THE ROLE OF GLUTATHIONE ON THE AROMATIC EVOLUTION OF DRY WHITE WINE

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Introduction:

The addition of peptides or sulfur-containing amino acids to fruit juices to prevent browning and deterioration of their aromas has been studied for a long time and successfully tested (Perl and Freidman; Main *et al*., 1993, 1997).

Just like fruit juices, certain dry white wines can develop a faulty aroma during aging that can be characterized by a loss of young fruity aromas and by the occurrence of heavy nuances reminding wax and naphthalene. Most of the time, this early aging of dry white wine aromas is also accompanied by color evolution, most particularly by a yellowing.

The interest that research showed for this subject was caused by the frequency of this flaw in dry white wines and by the subsequent commercial loss. The contribution of 2-aminoacetophenon to atypical aging (ATVA) of dry white German wines was clearly demonstrated (Rapp *et al.,* 1993). Soil and weather factors favoring the occurrence of this defect in wines (Kohler *et al.,* 1995 ; Rapp *et al.,* 1998) as well as the compound biosynthesis path have been the focus of numerous studies (Gener *et al.,* 1998). Processes aiming at the prevention of 2-aminoacetophenon formation in wine are currently being studied (Rauhut *et al.,* 2001).

3 hydroxy-2(5H) furanone (sotolon) was also found in dry white whites suffering from early aroma aging. If, on one hand, the contribution from this component in the aroma of yellow wines of Jura and in the wines of Xeres (Dubois, 1976; Guichard et al., 1993) as well as the "dried figs" and rancio notes it can confer to natural sweet wines and Port (Cutzach, 1998) is well established, on the other hand the role of sotolon in aroma atypical aging in dry white wines has never been studied before. We searched and dosed this compound in various dry white wines with a defective aroma evolution. The amounts of sotolon, even though they were much lower than those described in sweet wines made under oxidative conditions, can go beyond the sensory threshold (8ug/L). Several chemical pathways of sotolon formation have been described in the literature. Three of these pathways might intervene in dry white wines: this component can form from threonine in presence of glucose, oxygen and in acid environment (Takahashi *et al.,* 1976), or with aldo condensation of glutamic acid via oxoglutaric and pyruvic acids both in presence or absence of glucose (Kobayoshi,1989; Cutzach, 1998). A third chemical pathway of sotolon formation has been described more recently in lemon juices (Konig *et al.,* 1999). The authors describe the occurrence of sotolon in presence of ethanol, ascorbic acid and oxygen. It is interesting to note that the formation of sotolon in this type of drink is systematically considered as a defect.

The alteration of aromas and colors of fruit juices and the atypical aging of dry white wines thus appear to be quite similar. That's the reason why we thought that methods used in fruit juices to prevent this deviation, i.e. the addition of peptides or sulfur-containing amino acids, might also be effective in dry white wines. In this paper, we will present the first results concerning the role of glutathione on the aromatic evolution of dry white wines.

1:-Glutathione in musts and white wines

1-1: Glutathione, a natural component of wine must

The glutathione is a main natural component of many plants and food products (Friedman, 1994; Noctor *et al.,* 1998; Son *et al.,* 2001). This tripeptide is described as a good inhibitor of enzymatic and non-enzymatic browning of fruit juices and other food products (Molnar-Perl and Friedman, 1990; Friedman, 1994, 1996). It also prevents the formation of free radicals and acts as a cell detoxifier. It is thus used in the pharmaceutical industry (Ho *et al.,* 1992; Jones *et al.,* 1992).

The presence of large quantities of glutathione in the grapes was shown by Cheynier *et al.,* (1989) and Liyanage *et al.,* (1993). The mechanisms of its accumulation in grapes are not well understood. It seems however that the vine nitrogenous nutrition plays a main role in this accumulation phenomenon. We compared the amounts of glutathione in musts with various assimilable nitrogen contents (assessed with the formaldehyde method). In nitrogen-deficient musts (less than 160mg/L), the glutathione content is systematically lower, under our extraction conditions, i.e. with no oxygen and in presence of large SO_2 amounts (10g/hL) (Table1).

	Must 1	Must 2	Must 3	Must 4	Must5	Must 6	Must 7	Must 8
Assimilable nitrogen (mg/L)	62	244	76	202	224	56	187	42
Glutathione (mg/L)	12	28	17	28	25	6	22	4

Table 1: Effect of the vine nitrogen nutrition on the glutathione content of white musts.

