# CHARACTERIZATION OF THE THIOL AROMATIC POTENTIAL OF A NEW RESISTANT GRAPE VARIETY: FLOREAL

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## Abstract

**Aims:** Due to climate change and the desire to decrease enological inputs (organic farming), the vineyard has to be modified and the selection of new resistant grape varieties as an alternative is researched intensively today. From January 2018, four new grape varieties that are resistant against mildew and odium have been added to the official catalogue and are now available for planting new vineyards in France: Floreal, Artaban, Vidoc and Voltis. Floreal wines have been described as "*very aromatic and very intense with specific notes of grapefruit*" during tasting. Unfortunately, there is no data, either qualitative or quantitative, in literature to describe the aromatic quality of this resistant grape variety.
Today we know that the olfactory descriptor of grapefruit is mainly characteristic of 3-mercaptohexan-1-ol (3MH). To reach a deeper understanding of the aromatic potential of Floreal grapes, a combined study of the effects of both viticultural (nitrogen foliar spraying) and enological (cold lees settling) parameters has been carried out.

**Methods and Results:** After pressing Floreal grapes, corresponding must (with or without cold settling) was inoculated with a high β-lyase activity dry yeast strain at 20 g/hL. Temperature of fermentation was maintained close to 16 °C and we monitored the kinetic of alcoholic fermentation by measuring the rate of $CO_2$ release. Following this, several parameters were quantified: cysteinylated and glutathionylated thiol precursors (during grape maturation and in the must), and 3MH (in the final wine) by SIDA-UPLC-MS/MS. An innovative analysis of both reduced and oxidized forms of 3MH and 3MHA has been also performed in order to indicate possible "wine oxidizability" of such a resistant variety.

**Conclusion:** First of all, Floreal wines have concentrations in 3MH and 3MHA close to 1300 ng/L (sum of both compounds) which is relatively low in comparison with Colombard or Sauvignon blanc from Gers or Loire Valley, respectively. Thus, Floreal wine aromaticity cannot be only explained by 3MH and 3MHA, and other powerful thiols may be implicated such as 4MMP, opening an avenue for identification of new aroma compounds.
A surprising and interesting result was the fact that cold lees settling did not significantly improve the level of both 3MH and 3MHA in Floreal wines, whereas this technological practice is commonly used for its positive effect in non-resistant varieties such as Sauvignon blanc.

**Significance and Impact of the Stud**y: Therefore, accurate characterization of this new grape variety and those that will be developed in the coming years represents a great challenge: adapting viticultural and enological practices to produce high quality wines in the future.

**Keywords:** Thiol precursors, 3-mercaptohexan-1-ol, nitrogen foliar spraying, cold settling

## Introduction

Varietal thiols such as the 3-mercaptohexan-1-ol (3MH) or its acetate, 3-mercaptohexyl acetate (3MHA) are powerful aroma compounds with characteristics of grapefruit and guava notes in many wines (Roland *et al.,* 2011). 3MH arises from odorless precursors present in grapes (Peña-Gallego *et al.*, 2012) that are cleaved during alcoholic fermentation by yeast through its β-lyase activity. Even if varietal thiols biogenesis is always under study by the scientific community, many viticultural and enological practices are known to improve and drive the wine aroma quality. Indeed, grape maturation and nitrogen fertilization are two important parameters required for reaching the maximum concentrations of thiol precursors in grapes. For instance, in Sauvignon blanc grapes, thiols precursors have been seen to accumulate during grape maturation in Australia (Capone *et al.,* 2011), as well as in France (Roland *et al.*, 2010a) or in Italy (Cerreti *et al.,* 2015). Nitrogen foliar spray increased the concentration of cysteinylated precursor of 3MH in grapes (Choné *et al.,* 2006) and boosted the production of both 3MH and 3MHA as reported in Côtes de Gascogne wines (Geffroy *et al.,* 2016). From an enological point of view, several practices such as cold settling, yeast strain, temperature of fermentation and nitrogen grape must composition all modulate varietal thiol release. Each of these enological practices are well documented in literature except for cold settling. Indeed, cold-settling at 4 °C for 7 days increased the production of varietal thiols in Sauvignon blanc wine without identifying biogenesis mechanisms that were involved (Roland *et al.,* 2010b).

