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AGROALIMENTARI
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**QUALI-QUANTITATIVE STUDY ON
CHEMICAL EVOLUTION OF ACUTISSIMINS, DURING
RAPID AGEING OF RED WINE WITH OAK CHIPS**

TUTOR:

PROF. Raffaele Romano

COORDINATORE:

PROF. Salvatore Spagna Musso

DOTTORANDA:

ILARIA BORRIELLO

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CHAPTER I

RED WINE PROCESSING AND AGEING

Introduction

1.Red table wine composition and characteristics

The composition of a wine is determined by the initial composition of the grapes and subsequently influenced by the cumulative effects of the particular reactions that it undergoes during the winemaking sequence.

The combination of these effects, the grape cultivar and composition at harvest, pre-fermentation handling, fermentation conditions, microbial activity, barrel ageing and other actions constitute the ‘style’ in which the wine is made.

Red wine styles can range from the methodical, traditional ones to proactive and adaptive ones, with yet others being some combination of the two. In some wine styles, the effects of one or more of the aspects of the style (such as tannin extraction from seeds during fermentation or oak component extraction from the barrel during ageing) can dominate the flavor, color or ageing potential of the wine rather than being in balance.

In other wine styles, more subtle contributions of several aspects are sought (by deliberately controlling conditions and in some instances minimizing them in an attempt to make those of the grape flavour of central importance and the wine enjoyable to drink when young.

The vineyard site, the choice of cultivar and the cultivation of the vine will predetermine the potential for flavour and aroma components, while it is the growing conditions of the season that will determine the actual concentrations of

these components that are available to the winemaker. In varietal wine styles this is of major importance and the subsequent wine making actions will generally be aimed at maximizing the extraction and recovery of these fruit characteristics.

The extent to which the fruit composition contributes to the wine also depends on the nature and extent of extraction and the chemical changes that can accompany the subsequent treatments and conditions to which it will be exposed.

These treatments begin with the nature of the juice, skin and seeds contacting prior to, during and after the alcoholic fermentation. The impact of the ageing condition varies with the cooperage type, the source and age of wood, the contact time that is permitted and, to a lesser extent, the temperature, humidity and their diurnal variation during the ageing period. The point of induction of malolactic-fermentation and the subsequent sulphur dioxide regimen employed will have significant effects on the extent to which microbial tones are a factor in the wine. The polymerization of pigments and certain aspects of oxidation in the finished wine are also related to the way in which the wine has been handled after fermentation and during ageing.

In the recent years there has been a disturbing trend which a number of wine writers and reviewers have confused oak aroma with wine quality, and the natural response by many winemakers is to pursue heavily oaked styles in order to have their wine favourably appraised. This is however, leading towards a single, oak-aged style for most red wines that threatens to dampen out the natural variations resulting from varietal, seasonal and regional characteristics.(Fermented Beverages, Author, 2005)

In the last 90 years the wine industry has seen a revolution in the production of winemaking. This is largely due to the scientific development of wine production. Modern winemakers can now achieve almost total control of every stage of winemaking. Competition in the wine industry, however, has led to the temptation to produce cheaper and larger volumes wine at the expense of quality. Winemakers now face the challenge of producing wine for a larger market without losing character and individual flavour of their wines. Annually about 26 billion litres of wine are produced from about 8 million hectares of vineyards across the world. There is, however, a decline in consumption, which has led to a worldwide oversupply of 15-20 % (Cape Wine Academy, 2001). Fierce competition for market share has led to increased diversity and innovation within the wine industry (Pretorius, 2000). Oak chips use and additions are one of these innovations used in the industry today.

1.1.Red Winemaking

Red wine is a macerated wine. The extraction of solids from grape clusters (specifically from skins, seeds and possibly stems) accompanies the alcoholic fermentation of the juice. In conventional red winemaking, extraction of grape solids is by means of maceration, which occurs during must fermentation. Other methods exist that dissociate fermentation and maceration, such as thermovinification. The localization of red pigment exclusively in skins, at least in the principal varieties, permits a slightly tinted or white wine to be made from the colorless juice obtained from a delicate pressing of red grapes. Wines for the elaboration of champagne are a good example. The designation blanc de blanc was created to distinguish white wines derived from white varieties and those

from red. Varietal nature is not sufficient for characterizing the origin of a red wine. Maceration intensity is of prime importance. The length and intensity of maceration are adjusted according to grape variety and the type of wine desired. In fact, maceration is a means by which the winemaker can personalize the wine. Primeur wines are made to be drunk young: their aromas and fruitiness greatly outweigh phenolic compound concentrations, but premium wines require a sufficient tannin concentration to develop properly during ageing. Grape quality directly influences grape skin maceration quality in red winemaking and is thus of the greatest importance. In fact, the grape skin is more affected than the juice by cultivation techniques, maturation conditions and sanitary state. Vintage and growth rankings are therefore much more clearly defined with red wines than whites. Must acidity and sugar concentrations can fluctuate by 50% and 15%, respectively.

Enologists readily define ‘good’ tannins as those that give wines a dense structure without aggressiveness, and ‘bad’ tannins as those characterized by vegetal and astringent herbaceous savors. This highlights the need to wait until the grapes reach full phenolic maturity, which may occur later than physiological ripeness. Grape composition and quality variability result in heterogeneous grape crops. Grape selection can compensate for this heterogeneity and tanks should be filled with a homogeneous single-variety grape crop that has the same sanitary state and level of maturity. Terroir, quality, vine age, rootstock, fruit loads, and a number of other factors should be taken into consideration. Appropriate vineyard management methods are increasingly being applied to achieve the low yields essential to ensure perfect grape ripeness and high quality. This batch selection, effected at filling time, must be maintained during the entire winemaking process,

until the definitive stabilization after malolactic fermentation. The best batches are then blended together to make a wine of superior quality. The complementary characteristics of the various batches often produce a blended wine that is superior in quality to each of the batches before blending. The grape crop should also be carefully sorted to eliminate damaged or unripe grapes.

At the winery, the grapes are spread out on sorting tables. A conveyor belt advances the crop, while workers eliminate bad grapes. A concern for perfection in modern winemaking has led to the generalization of such practices. Their effectiveness is even more pronounced when they are applied to grape crops of superior quality. Red grape crop heterogeneity requires specific winemaking techniques to be adapted according to the crop. Much remains to be learned in optimizing the various grape specifications. The generalization of malolactic fermentation is another characteristic of red winemaking. This phenomenon has been recognized since the end of the last century but, until the last few decades, it was not a consistent component of red winemaking. For a long time, a slightly elevated acidity was considered to be an essential factor in microbial stability and thus contributed to wine quality. Moreover, red wine must acidification was a widespread practice. Today, on the contrary, malolactic fermentation is known to produce a more stable wine by eliminating malic acid, a molecule easily biodegraded.

1.1.1 Treatments

Red grapes are certainly less sensitive to maceration and oxidation phenomena than white grapes, but microbial contamination is likely to occur in a partially crushed harvest, left in the vineyard, especially in the presence of sunlight. These

risks must be avoided. During mechanical harvesting, the grapes are transported in high-capacity containers. Speed and hygiene are even more important in this case, since the grapes are inevitably partially crushed with this method. More generally, a dumping trailer is used, which empties its load into the receiving hopper. In high-capacity installations, the bins are placed on a platform which dumps the grapes sideways thus avoiding excessive truck and tractor manoeuvring. The grapes are initially sorted when they arrive from the vineyard. The second sorting operation, after destemming, removes any small fragments of stems and leaves, etc. that were missed during the first sorting operation, and is followed by crushing. In small wineries, they may be installed directly above the crusher-stemmer and filled directly from the transfer vehicle. In general, a perpetual screw in the bottom of the hopper regulates throughput and it should turn slowly to avoid excessive crushing of the grapes. Throughput may be increased by using a larger diameter hopper. When buying grapes according to weight and sugar concentration, these values must be determined at the time of reception. Grape crop heterogeneity complicates the determination of the sugar concentration. The sample should therefore be taken after crushing and homogenization. The sanitary condition of the grapes may also be assessed at this stage by analyzing their laccase activity. At the outlet of the crusher—destemmer, a pump distributes the grapes to a given tank. Sulfur dioxide is added at this time and any necessary addition. Grape handling should be minimized, limiting transfer distances and maximizing the use of gravity. Rough handling is likely to shred or lacerate stem tissues, so that sap is liberated from vegetal tissue and later found in wine. The suspended solids concentration simultaneously increases; in fact, this measurement may be used to evaluate equipment quality. The most

quality-oriented solution consists of sorting and destemming the grapes by hand, then crushing them, if necessary, through a wooden screen, thus eliminating the need to crush them mechanically. Finally, the must is transferred without pumping.

1.1.2. Crushing

Grapes are traditionally crushed to break the skin in order to release the pulp and the juice. This operation is probably one of the most ancient harvest treatments. Partial crushing can be obtained by the traditional technique of treading the grapes. Highspeed centrifugal crusher-destemmers assure an energetic crushing. There are also many other systems between these two extremes. The consequences of crushing are as follows:

1. The juice is aerated and it is inoculated by yeasts. The fermentation is quicker and the temperature higher. In certain circumstances, a slower fermentation speed and lower temperatures can be obtained through not crushing.
2. Aeration can be harmful. In partially rotted grapes, it can provoke an oxidasic casse.
3. Crushed grapes can be pumped, and sulfiting is more homogeneous.
4. All of the juice is fermented; at the time of running-off, the press wine does not contain sugar.
5. Crushing has a significant effect in facilitating maceration and accentuating anthocyanin and tannin dissolution.

An energetic crushing intensifies this effect. Tannin concentrations proportionally increase more rapidly than the color. This increased maceration can be an advantage in certain cases but it tends to increase the herbaceous astringency and

disagreeable tastes of average varieties. Premium wine grapes are traditionally lightly crushed to burst the berries without lacerating the solid parts. Crushing is used to facilitate fermentation and avoid residual sugar in press wines. Methods other than crushing should be used to increase maceration (vatting time, pumping-over operations, temperature). They better respect wine quality. Even when carbonic maceration is not strictly used, winemakers may wish to avoid crushing the grapes for great wines with long vatting periods, to avoid brutal damage to the plant tissues through the perforations.

1.1.3. Destemming

This operation, also known as destalking, is now considered indispensable. Destemming has a number of consequences:

1. A primary and financially important advantage of this operation is the reduction of the required tank capacity by 30%. In addition, the pomace volume to be pressed is greater with a stemmed grape crop. Although the stem facilitates juice extraction during pressing, a higher-capacity press is required.
2. Fermentations in the presence of stems are always quicker and more complete. The stem facilitates fermentation not only by ensuring the presence of air but also by absorbing calories, limiting temperature increases. Fermentation difficulties are rarely encountered with stemmed grapes.
3. The stems modify wine composition. They contain water and very little sugar, thus lowering alcohol content. Moreover, stem sap is rich in potassium and not very acidic. Destemming therefore increases must acidity and alcohol content.

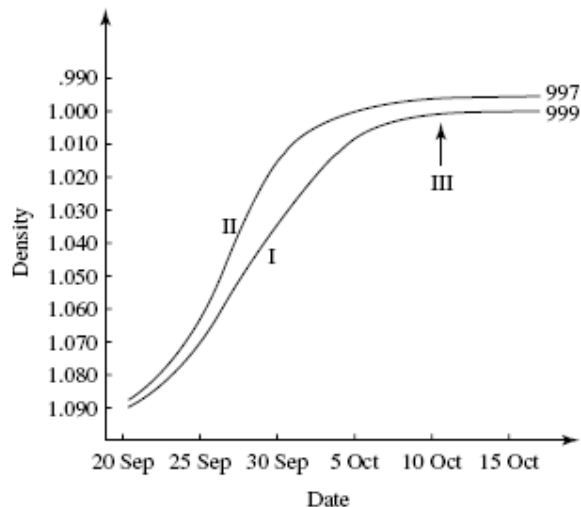


Fig. 1. Influence of stem on alcoholic fermentation (Ribereau-Gayon et al., 1976).

I: destemmed grapes. II: non-destemmed grapes. III: running-off date

4. With botrytized grapes, stems protect wine color from oxidasic casse. The laccase activity of *Botrytis cinerea* is most likely fixated or inhibited.
5. Destemming most significantly affects tannin concentrations.

Component	Juice	Juice + skins	Juice + skins + seeds	Juice + skins + seeds + stem
Color ^a :				
Intensity	—	1.81	1.40	1.17
Tint	—	0.39	0.43	0.48
Anthocyanins (g/l)	—	0.98	0.94	0.85
Tannins (g/l)	—	1.75	2.55	3.25
Total phenolic compounds (permanganate index)	5	32	47	56

^aIntensity = OD 420 + OD 520

Tint = OD 420/OD 520

(OD 420 and OD 520 = optical density, under 1 mm thickness, at 420 nm and 520 nm).

Table 1 indicates the approximate proportion of phenolic compounds supplied by the various parts of the grape cluster.

In this experiment, 54% of the total tannins come from grape skins, 25% from seeds and 21% from stems. Results may vary according to grape quality and grape variety.

Component	Destemmed	Not destemmed
Alcoholic strength (% vol.)	13.2	12.7
Total acidity (mEq/l)	86	78
Volatile acidity (mEq/l)	11.4	11.4
Total phenolic compounds (permanganate index)	38	58
Color ^a :		
Intensity	1.28	1.18
Tint	0.51	0.57

Table 2 Principal modifications of wine constitution caused by destemming.

Despite the increase in total phenolic compounds in the presence of stems, color intensity diminishes. This long-observed fact is interpreted as the adsorption of grape skin anthocyanins on the ligneous surface of the stems. This interpretation has been confirmed in a model solution containing anthocyanins and tannins; either a stem extract or the stems themselves is added. In the first case, the tannin concentration increases considerably, while the color intensity slightly increases. In the second case, the tannins increase but the color intensity decreases. Tannins play an important role in the color of mature wines. Although wines made from stemmed harvests have less color when young, they become more colored than their destemmed counterparts in the course of ageing.

6. The increased tannin and phenolic compound concentration of wines made from stemmed harvests can increase wine quality in certain cases, e.g. for young

vines and wines with insufficient structure without the stems. Yet grape stems are likely to give vegetal and disagreeable herbaceous tastes to wines.

In general, when finesse is favored, destemming is indispensable. In any case, the decision of a total or partial destemming must take into account stem quality, which is related to variety and maturity level. Crushing and destemming are generally effected by the same piece of equipment, but in certain cases it would be desirable to have the option of not destemming. For a long time, with conventional crusher–destemmers, crushing preceded destemming. Today, there is an increasing number of machines that eliminate the stems before crushing the grapes. The stems do not pass between the crusher rollers. In this manner, the risk of shredding the stems is lowered. This order of operation increases must quality, since stem shredding liberates vegetal vacuolar sap, which is bitter and astringent.

Tab.3 Influence of stem on wine composition (Ribereau-Gayon et al., 1976)

Component	Destemmed	Not destemmed
Alcoholic strength (% vol.)	13.2	12.7
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Color ^a :		
Intensity	1.28	1.18
Tint	0.51	0.57

1.1.4 Filling Vats and Related Operations

Grapes are usually received at a single winery location and transferred to the fermentation vats after destemming and crushing. Transfer pumps must do as little damage as possible to grape tissues and distances should be kept to a minimum, with as few bends as possible in the hoses. This operation can be carried out manually, without pumping. As the must increases in volume during fermentation, about 20% empty space should be left in each vat. A considerable volume of gas is released during fermentation, approximately 50 l of carbon dioxide per liter of must fermented. The grapes must be sulfited adequately and homogeneously during transfer to the vat . Several operations may be carried out during transfer of the grapes/must, or in the following few hours. Firstly, they may be inoculated with a fermenting must (a few percent corresponding to 10^6 cells/ml) or dried active yeast (LSA), *S. cerevisiae*, chosen from among the various commercial strains (over 100). The main qualities required are the aptitude to complete fermentation successfully and heat resistance. Winemakers must still ensure, however, that the strain selected is suitable for the type of wine being made.

Recommended doses of 10–25 g/hl correspond to inoculation with $2 \cdot 10^6$ – $10 \cdot 10^6$

cells/m. Indigenous yeasts must be inhibited by appropriate doses of SO₂ to ensure effective seeding. Dried yeasts must be reconstituted prior to use, by mixing them into a mixture of must and water (1:1) at 40°C. Acidity can be corrected during the initial transfer into vat or at a later time. Tannin addition during fermentation are capable of improving body and tannic structure, but also of stabilizing color by promoting condensation of anthocyanins and tannins. It is thus useful to add tannin early in the fermentation process, when the tannins have not yet been extracted from the grape seeds, so that they can react with the anthocyanins released early in vatting. According to some authors, the results are uneven due to the low solubility of the tannins and the difficulty of mixing them into the must (Blouin and Peynaud, 2001), so it is preferable to add the product after running-off. High doses (20–50 g/hl) are required to raise the initial tannin levels by approximately 10%. Another operation currently attracting some interest is the addition of pectolytic enzymes to promote extraction of phenolic compounds, for the purpose of obtaining wines with a higher tannin content, but less astringency and bitterness (Blouin and Peynaud, 2001). Care must be taken in traditional red winemaking to avoid producing off-aromas. Decreasing the quantity of must facilitates concentration of the phenolic compounds during vatting. This operation is generally carried out after the vat has been filled and the juice has been separated from the pomace. Water is eliminated (e.g. by reverse osmosis or vacuum evaporation) at the same time. The results are very similar but these methods maintain the natural grape sugars. These techniques are capable of concentrating the must by 5–10%, or even as much as 20%. Excessive concentration of the must changes the flavor balance of the wine completely and it is preferable to adapt vineyard management methods and reduce yields on the

vine to achieve similar results. After that there is the beginning of fermentation phase.

Various types of fermentor exist. They are distinguished by the aeration level supplied to yeasts and the modulation of skin contact. Aeration helps to ensure a complete fermentation, and skin contact modulation influences maceration and phenolic compounds extraction. Fermentation releases gas within the must. The bubbles rising toward the surface of the fermentor entrain solid particles, which unite and agglomerate, forming the cap. The skin cap is maintained at the top of the fermentor by the pressure of the released gas. Pomace plays an important role. First and foremost, during maceration, it yields its constituents (anthocyanins and tannins). These compounds are indispensable components of the character of red wine. Yeast multiplication is also particularly intense within the pomace: $10\text{--}50 \times 10^6$ cells/ml have been observed in the juice at the bottom of the fermentor and $150\text{--}200 \times 10^6$ cells/ml in the juice impregnating the pomace. Bacterial growth is facilitated and contamination risks are high due to the large surface area of this spongy surface. As soon as the fermentation slows, the pomace cap should be regularly immersed to drown the aerobic germs. This operation, known as cap punching (pigeage), can only be carried out manually in small-capacity fermentors. If necessary, it can be mechanically effected with a jack or another piece of equipment.

Submerging the pomace cap also contributes to the extraction of its constituents. It also aerates the must and homogenizes the temperature. The tanks must be run off before the carbon dioxide stops being released. Afterwards, spoilage risks in the pomace cap are certain and the resulting press wine would have an elevated volatile acidity. To avoid pomace cap spoilage and to eliminate the laborious

work of regularly punching down the cap, systems have been developed that maintain the cap immersed in the must, for example, under a wooden hurdle fitted to the tank after filling.

The must in contact with air is permanently renewed by the released gas. Acetic acid bacteria have more difficulty developing in this environment. The compacting of the pomace against the wooden hurdle does not facilitate the diffusion of its constituents, and several pumping-overs are therefore recommended to improve maceration.

Today, most red wines are fermented in tanks that can be closed when the carbon dioxide release rate falls below a certain level. The complete protection from air permits maceration times to be prolonged, almost as long as desired. The tank can be hermetically sealed by a water-filled tank vent or simply closed by placing a cover on the tank hatch. The tank should therefore be completely filled with wine or a slight pumping-over operation should be carried out twice a day to immerse the aerobic germs. For a long time, the major inconveniences of the closed fermentor were a considerable temperature increase and the absence of oxygen. As a result, fermentations were often long and difficult, and stuck fermentation occurred frequently. Today, these two inconveniences are mitigated by temperature control systems and pumping-over operations with aeration, permitting the dissolution of the necessary oxygen for a successful fermentation. In conclusion, actual fermentor design avoids alcohol loss by evaporation. Press wine quality is greatly increased, while the laborious work of cap punching is eliminated. This kind of tank has also been empirically observed to facilitate malolactic fermentation.

