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Effect of size and toasting degree of oak chips on the ellagitannins content and on acutissimin formation in wine model solution and in red wine

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Guardai il cielo: la via Lattea si stendeva sopra di me, mostrando l'immenso cammino che dovevamo percorrere. In un altro momento, questa immensità avrebbe suscitato in me una grande angoscia, una paura terribile di non poter ottenere niente, di essere troppo piccolo per quell'impresa. Ma quel giorno io ero una semente ed ero nato di nuovo. Avevo scoperto che, nonostante il conforto della terra e del sonno che stavo dormendo, la vita "lassù in cima" era molto più bella. E avrei potuto nascere sempre, ogni volta che avessi voluto, finché le mie braccia fossero diventate abbastanza grandi da stringere tutta la terra da cui provenivo.

Paulo Coelho, Il cammino di Santiago

Contents

Abstract

L'invecchiamento in botte è una pratica comune e consolidata, alla quale sono sottoposti i migliori vini. L'elevato costo delle botti di rovere e i lunghi periodi di stoccaggio del vino in cantina, insieme alla crisi finanziaria che negli ultimi anni si è abbattuta sull'Europa, sono le principali cause che hanno costretto i produttori ad utilizzare dei materiali alternativi più economici come, ad esempio, i trucioli di legno. Fino al 2006, in Europa l'aggiunta di trucioli di legno nel vino era illegale, mentre nei paesi vitivinicoli emergenti (Australia, Nuova Zelanda, California, Cile) questa pratica è consolidata già da prima. Di conseguenza, queste nazioni hanno conquistato il mercato di massa con vini più scadenti, ma con un buon rapporto qualità prezzo. Per rafforzare la competitività del mercato vitivinicolo comunitario, nello stesso anno, è stato emanato il *Reg. CE N. 1507/2006* che ha ammesso l'utilizzo dei trucioli di legno di quercia nell'elaborazione di un vino, purché l'utilizzo sia specificato in etichetta.

Durante l'invecchiamento con legno, il vino subisce delle importanti modifiche organolettiche, dovute in parte ad una progressiva solubilizzazione di molecole naturalmente presenti nella matrice legnosa come gli ellagitannini (vescalagina, castalagina, grandinina e roburina E).

Gli ellagitannini hanno una struttura chimica molto complessa, tale da coinvolgere queste molecole in reazioni di ossidazione e polimerizzazione. In particolare, la vescalagina reagisce con la (+)-catechina attraverso un meccanismo di sostituzione nucleofilica SN_1 per formare l'acutissimina A e/o B.

La concentrazione di ellagitannini nel legno dipende da diversi fattori naturali come, ad esempio, la specie botanica dell'albero e l'origine geografica dello stesso. In più, anche i trattamenti tecnologici come la tostatura, incidono sulla loro quantità nel legno.

Il progetto di ricerca ha avuto due obiettivi. In una *prima fase*, è stato valutato l'effetto della dimensione (2 mm e 8 mm) e del grado di tostatura (leggero, medio e forte) dei trucioli di legno sull'estrazione degli ellagitannini in soluzioni modello che simulano la composizione del vino, per 35 giorni. In più, è stato studiato l'impatto della concentrazione di (+)-catechina (0 mg/L, 50 mg/L e 200 mg/L) sul contenuto di ellagitannini e sulla formazione di acutissimina A e B.

In una *seconda fase*, sono stati quantificati gli ellagitannini e le acutissimine A e B in vino rosso "*Aglianico di Taurasi*" invecchiato per 35 giorni con gli stessi trucioli utilizzati nella preparazione delle soluzioni modello. Inoltre, i risultati sono stati comparati rispetto a quelli ottenuti analizzando tre differenti vini rossi italiani D.O.C. invecchiati in botte al fine di identificare differenze statisticamente significative tra vini invecchiati artificialmente e tradizionalmente.

È stato osservato che il grado di tostatura dei trucioli di legno ha inciso in maniera statisticamente significativa sulla concentrazione di ellagitannini nelle soluzioni modello. In particolare, in quelle a contatto con i trucioli leggermente tostati è stato rilevato il maggior contenuto di ellagitannini. Inoltre, differenze significative nel contenuto e nella cinetica di estrazione degli ellagitannini sono state rilevate in base alla dimensione dei trucioli di legno. La concentrazione di (+)-catechina non ha influito sull'estrazione degli ellagitannini, mentre la formazione delle acutissimine A e B è dipesa sia dal contenuto del flavanolo che dal grado di tostatura dei trucioli di legno. Il maggior contenuto di acutissimine A e B è stato rilevato nelle soluzioni modello contenenti 200 mg/L di (+)-catechina e trucioli di dimensione 2 mm con grado di tostatura leggero.