From 2018, Floreal was identified as a new resistant grape variety, commercially available, and that produces aromatic wines with specific notes of grapefruit. In order to characterize this specific grape variety, we conducted experiments to study: (i) the grape maturation of thiol precursors in Floreal grapes, (ii) the influence of nitrogen fertilization on thiol precursor levels and (iii) the impact of cold prefermentative settling on varietal thiol release. This study pursued a double challenge: (i) to complete the knowledge of thiol precursor biogenesis on a resistant grape variety and (ii) to propose technical solutions to winemakers in order to drive the wine aroma quality.

## Materials and Methods

### *Grape Maturation Monitoring and Nitrogen Foliar Spray*

On a specific plot of Floreal grapes in Pech Rouge (Gruissan, France), 8 rows of vines (length of 63.8 m/row and 567 studied plants) were studied; one half corresponded to the control whereas the other half was sprayed with nitrogen prior to veraison (Fertigofol Ultra© at 5 L/ha). Three weeks before harvest, 1 kg of grapes were picked at H-14, H-7 and H (H=date of harvest) on each vine row and directly stored at -20 °C before analysis. In a very close plot of a non-resistant variety (Grenache blanc), the same sampling was carried out (same dates) to compare both varieties.

### *Laboratory-Scale Vinifications*

After pressing Floreal grapes (150 kg), corresponding must was split to either perform a cold pre-fermentative settling (30 L) during 15 days at 2 °C or not. Before inoculation with a high β-lyase activity dry yeast strain (B4, Lallemand) at 20 g/hL, grape musts were divided again in two batches to either add settled lees or not. Each trial of fermentation was performed in triplicate using 1 L fermenter. Temperature of fermentation was maintained close to 16°C and the kinetic of alcoholic fermentation was monitored by measuring the rate of $CO_2$ release. Nitrogen supplements were added at 120 h and 169 h of fermentation by adding 100 mg/L of diammonium phosphate each time.

### *Analysis of Thiols Precursors*

Cysteinylated and glutathionylated thiol precursors of 3MH and 4-methyl-4-mercaptopentan-2-one (4MMP) were analyzed by SIDA-UPLC-MS/MS in Floreal grapes as already reported (Bonnaffoux *et al.,* 2017).

### *Analysis of Varietal Thiols*

Both 3MH and 3MHA were analyzed by SIDA-UPLC-MS/MS (Roland *et al.,* 2016) in wines after the end of alcoholic fermentation. Total 3MH and 3MHA corresponds to the sum of free and oxidized forms of each compound and suppresses any analytical bias due to wine oxidation.

## Results and Discussion

***Grape Maturation Monitoring and Nitrogen Foliar Spray***

During Floreal grape maturation, cysteinylated precursor of 3MH (called Cys3MH) ranged from 2.7 to 4.5 µg/kg ($p > 0.05$; F-Test) whereas the concentration of glutathionylated precursor (G3MH) decreased significantly from 300 µg/kg to 245 µg/kg ($p < 0.05$; F-Test) (Figure 1).

The decrease in G3MH during grape maturation could be linked with the biogenesis of dipeptidic precursors (Capone *et al.,* 2011b; Bonnaffoux *et al.,* 2017, 2018) or others unknown structures in the berry. On the contrary, thiol precursors in Grenache blanc grapes were relatively stable over the last 3 weeks of maturation with levels in the vicinity of 1.5 µg/kg and 150 µg/kg for Cys3MH and G3MH, respectively ($p > 0.05$; F-Test).

Recently, 64 red and white resistant varieties (excepting Floreal) were characterized and average concentrations for both G3MH and Cys3MH were found to be 236 µk/kg and 14.6 µg/kg, respectively (Nicolini *et al.,* 2020). By comparison, thiol precursor content in Floreal grapes is clearly in the same range of resistant varieties and even higher than those measured in Grenache blanc (non-resistant control of our study) (Figure 1).