1.1.5 Fermentation

In certain cases, red wine fermentations can occur at 30°C without cooling. In any case, cooling is simplified: a cool liquid is circulated within the double wall of the tank or in an integrated thermal exchanger. Today, it has been observed that these tanks insufficiently warm the fermenting must when the ambient temperature is too low. This phenomenon is accentuated in cases where the tanks have been placed outside to lower the cost of investment. As soon as fermentation stops, the tank temperature rapidly decreases to the ambient temperature; as a result, maceration phenomena, which are influenced by temperature, are slowed. To master red winemaking, temperature-controlled (heating and cooling) stainless steel tanks are necessary. Recent developments in cooling equipment have led to renewed interest in wooden or concrete fermentation vats, where homogeneous temperatures are easier to maintain. The tank should be filled before the start of fermentation and for this reason the filling time should not exceed 12 hours. Tank shape is important for red winemaking. The exchange surface between the pomace and the juice should be sufficient. Tanks should not be too wide. In this case, pomace leaching is greatly reduced and pumping-over operations lose their effectiveness; air contact can also be excessive. Tank height should slightly exceed tank diameter. High-performance pumping-over systems can compensate for disproportionately high tanks to a certain extent. The necessary conditions for successful winemaking are known: they are adapted to the nature of the grape crop and are not difficult to carry out, as long as the appropriate equipment is available. Temperature control in particular has been essential. Although a moderate temperature (20°) is necessary to initiate fermentation correctly, the

temperature should not be excessive. Yeasts in their growth phase are particularly heat sensitive: when the initial temperature is between 26 and 28°C, the increase in temperature during the yeast growth phase makes stuck fermentation more common and increases the risk of producing excessive volatile acidity.

Establishing the temperature during fermentation is dependent on many factors concerning fermentation kinetics and skin extraction by maceration.

Stuck fermentations are likely to occur when the temperature exceeds 30°C. Slightly lower and relatively constant temperatures (25–28°C) are advised for musts with elevated sugar concentrations and in difficult fermentation conditions.

Premium quality wines capable of ageing require a maceration permitting considerable phenolic compounds extraction. Elevated temperatures play an essential role in this phenomenon. After a successful fermentation, the temperature can be raised to above 30°C to increase this extraction.

Today, must aeration or, more specifically, aeration of yeasts during their growth phase, along with temperature control, is the most effective way of helping difficult fermentations. It is carried out during pumping-over operations, or, possibly, by means of microoxygenation. Pumping-over with aeration is only beneficial at certain moments, but the pumping-over operation in general has other effects. It homogenizes the temperature, sugar concentrations and yeast population of the fermentor, compensating the effects of the more active fermentation in and just below the pomace cap. Above all, this operation facilitates extraction of compounds from the pomace (anthocyanins and tannins) and enhances maceration.

The must flows from a certain height into a container with a capacity of several hundred liters. The pressure of the falling juice produces an emulsion which

facilitates oxygen dissolution. Running the must over a flat surface is also recommended, to increase air contact. Specially equipped faucets intensify the emulsion. The aerated must is then pumped back to the upper part of the fermentor, soaking the pomace cap. However, the quantity of oxygen dissolved in must exposed to air is of the order of 6–8 mg/l and varies according to temperature. The quantities necessary to avoid stuck fermentation are approximately 10–20 mg/l, which can be obtained by pumping-over with aeration twice, 24 hours apart. Experience shows that this amount is sufficient. Nevertheless, a system permitting controlled oxygen addition (from a compressed gas bottle, for example) would be preferable. Indeed, this is the aim of process known as microoxygenation. The precise amount of oxygen necessary must also be determined. Of course, oxygen is added simply to assure yeast growth and survival, and a quantity greater than the optimum dose has no adverse effects on yeasts. Nevertheless, enzymatic oxidations in must may occur, despite the protection of carbon dioxide during fermentation. Tannins protect healthy red grape juice from excessive oxidation. For this reason, they better tolerate aeration than white grape musts, which are not generally pumped over .

Depending on operating conditions, pumping over effectiveness with respect to pomace extraction is extremely variable. Close monitoring is indispensable.

The frequency of pumping-over operations should be modulated. This operation contributes to the tannic structure of wine and favors the extraction of the highest quality tannins, making wine rich and supple, but an excessive tannin concentration can lead to hard, aggressive, disagreeable wines. Due to its simplicity and its favorable effects, pumping-over is an essential operation in red winemaking.

1.1.6 Maceration

Red wines are macerated wines. Maceration is responsible for all of the specific characteristics of sight, smell and taste that differentiate red wines from white wines. Phenolic compounds (anthocyanins and tannins) are primarily extracted, participating in the color and overall structure of wine. Yet aromas and aroma precursors, nitrogen compounds, polysaccharides (in particular, pectins) and minerals are also liberated in the must or wine during maceration. The corresponding chemical elements come from the skins, seeds and sometimes the stems. Each of these organs supplies chemically and gustatorily different phenolic compounds. The gustatory differences are confirmed by tasting wines made in the presence of one or more of these organs. Stems give wine herbaceous flavors and seeds contribute to harshness. Skins contact alone produces a supple but incomplete wine that is too fluid in structure. Skins and seeds contact makes a more balanced wine. The phenolic compounds of each organ also vary according to variety, maturation conditions and other factors. Furthermore, in the same organ (for example, in the grape skin), herbaceous, vegetal and bitter substances along with leafy and grassy substances are located alongside phenolic compounds favorable to wine quality. Consequently, the maceration should be modulated and fractionated. Only useful grape constituents should be dissolved—those positively contributing to wine flavor and aroma. The extraction of these desirable substances should be maximal, if not total.

The concentration of substances in grape tissues detrimental to wine quality increases as grape quality diminishes. This phenomenon can be verified by chewing a grape skin after the pulp and seeds have been removed by pressing the

berry between the thumb and index finger. Initially, mild savors evolve toward mellow tannins. Afterwards, vegetal sensations become increasingly bitter and aggressive. The transition rate from pleasant to disagreeable sensations varies according to grape quality. The evolution of tannin quality can be evaluated during maturation in this manner. The same experience effected on seeds leads to similar results. Harshness and astringency diminish during maturation, while sensations of body and harmony increase. An abundance of pleasant-tasting substances useful for winemaking and a lack of unpleasant ones characterize the grapes of top-ranked growths. These characteristics typify mature years, i.e. great vintages. Such wines are capable of undergoing the most intense extractions and prolonged vatting times. The resulting high tannin concentrations are necessary to ensure their long-term ageing. Lesser quality red wines, made for immediate consumption, have relatively short macerations more flaws than qualities would result from longer macerations. The extraction of pomace constituents during maceration should therefore be modulated according to grape variety and quality and also the style of wine desired. Yet each grape crop is capable of producing a given type of wine, depending on natural factors (the terroir). Premium wines require a tannic structure which should not compromise finesse and elegance. These wines are difficult to produce and require grapes of superior quality benefiting from great terroirs and great growths. Light, fruity red wines are relatively easy to obtain—grape quality is not essential, but if grape quality (variety, maturity, sanitary state, etc.) is insufficient, tannic red wines rapidly become heavy, coarse and without charm.

A number of methods are available to the winemaker to adjust extraction levels during maceration. They essentially influence tissue destruction and favor the

dissolution of phenolic compounds. Techniques are continually evolving and engineers regularly propose new solutions. Current methods:

1. brutal crushing promotes the extraction of bitter and herbaceous substances.
2. percolation of must, on the contrary, favors supple and full-bodied tannins.

Constituent extractability of various organs varies with several factors (variety, maturity level, etc.). Enzymatic reactions, activated by grape enzymes, are involved in cell wall degradation. They favor the dissolution of their vacuolar contents.

In traditional winemaking, maceration occurs during vatting (*cuvaison*), while the pomace soaks in the juice. Alcoholic fermentation occurs in the juice, producing ethanol and raising the temperature. Both ethanol and temperature participate in the dissolution of pomace constituents.

There is a current trend to distinguish between the various types of maceration, other than standard extraction during fermentation:

- 1) High-temperature extraction prior to fermentation used in thermovinification either followed by normal fermentation, or separate fermentation of the juice.
- 2) Cool-temperature extraction prior to fermentation, aimed at enhancing aromatic complexity.

The start of fermentation is postponed by maintaining low temperatures and an appropriate level of SO₂, as well as by delaying inoculation with active yeasts. A more elaborate form of this technique consists of cooling the grapes to around 5°C, by injecting liquid CO₂ or dry ice, and maintaining this temperature for 5–15 days. The temperature shock bursts the grape skin cells and releases intensely colored juice (Blouin and Peynaud, 2001). Once the must has been heated to

normal temperature, fermentation proceeds as usual. The purpose of this technique is to obtain wines with high concentrations of phenolic and aromatic compounds. The results of this rather laborious method are not universally appreciated.

3) Post-fermentation vatting is required by the best premium quality red wines to prolong skin contact after the end of fermentation, sometimes combined with an increase in temperature (final, high-temperature maceration).

1.1.7 Principles of Maceration

Maceration is controlled by several mechanisms

1. The extraction and dissolution of different substances.

Dissolution is the passage of cell vacuole contents from the solids phase into the liquid phase. This dissolution depends first of all on vine variety and grape maturity levels. This is especially important for anthocyanins. In certain cases, strongly colored musts are obtained immediately after crushing. In other cases, a period of 24–48 hours is required. Tissue destruction through enzymatic pathways or crushing facilitates dissolution. The more intense the crushing, the more dissolution is favored. Finally, dissolution depends on the various operations that participate in tissue destruction: sulfiting, anaerobiosis, ethanol, elevated temperatures, contact time.

2. Diffusion of extracted substances.

Dissolution occurs in the pomace, and the impregnating liquid rapidly becomes saturated with extracted substances; exchanges therefore stop. Further dissolution is dependent on the diffusion of the extracted

substances throughout the mass. Pumping-over or punching down the pomace cap renews the juice impregnating the pomace cap. This diffusion is necessary for suitable pomace extraction. It homogenizes the fermenter and reduces the difference between the phenolic compound concentrations of free-run wine and press wine.

3. Refixation of extracted substances on certain substances in the medium: stems, pomace, yeasts. This phenomenon has been known since Ferré's (1958) observations.
4. Modification of extracted substances. This hypothesis still requires further theoretical interpretations. Anthocyanins may temporarily be reduced to colorless derivatives (Ribéreau-Gayon, 1973). The reaction appears to be reversible, since the color of new wines exposed to air for 24 hours increases, with the exception of those made from rotten grapes. Anthocyanin-Fe³⁺ ion complex formation may be involved in this color increase in the presence of oxygen. Ethanol may destroy tannin-anthocyanin associations extracted from the grape (Somers, 1979). In the same environmental conditions, free anthocyanins are less colored than tannin-anthocyanin combinations, which are formed again during ageing and assure color stability.

The quantity of anthocyanins and tannins found in wine depends first of all on their concentration in the grape crop. Ripe grapes are the first condition for obtaining rich and colored wines. However, only a fraction of the phenolic compound potential of the grape is found in wine. Their concentration depends not only on the ease of phenolic compound extraction but also on the extraction methods used. The phenolic compound concentrations of various components of

grape clusters and wine have been compared. Approximately 20–30% of the phenolic potential of grapes is transferred to wine. The loss is significant and efforts have been made to improve this yield but, due to the complexity of this phenomenon and the molecules involved, a simple solution is difficult to find. Finally, “bleeding” a vat is a way of raising tannin levels by reducing volume.

1.2.Wine ageing

During the period from the end of the fermentations until bottling, a wine is said to be ageing. Ageing duration is highly variable according to a wine's origin, type and quality. It must be long enough to stabilize the wine, as well as to prepare great wines for bottle ageing. Many changes occur in the composition of the wine during this period, accompanied by the development of color, aroma and flavor. The conditions under which wine is stored and handled, as well as the types of container used, have a very marked effect on these developments, which are closely connected with oxidation–reduction phenomena that take place in the wine. The ageing of red wine should be characterized by harmonious development of the various components of color, aroma and flavor. The color gradually changes from cherry red to deep red and then brick red. The oldest wines even take on an orange tinge. The flavor also evolves, becoming softer, with less astringency. There is, however, a risk that the wine may become thinner and dry out on the palate as it ages. Furthermore, the rate at which these changes occur is different for each wine, depending on both outside conditions and the wine's specific composition:

1. External conditions include oxidative phenomena (O_2 and SO_2), temperature and time. A great deal of research has focused on the ageing of wines prior to bottling (Pontallier et al., 1980; Pontallier, 1981; Ribéreau-Gayon et al., 1983; Glories, 1987; Chatonnet et al., 1990, 1993b; Vivas and Glories, 1993a, 1993b, 1996).
2. The way a wine ages depends on its phenol composition, characterized by the total quantity of phenols (OD 280), the ratio of the various pigments

(tannins/anthocyanins) and the type of tannins (seed tannins consisting of procyanidins polymerized to varying degrees and skin tannins with more complex structures) The presence of polysaccharides of both plant and yeast origin also affect ageing potential. Anthocyanins and tannins extracted from grapes are involved in various reactions that depend to a great extent on external conditions and produce a variety of compounds These reactions include degradation, modification, and stabilization of the color, polymerization of tannins and condensation with other components. These reactions are summarized in Figure 2

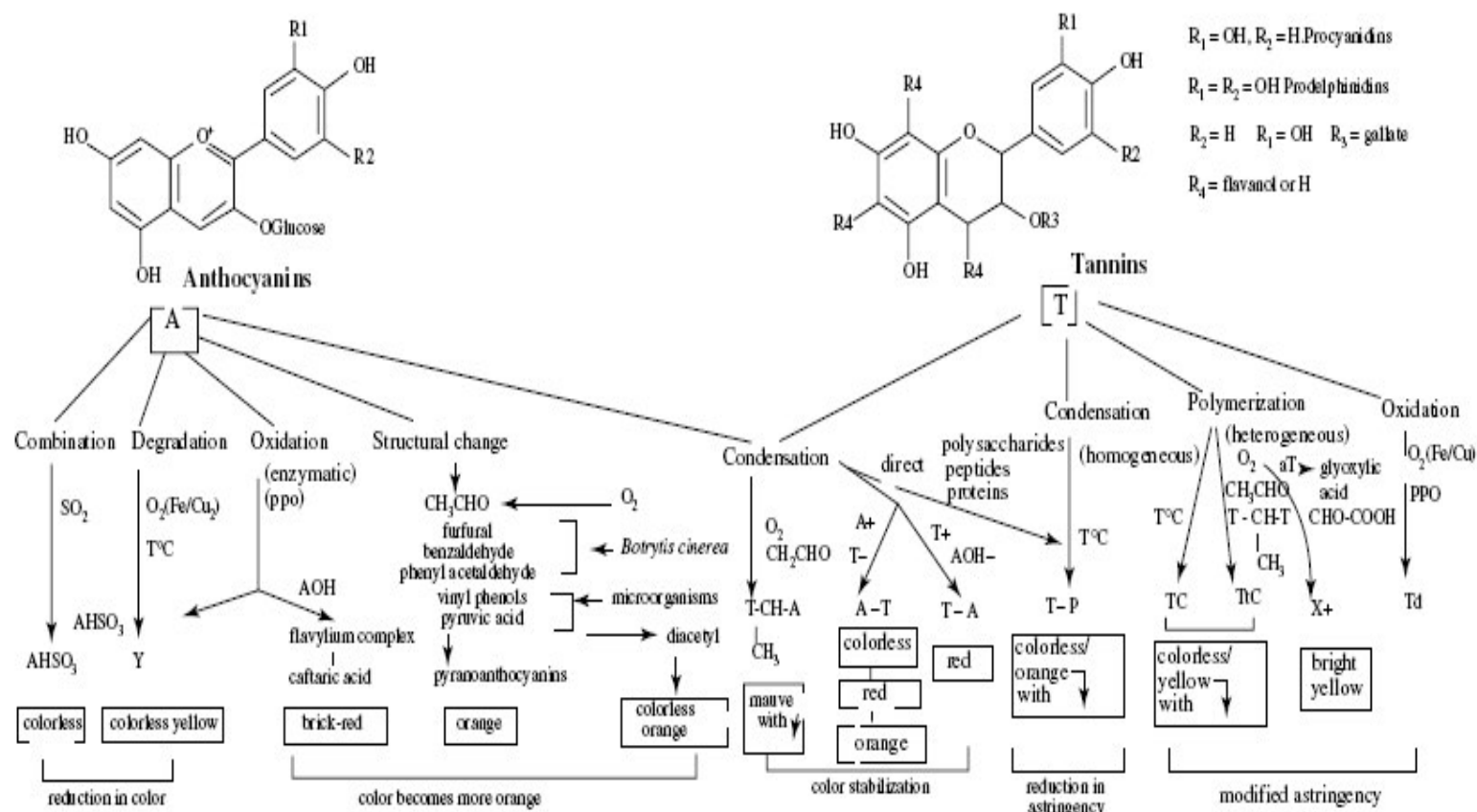


Fig2. Changes in phenols (A, anthocyanins; T, tannins) in red wine during ageing.

Impact of these reactions on organoleptic characteristics. (Glories, 2003, unpublished) \downarrow = precipitation; Y = anthocyanin degradation products (phenol-acids); TP = tannin-polysaccharide and tannin-protein combinations T-A = tannin anthocyanin combinations TC = condensed tannins; TtC = highly condensed tannins; X+ = xanthylum structure; aT = tartaric acid; Td = degraded tannins; ppo = polyphenol

The main consequences of these reactions involving phenols in red wines are changes in color intensity, a tendency to develop a yellow–orange hue (generally accompanied by loss of color) and various modifications in the tannins, responsible for their gradual softening.

1.2.1 Traditional Ageing Treatments

Use of Barrels

During wine ageing various stages take place. After the elaboration of wine (young wine) there follows a period of maturation, which encompasses all the changes that occur between alcoholic fermentation and bottling. During maturation the wine may be put into oak barrels. Wine stored in contact with oak wood undergoes important modifications. The wine fermentation compounds evolve depending on the type of wine and the conditions of ageing. Oak wood confers numerous specific compounds to wine, many of which are formed during toasting of the wood. Since barrel oak wood is a porous material, barrel storage allows a wine to undergo processes associated with so-called ‘low oxidation conditions’ (Vivas & Glories, 1993). Wine can also acquire complex aromas as well as stabilize its colour and it spontaneously clarifies in barrels (Jackson, 1994). Extraction of volatile compounds from oak barrels depends mainly on the quantity of compounds that are potentially extractable, on the contact time between wine and oak wood and on the wine composition. However, compounds extracted by wine from barrels undergo transformations, mainly microbiological ones, which modify the concentration of these substances in wine over time (Spillman, Iland, & Sefton, 1998). In addition, wine compounds can be sorbed by

wood and by wine lees (Chassagne, Guilloux-Benatier, Alexandre, & Voilley, 2005), so that this factor can also have an influence on wine volatile composition. A non-insignificant problem is that in wines aged in barrel, mainly in barrels which have been reused, ethylphenols could be formed and these are undesirable compounds for wine quality since they confer unpleasant odours to wine. These compounds have a microbiological origin; some yeasts capable of contaminating wood (*Brettanomyces*/*Dekkera* genera) decarboxylate cinnamic acids and form these phenols in wines (Chatonnet, Dubourdieu, Boidron, & Pons, 1992). All these processes give some idea of the enormous complexity of factors, which can have an influence on the final quality of wine aged in barrels. At present, for cost-effective reasons, alternatives to the oak barrel are being looked at to carry out the wine-ageing process. Consequently, alternative forms have been tried in the past few years to try to create conditions similar to those provided by oak barrels. This technique consists of adding oak chips to wine in order to impart a woody aroma and taste to the wine. During this process wine can also be micro-oxygenated. Micro-oxygenation is the controlled introduction of oxygen into wine, mirroring the slow oxidation found with barrel ageing.

