PRINCIPLES AND MANAGEMENT OF AROMATIC AND PHENOLIC WINE COMPOUNDS

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Introduction

The quality of red wines hinges specifically on the balance between diverse aromatic notes and a complex mouthfeel. In this context, aromatic and polyphenolic compounds are of preeminent importance for winemakers. Grape quality will be determining by means of its aromatic and polyphenolic potential, and the vinification procedures will have to be adjusted in order to express this potential according to the wine type desired. This integrated and careful oenological practice is especially valuable because of the market segmentation of Cotes du Rhone wines into "fruity" and "full-bodied" types. This market segmentation is based on precise analytical criteria. Among other criteria, fruity wines need to have a Colour Intensity (CI) below 5 and a Total Polyphenol Index (TPI) below 40. In contrast, full-bodied wines need to have a minimum of 5 and 40 in terms of CI and TPI, respectively. This article reviews current knowledge of the aromatic and polyphenolic composition of grapes and wines, and discusses factors and techniques allowing their transfer from grape to wine. Finally, several factors influencing the tannic and aromatic perception of wines will be considered with regard to their potential use for obtaining fruity and full-bodied wines.

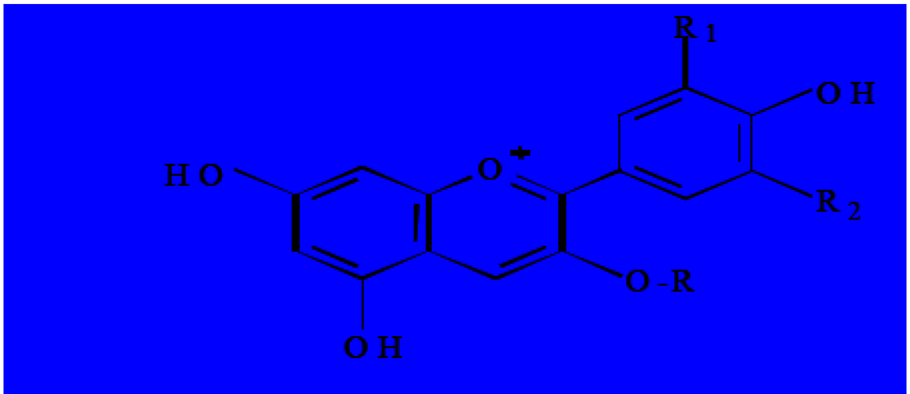
Polyphenol and aroma composition of grapes

- Principal polyphenols

Grape polyphenols can be divided into 2 categories: Flavonoids (proanthocyanidins or condensed tannins, anthocyans, essentially flavonols) and non-flavonoids (phenolic acids and stilbenes). This review will only cover anthocyans and tannins, which are the principal polyphenols of relevance in red wines because of their contribution to wine colour and astringency, respectively, but also because of their tendency to react and produce derivatives, which ensure colour stability and suppleness. Phenolic acids can contribute to perceived wine bitterness (Noble, 1994) and flavonols are involved in co-pigmentation reactions (Darias-Martin, 2002), which may increase colour intensity of wines.

- Anthocyans

Anthocyans are pigments, which are located in the cellular vacuoles of the grape berry hypodermis and in the pulp of teinturier-grapes ("dyers", such as *Vitis vinifera* var. Alicante Bouschet). Anthocyan content and composition differs between varieties. *Vitis vinifera* anthocyans are malvidin glycosides, peonidin, petunidin, delphinidin and cyanidin whose different hydroxylation (OH) and methoxylation (OCH_3) levels are shown in the following table.



	R1	R2
Malvidin	OCH_3	OCH_3
Peonidin	OCH_3	H
Petunidin	OCH_3	OH
Cyanidin	OH	H
Delphinidin	OH	OH

These five molecules can be found in the acylated form resulting from the esterification with acetic, *p*-coumaric or caffeic acid **(Figure 1)**. The absorption maxima (A_{max}) of anthocyans depend on the degree of substitution of the aromatic rings, as well as the esterification. Some of these molecules can confer violet tones.

