attributed to different wines being used in the investigation. This winery has been known to take every practice to control and minimize sulfur off-flavour in the wine, and sulfur off-flavour was seldom noticed in their wines. Regardless, wine closure did not affect DMS concentration in Pinot Noir and Chardonnay wine at any of the storage time.

Methyl thioacetate concentration in both wines decreased quickly during ageing due to hydrolysis under acidic conditions. The hydrolysis of methyl thioacetate leads to the formation of methanthiol. Closure type had no impact on the concentration of methyl thioacetate. A similar decreasing trend was observed for ethyl thioacetate in Chardonnay wine, where wine closure again had no impact on its decrease. Ethyl thioacetate was not detected in the Pinot Noir.

Methanthiol concentration was very low in both Pinot Noir and Chardonnay wines. Methanethiol is very reactive and sensitive to oxygen. It decreased very rapidly during the first 12 months, especially in Pinot Noir. An inverse relationship was observed between OTR and MeSH concentration. The closures with higher OTR corresponded to lower MeSH concentration. For both the synthetic and LDPE closures, methanethiol was completely lost after 12 months of storage in Pinot Noir wine.

Table 5. Acetaldehyde concentration (mg/l ± standard deviation) after 36 months storage in Pinot Noir and Chardonnay wines

Closure	Pinot Noir	Chardonnay
Synthetic	3.48 ± 0.17^{ac}	16.13 ± 1.01^{a}
Cork-up	3.18 ± 0.24^{ab}	15.74 ± 0.64^{a}
Cork-down	3.09 ± 0.36^{ab}	12.41 ± 1.51^{b}
Saran	2.67 ± 0.20^{b}	15.89 ± 0.90^{a}
LDPE	3.95 ± 0.12^{c}	18.57 ± 0.62^{c}
Saran–tin	3.04 ± 0.21^{ab}	12.95 ± 0.90^{b}

Different superscript letters indicate significant differences between sample means (Tukey HSD 95%, standard deviations were calculated from six replicates).

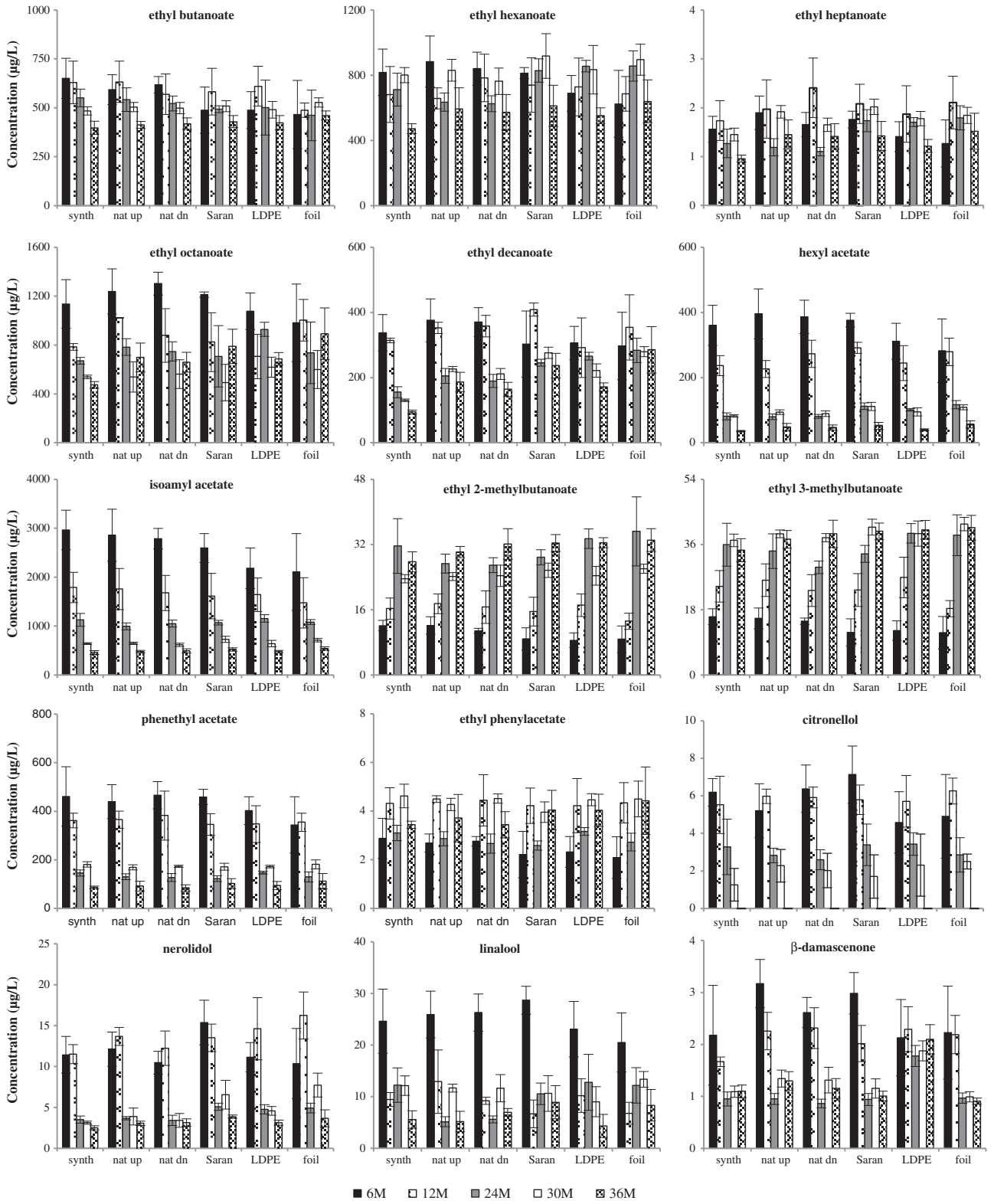

Figure 1. Other volatile compounds in Chardonnay wines during ageing (µg/l)

Both Saran and Saran–tin retained much higher methanethiol due to their low oxygen diffusion. This finding was consistent with previous results that volatile thiols are highly related to dissolved oxygen in the wine.[25,26] A similar trend was also found in Chardonnay wine. The synthetic and LDPE closures tended to have lower concentrations than Saran and Saran–tin. However, the decrease of methanethiol in Chardonnay was slower than Pinot Noir. In spite of the decrease of methanethiol

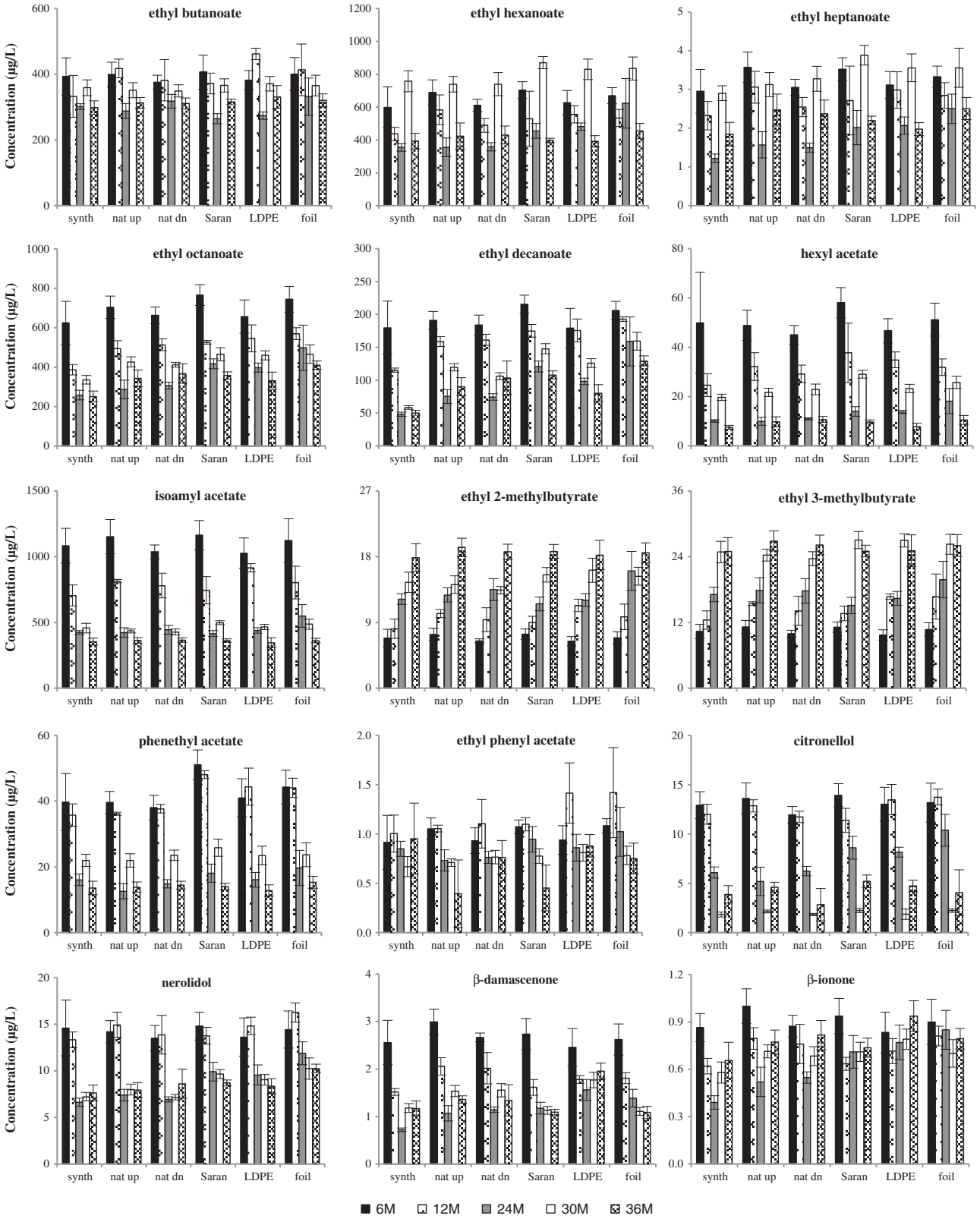

Figure 2. Other volatile compounds in Pinot Noir wines during ageing (µg/l)

during storage, accumulation of DMDS and DMTS were not detected in this study. Again, this result could due to low sulfur compounds in these wines; it could also be possible that the methanethiol was bound to other oxidative species such as quinines in wine instead of forming DMDS and DMTS.