In this PhD work, we look at the works carried out on these aspects that influence the quality of wine aged in oak barrels up to the present day. In order to illustrate the evolution of a wine so treated. Firstly, the influence of the wine composition on the extraction of volatile compounds from the wood is considered. Secondly, the influence of the oak wood composition on wine quality is studied. Thirdly, the influence of storage time on the volatile composition of wine is discussed. Fourthly, the formation of ethylphenols in wine aged in oak barrels is presented. Fifthly, the sorption of some wine volatile compounds by wood and by lees is

looked at. Finally, the use of modern methods for wine ageing is examined and described in order to explain the aim of this work and the changes observed in a wine aged with oak chips which are one of these new ageing methods.

1.2.2 Influence of wine composition on extraction of volatile compounds from wood.

Few studies exist about this question and some have been made with synthetic wines with alcohol levels unreachable in wine. Maga (1989) investigated the extraction of cis- and trans-oak lactones, quantified together, from American oak wood (*Quercus alba*) macerated in model wines with different concentrations of ethanol (0, 10, 20, 40 and 60%). In this study, the highest concentration of oak lactones was reached in the samples of 40% ethanol. Likewise Puech (1987) found that the extraction of phenolic aldehydes from Bulgarian oak shavings (*Quercus sessilis*) was higher in a hydroalcoholic medium at 55% than in a medium with 10% of ethanol. More recently, Ortega-Heras, González-Huerta, Herrera, and González-Sanjosé (2004) found that the variety of grape has an influence on the extraction of compounds from the barrel by the wine. Garduño-Cerdán, Torrea-González, and Ancín-Azpilicueta (2004) studied the extraction process of volatile compounds from oak wood during the ageing of wines with different alcohol levels and pH (Merlot wine: alcohol level, 13.6% v/v and pH, 3.7; Cabernet Sauvignon wine: alcohol level, 12.3% v/v and pH, 3.45). In this study, it was found that a greater alcohol level favoured the extraction of volatile compounds from oak wood to a significant extent (Fig. 3).

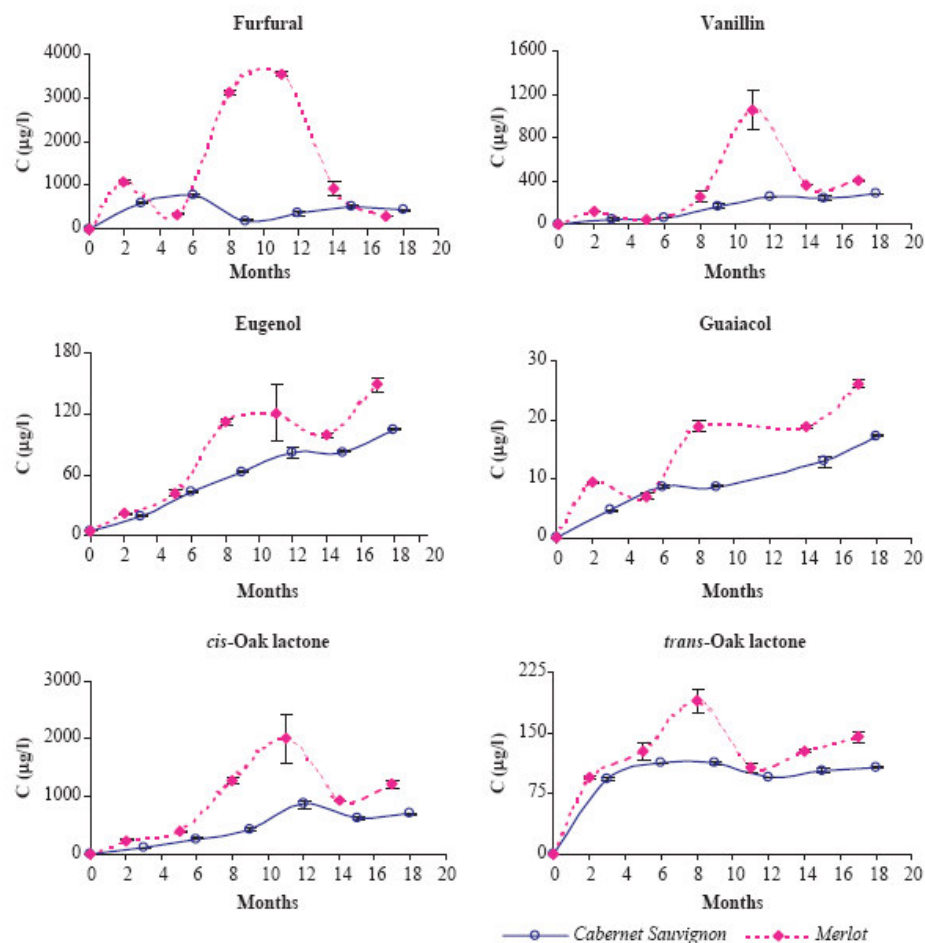


Fig.3 Evolution of the concentration (mg/l) of volatile compounds for Cabernet Sauvignon wine (alcohol level, 12.3% v/v; pH, 3.45) and Merlot wine (alcohol level, 13.6% v/v; pH, 3.7) during maturation in oak barrels (From Garde-Cerdán et al., 2004).

Similarly, it was observed that the pH of the wine had less influence on the extraction process than the alcohol level because the accumulation of oak compounds was higher in Merlot wine, with higher alcoholic concentration, than in the Cabernet Sauvignon wine, with lower pH. The concentration of SO₂, additive normally used in vinification, can also affect the concentration in wine of compounds with carbonyl groups coming from oak wood. Ancín, Garde, Torrea,

and Jimenez (2004) found that SO₂ combined with 5-hydroxymethylfurfural, vanillin, syringaldehyde and coniferaldehyde delaying their free occurrence

1.2.3 Composition of oak wood

The oak wood composition depends principally on the oak species and its geographical origin, as well as on the seasoning and toasting of the wood and the number of times the barrel has been used. The species of oak traditionally used for the ageing of alcoholic beverages are *Q. alba* (the so-called American oak), and *Q. sessilis* and *Quercus robur* (the so-called French oaks). The first two species are mainly used for the ageing of wine while *Q. robur* is used for the ageing of cognac. Several studies have been carried out to characterize the wood from different oaks. These studies show that this wood presents a wide variability as its composition varies depending on its geographical origin (Ancin et al., 2004; Mosedale, Puech, & Feuillat, 1999), its species (Feuillat, Keller, Sauvageot, & Puech, 1999; Mosedale, Feuillat, Baumes, Dupouey, & Puech, 1998) and on the tree itself (Doussot, Pardon, Dedier, & De Jeso, 2000; Masson, Moutounet, & Puech, 1995; Mosedale & Ford, 1996). Consequently, the cooperage oak selection should not be based only on oak species but rather on the combination species-origin. The only generalization that can be made on this subject is that American white oak species give a greater quantity of cis-oak lactone to the wine compared to European oaks. Several studies confirm this affirmation (Díaz-Plaza, Reyero, Pardo, Alonso, & Salinas, 2002; Garde-Cerdán, Rodríguez-Mozaz, & Ancin-Azpilicueta, 2002; Gómez-Plaza, Pérez-Prieto, Fernández-Fernández, & López-Roca, 2004; Marco, Artajona, Larrechi, & Rius, 1994; Pérez-Prieto, López-Roca, Martínez-Cutillas, Pardo-Mínguez, & Gómez-Plaza, 2002;

Towey & Waterhouse, 1996). Consequently, the ratio cis/trans can be used to distinguish those wines aged in American oak barrels and those wines aged in French oak barrels (Waterhouse & Towey, 1994). After being cut down the wood is submitted to a drying process to ensure the mechanical resistance of the barrels. During natural seasoning of the staves, the concentration of volatile compounds coming from the wood can change. Thus, the concentration of phenolic compounds and oak lactones increases during seasoning (Cadañá, Muñoz, Fernández de Simon, & García-Vallejo, 2001, Chatonnet, Boidron, Dubourdieu, & Pons, 1994). In order to give form to the barrels, oak wood should be heated. In cooperage, three types of toasting are used: light, medium and heavy. This stage is considered as having the most important influence on the chemical composition of oak wood. The thermal treatment generates thermo degradation of some components of oak wood, which produces numerous volatile compounds. Furanic compounds are formed through thermal degradation of carbohydrates; volatile phenols come from the thermal degradation of lignin and oak lactones are products of the dehydration of the acids present in wood. Chatonnet, Boidron, and Pons (1989) found that medium toasting corresponds to the maximum synthesis of the volatile compounds. The number of times the barrels are used is another factor that determines the oak wood composition. Due to the fact that the pool of oak extractives in a barrel is finite, the quantity of these compounds and their rate of extraction generally diminishes with the utilization of the barrel over successive years (Garde-Cerdán, Rodríguez-Mozaz et al., 2002; Pérez-Prieto et al., 2002; Towey & Waterhouse, 1996). As can be seen on Table 4, the compounds which tended to become particularly exhausted due to barrel use were furfural, followed by volatile phenols, with the exception of eugenol, as this compound is also found

in wine; the oak lactones, in general, were less exhausted than the above-mentioned compounds.

Compounds	New barrels ^a		Barrels with 3 uses ^b		Barrels with 5 uses ^c	
	American oak	French oak	American oak	French oak	American oak	French oak
Furfural	~4500	~4700	n.s.	n.s.	90	148
Vanillin	n.s.	n.s.	~90	~50	15	30
Eugenol	~22	~22	n.s.	n.s.	20	18
Guaiacol	~53	~49	~10	~10	6	5
4-Methylguaiacol	~15	~15	~8	~8	0.05	0.03
cis-Oak lactone	~225	~120	~120	~80	129	66
trans-Oak lactone	~40	~90	~10	~25	26	41
n.s., not study.						
^a Chardonnay wine aged in barrels for 7 months (From Towey & Waterhouse, 1996).						
^b Monastrell wine aged in barrels for 6 months (From Pérez-Prieto <i>et al.</i> , 2002).						
^c Blend wine (Tempranillo 41% and Cabemet Sauvignon 59%) aged in barrels for 12 months (From Garde-Cerdán, Rodríguez-Mozaz <i>et al.</i> , 2002).						

Tab.4 Mean concentrations (mg/l) of volatile compounds in wine aged in barrels with different number of uses (new barrels, barrels with three uses, barrels with five uses)

Different studies (Garde-Cerdán, Torrea-Goñi, & Ancín- Azpilicueta, 2002; Garde-Cerdán, *et al.*, 2004; Jarauta *et al.*, 2005; Puech, 1987) have found that the concentration of phenolic aldehydes is maximal after 10–12 months of ageing. Of this group of compounds, vanillin is the most important and it can have a great influence on wine aroma as its perception threshold is low. Just as happens with the furanic aldehydes, for short ageing periods, vanillin accumulates in wine (Table 4), because at the beginning its extraction is high, due to the difference of concentration between the wine and the wood (Garde-Cerdán & Ancín-Azpilicueta, 2006; Gomez-Plaza *et al.*, 2004). However, when the ageing period is long, it can be transformed into vanillyl alcohol so that the concentration of vanillin can decrease or show slight fluctuations. Phenolic alcohols are more stable than the above-mentioned compounds, since they do not seem to undergo transformation during ageing (Garde-Cerdán & Ancín-Azpilicueta, 2006;

Go´mez-Plaza et al., 2004; Jarauta et al., 2005). Therefore their concentration in wine depends on the rate in which they are extracted.

Perez-Prieto, Lopez-Roca, Martinez-Cutillas, Pardo-Minguez, and Gomez-Plaza (2003) found that 4-methylguaiacol needed three months to reach its highest concentration and guaiacol continued to be extracted for up to 9 months of ageing (Table.4). When the ageing period is longer, the concentration of phenolic alcohols increases slowly or remains practically constant. Phenolic alcohols, which possess toasty and spicy aromas, do not normally reach their threshold level in wines. Nevertheless, in a complex medium such as wine, these compounds may influence wine aroma at lower levels than their individual threshold values by means of additive, synergistic, or suppressive effects (Pe´rez-Prieto et al., 2003). Cis and trans-Oak lactones are found in wine in equilibrium with their acidic form and their corresponding ethyl ester (Waterhouse & Towey, 1994). The quantities of these compounds found in wine by Garde-Cerdan, Torrea-Gon´i et al. (2002) using twice-used barrels for 18 months were smaller than those published by Pe´rez-Prieto et al. (2003) who employed once-used barrels for 9 months ageing. The difference in the quantities extracted can be due to the exhaustion of lactones in the barrels. On the other hand, these compounds underwent small modifications in their concentration throughout ageing. Consequently, it would seem that transformation of these compounds is not as rapid as in the case of the furanic compounds. The cis isomer is regarded as among the most important of the volatile components of oak wood that are extracted into wine during barrel ageing. This compound is generally found at above its perception threshold.

1.2.4 Employment of new technologies for wine ageing

The use of oak barrels for wine ageing involves a high cost outlay for the wineries. Consequently, over the past few years alternatives to this traditional method have been looked for. Before looking at some of these alternatives it is important to stress that in Europe, at present, these new technology alternatives are permitted by law with limitation. However, in the rest of the wine-producing countries there are no laws about this option and so wineries are at liberty to choose the ageing method that best suits them. In recent years, the addition of oak chips has been used to introduce desirable oak aromas and flavours into wines. Since wood is being put into the wine and not wine into wood, the entire surface area of the wood is usable and not just 40% of it (Stutz, Lin, & Herdman, 1999). Perez Coello, Gonzalez-Vin~as, Garcia-Romero, Cabezudo, and Sanz (2000) added wood chips from American oak and French oak (Allier, Central France, and Vosges) at the beginning of the fermentation of Airen must. The amount of chips used was 4, 7 and 14 g/l of musts. These authors found that the minimum concentration of wood chips of American and Vosges oak necessary to reach the sensory threshold of cis oak lactone (90 mg/l in white wines; Chatonnet, 1991) was 5 g/l, whereas that of Central and Allier was approximately 8 g/l. With regard to the sensory analysis, they found that the woody character was practically undetectable at 4 g/l, unpleasantly strong at 14 g/l, and generally detectable and easily identified at 7 g/l. Gutierrez Afonso (2002) carried out a sensory study in order to compare wine from the Listan Blanco variety fermented in new French and American oak barrels and in tanks where oak chips were added, using two doses (4 and 8 g/l). This author found that oak chips produced in the wines a

greater intensity of wood aromas (coconut and vanillin) and a greater taste impact (bitterness and astringency) than oak barrels and he concluded that the use of each technique of elaboration should be recommended according to the sensory profile desired in the wine. In a recent study, Arapitsas, Antonopoulos, Stefanou, and Dourtoglou (2004) studied the extraction of volatile compounds by Asyrtiko wine aged during 14 days in contact with oak chips of two different sizes: 1 cm!1 cm!1 cm and 3.4 cm x 2 cm x 1 cm. The authors do not name the species of oak used. Of the compounds studied (furfural, guaiacol, eugenol, oak lactones, vanillin and syringaldehyde) only the final concentration of guaiacol showed differences in regard to the size of the oak chips. A higher level of this compound was found when the larger-sized chips were used for ageing the wine. Only one study has been found, made by del Alamo Sanza, Nevares Domínguez, Ca´rcel Ca´rcel, and Navas Gracia (2004) where the same wine is aged in oak barrels, with oak chips and with oak staves. In this study, the extraction of low molecular weight phenolic compounds, among these vanillin, was studied in a red wine aged during 12 months in three different ageing systems: 225 l barrels, 100 l stainless-steel tanks with oak chips and 100 l stainless steel tanks with oak staves. The quantity of oak chips and staves necessary to reproduce the surface/volume relation of a 225 l barrel are calculated accordingly from the 2.04 m² surface area of the barrels. Every system was manufactured from three different kinds of oak (American, French and Hungarian). The results obtained showed that vanillin was extracted more by the wine in contact with oak chips than by the wine in contact with oak staves and by the wine aged in oak barrels (Fig. 4). These latter two systems of ageing gave the wine very similar concentrations of vanillin. No studies have been found where the combined effect of the use of oak chips with micro-oxygenation

was studied in the wine volatile composition. This combined system of oak chips and small doses of oxygen imitates the conditions of traditional ageing more closely than just the use of oak chips.

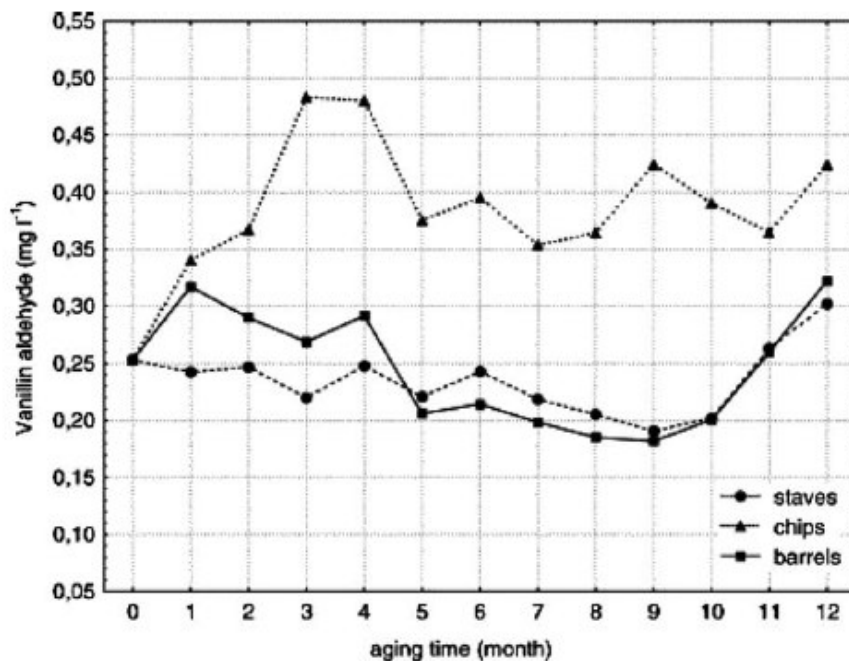


Fig. 4. Changes in the vanillin concentration (mg/l) during ageing of the same wine in three different oak ageing regimes (From del Alamo Sanza et al., 2004).

1.2.5 Pieces of Oak wood used in wine ageing: Typology and European Legislation

Today the addition of wood-shaving chips in the EU countries is forbidden for typical wine but allowed in commercial ones; although it is allowed in other countries, such as Hungary, Slovenia, Switzerland, Chile and Argentine (Spillman, 1999). From an economic point of view, the practices of using barrels or chips involve widely different costs; it is clear that the exclusive use of new barriques markedly affects the final price of a wine (Morris, 1992), while multi-year use can reduce the costs of containers (Spillman, 1999).



Oak Barrel



Oak Chips

1.2.6 Type of wood pieces

The different typologies of wood pieces applied in enology are shortly described below:

Chips and staves are the most commonly used but there are a rapid diffusion on international markets of new shapes called xoakers e cubes.

- Oak Chips are pieces of wood from 1 cm lenght (big) to rice seed (medium) or powder (small).



Oak Chips(big)



Oak Chips(medium)

- The oak staves are staves of 25 or 75mm large and 10mm deep and maximal length of 1m.



Staves

- Xoakers are wood spheres of 1inc.diameter.



Xoakers

- Oak cubes (beans) which are approximately 3/8" cubed The cubes are put in the stainless steel barrel like a tea bag and left to soak in the wine for however long the winemaker chooses.



Cubes

By the mid 1980s the use of new oak in inexpensive wine was taking hold, and wine drinkers around the world were assuming they would get the heady aromas and characteristics only oak can impart to a wine, even with the less expensive wines, but new oak costs. An American oak barrel costs about \$300-\$330. Oak staves should be properly seasoned for three years before they are used for barrels or cubes, sometimes referred to as beans. French oak barrels cost about double that, i.e., around \$600 per barrel. A barrel of wine holds approximately 300 bottles of wine, so the additional cost of oak barrels is \$1 to \$2 per bottle of wine.

This cost can be lessened over time. A winery might use a barrel for three of four years, and put 20% of a wine in new oak, 20% in one year old oak, etc., and then blend the wine to create the finished product. Using all new oak can destroy the natural characteristics of the wine, and create an overly oaked wine. The use of new oak must be judicious. Even though all new oak is not used, thereby lessening the cost, the maintenance of oak barrels is about \$50-\$60 a year per barrel, old or new. Another cost. So for these reasons there are the use of alternatives, which are oak staves, oak chips, and oak cubes (beans).