Per quanto riguarda la cinetica di estrazione degli ellagitannini nel vino, sono stati ottenuti risultati simili rispetto a quanto osservato per le soluzioni modello. Anche in questo caso, nei vini a contatto con i trucioli 2 mm leggermente tostati è stato rilevato il maggior contenuto di ellagitannini. In accordo con altri studi, in tutti i campioni di vino non sono state rilevate le acutissimine A e B. Considerando che in vini invecchiati in botte per 9 e 18 mesi sono stati rielvati i derivati dei tannini C-glucosidi, è possibile che le acutissimine A e B possano essere dei markers utili a discriminare vini invecchiati in botte o con trucioli di legno. Tuttavia, lo studio deve essere supportato da ulteriori indagini.

1.Introduction

There are many reasons and rewards for the study of wine. The grape has been said to be the only fruit that naturally preserves itself, and there is historical justification for the statement. At a time when our modern techniques of storing fresh food were undreamed of, and fresh vegetables and fruits were available only during the short local season, wine was indeed the gift of God. With only modest invention by ancient man, the grape and its associated yeast produce wine. Here was a food with a flavor like the fresh fruit which could be stored and transported under the existing conditions. At least part of the time it survived in drinkable condition from season to season or occasionally for many reasons.
The fact that wine produced euphoria was not lost on ancient man, and it became not only a regular part of the diet but also a social beverage used for feasting, celebrating, and entertaining guests. The grat variability possible in quality and type of wine naturally led to rating and selection. So wine early became an item of commerce, with appropriate quality judgments, records, and connoisseurship. As a result of all this, wine has deeply penetrated the social fabric and culture of times and countries from which we spring.
Wine is not just another product, and its worthiness for study is further illustrated by the extent of literature available. When Thudichum and Duprè wrote their scientific treatise on wine in 1872, they stated that they had consulted 200 of 600 extant works on enology. It is estimated that more than 20,000 books and pamphlets, in various languages, dealing specifically with wine or wine grapes have been published in this century.
Knowledge of wine contributes to an understanding of Greek mitology, Egyptian and Oriental art, excavations at Pompeii, early agriculture and its spread aesthetics and sensory appreciation, commerce, treaties, laws from Hammurabi to Prohibition to vehicle codes, and social interaction from ancient symposia throughout Shakespeare and Pepys to a modern diplomatic dinner.
To define wine simply is not easy, for the definition would vary according to the context and the attitudes of the definer and his audience. Wine is even differently in the detailed laws of various countries, and in popular usage wine means different things to different people. In Chinese the unqualified word for wine also mean “alcoholic beverage”; so beer becomes a “wine” and may be translated “appetite wine”. Perhaps this accounts for the fact that we speak of “rice wine”, a literal translation from Chinese. Even within the group of beverages which certainly are wine, as we use the term, there are many products with varied origins and uses. The most generally satisfying definition seems to be that wine is a beverage resulting from the fermentation by yeasts of the juice of the grape, with appropriate and legal processing and additions. Aging wine in oak barrel or with oak chips is an example of legal processing.

2. The present and the future of the International wine industry

2.1 Introduction

Wine is a unique commodity. Its production predates recorded history, as does the discovery of the healthful benefits of wine, now largely attributed to the antimicrobial activity of ethanol. Throughout antiquity the conversion of grapes into wine was considered a gift from the Gods and the best wines were thus reserved for the elite society. The image of wine as a beverage of the affluent persist today. Wine was also one of the first commodities to be bartered by early civilizations engaged in international trade. Then, as now, the most successful wine producers were those who grasped market forces of supply and demand, and whose products met the prevailing definition of quality.

Today, wine is an integral component of the culture of many countries,. a form of entertainment in others, and a libation of choice for advocates of its health benefits. Unlike many modern foods, wine's attractions rely not on bold consistent flavors, but upon a subtle array of shifting sensation that make its charm difficult to define. In essence, wine producers are selling a sensory experience to the consumer. Wine consumer in developed nations are typically prosperous, but wine is also consumed in impoverished areas where it is still safer to drink than the local water supply. Regardless of the region in which the wine is produced or the economic status of the consumer, all wines are expected to be pleasant experiences for the imbiber.

In past generations, the definition of quality was the preserve of the wine producer, and consumers who did not like a particular style of wine were often made to feel uncultured. But globalization and the accompanying rapid worldwide access to information has resulted in a more kwnowledeable and empowered consumer. Success as a wine producer in the twenty-first centuries requires a thorough appreciation of human behavior product choice.

Wine is again a unique commodity in this respect. The intrinsic sensory aspect of wine taste and aroma are only one component in the modern consumer definition of quality. Extrinsic factors such as bottle and label design and the perceived artistic talent of the winemaker are equally important motivators of human preference in wine selection. .In addition to a product that is enjoyable in all sensory aspects, consumers expect wines to be healthful and produced in an environmentally sustainable manner. In the future, these last two factors will become increasingly important economic driver of profitability.

