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SHORT COMMUNICATION

Varietal thiols levels and sensory effects in South African Colombard wines

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ABSTRACT

The levels of varietal thiols and the role these compounds play in Colombard wine have not been investigated in detail. This study assessed the levels of the varietal thiols 4-methyl-4-sulfanylpentan-2-one (4MSP), 3-sulfanylhexyl acetate (3SHA) and 3-sulfanylhexan-1-ol (3SH) and their sensory effects in 24 young South African Colombard wines. Levels of 3SHA and 3SH were, in general, in line with those previously reported in South African Chenin blanc and Sauvignon blanc wines. Levels of 4MSP were, in general, found at a narrower range than those reported for Sauvignon blanc wines. Twelve of these wines were also sensorially analysed by a panel of wine industry experts. Aroma descriptors, such as guava, passion fruit, sweat and tomato leaf, which have previously been linked to 3SH and 3SHA, were also found, especially in the wines containing higher levels of these two compounds. Good correlations between 3SHA and sweat and guava were found. This study contributes to the knowledge of varietal thiol levels and their role played in 24 Colombard white wines. It could also lead to wine producers adapting their winemaking practices to increase levels of varietal thiols if the aroma characteristics linked to these compounds are sought after in this single cultivar wine.

KEYWORDS: varietal thiols, Colombard, sensory profiling, chemical profiling

INTRODUCTION

The varietal thiols 4-methyl-4-sulfanylpentan-2-one (4MSP), 3-sulfanylhexyl acetate (3SHA) and 3-sulfanylhexan-1-ol (3SH) are present at extremely low levels in wine (ng/L) yet are potent odorants and have extremely low sensory thresholds (Lund *et al*., 2009; Tominaga *et al*., 1998; Chen *et al*., 2019). 3SHA has aromas of 'passion fruit', 'grapefruit' and 'box tree', while 3SH introduces 'passion fruit' and 'grapefruit' aromas to the wine (Tominaga *et al.*, 1998) and 4MSP exhibits 'box tree' and 'passion fruit' (Swiegers *et al*., 2007). Both 3SHA and 3SH consist of pairs of enantiomers (R and S forms), which have different perception thresholds and descriptors. The esterification of 3SH with acetic acid leads to the formation of 3SHA. (Coetzee and du Toit, 2012). Varietal thiols are present in grapes at very low levels and occur mainly as precursors in the glutathionylated or S-cysteinylated forms and dipeptidic derivatives and C_6-compounds for 3SH only (Thibon *et al*., 2016; Peña-Gallego *et al*., 2012; Capone and Jeffery, 2011).

Varietal thiols have been extensively investigated, and factors such as the influence of the origin of the grapes (Coetzee and du Toit, 2015), harvesting methods and transport (Allen *et al*., 2011; Capone and Jeffery, 2011), sulphur dioxide additions at harvest (Makhotkina *et al*., 2013), the role of different yeast strains (Harsch and Gardner, 2013; Swiegers *et al*., 2007), oxidation (Coetzee *et al.*, 2016), fermentation (Masneuf-Pomarède *et al.*, 2006) and storage temperature (Makhotkina *et al.*, 2012) have been investigated. Most of this work has been done in Sauvignon blanc wine, and it has, thus, been conclusively proven that these compounds play an important role in the passion fruit, guava, grapefruit and other descriptors linked to this cultivar wine (King *et al.*, 2011; Piano *et al*., 2015; van Wyngaard *et al*., 2014).

Varietal thiols have also been identified in Riesling, Gewürztraminer (Tominaga *et al.*, 2000), Trebbiano di Lugana (Mattivi *et al*., 2012), Petit and Gros Manseng (Dagan, 2006), Grillo (Fracassetti *et al.*, 2018), Chenin blanc (Coetzee *et al*., 2018; Wilson *et al*., 2019) as well as in Müller-Thurgau and Verdicchio among other monovarietal Italian wines (Carlin *et al*., 2022). Even though it was hypothesised by Du Plessis and Augustyn in 1981 that the 'guava' aroma in Colombard wine was due to varietal thiols, there has been very little research focusing on varietal thiols in Colombard wine. Tominaga *et al*. (2000) found varietal thiols in a very limited set of Colombard wines, with (Dournes *et al.*, 2022) reporting for the first time varietal thiol precursors found in Colombard juice, which indicates the potentially important role these compounds might play in the sensory attributes of this cultivar wines. However, the levels of varietal thiols in Colombard wines and the role these compounds play in the sensory attributes of these wines have, to the best of our knowledge, not been investigated before in detail. The main aims of this work were, thus, to investigate the concentration of varietal thiols in South African Colombard wines and the role these play in sensory descriptors linked to these cultivar wines.