The oak stave method is done by cleaning out the old oak barrel, and lining it with new oak staves. Depending on what type of oak is used, American or French or Hungarian (Not that common), the cost per barrel can be from \$50 - \$100.

The use of oak cubes (beans) which are fire toasted just like the staves, cuts the cost to about \$30 per barrel, i.e. stainless steel barrel.

The use of oak chips, which is an inferior product to cubes and staves, it is nonetheless used to cut costs. Who uses it and when is not always known, although some tasters can taste the use of chips immediately, because chips leave

a bitterness or harshness that cubes, staves, and barrels do not. Chips are often used in bulk wine to add color and structure only.

1.2.7 Legislative information

On december 2006 the European Union gave the go-ahead for its winemakers to start using oak chips, along with five other new enological practices. Introduced by European Regulation 2165-2005, the conditions outlining the implementation of some of the practices still remain to be defined.

With regard to oak chips, OIV (Organisation internationale de la vigne et vin) recommendations are being taken into account; however, individual states reserve the right to limit their use of oak chips to certain wine categories. Italy has indicated that it plans on extending usage to its DOC (Vino a Denominazione di Origine Controllata) wines whereas Spain is keen on preserving the traditional winemaking practices of its DO (Denominacion de Origen) categories. In France, the right to openly use oak chips for commercial purposes has long been awaited by vins de pays vintners and wine merchants. Over the past decade some producers have been flavoring their wines with chips under the cover of authorized experiments, a loophole enabling winemakers to add them to up to 20 percent of their production. European industry operators are confident that the new range of practices will offer a second wind to wines situated in the lower price brackets. (Web page)

Actually REGULATIONS (CE) N1507/2006 regulated the employment of oak wood pieces in wine making.

Concerning certain detailed rules implementing Regulation (EC) No 1493/1999 on the common organisation of the market in wine, as regards the use of pieces of oak wood in winemaking and the designation and presentation of wine so treated

Here are defined the requirements for use of pieces of oak wood in winemaking:

PURPOSE, ORIGIN AND AREA OF APPLICATION

Pieces of oak wood are used in winemaking, to pass on certain characteristics of oak wood to wine.

The pieces of oak wood must come exclusively from the *Quercus* genus.

They may be left in their natural state, or heated to a low, medium or high temperature, but they may not have undergone combustion, including surface combustion, nor be carbonaceous or friable to the touch. They may not have undergone any chemical, enzymatic or physical processes other than heating. No product may be added for the purpose of increasing their natural flavour or the amount of their extractible phenolic compounds.

LABELLING

The label must mention the origin of the botanical species of oak and the intensity of any heating, the storage conditions and safety precautions.

DIMENSIONS

The dimensions of the particles of wood must be such that at least 95 % in weight are retained by a 2 mm mesh filter (9 mesh).

PURITY

The pieces of oak wood may not release any substances in concentrations which may be harmful to health. This treatment is to be recorded in the register referred to in Article 70(2) of Regulation (EC) No 1493/1999.(fig...)

Indications authorised for use on wine labelling pursuant to Article 22(3)

"barrel fermented"	"barrel matured"	"barrel aged"
"oak-cask fermented"	"oak-cask matured"	"oak-cask aged"
"cask fermented"	"cask matured"	"cask aged" ' "

Tab.5. Indication on wine labeling after ageing with oak chips

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CHAPTER II

TANNINS AND PHENOLS IN WINE

During red wine production tannins can be extracted from different sources. During fermentation condensed tannins are extracted from the skins and seeds (Riou *et al.*, 2002; Sun *et al.*, 1999; Zimman *et al.*, 2004) or external seeds can be added during the fermentation to increase the condensed tannin concentration (Kovac *et al.* 1992, 1995). Most wines also receive some kind of oak contact during the maturation of the wine whether from barrel maturation, chips or staves. During this stage hydrolysable tannins are extracted (Puech *et al.*, 1999; Quinn and Singleton, 1985; Vivas and Glories, 1996) which, together with oxygen, can induce the indirect polymerisation of proanthocyanidins (Vidal *et al.*, 2004; Vivas and Glories, 1996). Phenols and more specific tannins are of great importance in wine. They play an important role in oxidation reactions, the maturation and ageing of wine, as well as the organoleptic properties. Tannins can be divided into two groups: 1) condensed tannins or proanthocyanidins that originate from the grapes and can be further divided into procyanidins and prodelphinidins, as well as 2) hydrolysable tannins that are extracted from wood.

At this stage ellagitannins are the only hydrolysable tannin that can be extracted from oak (Puech *et al.*, 1999), but gallotannins can be added to wine in the form of commercial tannin extractions from nutgalls. This report will give a short overview of the major phenols and condensed tannins present in wine and the influence of hydrolysable tannin extractable from wood during ageing.

2.1 Phenols in grapes and wine

The phenolic composition of wine depends not only on the phenolic composition of the grapes, but also on the winemaking conditions that influence the extraction of the phenols. Winemaking processes such as cold soaking, maceration temperature and punch downs influence the extraction of phenolics (Oberholster, 2003). The location of the phenols in the grapes is 1% in the pulp, 5% in the juice, 30-50% in the skins with the rest of the phenols in the seeds (Zoecklein *et al.*, 1995). Even during extended skin maceration only 50% of the available phenols in the skins are extracted, while 60% of the available phenols in the seeds are extracted during fermentation (Ribéreau-Gayon *et al.*, 1998; Zoecklein *et al.*, 1995). Phenols can be divided into two groups namely the non-flavonoids and flavonoids (Ribéreau-Gayon *et al.*, 1998).

2.1.1 Non-flavonoids

Benzoic acid and cinnamic acid derivatives are the main non-flavonoids present in grapes and wine (Figure 4). In grapes these non-flavonoids are usually bound to glucose and esters. Organoleptically they do not have any odour or taste. Non-flavonoid concentrations are in the order of 10-20 mg/L in white wines and 100-200 mg/L in red wines (Ribéreau-Gayon *et al.*, 1998).

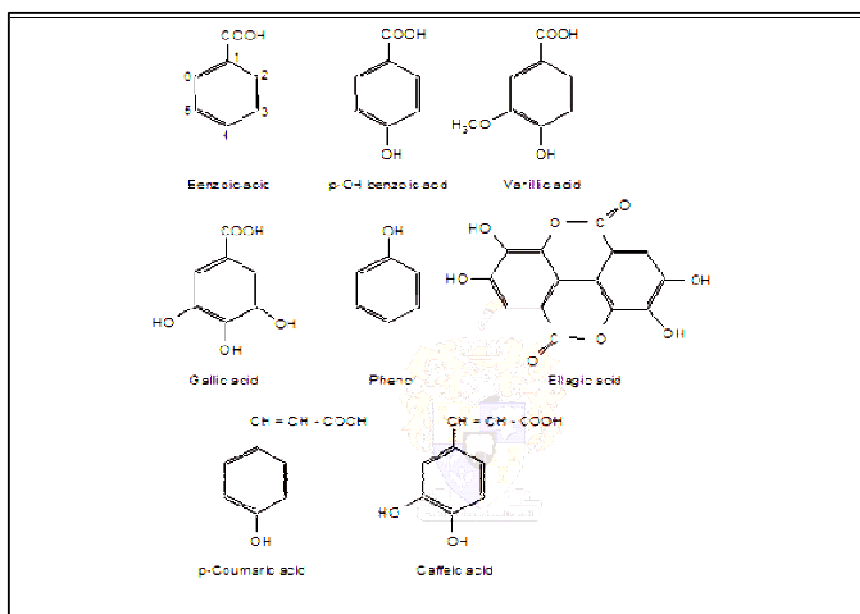


Figure 4 Examples of different non-flavonoids in grapes and wines (Ribéreau-Gayon *et al.*, 1998).

2.1.2 Flavonoids

Flavonoids are more complex than non-flavonoids and consist mainly of two benzene cycles bonded by an oxygenated heterocycle (Figure 5) (Hagerman, 2002; Monagas *et al.*, 2005; Ribéreau-Gayon *et al.*, 1998).

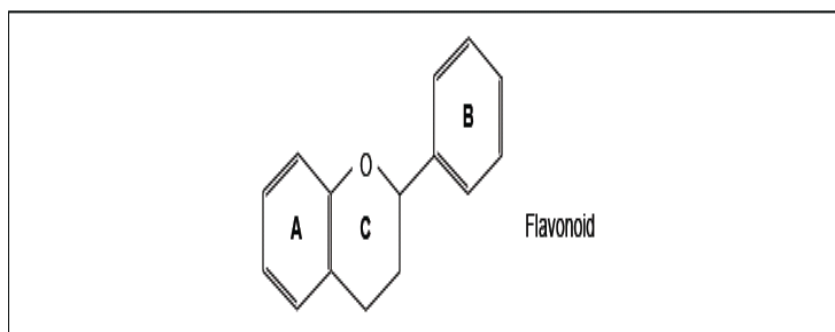


Figure 5 Flavonoid skeleton with the standard letters system.

2.1.3. Flavonols

Flavonols (Figure 6) are usually esterified to glucose at position 3 of the C ring. They occur mainly in the skins and are yellow in colour. They are efficient UV screens that can protect the bound pigment from photo-oxidative degradation (Sweeny *et al.*, 1981). In red wine, they usually disappear over time. Examples of important flavonols in wine are quercetin and kaempherol (Ribéreau-Gayon *et al.*, 1998).

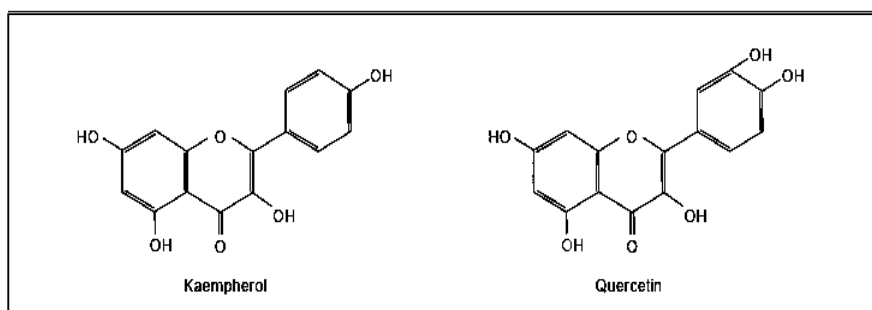


Figure 7 Examples of two flavonols, kaempherol and quercetin, in wine.

Flavan-3-ols

Flavan-3-ols are characterised by an OH group at position 3 of the C ring (Figure 7). Catechin and epicatechin are the natural occurring flavan-3-ols in grapes. When there are three OH groups on the B ring, gallocatechin and epigallocatechin

are formed. They can also have a gallic acid acylated at position 3 of the C ring and are then known as catechin-3-O-gallate or epicatechin-3-O-gallate. The polymers of flavan-3-ols are called proanthocyanidins or condensed tannins. Singleton and Esau (1969) found that the catechin and epicatechin concentrations in white wines range from 10-50 mg/L, while it may reach 200 mg/L in red wines.

2.1.4 Flavan-3,4-diols

Flavan-3,4-diols are characterised by an OH group at position 3 and 4 of the C ring (Figure 8). They react in the same manner as flavan-3-ols and can thus also polymerise to form condensed tannins (Ribéreau-Gayon *et al.*, 1998; Zoecklein *et al.*, 1995).

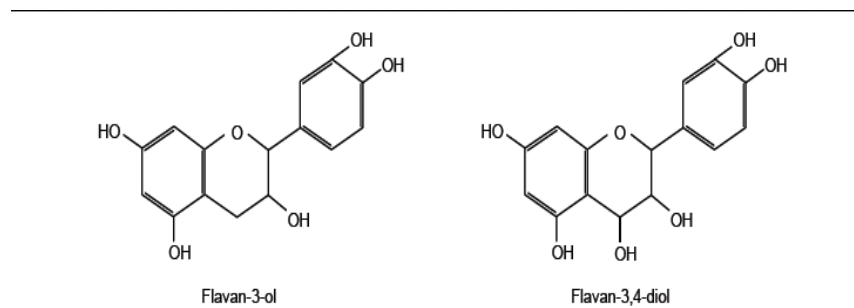


Figure 8 Basic structures of a flavan-3-ol and a flavan-3,4-diol.

2.1.5 Anthocyanins

When anthocyanidins are esterified to glucose, it is known as anthocyanins. This is the stable form that occurs in red grape skins and red wine. The anthocyanins are important for the red colour. Anthocyanin concentrations vary according to the wine age and cultivar. Usually it is present in concentrations between 100 and 1500 mg/L in wine (Monagas *et al.*, 2005; Ribéreau-Gayon *et al.*, 1998; Somers, 1971). Colour of wine is an important quality parameter and is normally

associated with the phenolic structure of the wine. Colour extraction normally reaches a maximum before the end of fermentation, but other phenols are still being extracted with extended skin maceration (Gil-Munoz *et al.*, 1999). Anthocyanins have a positive (+) charge on the C-ring, which is responsible for the reactivity of the anthocyanin and also for the absorption of green light, with red light being reflected. Wine pH has an influence on the charge and hence the colour of the anthocyanin. Depending on the pH, the anthocyanin can be in four different forms, these are: flavylium ion (red), carbinol base (colourless), chalcone (yellow) and quinoic base (violet). At a pH lower than 2.5 more than 50 % of the anthocyanins are in the red form (flavylium ion) and at a pH higher than 2.5 more than 50 % in the colourless form (carbinol base). At normal wine pH, about 25 % of the anthocyanins are in the red flavylium form. Other components in the wine (copigments) can also influence colour changes by copigmentation which results in a bathochromic shift (change to violet) and hyperchromic shift of the maximum absorbance (increase in intensity) (Ribéreau-Gayon *et al.*, 1998; Zoecklein *et al.*, 1995). Copigmentation is the association of anthocyanins with copigments to increase and stabilise their colour. These copigments include flavonoids, non-flavonoids, phenols, aminoacids and organic acids. Darias-Martín *et al.* (2001) investigated the copigmentation effect of caffeic acid and catechin on anthocyanins and wine colour. They found that caffeic acid addition enhanced the colour drastically and that catechin addition showed a slight increase over time when these copigments were added before fermentation. Malien-Aubert *et al.* (2002) explained the smaller increase in colour where catechin is added compared to the addition of caffeic acid. They found that when monomers and dimers bind to anthocyanin, yellow coloured xanthylium salts are formed that decreases the

red colour. They also showed that the stability of the anthocyanin increases with an increase in polymerisation of the copigment.

2.2 Tannins in general

The term tannin originates from the Celtic word which means oak. It is widely used in commercial leather tanning, protection of fishing nets and protection of metal drainage pipes. Saucier *et al.* (1998) defined tannins as secondary plant metabolites that are water-soluble polyphenols and have the ability to precipitate proteins and complex carbohydrates. This property is only found in polyphenols above a certain molecular weight (500-3000) (Puech *et al.*, 1999). Tannins in wine can be divided into two groups namely hydrolysable and condensed tannins, which originate from oak and grapes respectively.

2.2.1 Condensed tannins

Formation, components and structures

Condensed tannins are polymerised flavanol units. These flavanol units consist of catechin, epicatechin, galocatechin, epigallocatechin and epicatechin gallate (Figure 9) (Prieur *et al.*, 1994; Souquet *et al.*, 1996). When a third phenolic group is added on the B ring of catechin and epicatechin, galocatechin and epigallocatechin are yielded (Hagerman, 2002). Condensed tannins are also known as proanthocyanidins. Proanthocyanidins are classified either as procyanidins or as prodelphinidins. Procyanidins are catechin- and epicatechin¹³ based polymers, while prodelphinidin also contain galocatechin- and epigallocatechin units in addition to catechin and epicatechin (Hagerman, 2002).

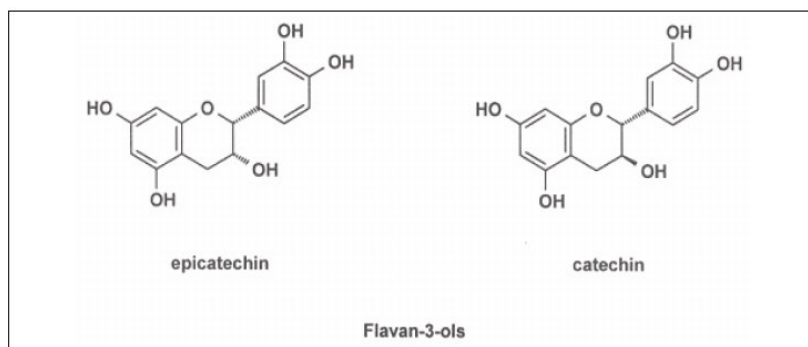


Figure 9 Structures of the monomeric flavan-3-ols, epicatechin and catechin.

There are a number of different ways that the flavan-3-ols and flavan-3,4-diols can polymerise to form condensed tannins. These are: direct polymerisation:

The flavonoids are linked by C4-C8 or C4-C6 carbon bonds to form a polymer (Prieur *et al.*, 1994). This reaction is dependent on temperature and the reaction rate increases with an increase in temperature. When dimeric procyanidins are formed with C4-C8 bonds it is identified as B1 to B4 and when C4-C6 bonds are formed it is identified as B5 to B8 depending on the combination of the subunits catechin and epicatechin (Figure 10). Type-A dimeric procyanidins can also form when in addition to the C4-C8 or C4-C6 bonds there is an ether bond between either the C5 or C7 and the C2 carbon (Ribéreau-Gayon *et al.*, 1998). Direct polymerisation is a slow process and no oxygen is required. The different linkages will also influence the three dimensional shape of the tannin as well as the way it interacts with other compounds (Allen *et al.*, 1997).

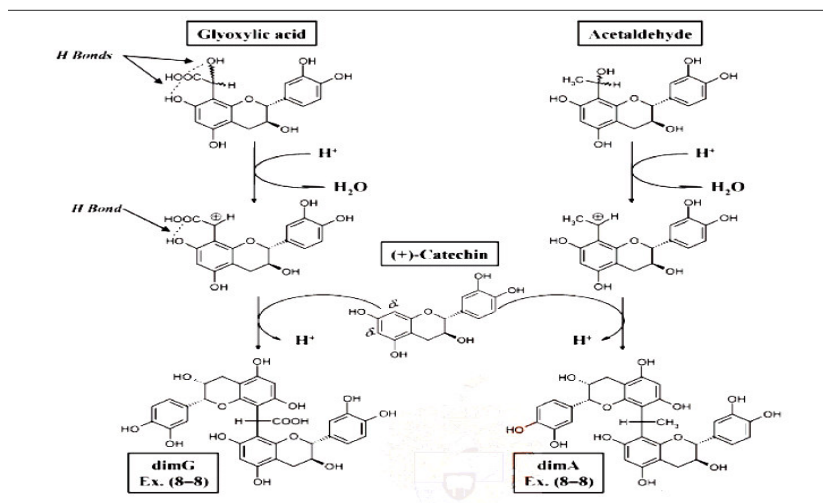


Figure 10 Direct C4-C8 (B1 to B4) and C4-C6 (B5 to B8) bonds.

Indirect polymerisation: Ethanol is oxidised to form acetaldehyde. The acetaldehyde polymerise with the flavonoids to form condensed tannins. This reaction is known as acetaldehyde-mediated condensation and an ethyl-bridge is formed between the flavonoids (Figure 11) (Vidal *et al.*, 2004). This form of polymerisation is much faster than direct polymerisation and can also take place between tannins and anthocyanins (Ribéreau-Gayon *et al.*, 1998). Tartaric acid can also be oxidised to yield glyoxylic acid, which then reacts with flavonoids to form polymers. This polymerisation reaction resembles that of acetaldehydemediated condensation and can be seen as a form of indirect polymerisation (Drinkine *et al.*, 2005; Fulcrand *et al.*, 1997).