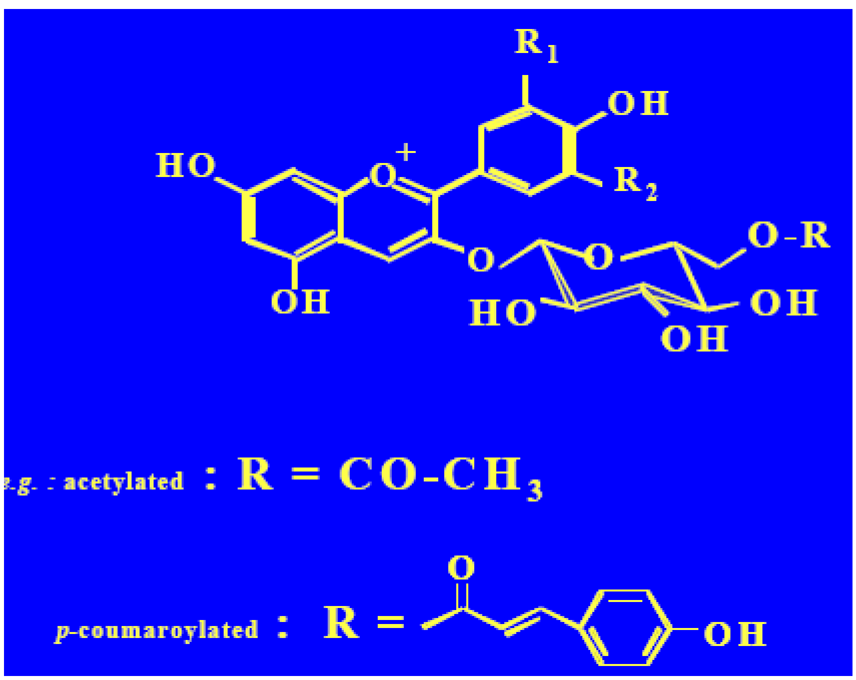


Figure 1
Estérification des anthocyanes

In addition to this structural complexity, anthocyans may exist in different isoforms (quinone base, flavylium ion = cation form, see Fig 1, hemiacetal base and its open form, the chalcone), which in solution exist in a pH dependent equilibrium. It is interesting to note that at the pH of red wine (3.5 on average) only about 20% of the anthocyans are in their flavylium form, which lends them red colour, while the colourless hemiacetal form is predominant. Different average total anthocyan contents have been measured in different varieties. Syrah has higher contents than Grenache (Puech 2000). Beyond the overall quantity, variations among the proportion of different anthocyan classes have been observed, as well: Grenache had 75% malvidin-3-glycoside while this molecule only represented 50% of the Syrah anthocyans (Puech 2000). It is also interesting to mention "plot effects" on the quantity of anthocyans but not their relative proportion, as demonstrated by the Grenache Observatoire (Puech 2002).

- Tannins

Proanthocyanidins or condensed tannins are polymers of flavonol monomers, that is, catechin, epicatechin, epicatechin gallate and epigallocatechin in the case of grapes. In the grape berry, these molecules are located in the pips (in the internal and external cell walls) or in the grape skin. The stems also contain tannins, but no more than 5% of the total grape tannins (Souquet et al 2000). The tannins are found in the cells either freely or associated, two structural states, which evolve during maturation (Cadot, 2004).
On structural level, tannins differ according to their localization in the berry. Pip tannins are characterized by epicatechin gallate levels above 20% (Prieur et al 94), and by a molecular size (or polymerization degree = pd) below 10 units on average. As for skin tannins, they are characterized by a lower epicatechin gallate content (approximately 5%) but especially by an epigallocatechin content of about 20%, which is a true marker for skin origin (Souquet et al 1996). Regarding their size, skin tannins are generally larger than pip tannins with an average pd of 30. The structural analysis of tannins remains the privilege of few basic research laboratories complicating the access to information. However, some differences between varieties have been demonstrated by combining results from several studies. Interestingly, it could be shown that Grenache and Syrah tannins differ by structure **(Table 2)**.