These issues are complex, requiring producers to understand latest developments in wide-ranging disciplines of science technology. The present-day wine industry is focused on optimizing the attractiveness of the product within the bottle. In the future, the industry will need to go beyond this, paying more attention to the extrinsic factors motivating product choice, while ensuring that production remains cost-effective and economically sound (Bisson et al., 2002).

2.2 The economics of wine productions

In contrast to other types of crops, grapes can be grown in diverse climate and solids. Although scientifically still unproven, environmental stress is believed to improve the sensory characteristics of grapes and wine, resulting in a better product. The French concept of *terroir* state that compositions of grapes produced in a specific growing region will be influenced by the local environment, which will carry through to the wines of the area (Laville, 1990). This concept also includes as an element minimal intervention in modification of growing environment so that the *terroir* may be evident. Thus, in contrast to other agricultural commodities, wine is marketed by the geographical location of production, and quality is associated with minimal wine yard inputs or manipulation.

Consumer except wine from a particular region to possess unique qualities that differentiate it from other wine of the same varietal from other regions. This peculiarity of the industry in a great economic equalizer across the globe. It means that wine perceived to be of high quality can be produced anywhere. Indeed, quality wine are currently being produced on all six arable continents, and affluent as well as emerging nations are active in the international wine trade. The heightened tourism that accompagnies the "discovery" of a new wine-producing regions is economically important to many countries. This "value added" economic aspect of wine production is remarkable, and the main reason that many governments support strong research programs in the development and improvement of their wine industries.

The wine industry is actually a composite of several individual economic or market segments. In the United States, roughly 70% of the market is comprised of the "economic" wine, those that retail for less than US$7 per 750-mL bottle. Wines range from economy to premium, ultra-premium and artisan, with wines in the latter two categories comprising only 2-3% of the market and commanding high prices.

However consumers have high expectations for product quality in all price categories. The actual sensory attributes desired may differ, but not the expectation of a pleasing sensory experience.
During the last third of the twentieth century the world wine market became significantly more competitive. Consumption declined in the traditional wine producing and consuming countries, while States, Australian, Chile, and prosperous consumer chose quality rather than quantity in consumption. In 2001, France, Italy and Spain combined to produce slightly more than half of all the world's wine, but in the past 30 years their own per capita consumption has fallen 40-50% (**Figure 2.1**), leading to an oversupply of "old World" wine (Protin, 1971). During the same period, US per capita wine consumption has almost doubled and, more important, US consumers have chosen to drink more expensive wine in a search for quality, a trend that seems to be true of European wine consumption as well. The New World producers have been quick to respond to global perceptions of quality, and have gained significant market share in the past 20 years, moving from 2 to 15% of the world export market, largely at the expense of the European producers (Aigrain, 2001).

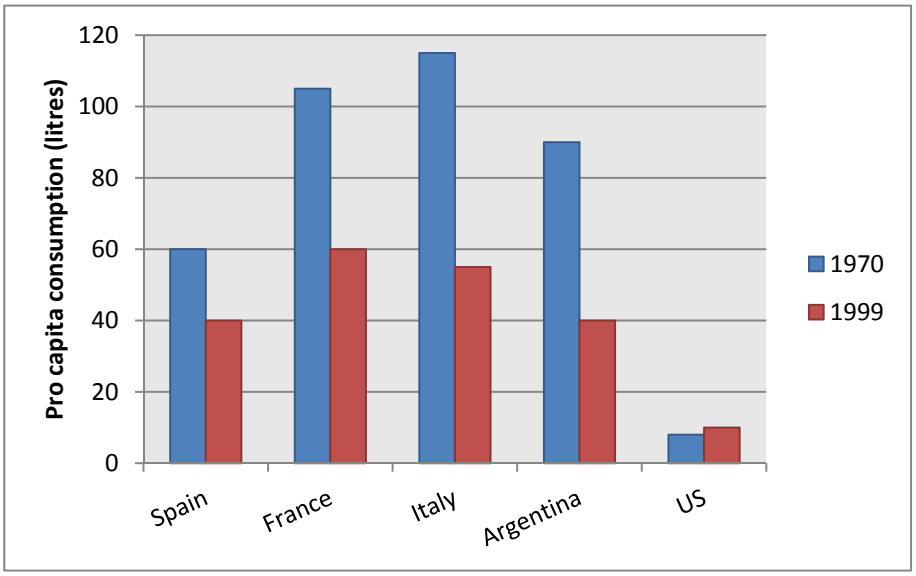

Figure 2.1. *Changes in total consumption of wine by country over the past three decades (Data from Bisson et al., 2002).*