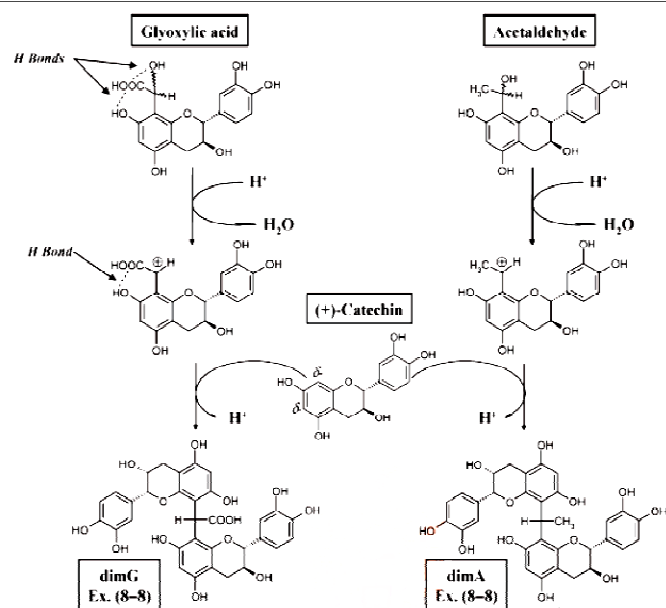


Figure 11 Indirect polymerisation and the formation of dimers (Drinkine *et al.*, 2005).

2.2.2 Condensed tannins (proanthocyanidins) in grapes

The concentration, nature and structure of the condensed tannins change with grape maturity and cultivar (Oberholster, 2003). The concentrations in red wine vary between one and four g/L and in white wine between 100 and 300 mg/L (Ribéreau-Gayon *et al.*, 1998). The highest levels occur just before colouration and then decrease up to *veraison* with a further decrease up to harvest (Harbertson *et al.*, 2002; Oberholster, 2003). Optimal ripeness does not occur simultaneously in the seeds and the skins and cooler conditions generally gives higher tannin and anthocyanin concentrations, providing the grapes get ripe (Oberholster, 2003). Skins and stems contain both procyanidins and prodelphinidins, while the seeds only contain procyanidins (Riou *et al.*, 2002; Souquet *et al.*, 1996). High condensed tannin concentrations in the skins are usually accompanied by a high

anthocyanin concentration. After *veraison*, the seed coats harden, which makes the polyphenols less extractable (Oberholster, 2003).

2.2.3 Extraction of grape phenolics

The main phenolic compounds in red wine are anthocyanins and proanthocyanidins (condensed tannins) (Riou *et al.*, 2002). Between one and four g/L proanthocyanidins are normally extracted from the grapes during fermentation (Ribéreau-Gayon *et al.*, 1998). The concentration, nature and structure of condensed tannins in wine vary with the type of technology used during winemaking. Vigorous crushing, mechanical punch down, pump over treatments, cold soaking and higher maceration temperatures all increase the extraction of phenolic compounds from the grapes to the must or wine (Oberholster, 2003; Sun *et al.*, 1999). Grape skins are the main source of extractable phenols, while only part of the phenols in the seeds are extracted. The seeds contribute only to the monomeric and oligomeric proanthocyanidins, but not to the polymeric proanthocyanidins (Sun *et al.*, 1999). General phenol extraction reaches a maximum at pressing and remains stable during malolactic fermentation and ageing. Anthocyanin extraction reaches a maximum after two to three days of fermentation and decreases during storage. During extended skin maceration, the anthocyanin concentration decreases, while the tannin concentration can still increase up to ± 36 days (Oberholster, 2003; Zimman *et al.*, 2004).

2.2.4 Tannin-anthocyanin interaction

Red wine colour becomes increasingly associated with polymeric material as the wine ages (Kennedy & Hayasaka, 2004). However, most of the observed colour in

red wine still needs to be characterised. Saucier *et al.* (2004) found that as red wine ages the observed colour increases in molecular weight. This is due to the polymerisation between anthocyanins and proanthocyanidins via indirect or direct polymerisation. The polymerisation of proanthocyanidins with anthocyanins also enhances the colour of the wine and protects it from oxidation (Saucier *et al.*, 2004). Polymerisation reactions depends on anthocyanidin composition and the ratio of proanthocyanidin and anthocyanidin (Timberlake and Bridle, 1977). Kennedy and Hayasaka (2004) concluded that ethyl-bridged anthocyaninproanthocyanin does not play a significant role in colour stabilisation and that ethyl-bridged pigments are unstable and rapidly converted to other pigmented material.

2.2.5 Organoleptic influence

Condensed tannins are responsible for some of the wine's major organoleptic properties namely astringency, browning and turbidity (Ricardo-da-Silva *et al.*, 1993). The astringency and bitterness of wine is mainly attributed to the presence of phenolics (Vidal *et al.*, 2004) and more specific condensed tannins. Astringency increases with an increase in the degree of polymerisation. Ethyl-bridged flavanols can also increase astringency and bitterness, provided they are present in sufficient amounts. An increase in the degree of trihydroxylation decreases the astringency, while an increase of gallottanin on increases the coarse perception of the proanthocyanidins (Vidal *et al.*, 2004). Pigmented polyphenolic compounds can also decrease the astringency if they are present at sufficient levels.

2.3.Hydrolysable tannins

2.3.1 Components and structures

Hydrolysable tannins contain a polyhydric alcohol (typically D-glucose) as a basic structural unit of which the hydroxyl groups have been esterified by gallic acid or hexahydroxydiphenic (HHDP) acid (Hagerman & Butler, 1991; Hagerman, 2002). These tannins are easily hydrolysed either enzymatically or in acid or base conditions to form free gallic acid or HHDP acid. The latter spontaneously hydrolyse to yield ellagic acid. Hydrolysable tannins can be classified as either gallotannins or ellagitannins, according to the type of acid formed (Puech *et al.*, 1999).

2.3.2 Tannins in oak wood

Of the ethanol-extractable phenols in oak wood, 90 percent are non-flavonoids which include lignin, gallic acids, ellagic acids, aromatic acids, aldehydes and hydrolysable tannins (Singleton & Esau, 1969). The non-volatile hydrolysable tannins account for the majority of these non-flavonoids and are 5 to 15 percent of the dry weight of oak wood (Puech, 1984). Quinn and Singleton (1985) found that the oak extracts of American and European oak species were very similar in the major components, but that American oak has a much lower phenolic content than European oak. They have also shown that ellagitannins are extracted into wine through oak chip or barrel ageing of wine.

2.3.3 Ellagitannins

Ellagitannins constitute up to 10% of the dry weight of oak heartwood. The two most common ellagitannins are vescalagin and castalagin (Figure 12). Vescalagin is in general more reactive than castalagin. Six other ellagitannins have also been identified namely roburins A-E and grandinin (Herv'e du Penhoat *et al.*, 1991). Ellagitannins can undergo intermolecular coupling with other hydrolysable tannins to yield dimers (Hagerman, 2002). According to Quinn and Singleton (1985), the tannins of cooperage oak are believed to be ellagitannins, whose products are gallic acid and ellagic acid. Ellagitannins influence the structure of phenolic compounds and the colour of red wine by speeding up the condensation of procyanidins, while limiting the degradation of procyanidins through oxidation and precipitation (Glories, 1993; Ribéreau-Gayon and Stonestreet, 1965; Vivas, 1993). The ellagitannins also have the ability to combine covalently to grape-derived nucleophilic species such as ethanol, flavanols, anthocyanins and thiols (Quideau *et al.*, 2005). Winemaking practices can influence the reactivity of ellagitannins. Puech *et al.* (1999) stated that the amount of time the wine spends in contact with oak would influence the amount of ellagitannins extracted from the wood. Vivas and Glories (1996) have shown that sulphur dioxide addition to wine limits ellagitannin oxidation by competing with the oxygen.

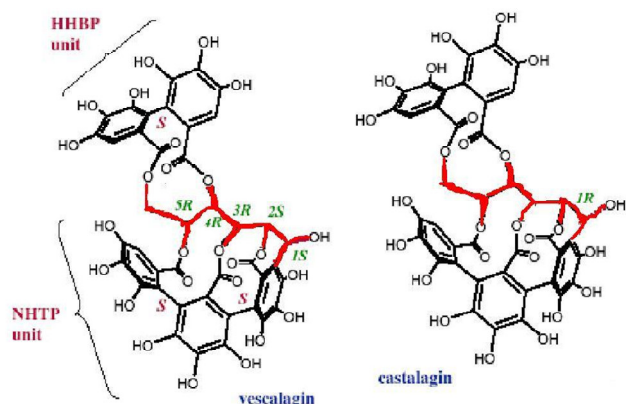


Figure 12 Structures of ellagitannins in extractions of oak heartwood (Ribéreau-Gayon *et al.*, 1998).

2.3.4 Reactions of hydrolysable tannins

The presence of several hydroxyl (OH) functions in the *ortho* position led authors to believe that hydrolysable tannins are involved in the oxidation processes in red and white wine (Moutounett *et al.*, 1989). According to Vivas and Glories (1996) when oxygen consumption between ellagitannins and catechin were compared in red wine, the oxygen consumption rate was much faster when the wine were supplemented with ellagitannins than supplemented with catechin. This is probably due to two hydroxyl functions existing in one mole of catechin, opposed to hydroxyl functions existing in one mole of castalgin or vescalagin. The higher oxidative ability of ellagic tannins generates peroxides faster and in larger quantities, which in turn produce larger quantities of acetaldehyde (the pivoting point of condensation between condensed tannins and anthocyanin) (Vivas & Glories, 1996). Vivas and Glories (1996) found that a solution containing a mixture of catechin and ellagitannins produces a large amount of peroxides, but far less than each component on its own. This is probably due to an inhibition

phenomenon, because the two substances are competing for the oxygen. They also found that ellagitannins consume most of the oxygen and hence protect catechin from oxidation.

2.3.5 Properties of hydrolysable tannins

The properties of the hydrolysable tannins vary widely due to differences in size, structure and configuration. Many of the properties of the hydrolysable tannins are due to the hydroxyl groups. Both the number and location of these hydroxyl groups will influence the properties of the tannin (Puech *et al.*, 1999). Hydrolysable tannin varies in size, type and binding reactions. The ester linkages make the tannins highly susceptible to hydrolysis. Puech *et al.* (1999) has shown ellagitannins to be very unstable in hydro-alcoholic solutions. Hydrolysable tannins are hydro soluble and dissolve quickly in wine (Moutounnet *et al.*, 1992). According to Puech *et al.* (1999), the solubility decreases with an increase in molecular weight, thus polymerisation lead to a decrease in solubility. Hydrolysable tannins are easily oxidised, thereby decreasing the oxygen availability for other reactions. They also chelate metal cations, which are catalysts for oxidation reactions. Hydrolysable tannins are acting as free radical scavengers and the possible combination with quinones also inhibit free radical formation (Puech *et al.*, 1999). Hydrolysable tannins can act as a passive defence against biological decay and have the ability to inhibit the growth of several wood decaying fungi (Scalbert *et al.*, 2005). There is no clear proof of any medicinal beneficial effect of oak tannins, because it is not sure whether the human body absorbs ellagic acid and gallic acid, but they have been shown to inhibit cancer formation (Gali *et al.*, 1992).

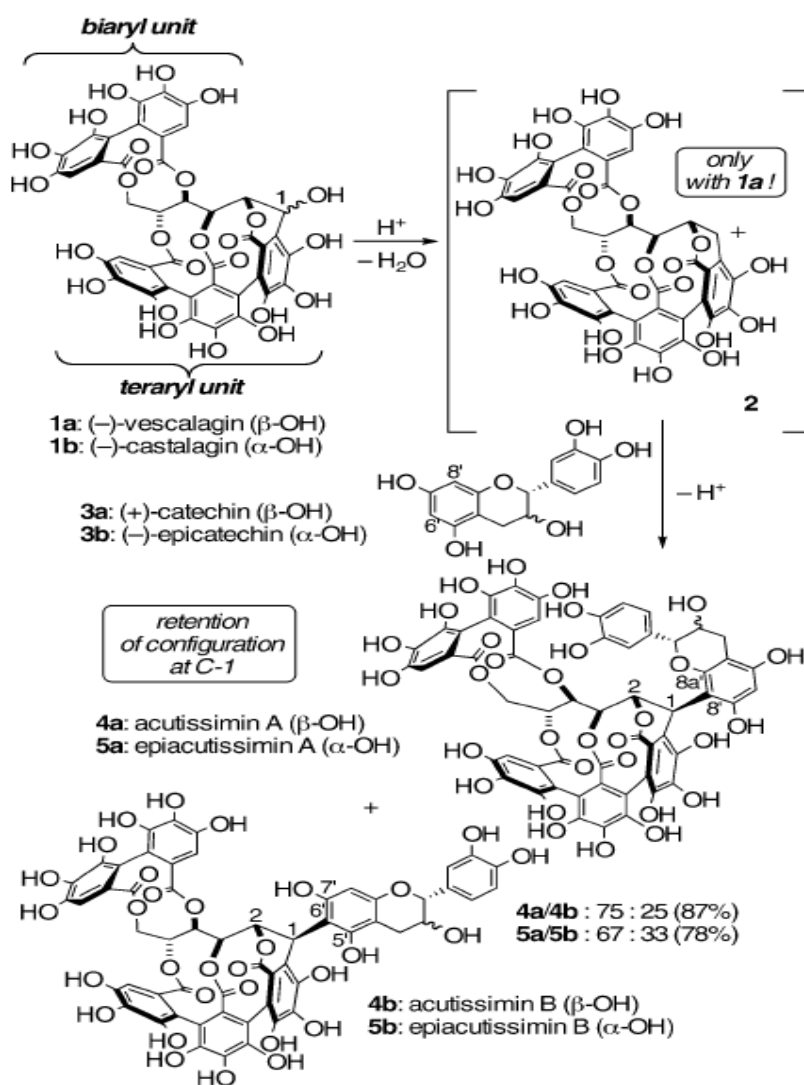
2.3.6 Influence of hydrolysable tannins on wine quality

There is great controversy on the organoleptic effects, especially taste properties, of hydrolysable tannins on wine. Quinn and Singleton (1985) postulated that ellagitannins account for much of the astringent taste and mouth feel of wine aged in barrels, but further investigation was needed. Somers (1990) suggested the concentrations of these compounds are too low to contribute to the taste of wine. Herv'e du Penhoat *et al.* (1991) confirmed the astringency of ellagitannins, but came to no conclusive results regarding the sensory impact of the ellagitannin oxidation products. Two authors came to the conclusion that ellagitannins are not responsible for the astringency in wine. Pocock *et al.* (1994) found that oak tannins were present in white wine matured in wood, but that these tannins were near or below their detection limit. Their tasting panel could, however, detect volatile oak constituent in wines that received only low dosages of oak extract. This suggested that oak-derived volatiles provided the primary sensory cue indicating that the wine received oak treatment. Puech *et al.* (1999) suggested that the direct effect of oak tannins on astringency remain uncertain, but only appear likely through a synergistic effect with other wine phenolic compounds. They also found that ellagitannins are astringent, but more bitter than astringent. The hydrolytic products of the hydrolysable tannins are not astringent or bitter (Quinn & Singleton, 1985). The presence of ellagitannins in oak aged wines is very low due to several reasons. Heating (toasting) of the barrels reduces ellagitannins. The wood structure is changed during toasting, which will influence the extraction of ellagitannins. Ellagitannins also undergo chemical transformation due to oxidation, polymerisation and hydrolysis in wine (Puech *et al.*, 1999). Vivas and

Glories (1996) determined that ellagitannins enhanced colour stability in wine and reduced astringency by enhancing tannin-anthocyanin reactions. Barrel fermentation and ageing on lees has shown to decrease the polyphenolic content of the wine due to the precipitation of tannins (Dubourdieu, 1992).

2.4 Flavano-ellagitannins: Acutissimins

The formation of Acutissimin A (4a) and related flavano-ellagitannins from flavan-3-ols and C-glycosidic ellagitannins carries out such as (-)-vescalagin (1a) and its C-1 epimer (□)-castalagin (1b) that feature an unusual open-chain glucose core C□C-linked to a galloyl-derived teraryl unit (Scheme 1).



Scheme 1. Formation of acutissimins.

[11] These C-aryl glycosides are characteristic metabolites of durable hardwood species. Acutissimin A (4a) was first isolated from the bark of the sawtooth oak (*Quercus acutissima*), [12] a pest- and disease free oak used as an ornamental tree in air-polluted urban areas in the United States. The compound was later found to be an inhibitor of human DNA topoisomerase II that is 250- fold more potent in vitro (concentration required for 100% inhibition, $IC_{100}=0.2$ μ M) than the clinically used anticancer drug etoposide (VP-16). [13] The chemistry of the formation of 4a is simple and involves an acid-catalyzed nucleophilic substitution reaction between either (–)-1a or (–)-1b at the C-1 center and (+)- catechin (3a) at its nucleophilic C-8 center (Scheme 1). Initial attempts by Jourdes and Quideau [12] to use this hemisynthetic approach to produce 4a from (–)-castalagin (1b) resulted in minute amounts (3.7%) of the desired product. The reactions has been carried out between purified (–)-1b and (+)-3a in tetrahydrofuran containing 1% trifluoroacetic acid at 60°C confirmed this failure. However, the use of (–)-1a instead of (–)-1b led, over a period of 7 h, to the clean formation of acutissimin A (4a) as the major product, together with its C-6 regioisomer acutissimin B (4b; Scheme 1). Both compounds have the same configuration at C-1 as 1a. This mixture was then separated by semipreparative HPLC and the individual isomers 4a and 4b were obtained in a 75:25 ratio and 87% yield. Interestingly, the isomers have been isolated in a similar ratio (81:19) from *Quercus acutissima*. [12] The identity of the hemisynthetic compounds was confirmed by comparison of ¹H and ¹³C NMR data as well as optical rotations. According to Jourdes study the initial failures to synthesize the target compound were essentially the result of incorrect selection of the starting C-glycoside epimer. (–)-Vescalagin (1a) is a much more efficient reaction partner than its α -anomer 1b. This difference in chemical

reactivity can be rationalized in terms of the difference in orientation of the reacting hydroxy group at C-1. In 1b, this OH group is a-oriented and embedded in the endo face of the molecule, whereas in 1a, it is b-oriented and points outward from the exo face of the molecule. The latter OH group is consequently more accessible and its oxygen atom probably has a more basic character than that of the OH group in 1b, which is also involved in intramolecular hydrogen bonding.[14, 15] They performed the same reaction with the flavan-3-ol(-)epicatechin (3b) and gave two new flavano-ellagitannins that were referred to as “epiacutissimins” A (5a) and B (5b) in a 67:33 ratio and 78% yield, again with retention of configuration at C-1 (Scheme 1). The position at which the flavanoid unit is connected to the C-glucoside C-1 center in these two regioisomers was confirmed by the observation of two- and three-bond couplings of H-1 with C-8' and C-8'a in the NMR HMBC spectrum of 5a, and with C-5' and C-6' in that of 5b. The stereochemistry at C-1 was deduced from the small NMR coupling constant between the glucose unit H-1 and H-2 protons; this weak coupling indicates that the dihedral angle between these two protons is close to 90° and such an angle is observed when H-1 is b-oriented.[12] In fact, all known naturally occurring C-1-substituted C-glycosidic ellagitannins have their C-1 substituent in this b orientation. It is likely that all these compounds are derived from(-)-1a and not from (P)-1b. A mechanistic description of the reaction follows a classical SN1-type pathway with protonation of the OH group at C-1 assisting the formation of a benzylic cation 2 (Scheme 1).[1] This stable carbocation intermediate is attacked by the flavan-3-ol units mainly from their C-8 center and, to a lesser extent, from their more encumbered and less nucleophilic C-6 center, with full diastereofacial differentiation (Figure 13).

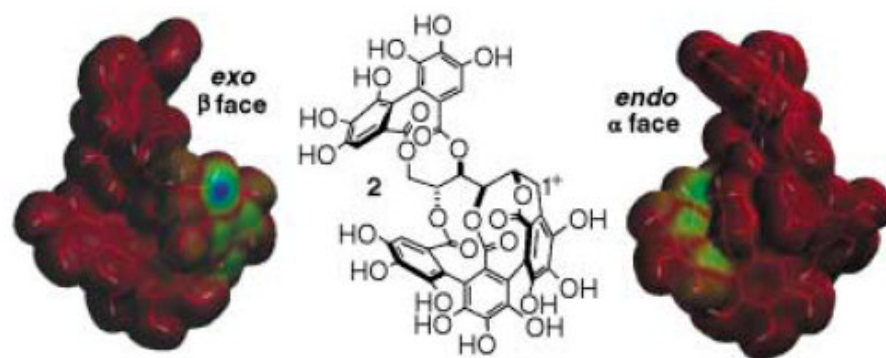


Figure 13. Diastereofacial differentiation of the benzylic carbocation intermediate.