We also show that in vines with symptoms of nitrogen deficiency (weak vigor, yellowing of leaves), an addition of ammonitrate (60 units) in June leads to a glutathione level in the must very similar to this of a control must, naturally rich in nitrogen (Table 2).

	Assimilable nitrogen (mg/L)	Glutathione (mg/L)
Deficient control	29	18
Deficient must with nitrogen supplementation (60 U)	174	120
Naturally rich in nitrogen	202	90

Table 2: effect of nitrogen addition to vines (June) on must assimilable nitrogen and glutathione levels.

The 2-S-glutathionyl caffeoyl tartaric acid (GRP) formation due to the reaction between glutathione with certain quinones during the must extraction was clearly described (Singleton *et al.,* 1984; Singleton *et al.,* 1985; Cheynier *et al.,* 1986). Another part of the glutathione is susceptible of being eliminated from the must by oxidation of this compound into disulfur (Adams *et al.,* 1995). These authors assess that half the glutathione present in the grapes is transformed into disulfur during pressing. Despite the high reactivity of glutathione with oxygen and the phenolic compounds in the must, we showed in white juices, extracted under real conditions, the presence of glutathione in its reduced form. The glutathione levels found in the various analyzed musts ranged from a few milligrams to twenty milligrams per liter (Table 3).

	Must 1	Must 2	Must 3	Must 4	Must 5	Must 6	Must 7	Must 8
Glutathione (mg/L)	11.5	5.8	3.1	24.5	2.5	6	18	7.3

Table 3: Examples of glutathione levels in various Sauvignon and Semillon musts (1999 and 2000 vintages).

As far as we know, the presence of glutathione in grape musts had never been described until now.

1-2: Evolution of glutathione concentration during alcoholic fermentation in dry white wines.

Glutathione, which represents more than 95% of the low molecular weight thiols in yeast cells (Elskens *et al.,* 1991), is essential to yeast growth (Murata et Kimura, 1986).

If we follow the evolution of the glutathione concentration in the must during alcoholic fermentation we first see a decrease. After 4 days of fermentation the glutathione level in the must increases again, which corroborates with the measurements of Park *et al.* (2000). This gradual increase in the glutathione concentration continues until after the wine sulfiting and stabilizes about a month after the end of alcohol fermentation. (Figure 1).

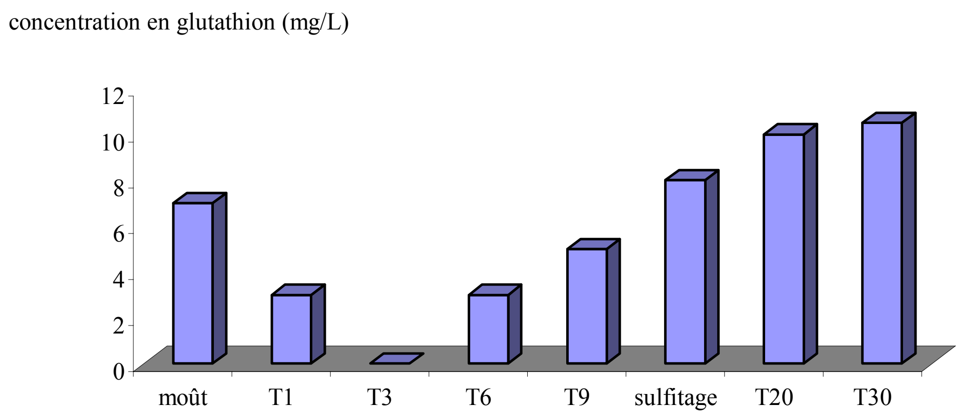

Figure 1: Evolution of the glutathione level in musts during fermentation

It is as if yeast used the glutathione available in the must during its growth phase and released it at the end of alcoholic fermentation. It is quite possible that glutathione is released at the same time as pools of amino acids are released, that is when the yeast autolysis begins.

In case of lee-aging, white wines gain a "reductive potential" at the beginning of aging process. Under certain winemaking conditions, there is apparently a correlation between the initial content of glutathione in the must and the levels found in the wine a month after the alcoholic fermentation (Table 4).