Factors other than enhanced product quality have fuelled the increase in US consumption of wine. In 1991, a study by Serge Renauld coined the term "French paradox" to describe the relationship between the high intake of fats in French diet and the low incidence of coronary heart disease. This well-turned phrase galvanized the attention of media, the public and other scientist. Although there are many differences between the American and the French diets, attention quickly focused on the disparity in wine consumption between the two countries. This was because of the long recognized mechanistic explanation for the paradox, which led many to doubt its validity, but Kinsella and colleagues (Kinsella et al., 1993) proposed that the natural antioxidants phenolic compounds of wine and fruits and vegetables of the Mediterranean diet might protect against heart disease (Renaud & Lorgeril, 1992). This conclusion was based partly upon a new theory advanced by Steinberg that linked oxidation in the blood to disease.
The power of the antioxidant hypothesis, coupled to the higher visibility of Renauld's report and strong pressure from public sector, promoted investigation into the chemical and biological activities of alcohol, dietary phenols and flavonoids. Today there is scientific evidence that moderate alcohol and/or wine consumption protect against the incidence of many diseases of modern society – cardiovascular disease, dietary cancer, ischaemic stroke, peripheral vascular disease, diabetes, hypertension, peptic ulcers, kidney stones and macular degeneration – in addition to stimulating resistance to infection and retention of bone density (German et al., 1997; De Lorimier, 2001). The benefits of antioxidants are more pronounced in red wines as these wines contain higher phenolic content, but white wines also offer some benefits to consumer.
The impact of the "French paradox"and the popularization of this study by the media had a pronounced impact on the international wine industry. Consumers were willing to pay more for a product with perceived health benefit, while still expecting a satisfying sensory experience. This market remains highly

competitive, as wine produced anywhere in the world may possess the same health-promoting effects. Some growing regions are even marketing their wine based on antioxidant content.

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3. Description and composition of the mature grape

3.1 The berry

The grape constitutes the raw material for producing wines. Its maturity level is the first factor, and certainly one of the most deciding ones, in determining wine quality. It is the result of all the complex physiological and biochemical phenomena whose proper development and intensity are intricately related to environmental conditions.

The grape is a berry, classed in a group of several seeded flesh fruits. The berries are organized into a cluster. Each berry is attached to the rachis by a small pedicel containing the vessel, which supply the berry with water and nutritive substances. Genetic factors and environmental conditions that characterize berry formation greatly influence its development and its composition at maturity.

3.2 Berry formation and the development stages of the grape

Fruit development is closely related to the modalities of ovule fertilization. Flowering correspond to the opening of the corolla and the ejection of the calyptras. The pollen liberated in this manner can reach the ovary and trigger its growth. Pollination is normally followed by fertilization, permitting the development of a berry possessing one to four normal seeds.

In the course of its development, from ovary to ripe fruit, the grape follows an evolution common to all berries. It is generally divided into three phases, taking into consideration parameters such as berry diameter, weight and volume:

1. An initial rapid growth or herbaceous growth phase lasting 45 to 65 days; depending on vine variety and environmental conditions. The intensity of cellular multiplication depends on the existence of seeds. Growth hormone concentration (cytokinins and gibberellins) correspond directly with the number of seeds. Cellular growth begins about 2 weeks after fertilization and continues until the end of the first phase. In the course of the first period, chlorophyll is the predominant pigment (Ito et al., 1969). The berries have an intense metabolic activity, characterized by an elevated respiratory intensity and a rapid accumulation of acids.
2. A slowed growth phase during which the apparition of color in colored varieties and a translucent skin in white varieties (véraison). It is an abrupt phenomenon at the berry level but takes place over several days when different berries of the same brunch are considered. It corresponds with the depletion of growth substance and an increase in the concentration of abscisic acid.
3. A second growth phase corresponding to maturation. Cellular growth resumes and is accompanied by diverse physiological modifications. The respiratory intensity decreases, whereas certain enzymatic activities sharply increase. This final period last 35 to 55 days, during which the grape accumulates free sugars, cations such as potassium, amino acids and phenolic compounds, while concentrations of malic acid and ammonium decrease. Grape size at maturity depend largely on these accumulation processes but also on the number of cells per berry.

3.3 Grape morphology

Each grape comprises a group of tissues (the pericarp) surrounding the seeds. The pericarp is divided into the exocarp (the skin), the mesocarp (the pulp) and the endocarp (the tissue that lines the seed receptacles containing the seeds but is not distinguishable from the rest of the pulp). The fruit is nourished by a branching vascular network of the rachins, which traverses the pedicels (**Figure 3.1**).

The skin of the grape forms a heterogeneous region constituted by the cuticle, the epidermis and the hypodermis. The cuticle is a continuous layer whose thickness varies depending on the variety. In the course of berry maturation and development, in becomes increasingly disorganized and its thickness diminishes. The cuticle is generally covered by epicuticular wax.

The epidermis is constituted of one or two layer of tangentially elongated cells whose thickness varies depending on the grape variety. The hypodermis comprises two distinguishable regions:

1. an outer region with rectangular cells;
2. a inner region with polygonal cells.

The pulp is composed of large polygonal cells, with very thin, distended cell walls. There are 25-30 cell layers, organized into three distinct regions.

Each normally constituted seed comprises a cuticle, an epidermis and three envelopes covering the albumen and embryo.

Grape berry consistency depends on skin and pulp cell wall thickness.