The concentration of H_2S in Pinot Noir was also affected by wine closure. Higher oxygen transmission liners resulted in lower concentrations of H_2S in the wine. The trend was especially obvious after 24 months of ageing, where the LDPE closure showed the lowest amount of H_2S. However, this trend was not obvious in Chardonnay wine, and the results were not consistent at all storage times. Other unknown factors may affect the accumulation of H_2S. Further study is needed to investigate the evolution of H_2S in wine.

Other Volatile Compounds

Acetaldehyde

Acetaldehyde in wine is generated through the oxidation of ethanol via hydrogen peroxide.[27] Its concentration is considered to be an indication of the oxidative status of the wine. At 36 months of ageing, both wines sealing under LDPE screw caps had higher concentrations of acetaldehyde than the other wines (Table 5), in agreement that high OTR can result in rapid oxidation of the wine during storage.[28–30]

Esters

Ester was the major class of volatile compounds analysed in this study based on their high concentration and importance for flavour contribution. Ethyl esters of butanoate, hexanoate, octanoate and decanoate, as well as isoamyl acetate and many other esters were quantified, and high concentrations were found for most of them. Esters contribute to the characteristic fruity aromas of wine. Ethyl esters had an overall decreasing trend during ageing due to the hydrolysis (Figure 1 and Figure 2). Rapid hydrolysis took place during the first 12 months, and then the hydrolysis rate slowed. However, the concentrations of ethyl 2-methylbutanoate and ethyl 3-methylbutanoate increased consistently in both wines during ageing. These branch-chained esters also contribute to the fruity aromas. The net loss of these esters probably will decrease the fruity aroma character (apple, banana and pineapple-like) in wine.[6,7]

Ethyl phenylacetate and 2-phenylethyl acetate have been identified as important flavour contributors to wine aroma. They are typically described as floral, cherry, stone-fruit, and dry plum-like. Ethyl phenylacetate maintained at the same level during storage, whereas 2-phenylethyl decreased.

Terpene alcohols

Terpene alcohols are responsible for characteristic floral and fruity aromas in wine. In this study, three terpene alcohols – linalool, nerolidol and citronellol – were quantified. All three terpene alcohols decreased with storage time, in agreement with literature reports.[9,10] Closure had no impact on nerolidol and citronellol. However, LDPE screw capped wine had lower concentrations of linalool at 36 months of storage (Figure 1 and Figure 2), possibly due to oxidation of linalool during bottle ageing.

C_{13}-norisoprenoids

β-Damascenone and β-ionone, two important C_{13}-norisoprenoids, were analysed in this study due to their low sensory thresholds.[31] This compound mainly comes from degradation of carotenoids in grapes.[32] β-Damascenone decreased significantly in the first 24 months, then remained relatively stable thereafter. The only closure found to affect its concentration in Chardonnay was LDPE, which maintained a higher concentration (Figure 1 and Figure 2). The same trend was observed in the Pinot Noir, where the LDPE samples had the highest concentration. This result is unexpected because it is not clear how higher oxygen will lead to a higher concentration of β-damascenone during ageing. But higher levels of β-damascenone have been recently linked to the development of dry fruit character more associated with oxidation notes.[33] β-Damascenone is important to wine flavour, contributing to a berry, ripe fruity flavour. β-Ionone has a distinct berry and violet-like aroma which could be very important to Pinot Noir wine based on its low sensory threshold and our in-house sensory evaluation. It had a concentration of 0.7 μg/l and remained at the same concentration during ageing in Pinot Noir wine. The results agreed with an accelerated ageing study that β-damascenone decreased after 1 week of ageing at 50°C whereas β-ionone remained constent.[34]

An informal sensory evaluation by 'experts' at the end of 3 years ageing did not detect any sulfur off-flavour in any of the experimental wines, but an 'oxidized' off-flavour was apparent in wines stored under LDPE and synthetic closures for both Chardonnay and Pinot Noir. Saranex and Saranex–tin capped Chardonnay wine was described to have better aroma, more crispy, fruity notes than the wines under other closures, although a well-trained sensory panel is needed to confirm the results.

Conclusion

Wine closure affects the SO_2, dissolved O_2, and volatile composition during in bottle ageing. The closure impact is probably due to the difference in initial dissolved O_2 inherent from the bottling processes, and oxygen ingression through closure. Low oxygen status achieved through screw cap closure with low OTR resulted in the wine with higher free SO_2, lower acetaldehyde, higher methanethiol and hydrogen sulfide. However, the higher concentration of methanethiol and hydrogen sulfide is due to slower decrease in bottle ageing rather than accumulation. DMS level was not affected by wine closure, and conversion of DMDS to methanethiol was not observed.

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The Importance of Analytical Testing in Winemaking

Regular testing during production can boost wine quality and consistency | BY RICKI HARTWELL

For thousands of years, vintners have harnessed a complex system of living organisms and biochemical processes to make wine. While the beverage has evolved over time and styles have diversified, the fundamental process of making a wine has stayed the same: Yeast ferment the sugar in grape juice, transforming it into ethanol, carbon dioxide, and heat.

The art of winemaking lies in knowing how to use different grape varieties, yeast strains, and production steps to create distinctive styles that are recognized for their aroma, taste, and appearance. Those traits, however, result from complex interactions among the growing conditions of grapes, their biochemical makeup at harvest, the reactions that occur during fermentation, and the biochemical development of must, juice, and wine during processing. Any imbalances in these interactions during production—from vine to glass—can alter the outcome and decrease the quality and palatability of a wine.

The wine market is highly competitive, and brand loyalty hinges on creating distinctive and enjoyable experiences again and again. Therefore, a winery's success comes from deftly orchestrating vinification to preclude imbalances. Ensuring customer satisfaction and building brand equity means making timely decisions that steer winemaking toward the exact experience a vintner aims to create.

Data Enables Time-Critical Decisions in Winemaking

When it comes to creating premium wines, there is no substitute for the experience and knowledge of a vintner. But complementing that expertise with a precise characterization of the biochemical changes occurring in a batch better informs decisions to optimize production, ultimately boosting wine quality and selling price. Analytical testing at all production stages is the key to such data-driven decisions. Sensitive, easy-to-use analyzers allow the vintner to monitor the material composition and conditions of biochemical reactions and identify when and how best to intervene. Imbalances can be anticipated and corrective action can be tailored to reestablish ideal conditions in a timely manner.

Analysis is crucial from the beginning of the winemaking process, even while grapes are still on the vine. A refractometer can be used to measure grape sugar content and thus determine the best harvest time. Sugar and organic acid content should also be measured in grapes brought in from external sources, as these parameters typically vary with growing conditions (e.g., temperature, soil type, rainfall). Different wine types and varietals build on different acid-to-sugar ratios, and a suboptimal biochemical starting point can lead to a stuck fermentation that falls short of reaching the necessary final gravity.

Dedicated electrodes can be used to accurately measure the pH, organic acids, and nitrogen content of must. The results can better guide the use of additives to promote fermentation and control pH, while preventing an imbalance in acidity that can derail the flavor, color, and microbial stability of the wine. Sulfur dioxide, which is used as an antioxidant and inhibitor of microbial activity, can be monitored to prevent an excess that dulls fermentation and lowers wine quality. Finally, hand-held devices can measure liquid turbidity and dissolved oxygen in barrels and bottles to ensure desired clarity and prevent excessive oxidation that discolors and degrades wine flavor.

Analytical needs vary from one winery to another. Therefore, the first step toward establishing a cost-effective analysis infrastructure is to systematically evaluate the type and frequency of testing that best serves production procedures.

Design an Analytical Testing Plan

A range of advanced, easy-to-use, and highly reliable analytical instruments make measuring critical winemaking

parameters straightforward. Designed to withstand the wear and tear of a manufacturing floor, these analyzers enable testing of a few to several hundred samples, either in a lab or directly at vines, vats, or barrels. The choice of instrument depends on three factors: the number of bottles produced at a winery, the frequency of measurements needed throughout the production process, and the vicinity of an accredited food analysis and safety lab. The latter point is important; waiting for results to return from an offsite lab can be the difference between a successful batch and one that is downgraded or lost. At a minimum, a wine producer should consider quantifying the parameters listed in Table 1 (see p. 36) on site, because changes usually require quick corrective action.