MATERIALS AND METHODS

1. Samples

Twenty-four wine samples from the 2017 vintage were collected (numbered 1 to 24) at South African commercial wineries. All samples were fermented dry and taken from tanks before bottling. The samples originated from 13 different cellars (two wineries presented only one sample each, with the rest donating two samples each), spanning four South African wine regions (Breede River Valley, Klein Karoo, Coastal and the Olifants River) and one geographical unit (Northern Cape) (SAWIS, 2018). The samples were stored in bottles at 4 °C before chemical and sensorial analyses, which took place one month after sampling.

2. Varietal thiol analysis

Measurements of three varietal thiols, 3-sulfanylhexan-1-ol (3SH), 3-sulfanylhexyl acetate (3SHA) and 4-methyl-4-sulfanylpentan-2-one (4MSP), were based on the method proposed by (Herbst-Johnstone *et al*., 2013) as adjusted by (Coetzee *et al*., 2018). For this method, ethyl propiolate (ETP) is used as a derivatising agent, and the thiol-ETP abducts are quantified by using GC-MS/MS.

3. Sensory analysis

Of the twenty-four Colombard samples, twelve were selected for sensory analyses (numbered 1 to 12). This sub-set of 12 wines was representative in terms of the levels of varietal thiols, which ranged from low, medium to high. An informal screening tasting conducted by the researchers/authors and a winemaker was conducted to ensure that the selected wines also spanned the same sensory space as the original sample set. The rate-all-that-apply (RATA) method was performed using an expert panel to assess wine aroma. RATA is a variation of the widely used check-all-that-apply (CATA) question format (Meyners *et al*., 2016). Although this method is frequently used with consumer panels, it is also suited to use with semi-trained, trained and expert panels ((Franco-Luesma *et al*., 2016)). The panel consisted of 23 men and seven women aged between 23 and 62. All panel members consisted of wine industry specialists, mostly winemakers. There were some smokers on both panels, but all panellists refrained from smoking on the morning of the sensory evaluation. No perfume or the consumption of coffee was allowed before or during the sensory evaluation sessions. The panel members were not paid for participating. No training of the panels took place.

Each panel performed RATA (Ares *et al*., 2014) on the same 12 randomly selected wines using the same list of aroma terms. The terms on the list were not randomised due to practical restrictions; the terms were organized per the aroma family. The list of descriptors was selected from descriptive analysis data of van Rooyen, 2019, which were performed on similar wines from the same cultivar and vintage. The list of terms of the 12 wines used in this study was confirmed afterwards by a group of five experts, two winemakers,

two sensory scientists and a research oenologist. A specified set of terms was provided, and each panellist had to indicate whether the term applied to that wine sample. If the term applied to the sample, the panellist would rate its intensity on a five-point scale, anchored at 1 (very low intensity) up to 5 (very high intensity). Every panel member evaluated each of the 12 wines twice. There was a 15-minute break between the evaluation of the duplicate sets. The panel members did not know that they had tasted the same wines a second time.

The sensory evaluation sessions were conducted in cellar tasting rooms with temperature control (20 ± 2 °C). Disturbances such as sounds, odours, light stimuli and interactions were kept to an absolute minimum. The wine was evaluated in international wine-tasting glasses (ISO NORM 3591, 1977). Each glass contained 25 ml of wine that was poured 20 min prior to evaluation and then covered with a petri dish. Each wine received a unique three-digit code, and the wine samples were presented in a randomised order to the panellists using a Williams–Latin square design.