	Polymerisation degree		% epigallocatechin		% epicatechin gallate	
Varieties	Pip	Skin	Pip	Skin	Pip	Skin
	Degré de polymérisation		% d'épigallocatéchine		% d'épicatéchine gallate	
Cépages	Pépin	Pellicule	Pépin	Pellicule	Pépin	Pellicule
Grenache noir [1]	12	35	0	30	38	8
Syrah [2]	10	30	0	22	20	5

1 Souquet, Engineering Thesis, CNAM, 1997
2 Vidal et al 2003

Table 2: Structural characteristics of Syrah and Grenache Noir tannins.

- Aromas

Two large families of aroma compounds are present in grapes: free aroma compounds, which are aromatic by themselves, and aroma precursors, which represent an aroma reservoir to be revealed during the various stages of vinification and wine ageing. Few wine grapes are rich in free aromas. The main varieties of the Rhone valley, Grenache and Syrah, have few aroma compounds, but contain numerous aroma precursors. Even though aroma compounds can be found throughout the entire grape berry, they are most abundant in the skin. Glycosidic precursors are among the most studied of these different molecules (Rémi Schneider, 2003). Generally speaking, glycosides consist of a glycosidic part and an aglycone.

The glycosidic part will be composed at least of one glucose molecule, one apiose, rhamnose or arabinose molecule, which can contribute to structural complexity of this sub-structure. The aglycones consist of volatile compounds divided into different classes according to their structure (aromatic, aliphatic and C6 alcohols, phenols, terpenes and C-13 norisoprenoids). Because of the limits of current detection methods for glycosidic precursors, only little information is available about this parameter so important for grape quality. The most valuable analytical technique combines a tedious sample preparation protocol (extraction, separation, enzymatic treatment) and GC separation with detection by mass spectrometry or flame ionization. Unfortunately, this approach is restricted to basic research laboratories, which not only have the required cutting-edge equipment but also the necessary scientific know-how to interpret the results. Faster methods have been developed. The GG method (Iland et al 1996) was developed by the Australians but high polyphenol glycoside contents (anthocyans and flavonols) interfere with the results. ITV and INRA have suggested a new and promising method by FTIR-spectroscopy, which still requires further work. Thus, the results obtained so far are built on basic research. Grape varieties not only differ on the basis of their glycosidic precursor amounts, but also because of the distribution of the different aglycone classes associated with them **(Figure 2)**.

A thesis supported by Inter Rhône intends to better characterize the aroma potential of Grenache and Syrah. Albeit containing comparable amounts of glycosidic precursors, Syrah sets itself apart by a higher relative content in C-13 glycosylated norisoprenoids and a lower glycosylated phenol content as compared with Grenache (Marie Ségurel, thesis in process).