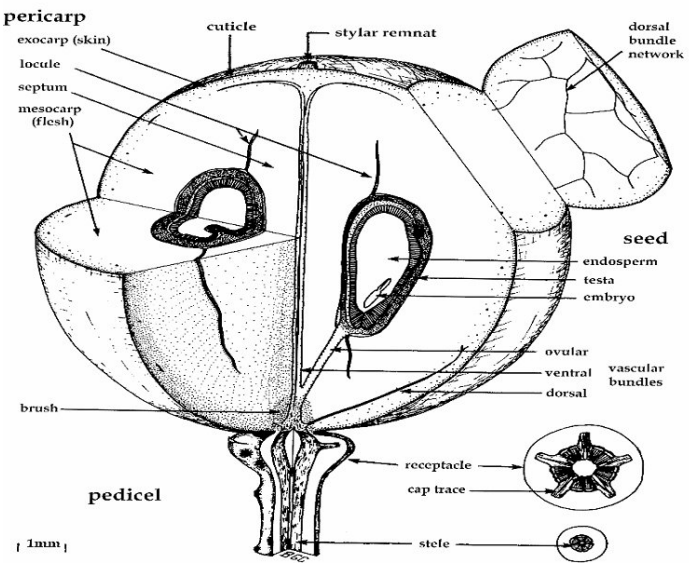

Figure 3.1. *The grape morphology.*

The stalk rachis represent around 3-7% of the weight of a ripe grape cluster. Its chemical composition is similar to the composition of leaves. Its contains little sugar (less than 10 g/kg) and an average acid concentration (180-200 mEq/kg). These acids are in the form of salts, due to the large quantity of cations present. Stalks are rich in phenolic compound. They can contain up to 20% of the total phenolic compound concentration of the grape cluster, event thought they represent a lower proportion of the total weight. These phenolic compounds are more or less polymerized and have a very astringent taste.
The stalk attains its definitive size around the véraison. Although it loses most of its chlorophyll, it remains green during the maturation. It is often completely lignified well after maturity.
The seeds represent 0-6% of the weight of the berry. They contain carbohydrates (35% on average), nitrogen compounds (around 6%) and minerals (4%). An oil can be extracted from the seeds (15-20% of the total weight) which is essentially oleic and linoleic acid. The seeds are an important source of phenolic compounds during red winemaking. Depending on the varieties, they contain between 20 and 55% of the total polyphenols of the berry.
The seed attain their definitive size before véraison. At this time, they have reached physiologicalmaturity. During maturation, the tannin concentration of the seeds decrease whereas their degree of polymerization increases. Conversely the nitrogen compounds are partially hydrolyzed. The seeds can yield up to one-fifth of its nitrogen to the pulp, while still remaining richer in nitrogen than the other solid parts of the grape cluster.
Depending on the grape variety, the skin represent from 8 to 20% of berry weight. Being a heterogeneous tissue, its importance depends greatly on the extraction method used. Separating the skins by pressing the grapes results in the extraction of the pulp and seeds. The sugar concentration of skin cells is very low. For the same weight, the skin is as rich in acids as the pulp but citric acid is predominant. Malic acid, in significant quantities in the skin of skins of green grapes, is actively metabolized in the course of maturation. The majority of tartaric acid is esterified by phenolic acid (cafeic, coumaric). A significant quantity of cations cause the salification of these acids. The contents of the skin cells always have a higher pH than the pulp.
The skin is especially characterized by significant quantities of secondary products of major enological importance (phenolic compounds and aromatic substances). It accumulates these substances during maturation.
The following phenolic compounds are present in the grape skin at maturity: benzoic and cinnamic acid, flavonols and tannins. They are distributed in the cells of the epidermis and the first sub-epidermal layers in both white and rape grapes. In addition, the red grape skin contains anthocyanins, essentially located in the hypodermal cell layers. Anthocyanin composition varies from cultivar to cultivar, depending on the anthocyanidin substitution and heterosidic nature of the cultivar.
The ripe grape skin also contains considerable amounts of aromatic substances and aroma precursors. Other chemical families of aromatic substances may also be contained in the skin. Finally, the skin is covered by epicuticular wax, essentially constituted of oleanolic acid.