4. Statistical analysis

Two-way ANOVA was performed on the sensory results obtained by RATA following a similar protocol as described by Franco-Luesma *et al*. (2016). The sensory judge was handled as a random factor, and the wine was used as a fixed factor. The significance threshold for all the ANOVA done was set at $p \leq 0.05$. The Fisher's LSD post-hoc test was used to perform pairwise comparisons when a significant ANOVA result was obtained. Panel performance was measured as described by Tomic *et al.* (2010). Principal component analysis (PCA) was performed on the correlations matrix of the sensory data to obtain a multivariate sensory map and to investigate correlations between samples and attributes. PanelCheck® (Version 1.4.0, Nofima, Ås, Norway) and XLSTAT (XLSTAT, 2020, Addinsoft Inc., https://www.xlstat.com) were used to perform the statistical analysis.

RESULTS AND DISCUSSION

1. Varietal thiol levels

As hypothesised by (Du Plessis and Augustyn, 1981), young South African Colombard wines seem to contain varietal thiols at levels above their sensory thresholds of 60 ng/L for 3SH, 4.2 ng/L for 3SHA and 0.8 ng/L for 4MSP (Tominaga *et al*., 2000), respectively. Table 1 shows the levels of the varietal thiols 3SH, 3SHA and 4MSP for each individual Colombard wine.

Excluding the Colombard wine with the highest 3SH level (Wine 1), the range of 3SH levels present in the Colombard wines (Table 1) was similar to that found in young South African Chenin blanc wines (380 ng/L to 2928 ng/L) (Wilson, 2017) and young South African Sauvignon blanc wine (500 ng/L to 3500 ng/L (Van Wyngaard, 2013). The average level of 3SH measured in the 24 Colombard wines (1966 ng/L) was more than double the average of the 3SH (883 ng/L) found in 48 young Chenin blanc wines reported by Wilson (2017). The Colombard wines in this study, as was the case in the study of Wilson (2017), thus, had a wide range of 3SH levels.

The 3SHA levels (Table 1) in the young Colombard wines, again excluding the wine with the highest level of 3SHA (Wine 1), were higher than the 3SHA levels of young Chenin blanc wines (ranging from not detected to 305 ng/L, with an average of 31 ng/L; Wilson, 2017) and the 3SHA levels of Sauvignon blanc wines (ranging from 10 ng/L to 710 ng/L; Van Wyngaard, 2013). The range of the varietal thiol 3SHA in Colombard wine was, thus, found to be wider, and the average level was higher than that found previously in Chenin blanc wines (Wilson, 2017) and Sauvignon blanc wines (Van Wyngaard, 2013).

The 4MSP levels in the Colombard wines were present in a much narrower range (8.9 ng/L to 13.4 ng/L) compared to young South African Sauvignon blanc wines (Coetzee *et al*., 2018). Although certain Sauvignon blanc wines had higher levels of 4MSP than the Colombard wines assessed in this study, the latter wines had an average level of 4MSP that was almost double that observed in the Sauvignon blanc wines. Although the highest level of 4MSP for young Sauvignon blanc wine was, thus, higher than for young commercial Colombard wine, none of the young commercial Colombard wines had a 4MSP level of below 8 ng/L. In the young Sauvignon blanc wines (Coetzee *et al.*, 2018), 16 out of the 20 wines had a 4MSP level of less than 8 ng/L. This may suggest that, although South African Sauvignon blanc wines could contain higher 4MSP levels than South African Colombard wines, the latter may have 4MSP levels in a closer range. The level of varietal thiols in South African Colombard wines seems to be relatively high when compared to Colombard wines from Jurançon in the Southwest of France (Tominaga *et al.*, 2000). Tominaga *et al*. (2000) found that 4MSP was not detectable in French Colombard wines, and the levels of 3SH (432 ng/L to 1053 ng/L) and 3SHA (21 ng/L to 63 ng/L) were lower in those wines analysed compared to the South African Colombard wines assessed in our investigation. However, it should be noted that (Tominaga *et al.*, 2000) had a very limited sample set of only two wines.