This diastereoselectivity can be considered surprising in view of the S_N1 -type mechanistic description proposed in Scheme 1.[1b, 6b] A computer-aided examination of the benzylic carbocation intermediate A furnished a clear rationalization of this stereochemical control (vide infra). This efficient nucleophilic substitution reaction between 1 and ethanol provided Jourdes and colleagues with the motivation for examining other nucleophiles present in wine. They examined the possibility of generating acutissimins from oak-extracted 1 and/or 2 and (+)-catechin (10 a) during wine ageing in oak barrels.

The formation of acutissimin A (11 a) as the major product is a consequence of the higher nucleophilic character of the more accessible catechin C-8' center.[21] To provide evidence of the presence of the acutissimins in wine, they did by analyzing a sample of red wine that had been aged for 18 months in oak barrels. Not only were the two acutissimins A and B (11 a/b) detected, but also the two new “epiacutissimins” A and B (12 a/b). A HPLC/ESIMS-based quantitative determination of their occurrence in the same sample indicated content values of 0.4 mg/L□1 for 11 a, 0.28 mg/L□1 for 11b, 0.30 mg/L□1 for 12 a, and 0.35

mg/L¹ for 12b.[22] Although they appear to constitute relatively minor components in wine, their occurrence is another proof of the participation of oak C-glycosidic ellagitannins in the elaboration of the chemical profile of wine. Of relevant note is the detection of both (-)-vescalagin (1) and its epimer 2 at concentrations of 2mg/L¹ and 8 mg/L, respectively, in the wine sample that Jourdes and Quideau analyzed. Other analyses have indicated amounts comprised between 0 and 7 mg/L¹ for 1 and between 5 and 21 mg/L for 2.[16a,b] The fact that 2 is always found in higher amounts than 1 can be explained by their difference in chemical reactivity. Two compounds, named (-)-vescalin (18) and (+)-castalin (19), according to Quideau studies [12c] are also relevant, since they are also extracted from oak by the wine during ageing.[16b] Their occurrence in oak presumably results from hydrolytic cleavage of the 4,6-HHBP unit of 1 and 2, and their presence in wine can additionally be due to the same hydrolysis taking place in the wine itself.

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3.OBIECTIVES

The recent studies carried out by Spain and France on rapid ageing of wine with pieces of wood represents one of the first step to demonstrate oak pieces influence on wine composition and evolution during ageing process.

The aims of these experimentations were mainly due to approve or avoid their use on quality wine and on typical wine.

So this PhD research started from this studies, and had the proposal of assess the presence of new compounds “Acutissimins”, discovered and initially analysed by two French researcher, Jourdes and Quideau, during wine shortening ageing period in presence of oak chips

We had also the objective of follow Acutissimins chemical evolution in and of quantify them .

In this purpose have been used for the experimentation alcoholic acid model solutions which simulates wine and commercial red wine.

CHAPTER 4

MATERIALS AND METHODS

Flavano-Ellagitannins “Acutissimin A and B” hemisynthesis has carried out through, 12% v/v Ethanolic, aqueous solutions containing 5g/L Tartaric acid pH 3.2 added with oak chips and catechin (Puech, J.). The first step of this research has been conducted on 100 ml of solution. Different model solutions, were prepared: with an high level 1000 mg/l, intermediate level 500 mg/l and low level 100 mg/l of catechin and with a constant content of oak chips 1% of total as showed below:

- Sol. 1000 ppm catechin-1% Oak chips (1000/1).
- Sol. 500 ppm catechin-1% Oak chips (500/1).
- Sol. 100 ppm catechin-1% Oak chips (100/1).

Then we analyzed hydroalcoholic solutions with different content of oak chips(5%and 10%) and 500 or 100 mg/l of catechin.

- Sol. 100 ppm catechin-5% Oak chips (100/5).
- Sol. 500 ppm catechin-5% Oak chips (500/5).
- Sol. 100 ppm catechin-10% Oak chips (100/10).
- Sol. 500 ppm catechin-10% Oak chips (500/10).

Oak chips are made from American oak treated with high toasting temperature, they are purchased from an Italian industry of Enological products.

All solutions were stored at $16^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

The evolution of chemical reaction has been clearly demonstrated trough acutissimins identification and quantitative evaluation of catechin content.

The Acutissimins hemisynthesis have been monitored over a period of 25 and 42 days by HPLC gradient reversed fase analysis.(AGILENT 1100 SERIES, Column Phenomenex Gemini C18 150 * 4,6 mm, 5 micron i.d. Detector UV-VIS, DAD).

HPLC working conditions

As eluting phases has been used a water and Trifluoroacetic acid (TFA) at 0,5% solution (Sol. A) and methanol at 0,5% of TFA (Sol. B). Absorbance traces are obtained at 230, 280 and 308 nm (9). The eluting conditions are illustrated below:

time (min)	% B
0	0
20	10
30	50
35	55
40	60
55	100
60	0

Acutissimins was identified by means with LC-MS analysis and quantified by using a calibration curve of catechin as external standard.

4.1 Wine Samples Analysis

Although has been simulated a short ageing process of commercial red wines with oak chips hardly toasted, added in the same surface/volume ratio of a 225 l oak barrel. Ageing process has been monitored over a period of 6 weeks, during this time every week, Acutissimins, Color values, totals polyphenols content, antocyanins, polyphenols by HPLC and legislative parameters as alcoholic grade, sulphur dioxide value ,pH value, Total and volatile acidity, sugars were determinated.

4.1.1 Acutissimins Extraction

The extraction of the flavano-ellagitannins was accomplished according to the method of Quideau et al. A sample (50 ml) of a red wine aged with oak chips was evaporated under vacuum and the resulting viscous dark residue, was dissolved in water 5 ml . This solution was loaded on column (150 mm x 40 mm) that has been packed with some Amberlite XAD-7 HP resin, previously swelled in methanol overnight.

An aqueous acidic solution [H₂O–HCOOH (99,6:0,4), 250 ml] was first used to wash out tartaric acid and sugars, and 20% methanolic aqueous solutions [H₂O–MeOH–HCOOH (79,6:20:0,4), 250ml] was then used to elute the ellagic fraction. This fraction was evaporated under vacuum to furnish a dark oink residue, retaken in water (400µl) and 20µl was injected into HPLC-MS system.

4.1.2 Acutissimins determination

The flavano-ellagitannins formation has been shown through HPLC-MS analysis.

Working conditions

HPLC AGILENT 1100 SERIES, Column Phenomenex Gemini C18 150 * 4,6 mm, 5 micron i.d. Detector LC-MS ESI-APCI. The flow was 1ml/min with elution program Water-Methanol-0.5% acetic acid which was the same of model solutions. Analysis was carried out in SIM modality.

Mass Spectrometry operating conditions

MSD-Spray chamber: Drying gas flow= 12 l/min, Nebulizer pressure=60 psi, Drying gas temperature=300 °C , Vaporizer temperature= 250 °C, Capillary voltage=2000 V, Corona current=2 µA, Changing voltage=2000 V.

Qualifier Ions for Acutissimins identification.

m/z=180 = Glucose

m/z=271 = Acutissimins-Ellagitannins

m/z=291 = Catechin

m/z=754 = Ellagitannins-Glucose

m/z=914 = Acutissimins-Catechin

m/z=934 = Ellagitannins

m/z=1025 = Acutissimins-Glucose

m/z=1205 =Acutissimins

Each of the four compounds has a molecular mass of 1205 Da, and their presence was validated by mass spectrometry and comparison of their chromatographic retention times and mass fragmentation patterns with those of the hemisynthetic compounds. Quantitative analysis has been executed using catechin as external standard.

4.1.4 Colour analysis

The spectral absorbance of red wines has a maximum at 520 nm (red colours) and a minimum in the region of 420 nm (brown colours). For younger wines 620 nm (blue colours) should also be taken into account (Ribéreau-Gayon *et al.*, 1998). Colour density represents the amount of colour, thus the sum of 420 nm, 520 nm and 620 nm measurements. The hue indicates the development of the colour towards orange, therefore the 420 nm value divided by the 520 nm value (Somers & Evans, 1977). Young wines normally have hue values in the order of 0.5 to 0.7, while older wines have values in the order of 1.2 to 1.3. White wines do not have

a defined maximum in the visible range and absorption ranges from 280 nm to 500 nm, but have a maximum in the UV range. Oxidised white wines can be measured using the 420 nm values (Ribéreau-Gayon *et al.*, 1998). Boulton (2001) described a method to determine the different fractions in which the colour can be classified namely: copigmented, free anthocyanins and polymeric pigments. When sulphur dioxide is added to wine, it binds quickly to the C4 site of the anthocyanin. This reaction produces a stable colourless bisulphite addition product (Timberlake & Bridle, 1967). When acetaldehyde is added to wine, it preferentially binds to sulphur dioxide and thus removes the bleaching effect that sulphur dioxide have on the colour. Using this method the modified colour density, in other words colour density without sulphur dioxide, and sulphur dioxide resistant pigments can be determined by measuring the red colour after the addition of excess sulphur dioxide. With these values the different fractions that contribute to the colour of red wine can be calculated (Boulton, 2001). The total red pigments of a wine can be determined when a spectrophotometric measurement is taken of the wine at a pH < 1.0 at 520 nm. At this low pH all the anthocyanins are in the coloured flavylum form. Thus the anthocyanins that are in the red form and other anthocyanins that are copigmented are measured (Boulton, 2001; Somers & Evans, 1977).

Colorimetric indexes.

Color intensity represents the amount of color. It varies a great deal from one wine and grape variety to another (0.3–1.8):

$$CI = OD\ 420 + OD\ 520 + OD\ 620$$

The hue indicates the development of a color towards orange. Young wines have a value on the order of 0.5–0.7 which increases throughout ageing, reaching an upper limit around 1.2–1.3.

$$T = \frac{OD\ 420}{OD\ 520}$$

Color composition, i.e. the contribution (expressed as a percentage) of each of the three components in the overall color:

$$\begin{aligned} OD\ 420(\%) &= \frac{OD\ 420}{CI} \times 100 \\ OD\ 520(\%) &= \frac{OD\ 520}{CI} \times 100 \\ OD\ 620(\%) &= \frac{OD\ 620}{CI} \times 100 \end{aligned}$$

The brilliance of red wines is linked to the shape of the spectrum. When wine is bright red, the maximum at 520 nm is narrow and well defined. On the other hand, it is flattened and relatively broad when wine is deep red or brick red. This characteristic may be shown by the expression:

$$dA(\%) = \left(1 - \frac{OD\ 420 + OD\ 620}{2\ OD\ 520} \right) \times 100$$

The results are between 40 and 60 for a young wine.

Operating Conditions

The absorbance spectra of the wine (200–780 nm) was registered in a 1 mm-width quartz cuvette using a Shimadzu UV-VIS spectrophotometer (Shimadzu UV-1601) at a speed of 2400 nm/min. From the absorbance values at 420, 520 and 620 nm, the following colorimetric indexes were calculated: color intensity (CI), %red, %yellow, %blue, and %dA (pure red). The tint was calculated according to Sudraud.

Ionization Index

The ionization index (Glories 1978), based on work by Somers and Evans (1974), is used to define the percentage of free and combined anthocyanins producing color in wine. To calculate this value, the wine is bleached by an excess of SO₂, at the normal pH of wine ($\Delta d\alpha$), on the one hand, and at pH 1 ($\Delta d\gamma$), on the other hand. The ionization value is expressed by the ratio of these two figures.

The procedure takes place in two stages.

Initially, 10 ml of wine with a normal pH is mixed with 2 ml of water. The optical density value (d_1) is measured at 520 nm on a 1 mm optical path.

The same operation is carried out again, replacing the water with 2 ml of sodium bisulfite solution ($d = 1.24$), waiting 5 min and measuring optical density under the same conditions to obtain the value d_2 :

$$\Delta d\alpha = (d_1 - d_2) * 12/10$$

This value represents the optical density at 520 nm, including only those free (Al) and combined (TA) anthocyanins, colored at the pH of wine, that react with SO₂.

An identical measurement is made at pH 1.2, when 95% of the anthocyanins present are colored.

In a mixture containing 1 ml of wine, 7 ml of HCl (N/10) and 2 ml of water, the optical density is measured at 520 nm on a 1 cm optical path, giving the value d' . A second measurement is made, replacing the water with sodium bisulfite as before, giving a value d'_2 :

$$\Delta d\gamma = (d'_{1} - d'_{2}) * 100 / 95$$

The ionization index is given by the expression

$$I = \Delta d\alpha / \Delta d\gamma \times 100$$

The ionization index for young wines varies from 10 to 30% and increases throughout ageing, reaching 80 to 90% in old wines.

If the coloring matter in red wine consisted only of free anthocyanins (Al), given the usual pH (3.4–4.0) and free SO₂ concentration (10–30 mg/l), the coloring percentages would be lower (3–14%). The new pigments produced when anthocyanins combine with tannins are much less sensitive to bleaching by pH and SO₂, so the percentage of coloring increases. This phenomenon continues throughout the ageing process. However, it also provides an estimate of copigmentation of the anthocyanins.

4.1.5 Total phenols content

There are various ways to determine the phenol content of wines, of which the Folin-Ciocalteu value (Folin & Ciocalteu, 1927; Singleton *et al.*, 1999) and 280 nm absorbance (Somers & Ziemelis, 1985; Ribéreau-Gayon *et al.*, 1998) are the most reproducible. The Folin- Ciocalteu method uses oxidation-reduction reactions, where the phenolate ion is oxidised while phosphotungstic phosphomolybdic compounds are reduced to a blue molybdenumtungsten complex that is then measured at 760 nm. The phenol content is determined by using the standard gallic acid as reference (Singleton & Rossi, 1965; Singleton *et al.*, 1999).

Total polyphenols content of model solutions and of rapid aged wine was measured according to the Folin- Ciocalteu method.

A 1 ml sample of red wine, diluted 1/10 or 1/5 with distilled water, is mixed with 5 ml of Folin-Ciocalteu reagent, 20 ml of sodium carbonate solution (20%) and distilled water QS 100 ml. After 30 min the characteristic blue colouration was measured by a spectrophotometer UV-VIS (UV-1601 UV-Visible spectrophotometer “ Shimadzu) at 760 nm wavelength and total polyphenol content expressed as mg gallic acid/l.

$$\text{IFC} = (\text{OD} \times \text{dilution}) \times 20$$

The value is between 10 and 100.

4.1.6 Anthocyanins

Anthocyanins are present in different forms in wine namely: free pH-dependant anthocyanin forms, quinone (blue), flavylium (red) and carbinol base, anthocyanins associated with copigments and anthocyanins bleached by sulphur dioxide. Anthocyanins have a maximum absorption in the range of 520 nm. An estimation of the total anthocyanin concentration can be obtained by bleaching all the anthocyanins with sulphur dioxide. The difference between the bleached and not bleached measurements will give an estimation of the total anthocyanins (Ribéreau-Gayon & Stonestreet 1965; Somers & Evans, 1977).

Anthocyanins (At) are present in wine in different forms: free anthocyanins (Al) and anthocyanins combined with tannins (Ac), some of which are bleached by SO₂ (TA), while the rest is unaffected (TAT):

$$\text{At} = \text{Al} + \text{Ac} = \text{Al} + \text{TA} + \text{TAT}$$

There is no accurate method for assaying At, so this value may only be estimated. On the other hand, a global value for Al + TA may be determined (As), using chemical and chromatographic methods.

Total Anthocyanins

The chemical methods are based on the specific properties of anthocyanins: color variation according to pH and bleaching by sulfur dioxide (Ribereau-Gayon and Stonestreet, 1965).

The procedure requires the preparation of two samples, each containing 1 ml of wine and 1 ml of EtOH 0.1% HCl. Then 10 ml of HCl at 2% (pH = 0.7) is added to the first sample and 10 ml of buffer at pH 3.5 to the other. The difference in OD at 520 nm, Δd_1 , is measured on a 10 mm optical path. In comparison with a standardized anthocyanin solution, the concentration $A_s = A_l + TA$ is given by the following equation:

$$C(\text{mg/l}) = \Delta d_1 \times 388$$

Reagents

Alcohol acid solution

Buffer solution at pH=3.5

1NHCl solution

Operating conditions

1ml of wine sample was added with 1ml of alcoholic solution and 10 ml of Buffer solutions and it was the blank sample .

1 ml of wine sample was added with 1ml of alcoholic solution and 10 ml of 1NHCl solution and then this solution was analysed at 520 nm wavelength in a spectrophometer with the blank solution.

The absorbance at 520 nm is taken as a measure of total anthocyanins.

4.1.7 Polyphenols by high performance liquid chromatography

Wine phenolic compounds can be separated and measured by means of high performance liquid chromatography (HPLC). Normally this separation is done by reverse phase columns packed with spherical particles of silica bonded with octadecyl (C18) chains (Lamuela-Raventós & Waterhouse, 1994). Castellari *et al.* (2002) described a method using a monolithic column. The monolithic column reduced the time of analysis, due to a faster separation time, shorter wash time, quicker re-equilibration and higher flow rate. By using a slow gradient the monomeric pigments and non-pigmented phenols, as well as up to the trimeric procyanidins can be separated as individual peaks. A sharp increase in gradient after their elution, elutes the remaining phenolic material as a large peak that are defined as the non-pigmented (280 nm) and pigmented (520 nm) polymeric peak (Price *et al.*, 1995; Peng, 2002).

HPLC analysis of polyphenols was carried out on a Agilent 1100 liquid chromatography system equipped with a photodiode-array detector (DAD). Separation was performed on a reverse-phase Phenomenex Gemini C18 (150 × 4,6 mm, 5 µm) at room temperature. A gradient consisting of solvent A (water/trifluoroacetic acid 0.1%) and solvent B (Acetonitrile/trifluoroacetic acid 0.1%) was applied at a flow rate of 1 ml/min as follows

t (min)	%B
0	0
2	2
8	8
15	15
18	23
25	95
35	95
40	0

Two milliliters of wine extract in water-methanol solution , previously filtered through a 0.45 µm membrane, was injected onto the column. Diodearray detection (DAD) was performed from 280 to 600 nm. Quantification was carried out by peak area measurements at 280, 320 nm.

4.1.8 Determination of Alcoholic grade, sulphur dioxide, pH, Volatile acidity, Total acidity, Sugars.

All these determination have been made according to European Commission regulament 2676/90, EU directive 1493 (1999) and OIV reference methods.

4.1.9 Determination of Volatile Compounds.

Volatile compounds were extracted using by means solvent extraction.

100 ml of wine has been extracted twice with 50 ml of Dichloromethane, the organic layer was dried over Na₂SO₄. Than organic samples were concentrated in a Kuderna-Danish distillatory-concentrator to 1 ml under a gentle stream of nitrogen.

Each wine sample's extract was subjected to GC-MS analysis, using a Agilent 6890 GC, equipped with a HP5MS column (30m X 0,25 mm i.d and 0,25 μ m film thickness) and a Agilent 5973 Mass spectrometer detector. Samples 1 μ l were injected using split mode with a split ratio of 1:50. The injector temperature was 250°C.

The oven temperature programme was as follows: 45°C(held for 3 min) to 90°C at rate of 10 °C/min(held for 5 min), then to 150°C at 15°C/min and finally to 250°C at rate of 15°/min with a 5 minute of final isotherm.

Detection was carried out with a ionization at 70 eV and analysis of mass fragment from 30 to 400 UMA.

Identification was carried out by comparing retention time of standard compounds and Mass spectrometer Nist98 and Whiley library .