A good starting point is to design an analytical testing plan that maps the points in manufacturing where data is important to inform next steps. The plan should outline the type and frequency of measurements to be made at each point and actions to be taken based on results. As a whole, the plan dictates the number of samples to be analyzed daily, the variety of analyses needed, and the ideal timing for corrective action. That information helps decide which samples can be shipped to offsite labs and which instruments are needed on site.

Laying out a well-planned testing strategy with guidelines for subsequent actions is the foundation of a cost-effective and smart quality control infrastructure, and it simply makes good business sense.

In-Process Analytical Testing

A robust analytical testing strategy to monitor production is the cornerstone of any good wine business. The core objective of the monitoring is to minimize variability in parameters that impact the traits of a wine, keeping them in the narrow range characteristic of a particular wine style. The benefits of this in-process monitoring, however, go far beyond just "keeping chemistry in check." End-to-end analytical testing supports compliance with quality and safety standards, maintains a robust and efficient production, and builds brand reputation through consistently high-quality and enjoyable wines (Figure 1, below).

Compliant quality and safety. Global, national, and local regulatory bodies in the wine industry dictate procedures that are and aren't allowed in vinification. For example, European Union legislation permits the addition of lysozyme for fining, but it must not exceed 500 mg/L. Other regulations stipulate that certain components in wine be published on labels, such as sulfite residues exceeding 10 mg/L and percentage alcohol content. By continuously tracking the biochemistry of a wine under production, a vintner can optimize the use of additives and processing aids and ensure that the final product aligns with regulations. Furthermore, the data collected serve as an audit to trace problems to their origin, a survey of overall production constancy over time, and a tool to predict product quality.

Robust, efficient, scalable production. Commercially viable wines must achieve healthy profit margins in a highly competitive market. Even the best-tasting wine cannot succeed in today's market without the manufacturing scale and reproducibility to secure supply. Scaling up production to a commercially meaningful volume while preserving the defining qualities of a wine—sweetness, acidity, tannin levels, flavor, and body—is challenging and may require adjustments and rethinking. Critical parameters measured along the way, from vine to glass, are benchmarks for the scale-up process, helping to ensure that buildout of each manufacturing step leaves intact the biochemistry that achieves stylistic and quality goals. With the data collected, every optimization decision begins with a known biochemical profile for the wine. As adjustments are made over time, that profile becomes a unique biochemical signature of the wine, guiding production.

Continuity of brand. The brand of a company is a promise to customers about what they can expect from products and services. In the case of a winery, that promise is kept by delivering on expectations of the aroma, taste, and appearance of its wines. Those precise traits are repeatedly and consistently created through the meticulous control of production processes, so it stands to reason that any investment in facilitating that control—creating an analytical testing strategy and acquiring the necessary equipment—is an investment in brand. Analytical testing renders each production step transparent, and the insights obtained allow a vintner to better craft established and new wines. In short, a unique wine may be an asset to a winery, but a memorable wine that is enjoyed year after year is brand equity.

Monitoring key parameters during winemaking is indispensable to consistently producing memorable, quality wines.

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Table 1. Routine analytical testing throughout wine making.

	Sugar	pH	Titratable acidity	Ammonia nitrogen	Sulfur dioxide	Turbidity	Dissolved Oxygen	Benefits
Prefermentation	✓	✓	✓	✓				Regular measurement of sugar in grapes determines the best harvest time. Organic acids in the grape can impact pH, which, conversely, can lead to growth of spoilage organisms and can influence taste and color of wine. Yeast assimilable nitrogen fosters fermentation.
Fermentation (incl. maceration)		✓		✓	✓			Nitrogen nutrients are consumed, and pH can change. Left unattended, the changes can halt fermentation. Sulfur dioxide may be added to inhibit native yeast.
Clarification						✓		The turbidity of wine is a measure of microbial stability. Careful monitoring during the clarification process ensures removal of unwanted particles.
Racking		✓	✓		✓	✓	✓	Sulfur dioxide may be added to suppress bacteria in wine. Oxygen is measured to prevent excess exposure that can destroy flavor. The overall balance of other parameters is also monitored in preparation for aging.
Aging		✓	✓		✓		✓	Levels of pH and sulfur dioxide are adjusted to maintain microbial stability throughout the aging process, while sulfur dioxide can also provide protection against oxidation from excessive oxygen exposure in barrels. The acidity of the wine is checked regularly to balance taste.
Bottling and further aging		✓	✓		✓	✓	✓	After sterile filtering, parameters are checked before bottling to ensure they are within specification. Turbidity is measured to ensure clear wine without haze.
Available quantitative analysis methods	Refractometer	pH meter	Manual or automated titration	Selective electrode or spectrophotometry	Manual or automated titration; photometry; chromatography	Turbidity meter	Oxygen sensor	

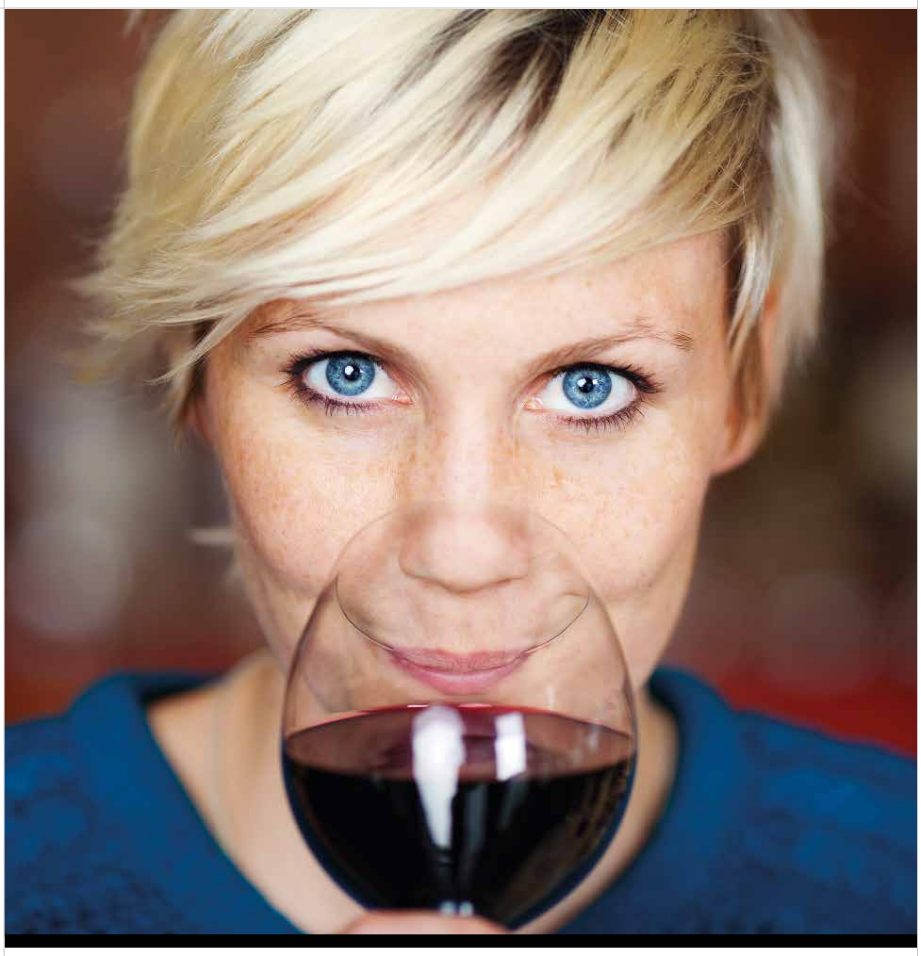
Always on Track to Meet Stylistic and Quality Goals

The long-term success of every business centers on giving customers a reason to return. In the wine industry, that means creating memorable and repeatable experiences through exceptional product quality, quality that not only meets food safety, regulatory, and import/export requirements, but also guarantees the flavor, aroma, color, and clarity traits that define a brand.

With a burgeoning global wine market and unprecedented choice for customers, the competition is intense. Successful wineries make every batch count. Successful wineries know in real time how grapes evolve into wine and steer the process to meet stylistic and quality goals for each blend and varietal. Additionally, successful wineries intervene at critical points to prevent that transformation from derailing. With advanced analytical tools to monitor winemaking, quality control becomes the gateway to higher-quality products, delighted customers, and stronger market positioning. ■

Hartwell is a senior product manager within the water lab products division at Thermo Fisher Scientific. Reach her at wlp.techsupport@thermofisher.com.

In The Lab



Extend the Shelf Life of Wines

Automated titration systems can improve sulfite monitoring

BY **GAYLE GLEICHAUF**

Ask wine connoisseurs about their favorite vintage and they'll probably mention the aroma from the uncorked bottle, the color in the glass, and the complex flavors. However, unwanted oxidation, discoloration, and microbial growth during production and after bottling can compromise all of these characteristics, putting revenues and reputations at risk.