2. Sensory

The intensity of the aromas described by the following attributes, 'guava', 'passion fruit', 'banana', 'oxidised apple', 'baked apple', 'stewed fruit', 'honey/caramel', 'gooseberry', 'litchi', 'sweat', tomato leaf' and 'cat pee', were perceived significantly different for the different wines, while pineapple, grapefruit, lemon, peach, pear, floral, fresh apple, cooked veg, tea/hay, grapey and fresh green were not significant.

LS means plots, obtained with ANOVA, for the average intensity scores of 'guava' and 'passion fruit', 'sweat', 'cat pee' and 'tomato leaf' and 'gooseberry' can be seen in Figure 1. It is, thus, clear that there were significant differences between the wines for these aromas, most of which are associated with varietal thiols (van Wyngaard *et al.*, 2014).

TABLE 1. Individual levels of 4MSP, 3SHA, 3SH (ng/L) in different 2017 Colombard wines.

Wine sample	Varietal thiol (ng/L)		
	4MSP	3SHA	3SH
Wine 1	12	1 721	6 862
Wine 2	11	508	3 443
Wine 3	10	941	3 281
Wine 4	10	783	2 328
Wine 5	10	790	1 957
Wine 6	9	305	956
Wine 7	10	661	2 458
Wine 8	11	192	1 285
Wine 9	11	543	2 315
Wine 10	10	1 145	2 806
Wine 11	10	957	2 878
Wine 12	12	653	2 103
Wine 13	10	1 140	2 224
Wine 14	12.	451	1 864
Wine 15	9	631	2 010
Wine 16	12	133	360
Wine 17	12	259	837
Wine 18	11	393	896
Wine 19	11	272	742
Wine 20	10	535	1 357
Wine 21	10	126	517
Wine 22	13	221	1 590
Wine 23	13	212	1 217
Wine 24	12	338	903
Min	9	126	360
Max	13	1721	6862
Mean ± SD	11	580 ± 391	1 966 ± 1 356

In the PCA bi-plot (Figure 2), almost 50 % (48.3 %) of the variance was explained by PC1 (29.4 %) and PC2 (18.9 %). Separation along PC1 was observed between the 'fruitier' aromas, such as 'sweat', 'guava', 'cat pee' and 'passion fruit' on the right, and the 'matured' and 'ripe' aromas of 'oxidised apple', 'stewed/dried fruit', 'banana' and 'baked apple' on the left. Descriptors that are typically associated with higher levels of varietal thiols (Coetzee and du Toit, 2015; King *et al*., 2011; van Wyngaard *et al.*, 2014) are found in the right-hand section of the plot. Wines 1, 3 and 10 correlated positively with the sensory attributes, 'tomato leaf', 'sweat' and 'cat pee', typically associated with varietal thiols. These wines also had the highest levels of 3SHA and, to an extent, 3SH (refer to Table 1). Wine 6 had a strong positive correlation with aromas of 'oxidised apple', 'baked apple' and 'stewed/dried fruit' and was not correlated with 'tomato leaf', 'sweat' and 'cat pee', while also having the lowest level of 3SH and 3SHA. It is well known that quinones produced during oxidation can bind to 3SH and 3SHA, rendering them less aromatic (Coetzee and du Toit, 2015; Coetzee *et al*., 2016).