5. RESULTS AND DISCUSSIONS

In figure 14 is illustrated a typical HPLC chromatographic profile at 230, 280, 308 nm of model solutions. It is possible to observe the presence of the peak of catechin and others three peaks, corresponding to new formation substances by catechin and woods reaction. In particular peaks n°2 and 3 had mass spectrum with fragment ions corresponding to acutissimins, instead for the peak n°1 the mass spectra corresponds to m/z ratio of wood ellagitannins

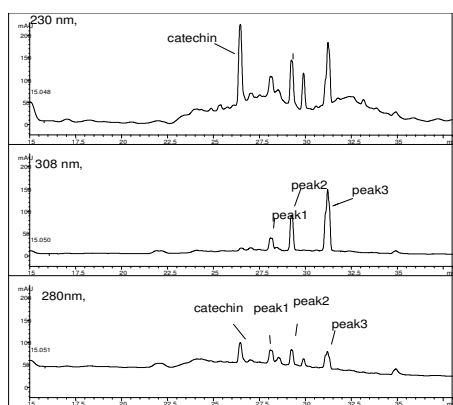


Fig.14 HPLC profile of model solutions (Peak 1:ellagitannins, Peak 2: Acutissimin B, Peak 2 Acutissimin A)

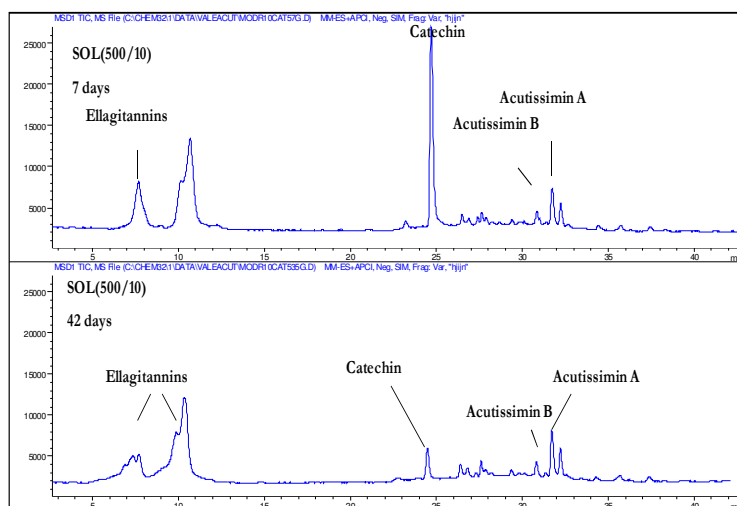


Fig.14 LC-MS profile of model solutions , identification of :ellagitannins, Catechin: Acutissimin B, Acutissimin A.

The first step of this research was the verification of hemisynthetic pathway of Acutissimins in acid alcoholic model solutions in presence of variable concentration of catechin and oak chips.

At the beginning Catechin amount was from 1000 mg/l to 100 mg/l in order to analyze how its amount influenced Acutissimins formation. The three solutions obtained were added with 1% of oak chips.

The maceration period in this first attempt to identify and quantify Acutissimins was of 25 days.

In the model solution 1000/1 Catechin amount was determined by means with HPLC measured at wavelength of 280 nm and it was quantified with external catechin calibration curve. It decreased from 1002 mg/l to 266 mg/l at wavelength of 280 nm (Figure 15).

This trend is due to catechin contribution in condensed tannins formation .

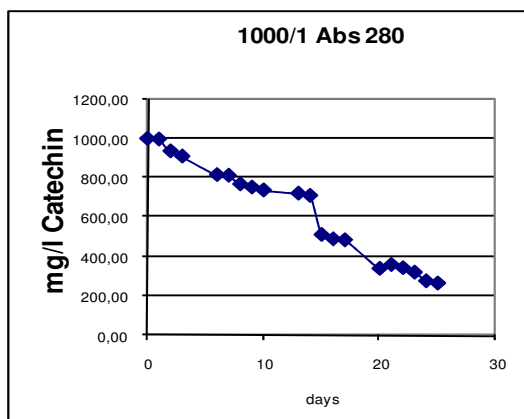


Fig.15 Catechin trend during 25 days of maceration in model solution 1000/1 .

Concerning neoformations peaks, it was revealed their presence from the first day of maceration

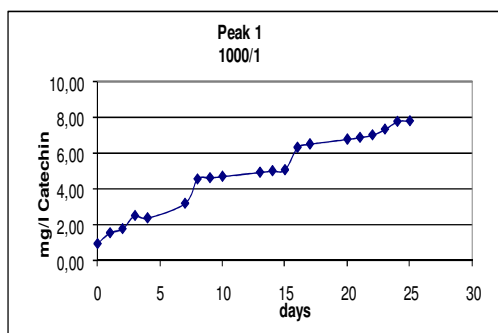


Fig.16 Peak 1 amount in 1000/1 solution during 25 days of maceration.

Peak 1 presence at the first day of maceration was about 0,92 mg/L but it reached a value of 7,83 mg/l at the end of maceration. Peak 2 and 3 reached the same amount(7,36 and 7,39 mg/l) at 25th day (Figure 17).

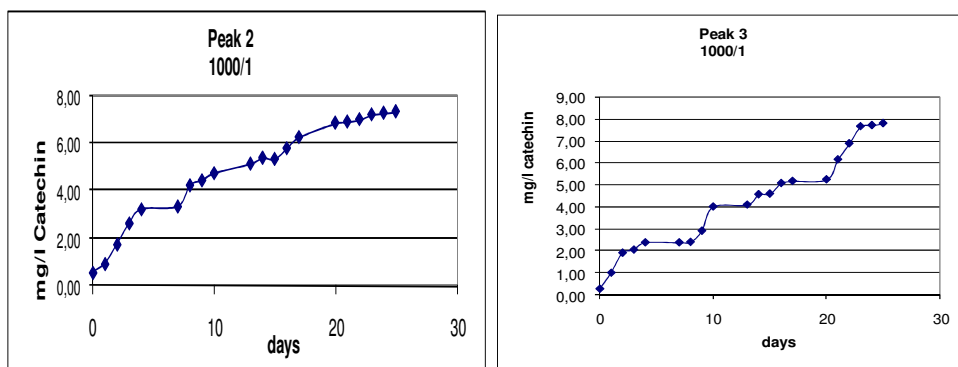


Fig.17 Peak 2 and Peak 3 amount in 1000/1 solution during 25 days of maceration.

In the solution 500/1 catechin amount decrease from 497 mg/l to a zero value(Figure 18)

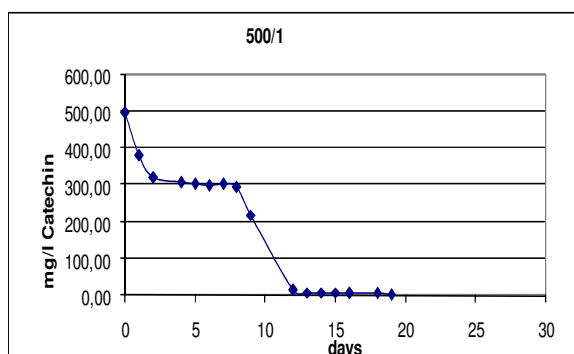


Fig.18 Catechin trend during 25 days of maceration in model solution 500/1

This decrease of Catechin is probably due to polymerization reaction and Acutissimins formation with ellagitannins released from the oak chips.

Peak 1, in this case, reached a final amount of 23,68 mg/l. Peak 2 and 3 also increased until the 25th day of maceration with the same trend of peak 1, as showed in the figure 19.

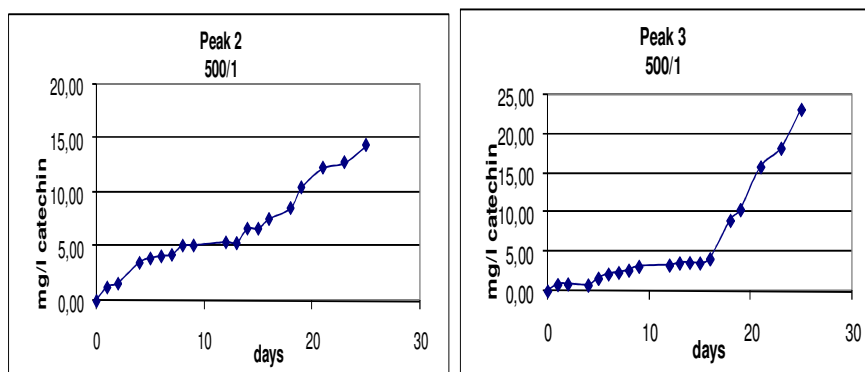


Fig.19 Peak 2 and Peak 3 amount in 500/1 solution during 25 days of maceration

In 100/1 solution catechin decreased from 99,5 mg/l to 0,25 mg/l at 23th day of maceration and to a value near to zero at the end of maceration(Fig.20)

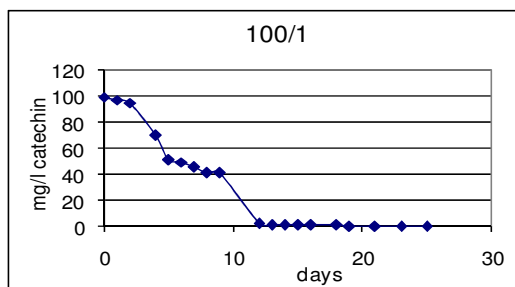


Fig.20 Catechin trend during 25 days of maceration in model solution 100/1

Neoformation peaks in this model solution reached an higher value than in the other solutions, in particular peak 1 had a value of 9,20 mg/l at the end of maceration time. Peak 2 and 3 reached values of 8,5 mg/l and 13,93 mg/l respectively(Figure 21).

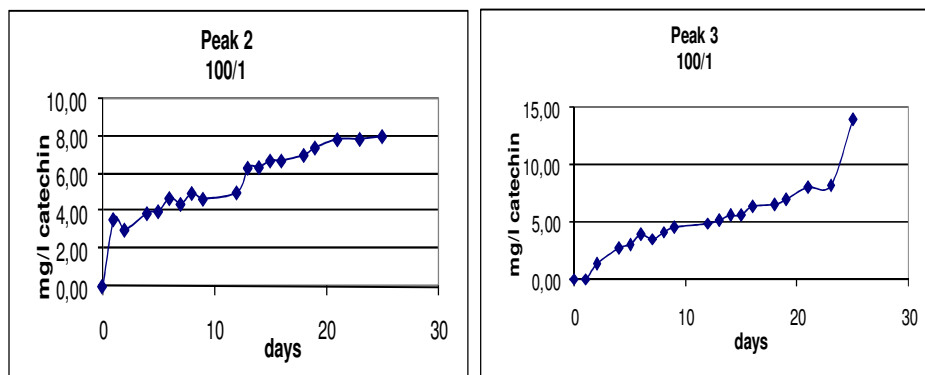
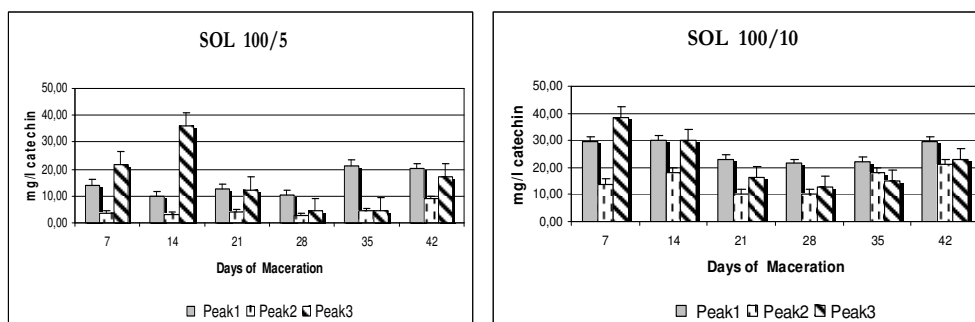


Fig.21 Peak 2 and Peak 3 amount in 100/1 solution during 25 days of maceration.

These results showed that a larger amount of catechin did not guarantee an higher content of neoformation peaks, in fact the best results were obtained in the circumstances of solution prepared with 500 and 100 mg/l of catechin

This reaction pathway was also defined by analyzing model solution for a long period, 42 days of maceration, and with different amount of chips added.

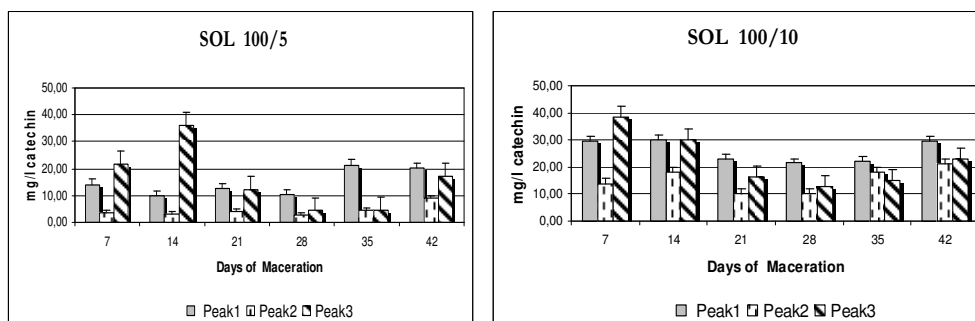
There were prepared four model solution with 100 mg/l of catechin and 5 or 10% of oak chips and 500mg/l of catechin and 5% or 10 % of oak chips. The peak 1 had an amount between 20,19 and 37,32 ppm at 42th day. Peaks 2 and 3 which had a fragmentation spectrum corresponding to acutissimin A and B respectively, had a variable values during maceration period. An higher ratio catechin/oak wood(SOL100/10-500/10) has promoted Acutissimins formation. In the model solution 500/10 has been found an high amount of acutissimins, -30,78 ppm for peak 2 and 31,68 ppm for peak 3 (Fig. 22a,b-23a,b).



a

b

Fig.22a,b. Neoformation peaks amount (mg/l catechin) during 42 days of maceration in solution 100/5 and 100/10 with american Oak chips.



a

b

Fig.23a,b. Neoformation peaks amount (mg/l catechin) during 42 days of maceration in solution 500/5 and 500/10 with american Oak chips.

By extending the maceration time the content of Acutissimins in model solutions increased and their presence were strongly associated to oak amount added in the solution, in fact the highest amount were registered in the 100/10 solution and even in the 500/10.

Trough HPLC-MS fitted with electrospray and APCI sorgent, has been evidenced the presence of fragments corresponding to m/z ratio of Ellagittanins and Acutissimins also in shortening aged wine extracts(figure 24).

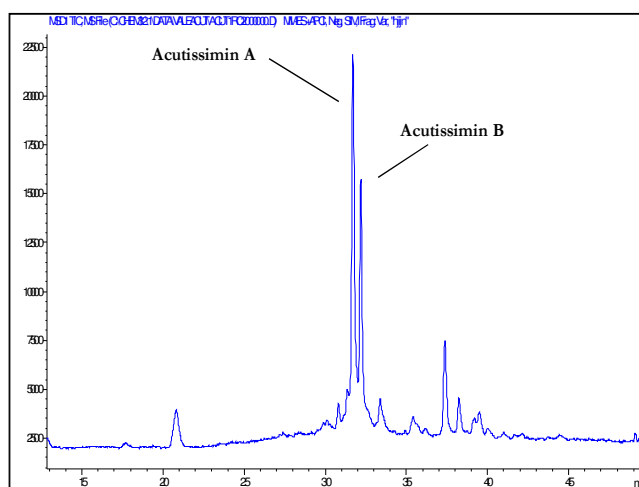


Fig.24.Flavano-Ellagitannins LC-MS APCI-ESI Chromatogram of wine aged with American oak chips

In Table 5 was monitored Acutissimins trend during rapid ageing. Their hemisynthesis in red wine aged with oak chips had a concentration between 0.10 - 0.36 mg/L.

t	acutissimin A	acutissimin B
Weeks	mg/l catechin	mg/l catechin
1	0,36 ± 0,030	0,11 ± 0,021
2	0,33 ± 0,040	0,18 ± 0,030
3	0,35 ± 0,045	0,16 ± 0,036
4	0,20 ± 0,048	0,13 ± 0,039
5	0,19 ± 0,048	0,23 ± 0,039
6	0,22 ± 0,051	0,15 ± 0,040

Tab.5 .Acutissimins trend in red wine 6 weeks aged with American oak chips.

Means of 5 replicates ±DS

Furthermore, we would like to emphasize here that any quantitative analysis by any available method of any compound at any given time in an ageing wine is rather pointless, for wine is a complex multicomponent reaction system that

slowly but continuously evolves under mildly acidic and oxidative conditions. As far as the flavano-ellagitannins are concerned, they are certainly further transformed in wine, but they will continue to form as long as the flavan-3-ols and the C-glycosidic ellagitannin that are present in the medium.

Wine treated with oak chips during ageing undergoes to a series of modification which are similar to transformations of a wine aged in a oak barrel.

Total polyphenols content, initially at 437 ppm, increased at the end of ageing period at 2126ppm. They reached these values as a consequence of ellagitannins formation but even for wood release of its compounds. It has been also evidenced a decrease of total free antocyanins content which reduced their values from 178ppm to 47,6 ppm (Figure 25). The ageing period leads to a wine colour variation, in fact it changes to yellow-orange tonality, red colour decreases from 77,54% to 10%(Tab.6.)

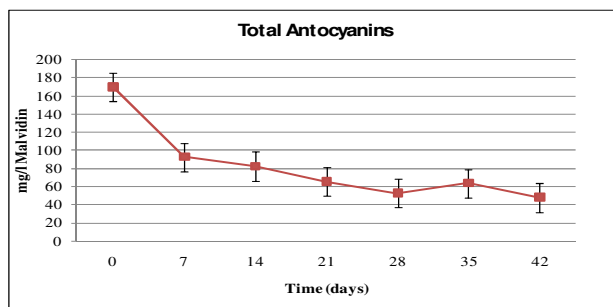


Fig. 25.Antocyanins amount during red wine shortening ageing.

Means of 5 replicates \pm DS

Parameters	Time(days)	
	0	42
pH	3,43± 0,08	3,38 ± 0,05
Total acidity (g/l Tartaric acid)	3,45 ± 0,07	5,21 ± 0,09
Volatile acidity (g/l acetic acid)	0,57 ± 0,06	0,90 ± 0,05
Alcoholic grade (%v/v)	11,00 ± 0,19	11,00 ± 0,17
Total polyphenols (mg/l Gallic acid)	713,57± 0,22	2.126,43± 0,44
Total anthocyanin (mg/l malvidina)	178,03 ± 0,38	47,6 ± 0,35
Intensity (u.a.)	2,42± 0,12	1,73 ± 0,12
Tonality(Abs 420/Abs520)	0,84 ± 0,12	0,92± 0,16
Red color purity (dA%)	77,54 ± 0,71	10,00 ± 0,75
%Yellow(% 420nm)	37,9 ± 0,23	42,13± 0,25
%Red(% 520nm)	43,93 ± 0,14	37,83 ± 0,21
%Blue(% 620nm)	18,6 ± 0,12	20,10 ± 0,18

Tab.6. Enological parameters of red wine aged with American oak chips.

Means of 5 replicates ±DS

Stabilization reactions affecting color, clarity and colloids, as well as modifications in the phenol structures (softening of the tannins), also occur in wine during ageing, while aromas develop.

Barrel ageing promotes these reactions and they could be accelerated in presence of oak chips in wine. The phenol composition of wine is considerably modified by barrel ageing, thanks to controlled oxidation. Color is intensified due to reactions between tannins and anthocyanins, as well as others involving ethanal. The free anthocyanins concentration decreases and the tannin structure evolves, as does its reactivity to gelatin. After ten months of barrel ageing, wines have better color than those aged in the vat , and this color remains more stable during bottle ageing. The flavor is also more attractive, characterized by softer tannins.

Piracci et al. looked at the use of wood (barrels, chips, or staves) in the vinification process and the conservation of wine, and stated that all cases were

characterized by a refinement and stabilization of wine color. Del Alamo et al studied the evolution of low molecular weight polyphenols in red wine ageing for different systems (barrels, oak chips, and oak staves) made of different oak species. The wine aged in contact with oak chips experienced faster ageing, loss of certain compounds, and a greater number of polymerizations than the wine aged in barrels. This result indicates that the typical reactions associated with ageing that result in a loss of anthocyanins took place more quickly in wine aged in alternative systems than in wines aged in barrels.

According bibliography color parameters of the commercial wine aged with oak chips in this research undergo to a positive evolution of color to yellow components at the end of the 42 days of maceration instead there was a reduction of red percentage color and a stabile evolution of blue color component as showed in the table below.