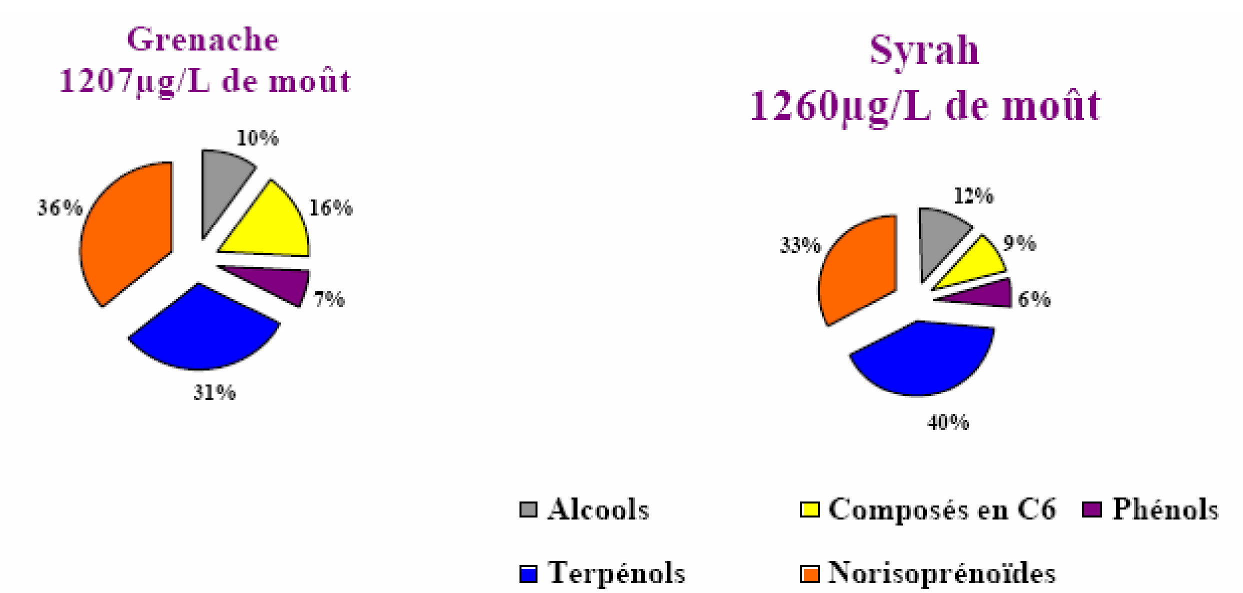


Figure 2

Distribution of different aglycone classes according to grape variety.

It is equally important to mention that differences could be shown between vintages (in 2002 values were lower than in 2001) and that the different precursor classes were affected to varying degrees. As well, "plot effects" could be demonstrated within the same thesis project **(Figure 3).**

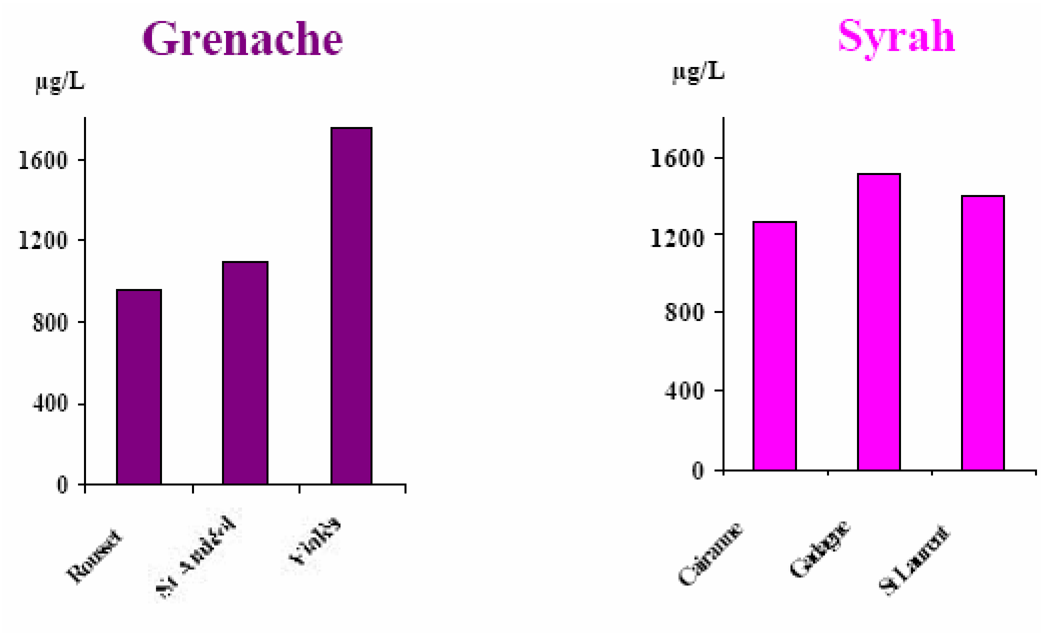


Figure 3

Differences among glycosidic precursors according to plots.