To prevent these undesirable processes and extend product shelf life, winemakers commonly add preservatives in the form of sulfites—sulfur-containing compounds such as hydrogen sulfite (HSO_3-), sulfite salts (SO_3^{2-}), and sulfur dioxide (SO_2)—that possess strong antioxidant and antimicrobial properties. Achieving the right balance of sulfites in wine is of utmost importance to protect product quality in line with stringent regulations. Increasingly, many wineries are recognizing the benefits of using automated titration systems that are capable of monitoring sulfite levels and delivering accurate and reliable results, quickly and cost-effectively.

The Importance of Monitoring Sulfites

Sulfites may be added at various stages of the wine production process, from the crushing of the grapes until just prior to bottling, depending on the type of wine being produced and the individual preferences of the winemaker. They may be present in wine as free sulfites (HSO_3^-, SO_3^{2-} or SO_2, depending on the pH) or bound to other wine components, such as phenols and carbonyl compounds.

For wineries, getting the level of sulfites right is of critical importance. If sulfite levels are too low, wine quality can be compromised, potentially resulting in the need to discard entire batches. Get sulfite levels too high, however, and wineries face a different set of challenges. Not only is the over-addition of sulfites costly, the presence of excess sulfites can delay key fermentation processes and have a detrimental impact on wine taste and aroma.

On top of this, sulfites are thought to cause allergic reactions in some people. Consumers who are particularly sensitive to sulfites may experience symptoms including skin rashes, stomach complaints, and breathing difficulties. Regulations around sulfite levels are in place to protect the public's health, and wineries cannot sell wines that don't meet these regulations.

Regulatory requirements for total sulfites (free and bound) in wine vary by region and product type. In the United States, wines cannot exceed total SO_2 levels of 350 mg/L, and any wines containing more than 10 mg/L sulfites must be labeled with a warning. In the European Union, tighter controls around sulfite use are enforced, with different limits depending on the type of wine. These regulations limit total SO_2 to 150 mg/L in most red wines and 200 mg/L in most white and rosé wines. Sparkling wines may contain up to 235 mg/L total SO_2, while certain sweet wines may contain higher sulfite levels up to a maximum of 400 mg/L. Similar regulations around sulfite levels are in place in other countries.

Determine Sulfite Levels

A wide range of methods are available to monitor sulfite levels in wine. These include distillation followed by acid/base titration, iodometric titrations, and enzyme assays involving colorimetric or spectrophotometric detection techniques.

The Monier-Williams method and the Ripper iodometric titration are two of the more widely used methods for the determination of sulfites in wine. The Monier-Williams method is a multi-step process that first involves capturing SO_2 in hydrogen peroxide by distillation. The sulfuric acid that's generated from this step is then titrated with sodium hydroxide to determine the concentration of SO_2. While the Monier-Williams method is a very precise technique for determining levels of sulfites in wine, the need to perform a distillation step often makes the use of this method for routine analysis applications impractical.

The Ripper titration is an alternative approach that enables sulfites to be measured directly, without the need for time-consuming distillation steps. Many wineries perform this iodometric titration manually, using starch as an indicator to monitor a color change end point. Levels of free SO_2 can be determined by acidifying samples prior to titration, while total SO_2 can be measured by first treating samples with sodium hydroxide, which releases the bound sulfites. After the bound sulfites are released, the titration proceeds as for the free SO_2.

If sulfite levels are too low, wine quality can be compromised, potentially resulting in the need to discard entire batches. Get sulfite levels too high, however, and wineries face a different set of challenges. Not only is the over-addition of sulfites costly, the presence of excess sulfites can delay key fermentation processes and have a detrimental impact on wine taste and aroma.

Despite this, using the manual Ripper titration to measure SO_2 can be challenging for a number of reasons. Given the need to monitor the color change associated with this titration method by eye, it can be problematic to accurately determine end points in red wines, as the dark color of the sample can make it difficult to identify the onset of the color change. This limitation means that measurements can often be inconsistent and unreliable, putting the quality and regulatory compliance of the end product at risk. Moreover, as operators must be fully engaged with the titration throughout the experiment, manual titrations can be very resource intensive. For wineries with limited resources or those deciding to scale up production, the need for a dedicated, trained operator, or team of operators (for large productions), to perform manual Ripper titrations can prove to be a bottleneck.

Using Automated Titrators to Measure Sulfites

Given the importance of monitoring sulfite levels to protect the quality of wine and extend product shelf life, winemakers are increasingly using automated titration systems to generate results faster and more efficiently. As automated Ripper titrations use electrodes to monitor potentiometric end points, rather than subjective color changes, they provide precise results regardless of which operator performs the test. Moreover, by generating accurate results that are right the first time, these systems are able to support rapid and more informed decision-making.

Modern automated titration platforms are also capable of performing testing with no manual intervention except for the

Extend the Shelf Life ...

initiation of tests with the push of a button, enabling wineries to undertake sulfite testing more efficiently. In addition, this ease of use frees up operators to work on other tasks, such as additional safety or quality tests, and gives wineries the flexibility and capacity to quickly scale up sulfite testing activities without having to significantly expand their teams. The latest automated platforms for sulfite testing extend beyond data collection to processing and analysis, enabling wineries to automatically calculate and store results in line with regulatory requirements, while avoiding the risk of transcription errors that can occur using manual workflows.

Additionally, some of the latest titration platforms enable wineries to program and save frequently used method details in the system for routine use by operators. These convenient and intuitive systems can help wineries work more efficiently by eliminating the time required to set up the relevant conditions before each test. More advanced platforms will allow system administrators to lock the pre-programmed tests, preventing them from being changed by unauthorized users. For laboratories with large workflows, these features can be highly beneficial in increasing productivity and delivering more consistent results.

The robustness of sulfite testing workflows is a key priority for many wineries, especially those with high-volume testing requirements. Recent improvements in the operational resilience of automated titration systems are helping to minimize maintenance requirements and simplify upkeep. Some modern automated titrators will even diagnose performance issues and guide operators through recalibration and maintenance steps using clear on-screen instructions. As sulfite testing is often undertaken by operators without any in-depth technical knowledge, the improved simplicity and ease of use of these systems can allow wineries to extend the interval between maintenance operations and get titrators back in action more quickly when issues do arise. These innovative features improve operational efficiency and productivity and help get wineries back to what they're good at: ensuring great wine makes the journey from vineyard to wine glass. ■

Gleichauf is an applications lab manager at Thermo Fisher Scientific. Reach her at gayle.gleichauf@thermofisher.com.

APPLICATION NOTE

Measuring pH in wine and juice

Key Words: pH, red wine, wine-making, juice, proteins, sulfides, tannins, polyphenols.

Goal

The following application note includes the recommended equipment, procedures and maintenance for accurate pH readings.

Introduction

Since pH plays a critical role in wine making, measurements are taken throughout the winemaking process, from juice to finished wine. Typical pH levels in wine range from 2.9 to 3.9. Various components of juice and wine can challenge the performance of the pH electrode, including proteins, sulfides, tannins, and polyphenols. This note includes recommended equipment, procedures, and maintenance to assure accurate pH readings.

Recommended Equipment

- Thermo Scientific™ Orion™ pH meter (or equivalent)
- Thermo Scientific™ Orion™ ROSS™ Sure-Flow™ pH Electrode (Cat. No. 8172BNWP), Orion Green pH Combination Electrode (Cat. No. GD9156BNWP), or equivalent
- Automatic Temperature Compensation (ATC) probe (Cat. No. 927007MD)
- Stirrer (Cat. No. 096019)
- Swing arm and electrode holder (Cat. No. 090043)
- pH electrode storage bottle (Cat. No. 910003)

Recommended Solutions

- pH 4.01 and 7.00 buffers (Cat. No. 910104, 910107)
- Electrode filling solution (Cat. No. 810007 for 8172BNWP, or Cat. No. 910008-WA for GD9156BNWP)
- Orion ROSS storage solution (Cat. No. 810001) or pH electrode storage solution (Cat. No. 910001)
- Deionized water (DI)
- pH electrode cleaning solution A (Cat. No. 900021-WA)
- pH electrode cleaning solution D (Cat. No. 900024)
- ~75% alcohol solution (methanol or ethanol in water)

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Meter Setup

Connect the pH electrode, ATC probe, and stirrer to the meter. Set measurement mode to pH. In Setup, set the stirrer speed to 3, pH resolution to 0.01, buffer set to USA and read type to auto or continuous.

Note: When the ATC is connected properly, the true temperature (not the default 25.0) will be displayed on the screen. The ATC will measure buffer and sample temperatures and will ensure precise automatic temperature-compensated readings.

Electrode Calibration

Before sample testing, perform a two-point pH calibration using pH 4.01 and 7.00 buffers. (See Analysis instructions below for details on test protocol). The electrode slope should be between 92 and 102%.

Sample Preparation

Place about 50 mL of sample in a small, clean beaker (about 100 mL size).

