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ENARTIS NEWS
WHITE AND ROSÉ THIOLIC WINES

The influence of thiols on wine aroma is the result of the combination of grapes and yeast. Yeast, with their enzymatic activities, can reveal certain thiols preferentially to others and thus give wine's aroma a pronounced tropical, citrus or herbaceous profile. However, yeast selection alone is not enough. The expression of a precise thiolic profile is the result of a more comprehensive strategy that creates the best conditions for the yeast to express its full potential.

YEAST AND THIOLS

Thiols are aromatic compounds named after the functional -SH group present in the molecule. They have very low aromatic perception thresholds and a wide range of descriptors (Table 1). This is why they significantly contribute to the aroma intensity and complexity of wines obtained from grapes rich in these compounds.

Sauvignon Blanc is certainly the star of the thiolic varieties but, as research progresses, it has been found that thiols are present in many grape varieties, both white and red (Table 2).

In grapes, thiols are present as odorless, non-volatile precursors, mainly as conjugates of cysteine and glutathione. Their transformation into odorous form occurs during alcoholic fermentation. These conjugates represent a source of nitrogen for the yeast (Figure 1). They are transported into the vacuole and cleaved to amino acids and free thiols by a pool of enzymes including ß-lyases, enzymes responsible for breaking the bond between cysteine and the thiolic compound. Amino acids are retained in the cell and used for protein synthesis while free thiols are released into the juice and contribute to wine aroma.

Yeast' ability to reveal thiols and to impact wine aroma depends on their enzyme activities and therefore on

	Thiolic compounds	Descriptors	Threshold (ng/L)	Concentration range (ng/L
MAIN VARIETAL THIOLS	4-methyl-4-Mercaptopentan-2-one (**4MMP**)	Box tree, blackcurrant, tomato leaf, cat pee	0.8	< 40
	3-mercaptohexyl acetate (**3MHA**)	Passionfruit, gooseberry, guava	4	< 2500
	3-Mercapto-hexan-1-ol (**3MH**)	Grape fruit, passion fruit	60	<19000
	3-mercaptopentan-1-ol	Grape fruit	950	90-300
	3-mercaptoheptan-1-ol	Grape fruit	35	25-75
	4-methyl-4-Mercaptopentan-2-ol (4MMPOH)	Citrus zest	55	< 90
MAIN NON-VARIETAL THIOLS	3-mercapto-2-methylpropanol	Broth, sweat	3000	25-10000
	Benzenemethanethiol	Gun powder, flint	0.3	10-40
	2-Furanmethanethiol	Coffè	0.4	0.4-62

Table 1: Main varietal and non-varietal thiols contributing qualitatively to wine aroma.

WHITE GRAPE	RED GRAPE
Sauvignon Blanc, Arneis, Carricante, Cataratto, Chardonnay, Chenin Blanc, Colombard, Cortese, Gewurztraminer, Grechetto, Grillo, Gros Manseng, Macabeo, Moscato, Muscadet, Petit Arvine, Petit Manseng, Gros Manseng, Pinot, Pinot Grigio, Riesling, Scheurebe, Semillon, Sylvaner, Tokay, Verdejo	Cabernet Franc, Cabernet Sauvignon, Grenache, Merlot, Pinot Noir, Sangiovese

Table 2: Grape varieties known to contain thiols.

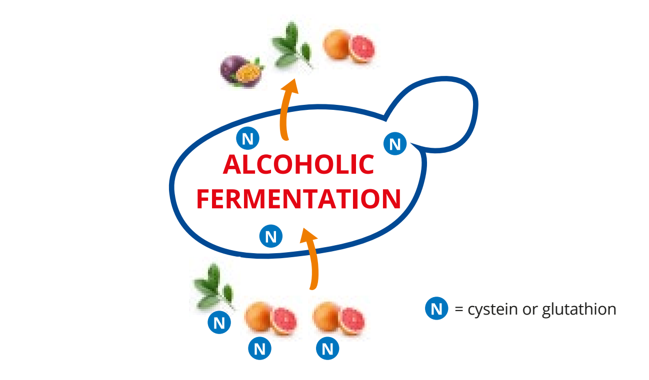

Figure 1: Mechanism of transformation of thiolic precursors into free thiols by the yeast.

their genetic makeup. An example is the IRC7 gene. This gene encodes the synthesis of a ß-lyase that is very performant in releasing 4MMP. The ICR7 gene exists in two versions: a long one consisting of 369 nucleotides and a short one of 331. Only the long IRC7 coding sequence produces an active ß-lyase therefore only yeast strains with at least one of the two genes in the long version are capable of liberating 4MMP and revealing the aroma of box tree, tomato leaf and blackcurrant produced by this thiol (Figure 2).