- Partial conclusion

Information about the aroma and polyphenol potential appears to be essential to better assess grape quality, but unfortunately, obtaining this information currently is complicated by the analytical methods, which can be difficult to implement. The previous summary reveals that the grape aroma and polyphenol potential varies according to grape variety, physiological state and vineyard location (terroir, vineyard management…). Interestingly, the major metabolic pathways for the biosynthesis of sugars, proteins and polyphenols are "in competition" in order to guide the relative grape composition towards a variety of molecules. An example would be the entirety of polyphenols, whose biosynthesis involves a common enzyme, phenylalanine ammonia-lyase (PAL). The PAL activity will favour polyphenol production over the synthesis of proteins when growth conditions are unfavourable.

Remarkably, the grape skin represents the main reservoir for aroma and polyphenolic compounds, which will influence the final wine quality.

Extraction factors

The main vinification challenge consists in extracting these compounds without negatively affecting the course of alcoholic fermentation. The successful production of a light and fruity or more structured wine relies on the proper control of anthocyanin and tannin extraction from the skin, but equally on the extraction of aroma compounds and the formation of a yeast fermentation bouquet. These two levels should be well managed according to various factors.

- **Colour and tannin extraction**

This extraction is always incomplete and is subject to strong variations. Generally, wines contain only 20 to 50% of the phenolic compounds originally present in the grape. The objective should not be the maximum extraction, but rather the optimum extraction of the highest quality compounds.

In order to obtain a light and fruity wine, it is necessary to rapidly extract the anthocyans while limiting tannin diffusion.

Diverse factors control the extraction and affect the various compounds to be extracted differently.

The first factor is the character of the raw material: the variety, grape maturity level and sanitary quality determine the content in polyphenols but also their extractability. The factors which determine the extractability of skin compounds are not well understood. The diffusion limiting cell wall polysaccharides and their partial hydrolysis during maturation are probably responsible for the extractability variations observed between different grape batches with identical polyphenol potential.

The second and main tannin extraction factor is alcohol.

In contrast to anthocyanins, which easily diffuse through aqueous media, tannins require the presence of alcohol for extraction. However, differences exist between skins and pips. Pip tannins diffuse less easily because of the cuticle. According to the presence of alcohol, three maceration stages can be distinguished:

- the prefermentation maceration (few hours to several days) occurs in the absence of alcohol,
- the alcoholic fermentation differs by the increasing alcohol concentration and by important yeast enzyme activities. Generally, it lasts three to ten days according to must fermentability and the yeast strain involved,
- the post-fermentation maceration takes place between 10 and 16% alcohol depending on the case. While generally lasting between 8 days to a month, some winemakers extend it to over two months.

Since alcohol increases over time during the fermentation, all those techniques which involve short skin maceration durations under alcoholic conditions allow to obtain less tannic wines. For example, thermovinification, cold or hot prefermentation maceration, utilization of pectolytic enzymes and the rapid expansion method (flash detente) immediately followed by pressing, are all procedures to reduce the maceration time and thus tannin quantity, while still extracting a maximum amount of anthocyans. In contrast, a long maceration will lead to significant extraction of skin tannins.

The temperature is also an essential factor. The range most frequently used for macerations is 28 to 30°C. Because the extraction is best between 30 and 35°C, a final hot maceration could be realized. However, these temperatures combined with the alcohol present and the reduced nutrient status, can stress yeast and even bacteria and may involve the risk of volatile acidity production and stuck fermentations. Below 25°C, extraction is slower. Rapid expansion (flash détente) followed by a maceration of three days or longer also allows to significantly increase extraction. Nevertheless, this method causes technical constraints since the must has 35°C after the treatment and absolutely has to be cooled in order to ensure a smooth fermentation. Enzyme addition is also necessary since significant pectin extraction and grape pectinase inactivation by the method lead to very turbid musts.

Continuous mixing of wine and skins is important in order to maximize the diffusion of skin compounds. In fact, the diffusion rate of a compound also depends of its concentration in the surrounding liquid. Without agitation, the must surrounding the skins will be concentrated, reducing diffusion. The agitation can be achieved by cap punching (pigeage), delestage, pumping over or other agitation methods, such as turbopigeage or the utilization of tanks with two stacked compartments of the Gimar-type, tanks using the fermentation gas, such as the Ganimede-type, or rotofermenters (or other movable types).