Analysis

Place the pH electrode, ATC, and stirrer into the electrode stand. Rinse each with DI water. Place probes and stirrer into the sample, immersing about 1-2 inches into the solution. Stir the sample continuously. When the meter indicates the reading is stable, record the pH to two decimal places (e.g., 3.39) and the temperature to one decimal place. (If using Autoread mode, the meter will lock on the final reading and automatically log the readings, when the log function is turned on in Setup). Between readings, rinse the probes and stirrer with DI water to remove any remaining sample.

Electrode Storage

After testing is complete, rinse pH electrode thoroughly with the ~75% alcohol solution or immerse for 5 minutes, then rinse thoroughly with DI water. Cover the fill hole and store pH electrode in a bottle of electrode storage solution. Change the storage solution biweekly or monthly. ATC should be stored dry.

Electrode Maintenance

Fill the electrode to the level of the fill hole each day, prior to testing. Weekly or biweekly, empty the fill solution and replace with fresh fill solution.

Electrode Cleaning

If the electrode begins to exhibit drift and/or is slow to respond, clean it as follows: clean the electrode with Orion Cleaning Solution A according to the instructions, to remove proteins and restore the pH membrane. If further cleaning is desired, use Orion Cleaning solution D to remove organic compounds and restore the pH membrane.

Quality Control (QC)

Recommended QC procedures may include: calibration, calibration verification, sample duplicates, and/or QC samples.

Results of Measuring the pH of Red Wine

Precision	8172BNWP	GD9156BNWP
pH of Red Wine, avg. (n = 10)	3.41	3.42
Standard Deviation	0.02	0.01
Avg. Temp. (°C)	21.9	21.9
Avg. Response Time	< 30 sec	< 30 sec
Accuracy		
pH 4 Buffer, avg. (n = 6)	4.01	4.01
Difference From Expected	0.00	0.00
Standard Deviation	0.01	0.01

Precision

Both Orion pH electrodes demonstrated excellent precision between test results for multiple replicates of wine and pH buffer as follows:

- Red wine - showing a standard deviation of <0.02 pH units
- pH 4 buffer - showing a standard deviation of 0.01 pH units.

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Accuracy

Both Orion pH electrodes demonstrated excellent accuracy for multiple replicates of pH 4 buffer, showing a difference from expected value of 0.00 pH units (reads exactly as expected).

Speed

Both Orion pH electrodes demonstrated excellent response time. The time to a stable reading averaged less than 30 seconds for wine samples and pH 4 buffer.

To purchase Orion meters, electrodes and solutions, please contact your local equipment distributor and reference the part numbers listed below:

Ordering Information

Product	Cat. No.
Meters	
Thermo Scientific™ Orion™ Versa Star Pro™ pH Benchtop Meter Kit with Stand, ROSS™ Sure-Flow™ pH Electrode, ATC Probe, Stirrer Probe, pH 4/7/10 Buffers and ROSS Storage Solution	VSTAR13
Thermo Scientific™ Orion Star™ A211 pH Benchtop Meter Kit with Stand, ROSS™ Sure-Flow™ pH Electrode, ATC Probe, pH 4/7/10 Buffers and ROSS™ Storage Solution	STARA2114
Electrodes	
Thermo Scientific™ Orion™ ROSS™ Sure-Flow™ pH Electrode	8172BNWP
Thermo Scientific™ Orion™ Green pH Electrode	GD9156BNWP
Solutions	
Thermo Scientific™ Orion™ pH 4.01 and 7.00 Buffers	910104 and 910107
Thermo Scientific™ Orion™ Filling Solution for 8172BNWP or GD9156BNWP pH Electrodes	810007 or 910008-WA
Thermo Scientific™ Orion™ ROSS™ Storage Solution or pH Electrode Storage Solution	810001 or 910001
Thermo Scientific™ Orion™ pH Electrode Cleaning Solution A or D	900021-WA or 900024

References

1. Zoecklein et al. *Wine Analysis and Production.* Chapman and Hall. 1995.
2. AOAC International. *AOAC Official Method 960.19, pH of Wines.* Official Methods of Analysis (OMA), 16th edition. 1999. www.aoac.org

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APPLICATION NOTE

Measuring the dissolved oxygen of wine in the bottle

Key Words: Wine quality, dissolved oxygen, DO sensor, optical sensor, portable meter, beverage testing.

Goal

The following application note describes how to reliably measure the oxygen content of wine directly in the bottle, using a Thermo Scientific™ Orion™ Optical Dissolved Oxygen Sensor with automatic temperature compensation and a Thermo Scientific™ Orion Star™ A223 Dissolved Oxygen (DO) Portable Meter.

Introduction

A bottle of wine's oxygen content has a great effect on its quality, stability and longevity. This is why monitoring and controlling the oxygen incorporation at different stages of the wine-making and bottling process is becoming a growing concern for wineries. Although oxygen is a part of the wine's natural aging process, adverse levels can cause discoloration to white wines and flavor degradation to both white and red varietals. To ensure the consumer is getting the highest quality product, measuring the concentration of molecular oxygen of wine after bottling is very important. By using an Orion Optical Dissolved Oxygen Sensor and an Orion Star A223 Dissolved Oxygen (DO) Portable Meter, reliable oxygen measurements can be made directly in the bottles of wine.

Equipment

- Orion Star A223 Dissolved Oxygen (DO) Portable Meter Kit – includes optical DO sensor, portable meter armor, field case and USB computer cable (Cat. No. STARA2235) or
- Orion Star A223 DO Portable Meter (Cat. No. STARA2230) or equivalent Orion portable DO meter
- Optical DO Sensor – includes calibration sleeve and stainless steel sensor guard (Cat. No. 087010MD)
- Silicone tubing

Solutions

- Deionized water (DI)

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Luminescence-Based Dissolved Oxygen Method

The oxygen content of wine must be monitored throughout the wine-making process. Using the optical DO sensor with built-in automatic temperature compensation and a portable meter, reliable measurements can be directly in the wine bottle.

DO Sensor Setup

Refer to the Optical DO Sensor User Guide for detailed assembly and preparation instructions for the optical DO sensor. Place DO sensor into the calibration sleeve. Remove the sponge from the bottom of the calibration sleeve. Moisten it with DI water, squeeze out excess water, and replace the sponge. Connect the optical DO sensor to the 9-pin MiniDIN input on the meter. Once assembled, the optical DO sensor can be used immediately.

Meter Setup

Turn the meter on. The meter should automatically detect the type of DO sensor and update the measure type to optical DO. Access the setup menu and update the channel settings to the following, as needed:

- Measure Mode: Auto
- Measure Unit: mg/L
- Resolution: 0.01
- Read Type: Auto Read
- Baro Pressure: Auto
- Salinity Correct: Manual (0.0)

Update the instrument settings to the following, as needed:

- Export Data: On
- Data Log: On
- Date / Time: Set current date & time

Sensor Performance Checks

A properly calibrated optical DO sensor should read between 98 and 102% saturation in the calibration sleeve. If not, recalibrate the sensor. The optical DO sensor should stabilize during calibration within 2 minutes when working properly. Make sure to thoroughly rinse and blot dry the optical DO sensor after measuring samples and before placing into the calibration sleeve (see Comments section). Refer to the optical DO sensor user manual if the sensor does not pass the performance checks.

Sensor Rinsing, Soaking and Storage

After each sample measurement, rinse the optical DO sensor thoroughly with deionized water and blot the sensor dry with a lint-free cloth. For short term storage, overnight or between measurements, keep the optical DO sensor in the calibration sleeve or a biochemical oxygen demand (BOD) bottle with water-saturated air. For long-term storage, keep the optical DO sensor in the calibration sleeve.

Sample Preparation and Preservation

No sample preparation required. Dissolved oxygen can be measured directly in the wine bottle.

Calibration

If not already done, prepare the optical DO sensor according to the DO Sensor Setup procedure. Perform a water-saturated air (Air) calibration with the optical DO probe in the prepared calibration sleeve. A stable reading of 100.0 % saturation should be displayed within about two minutes.

Analysis

Slide a ring of silicon tubing over the optical DO sensor, sliding it up the probe to just below the threads. For details see Notes below. Rinse the optical DO sensor with deionized water and blot excess rinse water off with a lint-free cloth. Place the optical DO sensor in the bottle. The silicone ring should make a seal with the bottle. Place the bottle on its side so the neck becomes flooded with wine, covering both the dissolved oxygen and temperature sensors on the optical DO probe. Initiate a reading using the Auto Read measurement mode by pressing the measure key on the meter keypad. For best results, take a second reading to ensure the dissolved oxygen measurement is fully stabilized, as it may take the optical DO sensor one to two minutes to fully stabilize in the wine sample. Use the second stable value for the oxygen content of the wine. Both readings will be saved in the meter data log.

Comments

It is important to thoroughly clean the optical DO sensor after sample measurement. Rinse with deionized water and thoroughly blot all excess water with a lint free cloth several times before putting the sensor in the calibration sleeve. Rinsing following the completion of all sample measurements should take 5 to 10 minutes. The Orion meter data log collects up to 1000 measurement sets with time and date stamp and the non-volatile meter memory preserves data, even with loss of power. Download Orion Star Com software to facilitate the transfer of the data log from the meter to a computer at **www.thermofisher.com/orionsoftware**. Use the Orion Star Com software to export data to a Microsoft® Excel® spreadsheet or as a comma separated value file (.csv) or print data to a network or local printer.