Foliar nitrogen fertilization considerably increased the thiol precursor concentrations in Floreal grapes three weeks before harvest as already reported in literature for Sauvignon blanc (Choné *et al.,* 2006). However, it did not prevent the dramatic loss of G3MH during maturation.

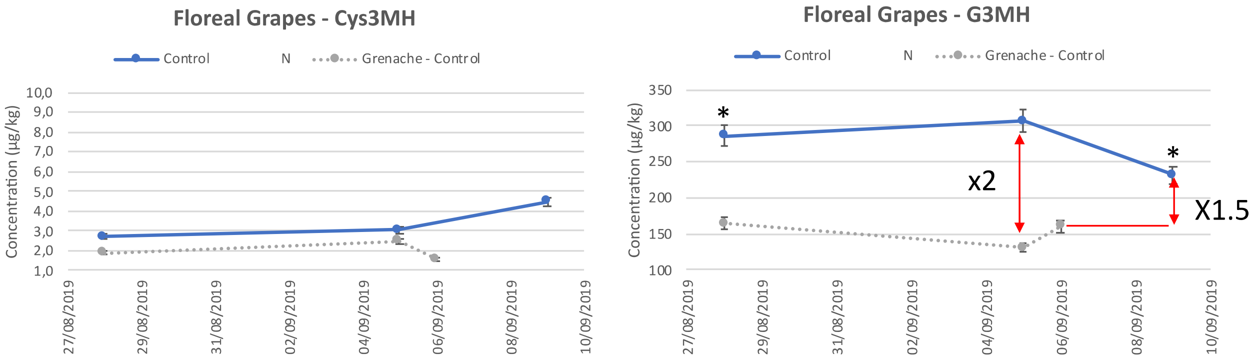


**Figure 1:** Thiol precursors monitoring in Floreal and Grenache blanc grapes during 3 weeks before harvest compared with one-way ANOVA then by Tukey HSD multiple comparison ($p < 0.05$). The symbols * indicates significant differences between the means at $p < 0.05$.

To go deeper, we decided to represent the sum of both Cys3MH and G3MH as Eq3MH according to this formula:

$$\text{Eq3MH } (\mu g/kg) = \left(\frac{[Cys3MH]}{MW_{Cys3MH}} + \frac{[G3MH]}{MW_{G3MH}}\right) \times MW_{3MH}$$

Eq3MH is representative of thiol aromatic potential of grapes and it is very useful for winemakers to pilot the quality of grapes. During maturation, thiol aromatic potential decreased dramatically for Floreal grapes during the last 3 weeks before harvesting whereas it remained stable for Grenache blanc (Figure 2). In literature, it is well known that thiol precursor levels increase during maturation of non-resistant grapes (Roland *et al.,* 2010a; Cerreti *et al.,* 2015; Capone *et al.,* 2010a). Nevertheless, one study showed that thiol precursors contents decreased in a Japanese hybrid grape called Koshu (Kobayashi *et al.,* 2010). Even if, the concentration of thiol precursors in grapes is always one order of magnitude higher than the highest concentration of 3MH found in wine, it is important to keep in mind this dramatic loss of G3MH during maturation. From technical point of view, it seems that earlier harvests could be recommended in order to segment the quality of grapes before crushing.