Finally, other techniques allow to adjust the extraction. For example, draining 10 to a maximum of 30% of the juice upon vatting, partial or no destemming, maceration with sulphite, blending all or part of the press wines, or the tanning technique, which also allows to adjust the abundance of wine polyphenols. If the seeds are lignified, they can be retained as an additional source of tannins.

- Liberation of skin aroma compounds and production of fermentation aromas

Besides extracting polyphenols, varietal and fermentation aromas have to be preserved. The appropriate conditions for this preservation are often contrary to the optimum extraction conditions. The level of maturity and the weather conditions during maturation also influence the aroma compounds. For example, this is the case for the exposure of grapes to sun. The temperature is certainly the major factor defining the aroma profile of wines. Above 30°C, this profile changes with a significant shift of fresh fruit aromas towards ripe fruit, jams, or fruit brandies. Below 25°C, fruit and fermentation aromas are well preserved.
Other factors are important: anaerobic conditions for ester synthesis, the must amino nitrogen content for the synthesis of higher alcohols and avoiding over-clarification.

Specific techniques such as carbonic maceration or the Beaujolais-vinification enable an anaerobic metabolism in the whole berries in the carbonic atmosphere. This technique produces particularly fruity wines, which emphasize the aroma expression of certain varieties, such as Syrah.

Wine polyphenol and aroma composition

- Polyphenols

Grape anthocyans and tannins are very reactive molecules, which - once extracted into the liquid - can lead to the formation of new molecule types. Of course, the reaction rate as well as the reaction pathways depend on the medium conditions (pH, temperature, oxygen…). Polyphenol chemistry is very complex and the diversity of the molecules formed increases the analytical difficulties. However, certain basic studies allow to shed some light on the multitude of polyphenolic structures. The studies by the group of Véronique Cheynier at the INRA of Montpellier are certainly among the most significant in this respect.

Two major evolution pathways of polyphenolic compounds in wines have been studied. A first pathway involves the acetaldehyde produced by yeast during alcoholic fermentation. This reaction pathway leads to the formation of tannins with ethyl bridges (Es-Safi et al 1996), oligomeric structures where the anthocyan monomers are linked by ethyl bridges (Atanasova 2002), and mixed molecules, in which tannin and anthocyan units are equally linked by ethyl bridges (Escribano et al 2001). As second reaction pathway relies on the direct condensation between tannins and anthocyans (Rémy 2000).
This reaction pathway is also related to the property of tannins to hydrolyse at wine pH. Incidentally, it has been shown that the average size of tannins had the tendency to decrease

over time (Vidal et al 2002), a result which is in disagreement with the general notion that tannins polymerise in wine during ageing. It would be much too difficult to present the structures in more detail, and the purpose of this section is rather to underline the fact that a large number of polyphenolic structures are formed in the wine, whose impact on wine colour and organoleptic character are however hard to assess because of the difficulty in analysing these molecules.

- Aromas

Different aroma types can be found in wines: those which come from the grapes, those produced by yeast activity, which are called fermentation aromas, and those revealed by the aroma potential, which is represented by the aroma precursors. We already mentioned that the amount of free aromas is very low in the majority of wine grapes. In their majority, the fermentation aromas are due to esters and higher alcohols and are characteristic for the yeast-must combination. In fact, the energy of glycolysis and respiration is used for the synthesis of fermentation aromas from the sugar, nitrogen, lipid and sulphur metabolism.

On the other hand, the aroma precursors will lead to the production of a series of odour compounds, which could lend specific notes to the wines. This property has been well documented for the glycosidic Grenache and Syrah precursors under accelerated ageing conditions (thesis of Marie Ségurel) since presence of 69 odour compounds could be demonstrated. Among these 69 compounds, 15 seemed to allow discrimination between Grenache and Syrah with regards to odour intensity. The glycosides can undergo hydrolysis by two major ways, potentially leading to the slow release of odour compounds: chemical and enzymatic hydrolysis. Chemical hydrolysis relies on sufficient acidity levels and is accelerated by temperature increases. Enzymatic hydrolysis relies on specific enzymes whose activity defines the rate of hydrolysis. This activity is lower in musts compared with wines because of enzymatic inhibition by glucose.