Quality Control (QC)

Recommended QC procedures may include: calibration, check of the thermistor (temperature sensor) response against a calibrated NIST-traceable thermometer, and measurement of a zero DO solution, such as 5% sodium sulfite.

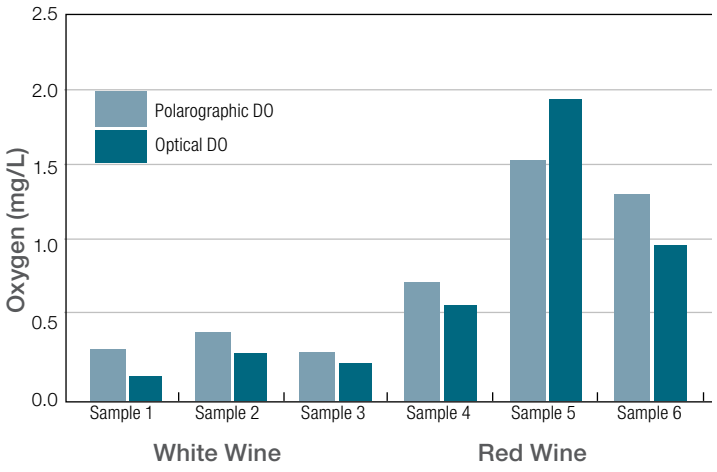
Notes

- Silicone tubing is necessary to make a seal so the bottle can be laid on its side, immersing the probe and temperature sensor in the sample while keeping all of the wine in and ambient oxygen out of the bottle. The optical DO sensor and temperature sensor are not immersed in the sample if the bottle is sitting upright. Use a piece of soft silicone tubing with an inner diameter of 1/2 to 5/8 inch and outer diameter of 5/8 to 3/4 inch with a wall thickness of 1/8 inch. Cut a ring of tubing that is 1/4 to 1/2 inch wide. Before measuring the wine sample in the bottle, slide the ring onto the probe and push it up to just below the threads.
- Keeping the calibration sleeve clean and free from water or sample droplets is essential to getting good calibration and read back values in water-saturated air. Rinse the optical DO sensor thoroughly with deionized water and wipe excess water with a lint-free cloth prior to putting the sensor in the calibration sleeve.
- Optical DO sensors do not require stirring or a sample stream for accurate measurements. The speed, accuracy, and precision of the optical DO sensor are equivalent or superior to the traditional polarographic sensor measurement.
- If readings are slow or inconsistent, ensure the temperature sensor is completely submerged in the sample.
- If the temperature sensor is not in the sample, the DO readings will be incorrect.
- The optical DO cap must be replaced every 365 days. The remaining optical DO cap life can be viewed in the channel setup menu. The meter will display an error message when the optical DO cap needs to be replaced.

Results

Dissolved Oxygen Readings in Wine Bottles

Oxygen (mg/L)				
	White Wine		Red Wine	
	Optical	Polarographic	Optical	Polarographic
Sample 1	0.35	0.17	0.80	0.64
Sample 2	0.46	0.32	1.61	2.01
Sample 3	0.34	0.26	1.39	1.04
Temperature (ºC)	21.5	21	21.3	21.1



Thermo Scientific™ Orion™ Optical DO Sensor

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Summary

Using an Orion Star A223 Dissolved Oxygen (DO) Portable Meter with an optical DO sensor enables wineries to continually produce high quality wines. Since the optical DO sensor allows the wine to be measured directly in the bottle, dissolved oxygen measurements can be made with speed and accuracy. The speed, accuracy and precision of the optical DO sensor is equivalent or superior to current DO measurement techniques.

To purchase an Orion Star A223 DO Portable Meter, Orion Optical DO Sensor and other related products, please contact your local equipment distributor and reference the part numbers listed below.

Thermo Scientific™ Orion Star™ A223 Dissolved Oxygen Portable Meter Kit

Ordering Information

Product	Cat. No.
Portable Meters	
Thermo Scientific Orion Star A223 Dissolved Oxygen Portable Meter	STARA2230
Thermo Scientific Orion Star A223 Dissolved Oxygen Portable Meter Kit with Optical DO Sensor, Portable Meter Armor, Field Case and USB Computer Cable	STARA2235
Thermo Scientific Orion Star A326 pH/DO Portable Meter Kit with ROSS Ultra Low Maintenance Gel pH/ATC Electrode, Optical DO Sensor, Portable Meter Armor, Field Case, Calibration Solutions and USB Computer Cable	STARA3265
Thermo Scientific Orion Star A329 pH/ISE/Conductivity/DO Portable Meter Kit with ROSS Ultra Low Maintenance Gel pH/ATC Electrode, Conductivity Sensor, Optical DO Sensor, Portable Meter Armor, Field Case, Calibration Solutions and USB Computer Cable	STARA3295
Optical DO Sensors	
Thermo Scientific Orion Optical DO Sensor with 3 Meter Cable	087010MD
Accessories	
Calibration Sleeve for Optical DO Sensors	087003
Stainless Steel Protective Sensor Guard for Optical DO Sensors	087002
RS232 Computer Cable	1010053

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APPLICATION NOTE

Measuring Clarity in Wine

Key Words
wine clarity, turbidity, beverage quality, fermentation, barrel testing, filtration, wine tank testing.

Goal

The following application note explains how to measure the turbidity of red, white and rosé wine samples using a Thermo Scientific™ Orion™ AQUAfast™ turbidity meter. The analysis of wine turbidity may be used to evaluate chill haze, protein stability, and wine clarity. In this note, the evaluation of wine clarity is described.

Introduction

Orion™ AQUAfast™ AQ3010 and AQ4500 Turbidity Meters allow quick and simple determinations of the clarity of white, rosé, and red wine samples. Understand how to measure the clarity or "turbidity" of various wine samples using the AQ310 model, or the infrared mode in the AQ500 model. As the light source is infrared, the turbidity measurement is independent of color.

Recommended Equipment

- Orion™ AQ3010 Turbidity Meter and Orion™ AC3V25 Turbidity Vials

OR

- Orion™ AQ4500 Turbidity Meter and Orion™ AC2T24 Turbidity Vials

Required Reagents and Solutions

- Orion AC301S Turbidity Standards (if using AQ3010)
- Orion AC45ST Turbidity Standards (if using AQ4500)
- Turbidity-free water (TFW), e.g., by filtration through 0.1 um filter, or equivalent water

Solutions Preparation

None.

Meter Setup

None.

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Meter Performance Check/Calibration Verification

Orion AC301S and AC45ST styrene divinylbenzene (SDVB) polymer turbidity standards never need mixing. Do not shake the standards as this will introduce bubbles and cause them to read inaccurately until the bubbles dissipate.

AQ3010

Check meter accuracy by reading one or more turbidity standards (included with the meter) at the level of interest. For example, read the zero (0.02) and the 20 NTU standard. The zero should read <0.1 NTU and the 20 NTU standard should read within ±10%, e.g., 18-22 NTU.

AQ4500

Review certificate of analysis of the turbidity standards and record the expected turbidity values for the IR Ratio mode.

Set the meter to the IR Ratio mode. Check meter accuracy by reading one or more turbidity standards at the level of interest. For example, read the zero (0.02) and the 1 NTU standard. The zero should read <0.1 NTU and the 1 NTU standard should read within ±10% from the expected value according to the Certificate of Analysis.

If the AQ3010 or AQ4500 meter performance check fails, take corrective actions as follows:

1. Wipe the vial carefully with a lint-free wipe to remove all fingerprints and liquid drips from the exterior, handle the vial by the cap only, and remeasure.
2. Tap the vial gently three times and let the vial sit for 60 seconds to allow for bubbles to release, then remeasure.
3. Using a clean vial (which reads <0.1 NTU when filled with TFW), pour a fresh portion of turbidity standard into the clean vial, wipe carefully, and measure.

Sample Vial (Cuvette) Storage, Soaking, and Rinsing

Store vials filled with TFW. Immediately after use, clean sample vials with laboratory detergent and rinse multiple times with TFW. Note: Standards may be stored in supplied glass sample vials until the standard reading is no longer in specification. See Meter Performance Check section for corrective actions when a standard reads out of specification.

Sample Storage and Preparation

In general, allow the samples to warm to room temperature before measurement. Mix the sample well, but do not introduce bubbles by shaking the sample. Use a little of the sample to rinse a clean sample vial twice. Mix the sample again and fill the rinsed vial.

Calibration - AQ3010

The meter is shipped precalibrated. The meter performance is very stable and does not require frequent calibration. If a standard reading is not within criteria, take all necessary corrective actions (as described in the Meter Performance Check section) to improve meter readings. If corrective actions fail and recalibration is necessary, perform the recalibration only on the points that failed and do so with fresh portions of standard poured into clean vials. Ensure that all fingerprints and liquid drips have been removed from the exterior of the vial with a lintfree wipe before using. Handle vials by the cap only.

Calibration - AQ4500

The meter is shipped precalibrated. The meter performance is very stable and does not require frequent calibration. If a standard reading is not within criteria, take all necessary corrective actions (as described in the Meter Performance Check section) to improve meter readings. If corrective actions fail and recalibration is necessary, perform the recalibration in IR Ratio mode (see the Initial Calibration section of the Meter User Guide and an example on page 3).