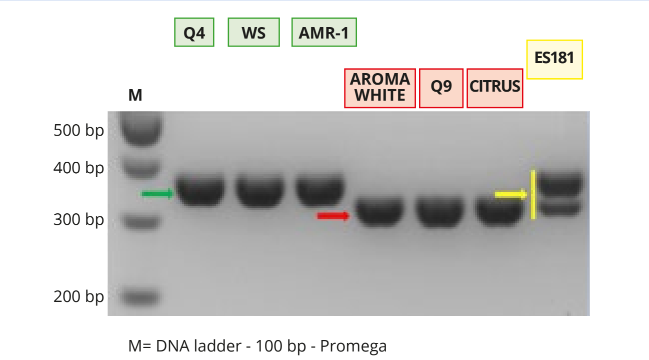

Figure 2: IRC7 genotype of some of EnartisFerm yeast strains. In green, homozygous long allele (IRC7L IRC7L) strains; in red homozygous short allele (IRC7s IRC7s) strains; in yellow, heterozygous (IRC7L IRC7s) strains.

Differently from the other thiols, the 3-MHA is not formed from a non-volatile precursor but results from acetylation of 3-MH by yeast alcohol acetyltransferase activity. Yeast strains with an overexpression of gene ATF1 encoding this enzyme will enhance tropical fruit aromas.

GUIDELINES FOR THIOLIC WINE PRODUCTION

From what has been said so far, the expression of the thiolic aromas is closely related to the enzyme activity of yeast. Genetically different yeasts show a different ability to release and convert volatile thiols and to modulate both the intensity and aromatic profile of wine accordingly. However, the result depends not only on the yeast, but also on other factors impacting thiol expression such as pre-fermentation cellar practices such as maceration and skin contact, fermentation temperature, yeast nutrition and antioxidant strategy.

Maceration

In the vinification of white grapes, maceration has the goal of increasing the extraction of thiol precursors and increase wine potential aromatic intensity and complexity. Thiol precursors have a different localization in the berry: the 3MH is mostly found in the skin while the 4MMP is equally distributed between skin and flesh. Therefore, skin contact and stabulation (prolonged settling on gross lees) have the effect of enhancing the tropical and citrus aromas.
Whatever the technique, the use of the maceration enzyme **EnartisZym Arom MP** accelerates thiolic precursor extraction thus reducing cold soak time, energy consumption and CO_2 emissions. Moreover, this enzyme improves wine protein stability, a common concern with thiolic varieties.
Care must be taken during skin contact and pressing to limit the extraction of polyphenols whose oxidation to quinones can cause loss of thiols during wine storage and bottling. Fining at juice stage can be helpful in reducing phenolics and producing a wine with a long-lasting aroma.

Plantis AF-L

Enartis, first promoter at the *Organisation Internationale de la Vigne et du Vin* (OIV) of the use of plant proteins in winemaking, offers a wide range of pea and potato protein based fining agents. Among the latest, Plantis AF-L is a pure pea protein in liquid solution stabilized with SO_2. Plantis AF-L improves juice and wine clarity whilst producing a small volume of lees and increases wine resistance to oxidation by removing iron and catechins. The liquid form makes it suitable for the addition with dosing pumps. Recommended dosage between 20 and 80 g/hL.

Juice Antioxidant strategy

According to the existing literature, some juice oxidation is beneficial when vinifying thiolic grapes. Thiolic precursors are not oxidizable because of the chemical stability of the bond between the sulfur amino acid and the thiol under oxidative conditions whereas the glutathionylated precursor of 3MH levels increase. The hypothesized reaction between glutathione, added or naturally present in the grapes, and the (E)-2-hexenal generated by enzymatic oxidation of lipids, during the pre-fermentative operations of winemaking, can lead to the higher formation of 3MH precursors.

According to Fracassetti et al. juice copper content limits this de-novo synthesis of 3MH. Copper could either bind glutathione in stable complexes or, under oxidative condition, oxidize it, making the reaction with (E)-2-hexenal impossible (Figure 3).

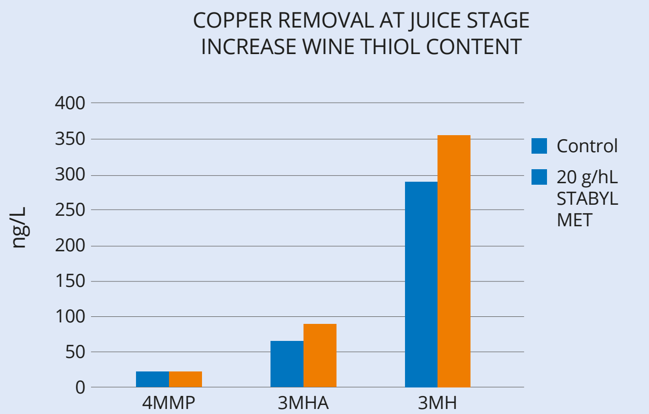

Figure 3: Copper removal at juice stage helps to increase wine thiol content. Sauvignon Blanc fermented with EnartisFerm Q4. Stabyl Met (fining agent containing PVI-PVP) was used during settling at 20 g/hL.

How to remove copper during settling?