All of this information is very important from a technological point of view. All methods increasing he solid-liquid contact for color extraction of aroma dissolution should be favored during winemaking.
The pulp represents the most considerable fraction of the berry in weight (from 75% to 85%). The vacuolar contents of the cell contain the grape must-the solid parts (cytoplasm, pectocellulosic cell walls) constituting less than 1% of this tissue. The must is a cloudy liquid, generally slightly colored, having an elevated density due to the chemical substances that it contains. Sugars are the primary constituents-essentially glucose and fructose. Fructose is always predominant (the glucose/fructose ratio is around 0.9). Saccharose, which is the migratory form of sugar in the plant, exist in only trace amounts in the grape. Other sugars have been identified in the grape: arabinose. Xylose, rhamnose, maltose, raffinose. The reducing sugar concentration in normal ripe grapes varies from 150 to 240 g/L.
Most of the acids of the metabolism are found in trace amounts in ripe grape pulp. Must acidity, an important element of enological data, is essentially constituted by three acids: tartaric, malic and citric acid. It can vary from 3 to 10 mg/L in sulfuric acid or from 4.5 to 15 g/L in tartaric acid, depending on the cultivar, the climate and grape maturity. Phosphoric acid is the preponderant inorganic anion.
The pulp is particularly rich in cations. Potassium, the principal element, is much more abundant tha calcium, magnesium and sodium. The other cations are present in much lower concentrations, with iron representing 50%of the remaining cations.
Must pH currently varies between 2.8 and 3.5.
The pulp contains only 20-25% of the total nitrogen content of the berry. The must contains 40-220 mM of nitrogen in its ammoniacal or organic form. The ammonium cation is the most easily assimilable nitrogen source for yeast and it is often present in sufficient quantities. The amino acid fraction varies from 2 to 13 mM in leucine equivalents (2-8 g/L).
At maturity, the grape is characterized by a low concentration in pectic substance with respect to other fruits. Pectins represent from 0.02 to 0.6% of fresh grape weight. Differences from cultivar to cultivar and from year to year can be significant. Only the free pectic fraction, associated to diverse soluble loses, it likely to be found in must. This fraction also contains small amounts of insoluble proteins.
The skin is considered to be the principal source of aromatic substances, but the pulp does contain significant concentrations of these compounds. The pulp is characterized in particular by the accumulation of a diverse variety of alcohols, aldehydes and esters which participate in grape aromas (Bayonove, 1993).
There is considerable heterogeneity between different grapes on the same grape cluster. Similarly, the diverse constituents of must are not evenly distributed in the pulp. As a primary technological consequence, the chemical constitution of the juices evolves in the course of grape pressing in white winemaking. The peripheral and central zones are always richer in sugar than the intermediary zone of the pulp. Malic and tartaric acid concentrations increase towards the interior of the berry. Potassium is distributed differently within the grape and often causes the salification of the acids, with the precipitation of potassium bitartrate, in the course of pressing.
Finally, the half of the grape opposite the pedicel is generally richer in sugar and poorer in acids than the proximal half.

3.4 Changes in the grape during maturation

The biochemical processes of maturation have traditionally been summarized in the transformation of a hard, acidic green grape into a soft, colored fruit rich in sugar and aromas. As already indicated, these transformation can only occur when the grape is attached to the rest of the plant. In this case, the increase in the concentration of a substance in the berry can be due to importation of this substance, on-location synthesis or water loss in the vegetable tissue. Conversely, its diminution can result from exportation, degradation or water gain in the tissue (Ruffner, 1982).
During maturation, the grape accumulates a significant quantity of solutes, principally sugars. In spite of berry enlargement (cellular enlargement), the percentage of solid material increases-indicating that solutes are imported in greater quantities than water. The amount of water that accumulates each day in the grape is the sum of phloem and xylem sap flux minus the water loss due to transpiration. At the start of maturation, the berries simultaneously import water with the sugars, but the amount of water transpired rapidly diminishes as the stomata degenerate; then, transpiration uniquely occurs across the cuticular wax. Sugar accumulation than occurs against the diffusion gradient, often up considerable concentrations corresponding to a substantial osmotic pressure. In addition, the xylem solute supply strongly diminishes after véraison. This phenomenon, due to a partial vascular blockage, has an impact on the accumulation of certain substances, especially minerals. Peripheral vessels then become responsible of most of the food supply to the grape.

The grape is more than an accumulation organ: it maintains an intense activity (respiration and biochemical transformations) during maturation. Véraison also correspond to the synthesis of new enzyme activities and the release of inhibition of other ones. These variations in gene expression cause profound change in grape metabolism (Robinson andn Davies, 2000).

3.4.1 Definition of maturity

In enology, pulp maturity correspond to an optimal sugar/acid ratio; skin maturity in the stage at which the phenolic compounds and aromatic substances attain a maximum concentration. These two kind of maturity can be distinguished, but the dissociation of the cell wall from the skin must be sufficiently advanced to permit easy extraction of these essential constituents (Abbal et al., 1992).
Grape maturation results from several biochemical transformations that are not necessarily related to each other. To simplify matters, the increase in sugar concentration and the decrease in acidity are monitored. The accumulation and refinement of white grape aromas and phenolic compounds in red grapes should also taken into account. The essential property of a quality wine-producing area is to permit a favorable maturation.
This corresponds with a harmonious evolution of the various transformations to reach the optimum point simultaneously at the time of the harvest.
In too cold of a climate, the maturation cannot b satisfactory, but in very warm climate the increase in sugar concentration can impose a premature harvest even though the other grape constituents are not at full maturity. Of course, environmental conditions are involved in these phenomena.