Analysis

Gently invert the filled sample vial a few times to mix the sample well without introducing bubbles. Wipe the sample vial to remove all traces of liquids and fingerprints, place into meter, and press the measure key. Record the reading. Press the measure key to take duplicate measurement(s). Continue until readings stabilize and results agree, for example, within 5% or ±0.02 NTU, whichever is higher.

Quality Control (QC)

Recommended QC procedures include: calibration verification, turbidity-free water analysis (optional), and sample duplicates.

Notes for Improved Accuracy of Low-level Samples

If improved accuracy is desired, pay close attention to:

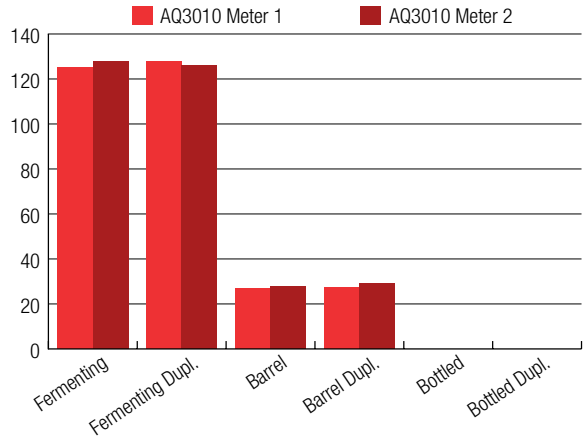
- The cleanliness of the sample vials.
- The quality of the TFW.
- The handling of the standards and samples.
- Use of matching vials.
- Storing clean vials filled with TFW.
- Use vials free of scratches or other imperfections.

For improved low-level accuracy, ensure that a clean vial filled with TFW reads < 0.1 NTU before using that vial to test highly filtered wine. If a clean vial does not read <0.1 NTU, discard it or set it aside for further cleaning. If no clean vials read <0.1 NTU, the TFW may need degassing or a cleaner source of TFW may be required. See ASTM D6855 Test Method for Test Method for Determination of Turbidity Below 5 NTU in Static Mode for more information about low level turbidity readings.

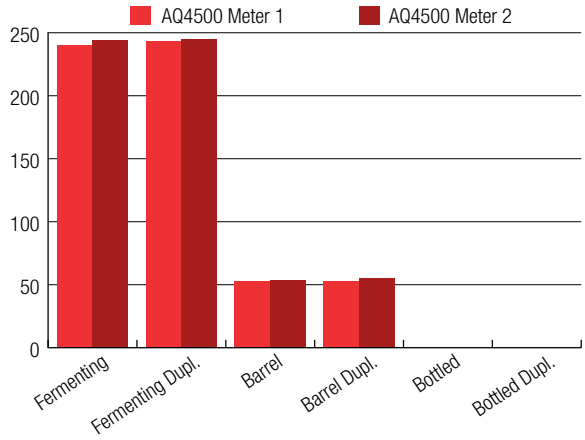
Results

Various wine samples, taken at different stages of the winemaking process, were tested for turbidity on the AQ3010 and AQ4500.

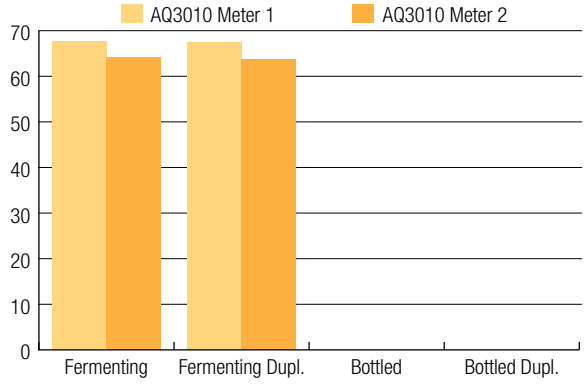
Turbidity of Red Wine (AQ3010)



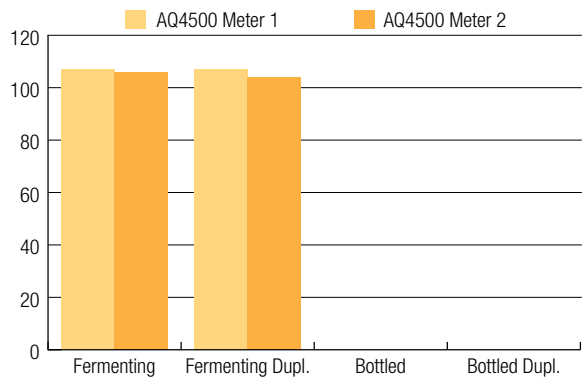
Turbidity of Red Wine (AQ4500)



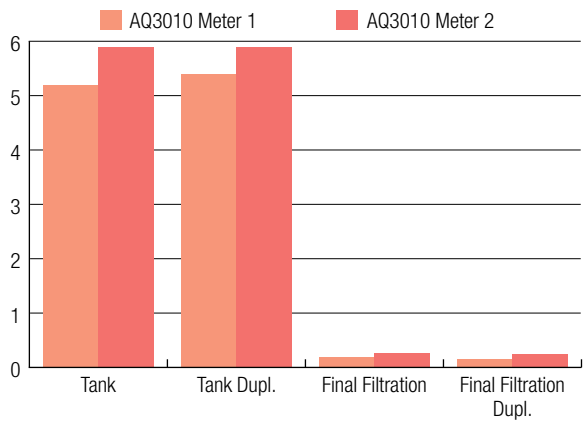
Turbidity of White Wine (AQ3010)



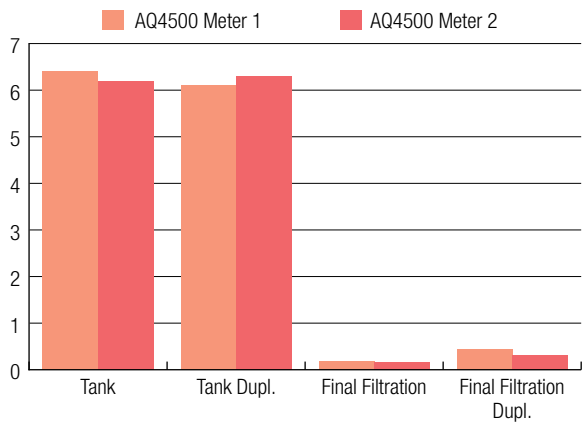
Turbidity of White Wine (AQ4500)



Turbidity of Rosé Wine (AQ3010)



Turbidity of Rosé Wine (AQ4500)



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Summary

The Orion AQUAfast AQ3010 Turbidity Meter allows accurate measurement of red, white, and rosé wines at various stages of the wine-making process. The infrared light source allows readings which are not affected by the deep color of red wines or the blush color of rosé wines.

The Orion AQUAfast AQ4500 Turbidity Meter allows accurate measurement of red, white, and rosé wines at various stages of the wine-making process. When measurements are performed in the infrared ratio mode, readings are not affected by the deep color of red wines or the blush color of rosé wines.

To purchase an Orion turbidity meter, or other related products, please contact your local equipment distributor and reference the part numbers listed below.

Results of Testing Turbidity Standards using an AQ3010 Meter

Expected Value	AQ3010 Meter 1	% Recovery	AQ3010 Meter 2	% Recovery
0.02NTU (<0.1)	0.00	NA	0.00	NA
20NTU	18.9	94.4%	20.1	100.5%
100NTU	96.3	96.3%	101	101.0%
800NTU	772	96.5%	798	99.8%

Results of Testing Turbidity Standards using an AQ4500 Meter

Expected Value	AQ4500 Meter 1	% Recovery	AQ4500 Meter 2	% Recovery
<0.1	0.00	NA	0.03	NA
0.93	0.95	102.2%	0.93	100.0%
9.54	9.30	97.5%	9.65	101.2%
99.4	99.6	100.2%	99.8	100.4%
708	742	104.8%	722	102.0%

Ordering Information

Product	Description	Cat. No.
Turbidity Meters	Thermo Scientific Orion AQUAfast AQ3010 Turbidity Meter	AQ3010
	Thermo Scientific Orion AQUAfast AQ4500 Turbidity Meter	AQ4500
Accessories	Thermo Scientific Orion Turbidity Vials, for use with the AQ3010	AC3V25
	Thermo Scientific Orion Turbidity Vials, for use with the AQ4500	AC2T24
Solutions	Thermo Scientific Orion Turbidity Standards (0, 1, 10, 100, 1000 NTU), for use with the AQ4500	AC45ST
	Thermo Scientific Orion Turbidity Standards, for use with the AQ3010	AC301S

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APPLICATION NOTE

Titratable acidity in wine by automatic titration

Key words
TA, wine, must, juice,titrametric, potentiometric, AOAC 942.15, Orion 8172BNWP, Orion 8102BNUMD, Orion Star T910, Orion Star T940.