- **STABYL MET:** fining agent containing PVI-PVP, selective for absorbing copper and iron.
- **CLARIL HM:** PVI-PVP and activated chitosan. Activated chitosan improves PVI-PVP effectiveness in removing copper.
- **ENARTISPRO XP:** inactivated yeast rich in mannoproteins and PVI-PVP. Beside removing copper, it improves wine sensory and stability.
- **ENARTISPRO FT:** inactivated yeast rich in mannoproteins and antioxidant peptides, and PVI-PVP. Beside removing copper, it improves wine sensory, stability and antioxidant protection.

As precursors are resistant to oxidation so the free thiols are easily oxidizable. Thiol protection is critical from the end of fermentation to bottling. In this phase, aging on lees, decreasing the temperature during storage, adding inactivated yeast and SO_2 are all practices that help to prevent thiol oxidation.

Yeast nutrition

As mentioned before, the biological reason why yeast cleave thiol-cysteine and thiol-glutathione complexes to the single amino acids and the free thiols is that the former represent a source of nitrogen. Supplementing grape must with an optimal nitrogen source, such as ammonium, could decrease thiol liberation. For this reason, autolyzed yeast-based nutrients are preferable in the early stages of fermentation while DAP addition should be postponed to 24-48 hours after yeast inoculation.

	NUTRIFERM AROM	NUTRIFERM AROM PLUS	NUTRIFERM ENERGY	NUTRIFERM ULTRA
APPLICATION	Supply of precursors for the synthesis of fermentation aromas	Supply of precursors for the synthesis of fermentation aromas	Reinforce fermentation capacity of yeast	Reinforce fermentation capacity of yeast
ORGANIC NITROGEN	4	6	4	5
AROMATIC PRECURSORS	5	6	3	3
STEROLS & FATTY ACIDS	3	3	4	6
MINERALS	3	3	3	4
VITAMINS	3	3	4	5
TIMING OF ADDITION	Yeast inoculation	Yeast inoculation	Yeast inoculation	Yeast inoculation
RECOMMENDED DOSAGE	30 g/hL	15-30 g/hL	10-30 g/hL	10-30 g/hL
SUITABILITY FOR ORGANIC WINE (EU REGULATION)	YES	YES	YES	YES

Table 3: Range of Enartis nutrients providing only nitrogen of organic origin (1 = lower content, 6 = higher content).

Fermentation temperature

Fermentation temperature has a great influence on aroma production. Warmer temperatures, around 20°C and above, favour the production of both esters and thiols but also cause a greater loss of aromatics by volatilization. At lower temperatures, close to 10°C, CO_2 stripping effect is limited but esters and thiols synthesis is reduced. Only the production of ethyl esters is marginally influenced by low temperature, and therefore wines fermented at 13-14°C benefit from the fresh and long-lasting fruity aromas produced by these compounds.

In conclusion, wine aroma is the result of the balance between synthesis and loss, synergy and antagonism between thiols and esters. In this competition between opposites, yeast plays a very important part, so it is difficult to draw firm assumptions. As a general guideline, when fermentation temperature is around 18-20°C acetate esters production boosts the tropical fruit profile of thiols; at temperature of 13-14°C a higher retention of moderate quantity of ethyl esters and thiols results in a fresher, greener and more citrus-like aromas.

ENARTIS GUIDELINES FOR THE PRODUCING DIFFERENT STYLES OF THIOLIC WHITE AND ROSÉ WINES

	Herbaceous profile	Tropical Profle	MIneral Profile	Thiol 360°
KEY POINTS	• Release 4 MMP • Minimize ester production • Minimize 3MHA production	• Maximize 3MHA • Boost tropical aroma with esters	• Minimize ester production • Enhance non-varietal thiol synthesis	• Maximize varietal thiol expression
MACERATION		EnartisZym Arom MP	EnartisZym Arom MP	EnartisZym Arom MP
SETTLING	EnartisZym RS or EZFIlter	EnartisZym RS or EZFIlter EnartisPro FT	EnartisZym RS or EZFIlter EnartisPro XP	EnartisZym RS or EZFIlter EnartisPro FT
YEAST	EnartisFerm Q4*	EnartisFerm Aroma White	EnatisFerm Q9	EnartisFerm ES181
YEAST NUTRITION	Nutriferm Energy or Nutriferm Ultra	Nutriferm Arom or Nutriferm Arom Plus	Nutriferm Energy or Nutriferm Ultra	Nutriferm Arom or Nutriferm Arom Plus
FERMENTATION TEMPERATURE	14-16°C	14-16°C	12-13°C	14-16°C
ADDITIONS IN FERMENTATION	EnartisPro Blanco		EnartisPro Blanco Incanto chips	

* Strain sensitive to copper. Treat juice with Stabyl Met if necessary.

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Australia Branch - 69 Chadstone Road, Malvern East, Victoria, 3145, Australia - Phone: + 61 (03) 9428 0037

New Zealand Branch - PO Box 4304, Marewa, Napier - Phone: + 64 (06)8434 413

www.enartis.com