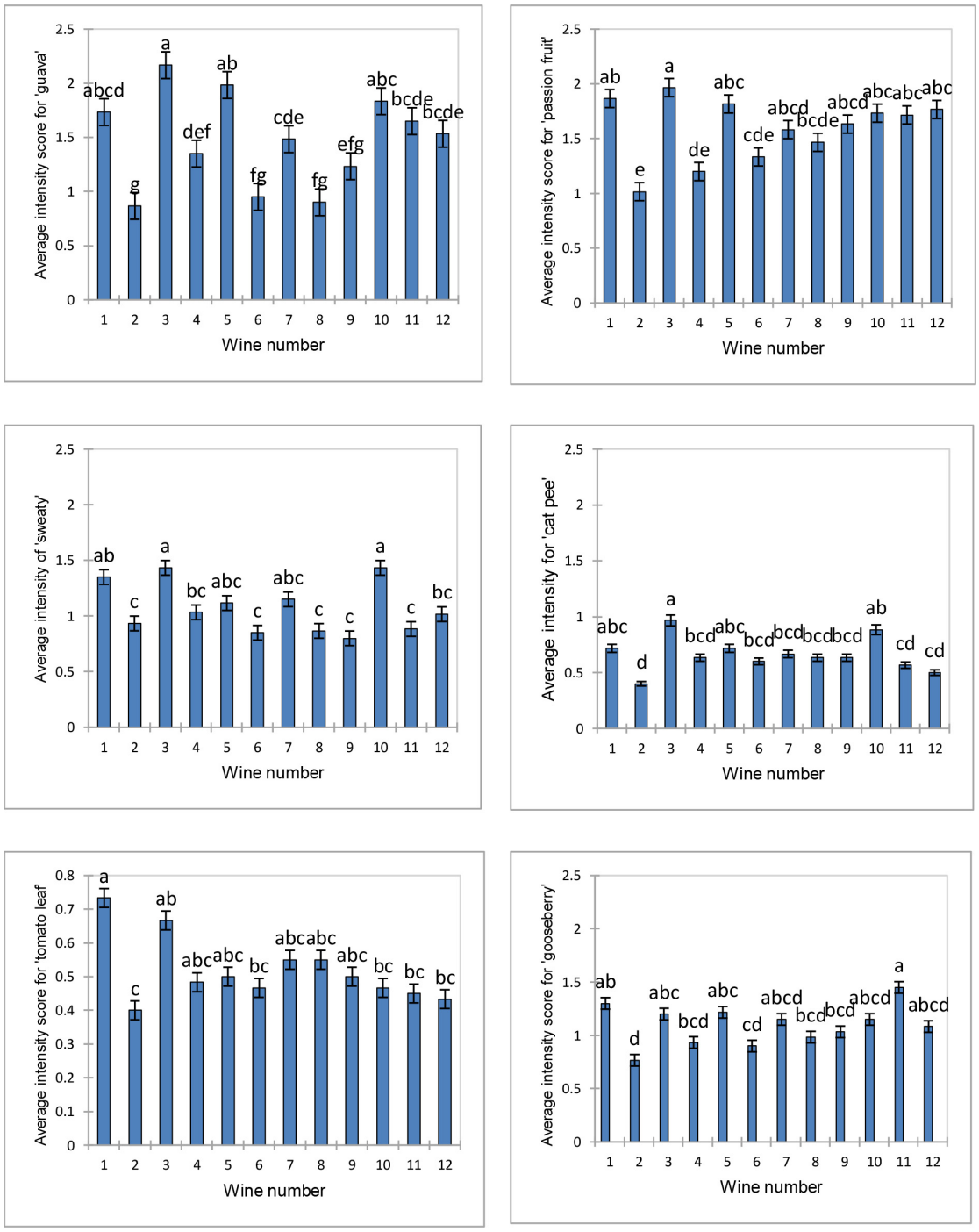


FIGURE 1. LS means plots illustrating aroma intensity differences for the guava, passion fruit, sweat, cat pee, tomato leaf and gooseberry attribute (scale 0–5) between wines, with significant letters from Fisher's LSD post-hoc test. Vertical bars denote 95 % confidence intervals.

Good Pearson correlations (Table 2), with relatively high correlation coefficients, considering that chemical data does not always correlate well with sensory data in wine, were observed between 'tomato leaf', 'gooseberry' and 'sweat' with 3SH. Even better correlations were observed between 3SHA and 'guava', 'passion fruit', 'gooseberry' and 'sweat' with 3SHA. This could be due to the lower perception threshold of 3SHA (Coetzee and du Toit, 2015), which elicited a large sensory response.