Introduction

Titratable acidity (TA), is a measure of the organic acid content in wine, juice, or must. These organic acids come from the grapes, the fermentation, and the bacterial activity. The acidity can affect the flavor, color, and stability of the wine. TA in wine, juice, or must is determined using the preprogrammed method T1A TA Wine. This method is a direct titration to a preset endpoint at pH 8.2 using 0.1M (0.1N) sodium hydroxide titrant. The method may be edited to perform titratable acidity in other samples as well.

Recommended equipment

- Thermo Scientific™ Orion Star™ T910 pH Titrator or T940 All-In-One Titrator or equivalent
- Thermo Scientific™ Orion™ ROSS™ Sure-Flow™ pH Electrode (Cat. No. 8172BNWP) or equivalent
- Orion™ Automatic Temperature Compensation (ATC) probe
- Analytical balance (for sample measurement by weight) or graduated 10 mL pipet (for sample measurement by volume)

Required reagents and solutions

- Purchased or prepared sodium hydroxide (NaOH) standard titrant solution, 0.1 M (0.1N)
- Reagent grade water (RGW)
- pH buffers: pH 4, 7, and 10

Optional:

- Potassium hydrogen phthalate (KHP) acidimetric standard

Use suitable personal protective equipment (PPE) as recommended by the Safety Data Sheets (SDS) for the chemicals utilized during this procedure.

Titrator setup

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Connect the Orion pH electrode, ATC, and the stirrer probe to the titrator. If not previously done, import the T1A T1A Wine preprogrammed method into the titrator from the Methods screen1. Rinse and fill the burette with 0.1M (0.1N) sodium hydroxide titrant. See the titrator user manual for details. If bubbles are visible in the tubing, dispense titrant (from the Burette screen) until the bubbles have been expelled. Tap tubing to dislodge bubbles that stick. Consider standardizing the titrant before titrating samples. See the following Titrant section.

T1A TA Wine Method: Preprogrammed parameters

Electrode	Parameter
Electrode Type	pH
Electrode Name	Edit as desired
Resolution	0.01
Buffer Group	USA

Titrant	Parameter
Titrant Name	NaOH
Titrant ID	Edit as desired
Conc. Input Mode	Standardization
Nominal Concentration	0.1M
Standardize Tech	Equivalence Pt.
Number of Endpoints	1
Results Units	M
Standardize Reaction Ratio	1
Standard Name	KHP
Standard Amount	Variable weight
Standard Molecular Wt	204.2
Standard Purity	100%
Pre-dose Titrant Volume	0 ml
Max. Total Titrant Volume	5 ml
Stand. Process Control	Routine
Pre-stir Duration	5 sec
Stir Speed	Medium

Titration	Parameter
Titration Technique	Preset End Pt.
Number of Endpoints	1
Endpoint Values	8.2
Titration Type	Direct
Blank Required	No
Result Units	g/L
Reaction Ratio	0.5
Sample Mol. Wt.	150.09
Sample Amount	Fixed vol, 5.0 mL
Pre-dose Titrant Volume	0 ml
Max total titrant volume	10 ml
Titration Process Control	Routine
Pre-stir Duration	5 sec
Stir Speed	Fast
Sample ID	Manual

Electrode preparation

Remove electrode from storage solution. Top up the fill solution to the bottom of the fill hole and leave the fill hole open during testing. Rinse thoroughly with RGW before and between titrations.

Sample preparation

Accurately measure 5.0 mL of wine, juice, or must into a clean 100 mL beaker. Add RGW to the 60 mL mark on the beaker. The sample is ready to titrate.

Sample titration

1. From the Home screen, select option to use a saved method, then select the T1A TA Wine reprogrammed method.
2. At the pre-titration screen, select the Calibrate option and calibrate the electrode with pH 4, 7, and pH 10 buffers.
3. After calibration, place the electrode, ATC, stirrer, and dispenser into the sample in the beaker. Ensure that the dispenser tip is inserted below the surface of the sample and start the titration.
4. When prompted, enter the exact weight of the sample.

Results

Sample	Results	RSD (n = 3)	% Recovery	Duration (min:sec)
Tartaric Acid Standard	7.563 g/L	0.25%	100.8%	2:33
Red Burgudy Wine	5.835 g/L	1.24%	NA	3:21
Red Burgundy Wine Spike	13.33 g/L	0.19%	99.9%	4:55

Range

This preprogrammed titration method covers a range of TA that may be expected in wine, juice, or must.

Method modifications

- **For other result units:** Edit the Titration section of the method and choose the desired unit.
- **For shorter titrations:** For routine titrations with well-established endpoint volumes, use a pre-dose to shorten the analysis time. Edit the pre-dose in the Titration section of the method. In general, set the pre-dose at a volume that is 0.5 mL less than the expected endpoint volume.

Titrant

Over time, standard titrant solutions age and can change concentration. For higher accuracy, determine the exact concentration by standardizing the titrant. It is common to standardize on a weekly basis, but other standardization frequencies may be suitable.

1. Standardizing the Titrant
   a. Weigh about 0.05 g KHP into a clean 100 or 150 mL beaker. Record the exact weight to the nearest 0.0001g. Repeat twice more for a total of three beakers of KHP. Add RGW to the 60 mL mark on each beaker and stir for about 2 minutes or so until the KHP is completely dissolved.
   b. If the KHP purity is not 100%, edit the Titrant section of the method to enter the actual purity
   c. Select the Titratable Acidity preprogrammed method on the titrator.
   d. At the pre-titration screen, select the Standardize option and follow the prompts to standardize the titrant.
   e. The new standardized titrant concentration will automatically be saved and used for subsequent T1A TA method titrations.
2. Certified Standardized Titrant Solutions
   a. Some customers may prefer not to standardize their titrant, instead choosing to purchase and use certified standardized titration solutions. In this case, edit the Titrant section of the method and enter the certified concentration and titrant ID (i.e., lot number, if desired).

Titrator and electrode care

Refer to the titrator and electrode user manuals for details on cleaning, storage, and maintenance recommendations to keep the titrator and electrode performing well. Main points for care are summarized as follows.

Daily Care	Weekly or Biweekly Care	As Needed
• If bubbles are visible in the titrator tubing, dispense titrant until bubbles have been expelled • Top up the electrode fill solution and leave the fill hole open during measurement • Rinse electrode well with RGW between titration cycles • Cover the fill hole and store electrode in storage solution overnight	• Drain and replace the fill solution of the electrode. • Change the storage solution in the electrode storage bottle • Consider standardizing the titrant on a weekly basis	• For slow or drifty electrode response, soak 15 minutes in 1% laboratory detergent while stirring. Rinse well with RGW afterwards • If still slow or drifty, use Orion pH cleaning solution D per instructions • See the user manuals for maintenance details

Notes

[1]Refer to the user manual for detailed instructions, if desired.

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To purchase Thermo Scientific laboratory products, please contact your local equipment distributor and reference the part numbers listed below:

Product	Description	Cat. No.
Titrator kits	Thermo Scientific™ Orion Star™ T910 Titrator Standard Kit with 8102BNUWP Thermo Scientific™ Orion™ ROSS Ultra™ pH Electrode and ATC Probe	START9101
	Thermo Scientific™ Orion Star™ T910 pH Titrator Sure-Flow™ Kit with 8172BNWP ROSS™ Sure-Flow™ pH Electrode and ATC Probe	START9102
	Thermo Scientific™ Orion Star™ T940 All-In-One Titrator Standard Kit with 8102BNUWP ROSS™ Ultra pH Electrode and ATC Probe	START9401
	Thermo Scientific™ Orion Star™ T940 All-In-One Titrator Sure-Flow™ Kit with 8172BNWP ROSS™ Sure-Flow™ pH Electrode and ATC Probe	START9402
Titrators	Thermo Scientific™ Orion Star™ T910 pH Titrator without electrode	START9100
	Thermo Scientific™ Orion Star™ T940 All-In-One titrator without electrode	START9400
Electrodes	Thermo Scientific™ Orion™ ROSS™ Sure-Flow™ pH Electrode	8172BNWP
	Thermo Scientific™ Orion™ ROSS Ultra™ pH Electrode	8102BNUWP
	Automatic Temperature Compensation (ATC) Probe	927007MD
pH Buffers	Orion pH 4.00 Buffer, NIST traceable, 475 ml	910104
	Orion pH 7.00 Buffer, NIST traceable, 475 ml	910107
	Orion pH 10.00 Buffer, NIST traceable, 475 ml	910110
Reagent Grade Water	Thermo Scientific™ Barnstead™ Smart2Pure™ 12 UV Water Purification System	50129890*
Reagents	0.1M (0.1N) Sodium Hydroxide Titrant	
	Potassium Hydrogen Phthalate, primary or acidimetric standard grade	
Accessories	100 or 150 mL beakers	

*Please contact your local Thermo Scientific representative for support on ordering water quality products. For more information, visit thermofisher.com/waterquality.

References

- Acidity (Titratable) of Fruit Products, Method 942.15. Official Methods of Analysis (OMA). AOAC International, 2275 Research Blvd, Ste 300, Rockville, MD 20850-3250. USA.
- Patrick Iland et al. (2004). Titratable Acidity. In: Chemical analysis of grapes and wine: techniques and concepts, 2nd ed. Patrick Iland Wine Promotions PTY LTD, pp. 39 - 43.

Find out more at **thermofisher.com/titrator**

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