3.4.2 Evaluation of the state of maturity. Maturation Index

Grape monitoring during maturation helps vineyard managers to set the harvest date and maximize the efficiency of their harvest teams according to the ripeness of different cultivars and diverse parcels.
Determining the grape sugar concentration is essential. It is most often effected by an indirect physical measure such as hydrometry or refractometry. The results are expressed in various units, depending on the instruments used.
The relationship between must density and alcohol content is always approximate, since sugar is not the only chemical must constituent that affects density (Blouin, 1992; Boulton et al., 1996).
Empirical observation of the inverse variation of sugars and acidity during maturation led to the development of a sugar/acidity ratio, called the maturation index. This index is very simple but it should be used with precaution, since there is no direct biochemical relationship between sugar accumulation and acidity loss. More specifically, a given gain in sugar does not always correspond with the same drop in acidity. This ratio is not suitable for comparing different varieties, since varieties exist that are rich both in sugar and in acids.
Attempts have been made in the past to describe the state of maturity, taking into account the respective variations of malic and tartaric acid on the accumulation of cations, but none of the indices developed have significantly improved the evaluation of the maturity level.
More recently, researchers have focused on the evolution of phenolic compounds during maturation, but the technique of separating the skin from the seeds is awkward and exacting limiting its practical applicability.
Unfortunately, no simple methods currently exist that permit an aromatic substance maturation index. Tasting the grape remains, in this respect, the only available criterion for judgments but this does not estimate the subsequent revelation of other aromas.
Micro-imagery by nuclear resonance has recently been shown to give detailed information on the chemical composition and degradation level of grape cell walls (Pope, 1993) but this technique will remain reserved for scientific experimentation for a long time.
Fourier transform infrared spectrometry, which has recently been developed, shouldmake it possible to assess grape quality more accurately (Dubernet et al., 2000). This method is easy to implement and does only require prior filtration of the samples. It provides a satisfactory evaluation of the potential alcohol, total acidity, pH and nitrogen content, as well as the color index for black grapes, in a single operation. This new techniques, however, only gives reliable results after a long, laborious calibration process using samples analyzed by standard methods.

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4. Red winemaking

4.1 Introduction

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 mean of maceration, which occurs during must fermentation.
The localization of red pigment exclusively in skins, at least in principal varieties, permits a slightly tinted or white wine to be made from colorless juice obtained from a delicate pressing of red grapes.
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.
Grape quality directly influence 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.
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 grape crop should also be carefully sorted to eliminate damaged or unripe grapes. This operation can be effected in the vineyard during picking or in the winery at harvest reception.
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.

4.2 Basic procedures of wine production

Vinification formally begins when the grapes, or juice, reach the winery. The basic steps in the production of table wines are outlined in **Figure 4.1**.