Glutathione in the must (mg/L)	9	5	4	17	2
Glutathione in the corresponding wine (mg/L)	11	7	6	22	3

Table 4: Effect of must glutathione content on the young wine glutathione content

Still, these observations need to be confirmed.

1-3: Evolution of glutathione content in dry white wines during aging.

The enrichment in glutathione at the end of alcoholic fermentation confers them a "reductive capacity. If our hypothesis is correct, it could protect the aroma against an early evolution. Most long-ageing white wines are matured during several months in barrels, most of the time on total lees, before being bottled.

Figure 2 shows the evolution of the glutathione content of a Sauvignon wine matured during 10 months in new or old barrels and on total lees or without lees.

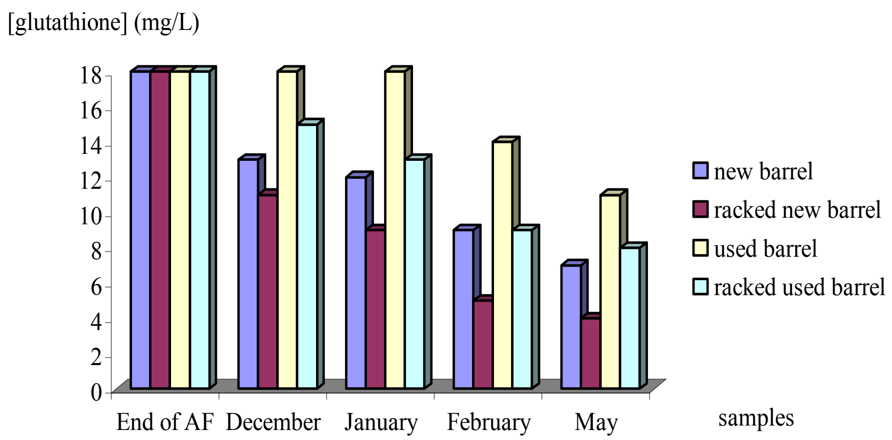

Figure 2: effect of various barrel aging methods on the evolution the glutathione content in wines.

When lees are removed from the wine the glutathione content decreases rapidly and in significant proportions during aging. In new barrels this effect is even greater due to the oxidation phenomena. Whatever the aging method, a significant decrease of the "reductive capacity" of the wine is observed. However on-lees aging of wines allows a better preservation of the glutathione content. Under these conditions the decrease of the “reductive capacity” of the wine is both slower and lighter.

2- Glutathione content in wines and their aromatic evolution:

2-1: Impact of the glutathione content of wines during aging on their aromatic evolution.

We showed that according to the method chosen for aging, the wine reductive capacity, assessed by its glutathione level, is more or less well preserved in barrels. One of the empirical roles played by lees for long-aging winemaking is to protect the fruity aroma of young wines by limiting their oxidation. These well proven observations made us think that the glutathione release by the lees during wine aging might partly explain this phenomenon.

In a 2000 Sauvignon white wine, matured in new and used barrels, on total lees or without lees, we studied the simultaneous evolutions of glutathione, volatile thiols (4-methyl-4-mercaptopentanone, 4MMP and 3-mercaptohexanol 3-MH) - aroma key compounds of Sauvignon wines (Dubourdieu *et al.,* 2000) - sotolon and 2-aminoacetophenone - markers of atypical ging of dry white wines.

As we previously observed it, the wine glutathione level decreases very significantly without lees, especially when wine is matured in a new barrel.

Glutathione contents (mg/L)

	Used Barrel	Racked Used Barrel	New Barrel	Racked New Barrel
End of AF	6.3	6.3	6.3	6.3
November	7.5	5.2	6.7	4.1
April	5.8	3.1	4.8	2

Table 5: Evolution of glutathione content in Sauvignon wine during barrel-aging

The main markers of the fruity aroma of Sauvignon wines, namely 3-MH and 4-MMP, show similar evolutions. (Table 6)

		4-MMP (ng/L)	3-MH (ng/L)
Used Barrel	End AF	11	1501
	November	13	1508
	April	13	1318
Racked Used Barrel	End AF	11	1501
	November	11	1144
	April	10.1	717
New Barrel	End AF	10	1406
	November	8.7	1240
	April	8.3	1235
Racked New Barrel	End AF	10	1406
	November	8.7	1231
	April	5.5	520

Table 6: Evolution of 4-MMP and 3-MH content in Sauvignon wine aged in barrel under different conditions

After 8 months of aging (April), the 3-MH level, volatile compound with a grapefruit note, significantly decreases when the wine is matured without lees. 4-MMP levels are more stable. Still they are divided by two in the racked and new barrel, that is the same aging condition that favors the greatest alteration of the wine reductive capacity. These results clearly show the protective role played by lees on the fruity aroma of young wines.