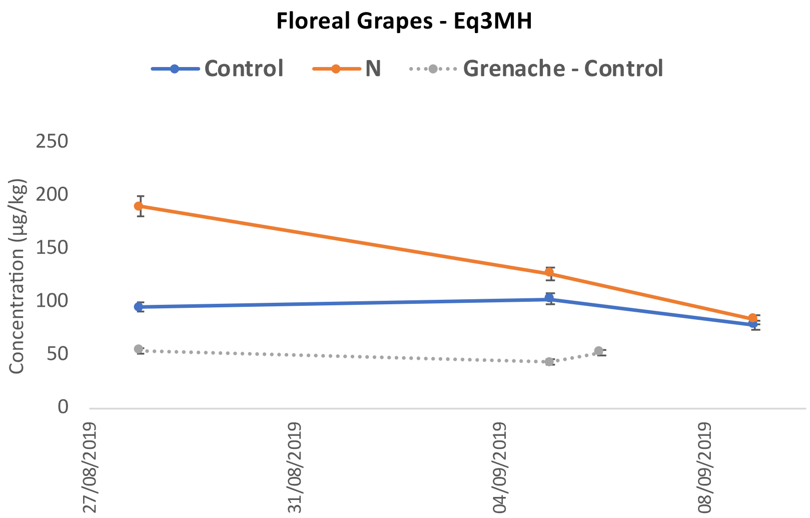


**Figure 2:** Evolution of thiol precursors represented as Eq3MH for both Floreal and Grenache blanc grapes compared with one-way ANOVA then by Tukey HSD multiple comparison ($p < 0.05$). The symbol * indicates significant differences between the means at $p < 0.05$.

***Laboratory-Scale Vinifications***

First of all, total 3MH level is on average close to 1300 ng/L in all experimental wines (Figure 3). For total 3MHA, minor traces were detected but not quantified. Usually, 3MHA corresponds to more or less 10% of 3MH in wines from non-resistant grapes, depending obviously on the yeast strain. Floreal wines did not contain a similar ratio of 3MHA but no hypothesis can be put forward for the moment.

Cold pre-fermentative treatments of grape juice are well known by winemakers to enhance varietal thiol levels in wine. In our study, experimental wines obtained from cold settled juices have similar 3MH levels ($p > 0.05$; F-Test) than control ones (Figure 3). This is the opposite tendency to those normally observed and raises the question of the validity of this enological practice for the vinification management of the new varieties.

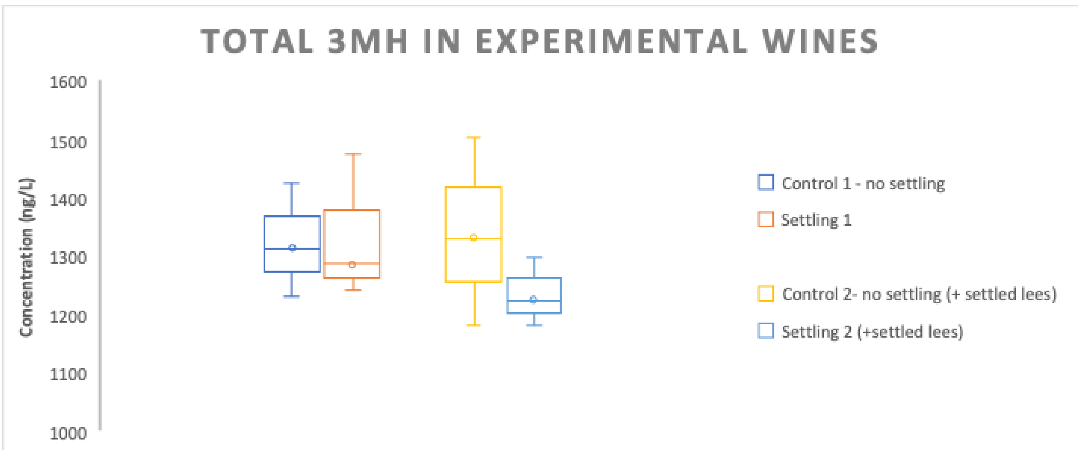


**Figure 3:** Total 3MH measured in Floreal experimental wines (laboratory scale vinification 1 L, triplicate for each trial) obtained by different pre-fermentative protocols: With or without cold settling (Statistics: one-way ANOVA then by Tukey HSD multiple comparison ($p < 0.05$). The symbol * indicate significant differences between the means at $p < 0.05$.).

## Conclusions

Floreal grapes have considerable thiol precursor concentration that could be raised by nitrogen foliar spraying before veraison. During grape maturation, thiol precursor levels decreased dramatically, suggesting earlier harvest dates could preserve a greater aromatic potential.

Experimental Floreal wines have a total 3MH concentration in the range of 1300 ng/L but surprisingly, contained just traces of 3MHA. Cold pre-fermentative settling of juices did not improve the 3MH as commonly expected. Viticultural and enological practices have to change in order to follow the evolution of raw material and to obtain high quality wines in the future.

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