It has been shown that, in Sauvignon blanc wine, the aroma descriptors of 'guava' and 'passion fruit' are conclusively associated with the varietal thiols 3SH and 3SHA (Tominaga *et al.*, 1998; van Wyngaard *et al.*, 2014). It, therefore, seems, that based on the sensory evaluation and

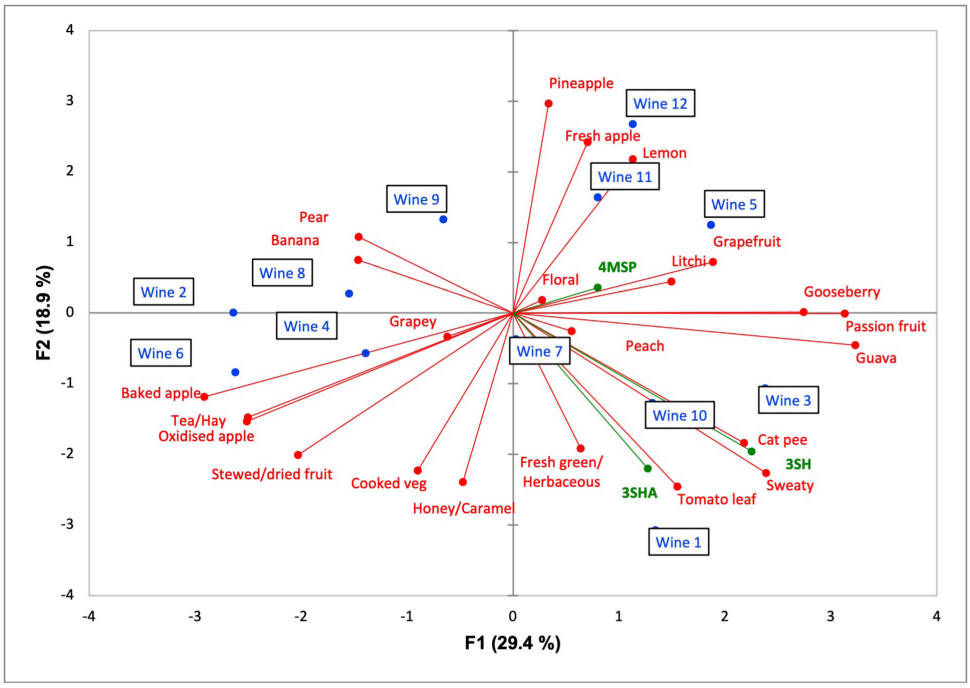


FIGURE 2. PCA biplot showing the correlations between the sensory and varietal thiol data of the twelve Colombard wines tested.

TABLE 2. Pearson's correlations between concentrations of the three varietal thiols measured in the 12 wines that was sensorially evaluated and their sensory descriptors.

Varietal thiol	Guava	Passion fruit	Tomato leaf	Gooseberry	Sweaty
4MSP	0.02	0.27	0.27	0.19	0.05
3SHA	0.69*	0.56	0.57	0.66*	0.73**
3SH	0.36	0.31	0.63*	0.40	0.55

Significant correlation coefficients are indicated in bold; *$p < 0.05$ and **$p < 0.01$.

analyses of the wines, the varietal thiols 3SH and 3SHA are also responsible for the aroma descriptors linked to these compounds in South African Colombard wine.

CONCLUSION

The fact that the varietal thiols 3SH and 3SHA in Colombard wines occur above their detection threshold is an interesting proposition. It was shown that young South African Colombard wines might contain the same or an even higher range of varietal thiols than those found in young South African Chenin Blanc and Sauvignon blanc wines.

This research seems to indicate that a correlation exists between Colombard wines with a high level of varietal thiols and more intense aromas of 'guava', 'sweat', 'passion fruit' and 'cat pee' descriptors. This knowledge may be valuable to winemakers when aiming to produce Colombard wine with a certain sensory characteristic. If intense 'guava' and 'passion fruit' aromas are required, different techniques can be used to produce Colombard wine with higher levels of varietal thiols. These methods could include suitable site selection for Colombard vineyard establishment, yeast selection and fermentation at higher temperatures. Certain areas might be better suited to delivering Colombard wines with significantly higher levels of varietal thiols, and Colombard could, therefore, be planted in these areas. With the high volumes of Colombard wines produced annually in South Africa, further research on methods to increase the levels of varietal thiols in Colombard wine might have a large economic impact on the producers of grapes and wine alike.

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