Days	Red Wine aged with American Oak chips									
	%Yellow		%Red		%Blue		Intensity(ABS)		Tonality(ABS)	
	means	dS	means	dS	means	dS	means	dS	means	dS
0	37,93	1,11	43,93	7,10	18,66	7,54	3,17	1,80	0,83	0,16
7	39,40	1,34	39,35	3,60	21,72	3,27	2,77	2,32	1,01	0,09
14	39,64	1,80	37,86	0,61	23,22	0,92	2,84	2,43	1,02	0,09
21	41,80	0,60	37,83	0,33	20,38	0,41	2,77	2,31	1,03	0,09
28	40,92	0,85	37,42	0,28	22,06	0,24	3,04	2,62	1,08	0,12
35	40,58	0,82	38,01	1,21	21,80	0,96	2,95	2,43	0,93	0,10
42	42,13	1,11	37,83	0,52	20,10	1,68	2,93	2,41	0,92	0,13
49	42,73	2,79	37,56	0,61	21,03	0,95	3,16	2,87	0,91	0,16

Tab 7.Evolution of wine color parameters during 42 days ageing

Means of 5 replicates \pm DS

The results obtained over the 6 weeks of ageing indicate that color intensity decreased with ageing time and that the highest decrease was experienced by wine stored 21 days with of American oak chips This change in color could be explained by the leaching of wood compounds that either have color or that

condense with colored compounds(Figure 26). The higher intensity of color in wine treated with chips is mainly due to the yellow and blue components. Both color components are clearly higher in wine stored in alternative systems than in barrels. This result agrees with tonality data, where it is evident that wine stored with chips has the highest yellow/ red ratio until 28 days of ageing.(Figure 27)

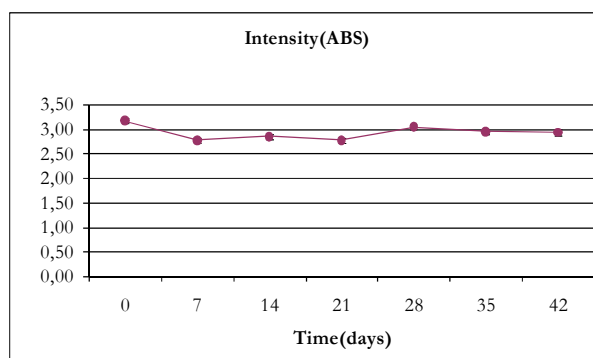


Fig.26.Intensity (Abs)measured in a commercial wine aged with American oak chips highly toasted.

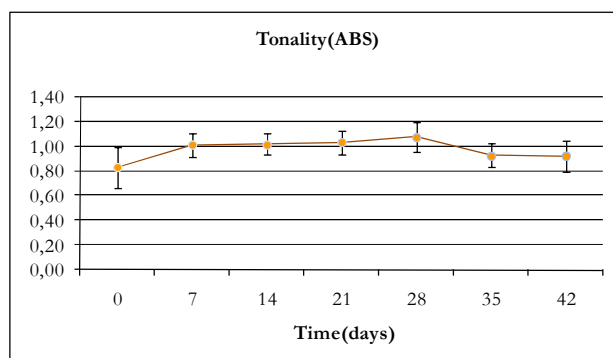


Fig 26. Tonality values measured in a commercial wine aged with American oak chips highly toasted.

During ageing wine experienced a large number of chemical changes and the component most affected from this reactions are polyphenols.

Polyphenols were analyzed as total in order to define their evolution during shortening ageing, although we examined their single amount by means with

HPLC system. Derivatives of benzoic and cinnamic acids are the main phenolic compounds that give the typical character to the aged wine. Phenolic compounds contribute directly or indirectly to colour astringency, bitterness, and aroma .

Results of routine wine analyses obtained at the beginning and at the end of ageing process are shown in the graphics below.

Chlorogenic acid, Gallic acid, Cinnamic acid, p-cumaric acid, vanillic acid, caffeic, hydrocaffeic acid, acid syringic acid and ellagic acid, catechin and epicatechin were the most representative compounds.

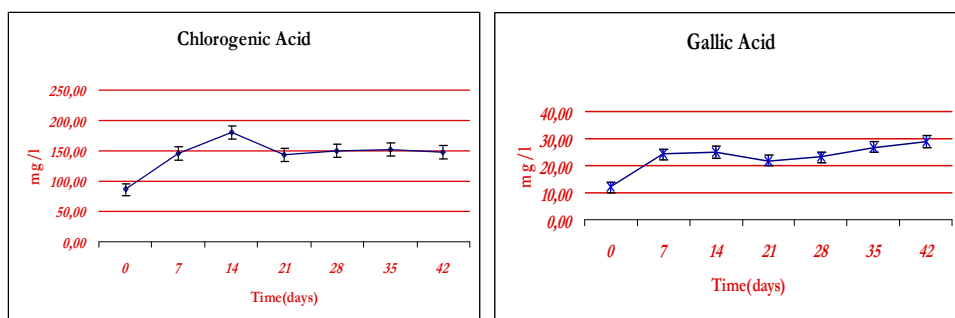


Fig.28 Chlorogenic and Gallic acid trend in wine shortening aged with oak chips.

The concentration of Chlorogenic acid and Gallic acid increased during shortening ageing, in particular the increasing value of gallic acids it is due to the constantly extraction of hydrolizable tannins from oak chips(Fig.28).

The changes in ellagic acids concentrations were in agreement with the contribution to Acutissimins formations. It reached the highest amount after 21 days of ageing.at the same time there were the catechin and epicatechin reduction even for flavano-ellagitannins hemisynthesis.

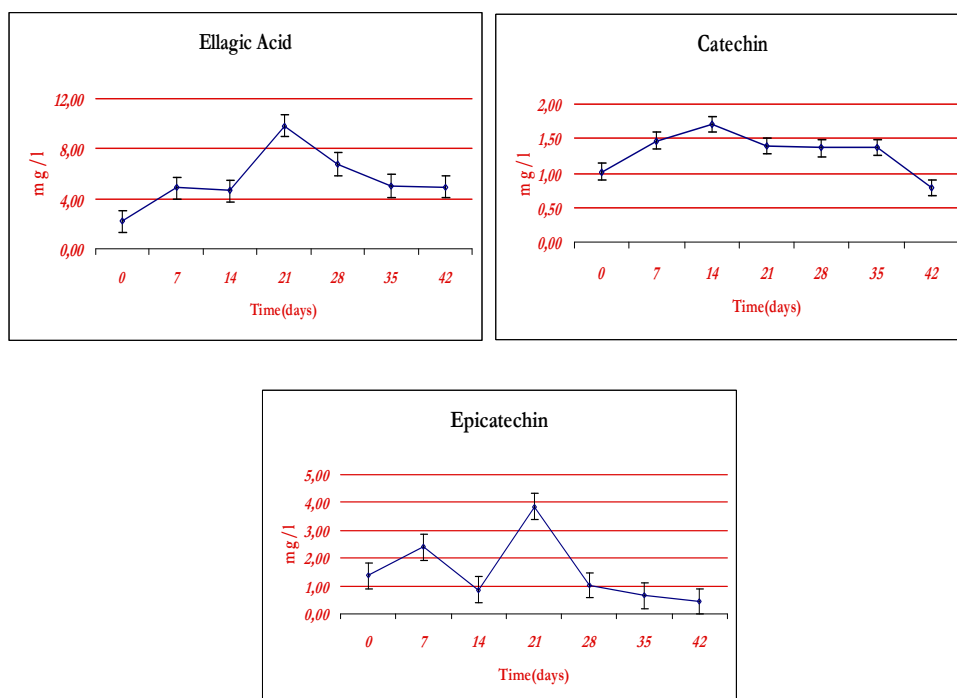


Fig.29 Phenols trend in shortenin ageing with oak chips of red commercial wine.

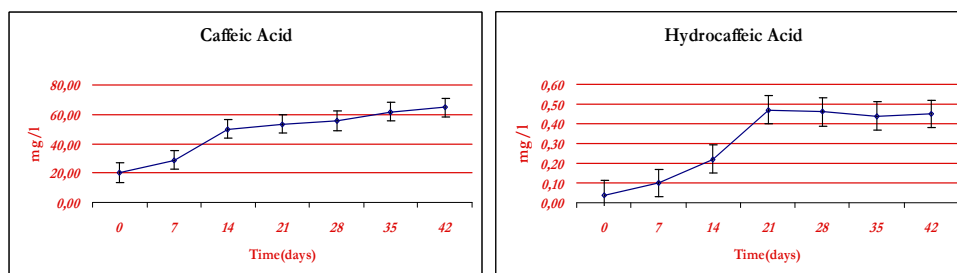


Fig.30. Phenols trend in shortenin ageing with oak chips of red commercial wine

In figure 30 caffeic and hydrocaffeic acids showed a positive trend along 42 days of ageing. Changes in o-cumaric, p-cumaric, salicic acid content are shown in figure 31.

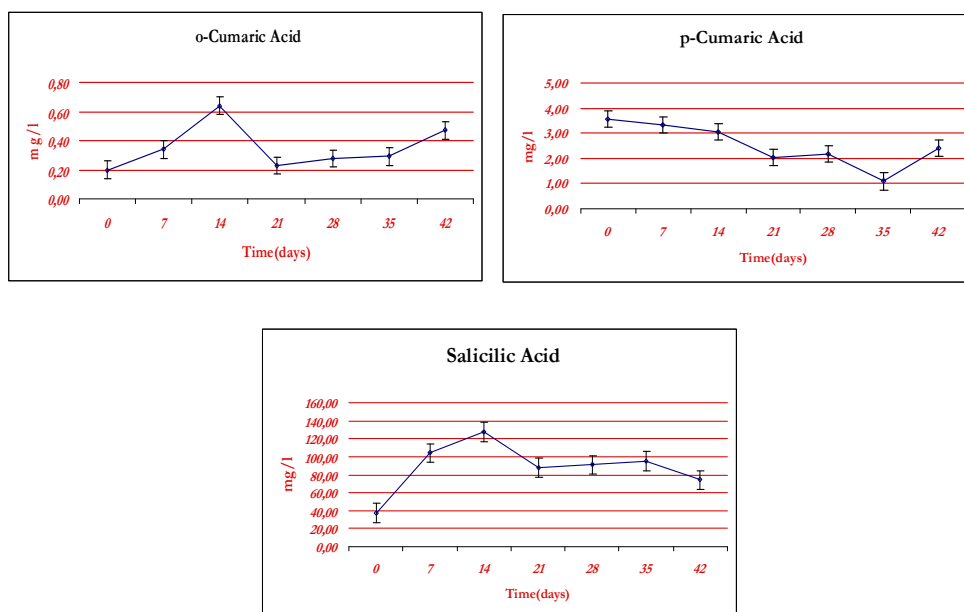


Fig.31. Phenols trend in shortenin ageing with oak chips of red commercial wine

The presence of vanillin that can strongly influence wine aroma and thus it is the important phenolic compound of ageing of beverages in toasted wood barrels although, in the case of wines. In our study, its content constantly increased in time after the initial equilibration and so our results confirm other bibliographic results on its amount in wines aged with alternatives methods.

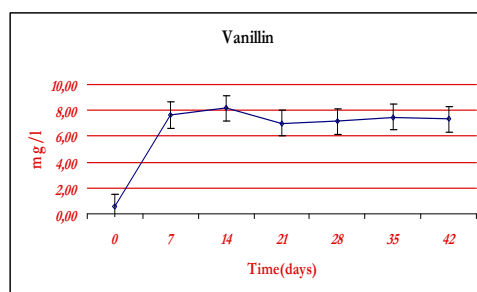


Fig.32. Vanillin content in wine aged with American oak chips.

The toasting intensity of the wood on the phenolics and vanillin determines a rising values of these compounds in wine of this two compounds, vanillin is the most important and it can have a great influence on wine aroma as its perception threshold is low. Just as happens with the furanic aldehydes, for short ageing periods, vanillin accumulates in wine, because at the beginning its extraction is high, due to the difference of concentration between the wine and the wood.

Evaluation of Volatile Compounds

The extraction of volatile compounds from oak into wine during aging, depends both on the pool of potential extractable compounds originally present in the staves of the barrels or in this case in the quantity of chips added into wine

The factors affecting the pool of oak extractives are mainly: the species and geographical origins of the oak wood, the seasoning of the staves and the toasting of the wood pieces.

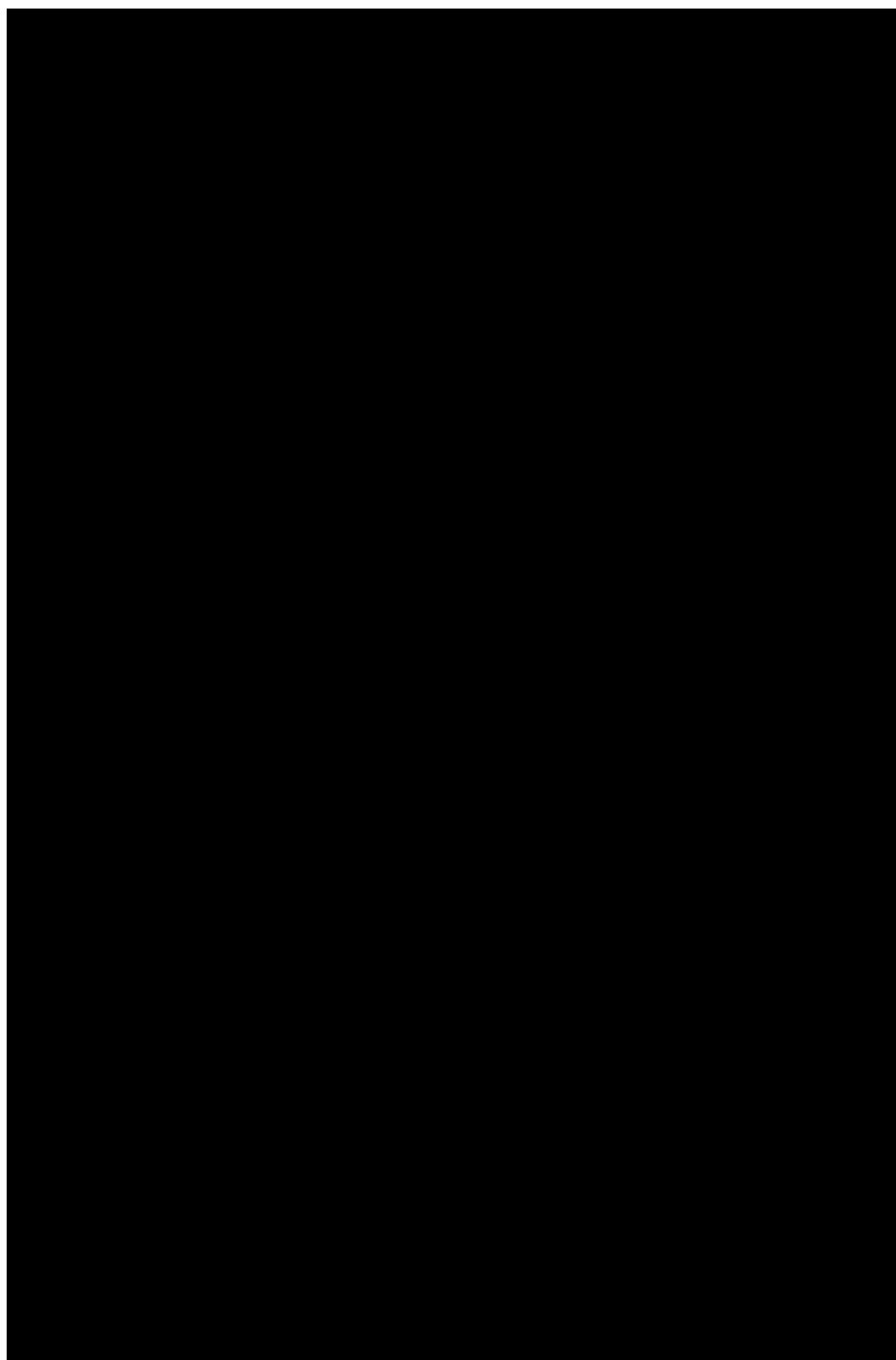
Oak wood is mainly composed of three large insoluble polymers-cellulose, hemicellulose and lignin. It also contains other compounds of lower molecular weight, such as volatile and non-volatile acids, sugars, steroids, terpenes, volatile phenols, lactones which can be extracted in wine. Although aging conditions such as temperature, length of time and humidity affect the characteristics of wine.

The aromatic profile of the commercial wine aged with the oak chips of this research is showed in table 8 below.

49 different volatile compounds was identified.

The most abundant were the typical aromatic compounds of red wine such as 3-methyl-Butanol, 2-methyl-Butanol and the Phenylethyl Alcohol which conferred the fruity and rose flavor to wine. Their percentage increased after the first week of aging and then remained constant. Ethyl-esters are syntetized by yeast during fermentation .At present little is known about their evolution during aging of wine with pieces of wood. This compounds are in equilibrium with their corresponding acids with ethanol, so alcohol level of wine could affect this equilibrium and as a consequence, their concentration during aging. It has also been observed that the

evolution of esters during aging can be influenced by pH of wine (Garolfo & Piracci, 1994; Ramey & Ough, 1980).



Tab.8 Volatile compounds(%) of red wine shortening aged with oak chips.

Diethyl butanoate, succinate and lactate are largely fermentation products , but oak aging has been shown to increase levels of these compounds to a certain degree. In our experimental samples their percentage decreased respect the wine without oak chips but reached a 5% at the end of aging period.

It is useful to consider the oak compounds in related groups –volatile phenols (guaicyl and syringyl based compounds), furan based compounds and the oak lactones. Guaiacol is produced by the lignin's breakdown during wood toasting and it is responsible for the burn overtones of wine aroma (Aiken & Noble 1984, Chatonnet, 1998). The wine rapid aged of our research experienced an increasing amount in percentage of syringaldehyde until along the 42 days of aging. It is related to the Vanilla character of wine(Chatonnet et al 1998)

Esahydropirene, a typical product of burned wood, has been released during all the aging period, and reached an high value at the end of maceration.

Furaldehyde that originates from degradation of monosaccharides produced by partial hydrolysis of hemicelluloses, contributes the character of dried fruits and burned almonds to wine. It's amount was about 0,70% of all volatile compounds and remained constant during aging.

Vanillin emanates from lignin degradation, represents the 0,26% of total volatiles and reached this value after 42 days of aging. It influences wine aroma directly and pleasantly by attributing a character of vanilla.

According to bibliographic studies furfural syringaldehyde and vanillin presents a rapid extraction rate in wines treated with oak chips and also their amount is higher than in traditional barrel aged wine. Although this behavior is due to the weight of pieces of wood added and not to their surface of contact.

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6.CONCLUSIONS

This PhD research started from the actual problem of using pieces of wood in wine aging, and underlined the complexity of chemical evolution which a wine undergoes during aging.

The presence of Acutissimins, first examined as anticancerogenic molecules, has been well demonstrated by using the study of a hydro alcoholic model system.

Flavano-ellagitannins hemisynthesis begins since the 7th day of maceration of wood in ethanolic medium . At the end of maceration, there is an high amount of acutissimins coupled with an higher amount of catechin. Instead at the beginning (7th day) their formation is influenced by oak quantity added to solutions.

Higher amount of catechin led to lower values of Acutissimins .

The best results have been reached with 100 or 500 mg/l of catechin and in particular with 10 % of oak pieces added.

The wine aged with Oak chips presents an acutissimins amount lower than in the model solutions and this value still remains under 0,2 mg/l, according to Jourdes bibliographic indication.

Colour parameters confirmed the rapid evolution of wine with the typical features of aged wine as red-yellow colour, low intensity value and high tonality. The aromatic profile of wine aged with oak chips, also is characterized from vanillin and aldehydes extracted from wood and had the burned aroma compounds which are so common in the barrel aged wine.

This qualities have been reached in 42 days instead 12 or 18 months as in the case of traditional aging.

Acutissimins determination in a rapid aged wine confirm the hypothesis of a general chemical transformation pathway of wine c-glucosydic ellagitannins not only in a wine aged in a traditional way (18 months in barrel) as Jourdes and Quideau demonstrated, but also in a commercial wine aged with oak chips.

This research could be considered one of the first Italian attempt to study alternatives aging techniques in winemaking, by demonstrating Acutissimins presence which could be positive in a nutritional way, we also define the relationship of a rapid aged wine quality aspects to the traditional one.

Special Thanks

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