- Partial conclusion

It could be clearly shown that the grape polyphenol and aroma potential has an effect on wine quality through a number of molecules, which are extracted during the various vinification stages. However, it also influences the potential for further development of the organoleptic characteristics.

The interactions between different molecule groups, which contribute to wine complexity, also have to be considered. Two examples, which reveal the complexity of wines, can be cited. The first example involves phenolic wines and demonstrates the link between polyphenols and odour compounds.

In fact, ferulic and coumaric acids, both non-flavonoid polyphenols, are precursors for volatile phenols (ethyl phenol and ethyl guaiacol), odour compounds, which are responsible for a significant aroma defect in wines containing yeast of the genus *Brettanomyces*. The second example was chosen to illustrate the influence of the wine matrix on the perception threshold of odours. Because of the molecular interactions among odour compounds and polyphenols or polysaccharides, the perception thresholds can vary as well-known for other agri-food products.

Factors influencing the tannic and aroma perception of wines

According to segmentation criteria, a wine for the “fruity” range must have few tannins, which have to be integrated. The astringency should not dominate. A “full-bodied” wine must have a higher tannin content, which should produce a good sensation of mouthfeel and wine concentration without however displaying an excessive astringency.

The astringent properties of tannins are attributable to their capacity to precipitate saliva proteins thus leading to a reduced lubrication of the mouth. Numerous parameters, including tannin structure, have an effect on the perception of astringency. Recently, knowledge about the effect of the structure of tannins on their astringent qualities has progressed significantly. Previously, it was thought that astringency increases with size up to a certain value (pd=7) and then decreases as tannins augment in size based on the assumption that these polymers would precipitate. This hypothesis recently has been rejected by Vidal et al (2003), who demonstrated that astringency increased with the polymerisation degree of tannins regardless of size. The percentage of galloylation (higher in the pips) intensifies astringency while it is reduced by the trihydroxylation level (specific for the skins). During ageing, the average degree of tannin polymerisation decreases because of tannin chain ruptures caused by acid hydrolysis and, specifically, recombinations with anthocyans. These phenomena could explain the decrease in astringency observed during wine ageing. Besides, a recent study has shown that these newly formed tannins were less astringent than the tannins initially released (Vidal et al 2004). Ageing allows for the recombination of wine polyphenols depending on the temperature, oxygen and the duration, and will lead to a decrease in astringency. This decrease can also be linked to the loss of solubility and the precipitation of tannin complexes over time. Wine ageing in barrels or large oak casks, as well as the microoxygenation technique allow to adjust the oxygen parameter.

However, the astringency of tannins is not exclusively determined by their molecular structure. Other biochemical and physical factors also play a role.

Firstly, wine constituents (polysaccharides, proteins, glycerol, tartaric acid, ethanol, oak compounds) modulate the perception of tannins. The polysaccharides, for example, interfere by establishing interactions with tannins, thus limiting tannin association with proteins. Wine ageing on yeast lees can thus increase mouthfeel and decrease harsh tannins. Alcohol and acidity will intensify perceived astringency, while residual sugars and glycerol will reduce it. During long macerations, tannin perception augments over time up to 10 to 12 days of maceration. Then, if the skins are sufficiently ripe, tannin perception will decrease with an intensification of tannin suppleness.

Oak compounds also influence the astringency because of the release of ellagitannins from oak, but they also significantly increase the impression of wine “sweetness”, which softens tannin perception.
Finally, the tasting parameters, wine temperature, environment, psychological state and food, influence the perception of astringency strongly.

Conclusion

Knowledge of the grape aroma and phenol potential as well as grape maturity are essential. In this article we have described numerous parameters and techniques allowing to control astringency, colour and aroma of wines. The choice and combination of these different parameters should allow to adjust the vinification in order to elaborate wines, which respond to the objectives of segmentation. Certainly, this choice will depend on other parameters (type of winery, equipment, appellation of origin, regulations…), as well.

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