It seems that the presence of lees during the barrel-aging of dry white wines helps delay the occurrence of their aromas atypical aging. The sotolon and amino-acetophenone levels observed in the various treatments of our trial confirm it. (Table 7).

	B 1 wine	B 1 racked wine	B new	B new racked
Sotolon (µg/L)	1	2.6	4	8.2
2-aminoacetophenone (ng/L)	< 20	75	80	128

Table 7: level of Sotolon and 2-aminoacetophenone after aging.

The sotolon, which presence in dry white wines had never been shown until now, was detected in each of the treatments of this study. Again the wine sotolon level in the is highest in the wine aged in new barrel without lees. And it is the same with the 2-aminoacetophenone level. However, unlike sotolon, the sensory threshold of this compound has never been reached during our study.

These results show that lees may help to limit the formation of sotolon and 2-aminoacetophenone during aging and helps to prevent early evolution of dry white wines aromas.

We show that the most favorable conditions to preserve the aromatic characteristics of dry white wines are those that limit the glutathione level decrease. The capacity of the lees to combine oxygen (Salmon *et al.,* 1999) apparently explains their protective role against glutathione and sulfur aromas.

2-2: Effect of the addition of glutathione during bottling on the aromatic evolution of wine.

We observed that the various different stabilization processes to which the wine is submitted before bottling (fining, cold treatment and filtrations) may lead to a significant decrease of the glutathione level. When atypical aging does not occur during aging, it can sometimes occur very rapidly after bottling

We compared the aromatic evolution of a 1995 Sauvignon, to which was added or not 10mg/L of glutathione at bottling. The levels of volatile thiols, sotolon and 2-amniocetophone were measured after 3 years in bottle and also the intensity of yellow color (OD 420)

	Wine	Wine with added glutathione (10mg/L)
OD 420	0, 203	0, 136

Table 8: Measurement of the yellow tint after 3 years in bottle.

It is clear that the addition of glutathione at bottling reduces significantly the yellowing of wine. These results confirm the capacity of glutathione to help inhibit the enzymatic and non enzymatic browning phenomena of fruit juices (Molnar- Perl and Freidman, 1990 ; Freidman, 1994, 1996).

In the presence of glutathione the fruity aroma of the young wine, assessed with the 3-MH measurement, is also better preserved (Table 9).

	Wine	Wine with added glutathione (10m/L)
3-MH (ng/L)	320	445

Table 9: Level of 3-mercapto-hexanol in wines after 3 years in bottle..

Also like we observed it during aging, the occurrence of atypical aging in dry white wines was clearly delayed when glutathione was added at bottling (Table 10)

	Wine	Wine with added glutathione (10m/L)
Sotolon (µg/L)	9	3
2-aminoacetophenone (ng/L)	215	125

Table 10: Level of sotolon and 2-aminoacetophenone after 3 years in bottle

The sotolon concentration of the control wine went beyond the sensory threshold (8g/L). This sample also had two times more the amnioacetophenone level that the wine with added glutathione. These results confirm those of wine tasters who identify atypical aging aroma in the control wine whereas the wine supplemented with glutathione at bottling is judged as being much fresher.

Conclusion:

Between 0 and 30 mg/L, the glutathione levels in Sauvignon musts are influenced by the vine nitrogenous nutrition. The musts with the lowest levels of nitrogen also contain less glutathione. At the beginning of alcoholic fermentation and during yeast growth, the glutathione disappears almost completely. It increases again at the end of alcoholic fermentation and during the first month of lees-aging. During barrel aging, the presence of reductive lees limits the decrease of glutathione and varietal volatile thiolés levels in Sauvignon wines. Simultaneously lees help prevent atypical aromatic aging of dry white wines (sotolon and 2-aminoacetophenone). At bottling, the addition of 10mg/L of glutathione limits the yellowing of wine color, the erosion of varietal aromas, and the occurrence of atypical aging.

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