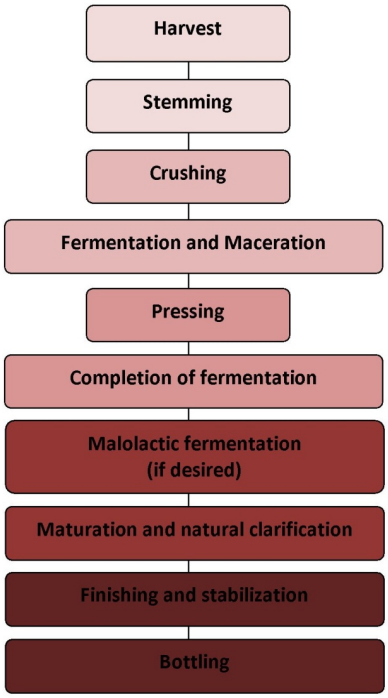


Figure 4.1. *Flow diagram of winemaking.*

The first step involves removing the leaves and other extraneous material from the grapes. The fruit is then crushed (or pressed) to release the juice and begin the process of maceration. Maceration facilitate the extraction of nutrients, flavorants, and other constituents from the pulp, skin, and seeds. Initially, hydrolytic enzymes released from ruptured cells promote this liberation. The cytotoxic action of pectic enzymes further promotes the release of cellular constituents into the must (grape macerate). Enzymes released or activated by cell death may also activate the syntheses of flavor compounds and hydrolyze macromolecules to form utilizable by yeast and bacteria.
For red wines, maceration is prolonged and occurs simultaneously with alcoholic fermentation. The alcohol generated by yeast action enhances the extraction of anthocyanin and promotes the uptake of tannins from the seeds and skins (pomace). The phenolic compounds solubilized give red wines their basic properties of appearances, taste, and flavor. They are also required to give red wines their aging and mellowing characteristics. In addition, ethanol augments the liberation of aromatic ingredients from the pulp and skins. After partial or complete fermentation, the free-run is allowed to flow away under gravity. Subsequent pressing extracts press fractions (most of remaining juice). Press fractions may be incorporated with the free-run in proportions determined by the type and style of wine desired. Fermentation may start spontaneously, due to endemic yeasts derived from the grapes, but more frequently from crushing equipment. Standard practice, however, is to inoculate the juice or must with a yeast strain of known characteristics. Yeasts not only produce the alcohol, but also generate the general bouquet and flavor attributes that typify wines.
After completing alcoholic fermentation, the wine may be treated to foster a second, malolactic fermentation. Malolactic fermentation is particularly valuable in cool climatic regions,where a reduction in acidity ameliorates wines benefit from malolactic fermentation. In warm viticultural regions, malolactic fermentation is often unneeded and undesirable. Its development is usually discouraged by practices such as the addition of sulfure dioxide, early clarification, and storage under cool conditions.
Newly fermented wine is protected from, or givin only minimal exposure to oxygen during maturation. This limit oxidation and microbial spoilage. During storage, excess carbon dioxide escapes, yeasty odors dissipate, and suspended material precipitates. Changes in aroma and development of an aged bouquet may begin during maturation. Exposure to air is usually restricted to that which occurs during racking, or battonage. Such slow or limited exposure can help oxidize hydrogen sulfide and flavor color stability in red wines. After several weeks or months, the wine is racked. Racking separated the wine from solids that settle out during spontaneous or induced clarification. The sediment consists primarly or yeast and bacterial cells, grape cell remains, and precipitated tannins, proteins and potassium tartrate crystals. If left in contact with wine, they can lead to the production off-odors, as well as favor microbial spoilage.
Prior to bottling, the wine may be fined to remove traces of dissolved proteins and other material. Otherwise, they could generate haziness, especially on exposure to heat. Fining may also be used to soften the wine's taste by removing excess tannins. Wine are commonly chilled and filtered to further enhance clarification and stability. At bottling, wines are generally given a small dose of sulfur dioxide to limit oxidation and microbial spoilage.
Newly bottled wines are normally aged at the winery for several moths to years before release. This period permits wines blended shortly before bottling to "harmonize". In addition, it allows acetaldehyde produced following bottling to be converted to nonaromatic compounds. Thus, the "bottle sickness" induced by acetaldehyde usually dissipates before the wine reaches the consumer.

4.3 Preparation of the must

Stemming and crushing are typically conducted as soon as possible after harvesting. During the harvest, some grapes are unavoidably broken and their juice released, whereas other may be bruised. Thus, oxidative browning often begins before the grapes reach the winery and crushing begins. The juice also becomes field-inoculated with the yeast and bacterial flora present on grape surfaces. If the berries are harvested during the heat of the day, undesirable microbial contamination can rapidly develop. To minimize this occurrence, grape may be sulfited after harvest or harvested during the cool parts of the day. Left in containers, harvested fruit quicly warm due to the endogenous metabolic activity of the grapes and the insulating influence of the volume. This can aggravate contamination by speeding microbial activity.

4.4 Destemming

The present-day trend is to separate the process of stemming and crushing physically, if not temporally. The removal of stems, leaves and grape stalks before crushing has several advantage. Notably, it minimizes the uptake of phenolics and lipids from wine parts. The extraction of stems phenols is of potential value only when dealing red grape varieties low in phenol content, such as in "Pinot noir". Stem

phenols are intermediate in astringency and bitterness, relative to the less strident tanstes of skin tannins and the more assertive seed tannins. The phenolic extracted from stems include catechins, flavonols, and caftaric acid (Sun et al., 1999).
In the past, stems were often left with the must throughout fermentation, especially in the production of red wines. Occasionally, some of the grape cluster were left uncrushed (Henderson, 1824) – thus, permitting partial carbonic maceration. The presence of stems made pressin easier, presumably by creating drainage channels along which the juice or wine could escape. Modern improvements in press design have made stem retention unnecessary, unless specifically desired. The enhanced tannin content derived from prolonged stem contact grape wine, made during poor vintage years, extra body and improved color density.
In addition to augmenting phenol extraction and facilitating pressing,maceration with the stems may increase the rate of fermentation. This effect appears to be due to the enhanced uptake of oleanolic acid (Bréchot et al., 1971). It supplements the amuon of long-chain unsaturated fatty acids available to yeast. It especially helps them complete fermentation under cool cellar conditions.
Leaf removed before crushing helps limit the production of C_6 aldehydes and alcohols. They are produced during the enzymatic oxidation of linoleic and linolenic acids extracted from the leaf cuticle. Leaf aldehydes and alcohols can taint wine with a grassy to herbaceous odor. Nonetheless, they may contribute to the typical aroma of some wines in small amounts. High leaf content in the must may also result in the excessive uptake of quercetin (Somer and Ziemelis, 1985).
For convenience and efficiency, the same piece of equipment often performs both stemming and crushing. Stemmers usually contain an outer perforated cylinder that permits the berries to pass through, but limits the passage of stems, stalks, and leaves. Often there is a series of spirally arranged arms, possessing flexible paddle ends, situated on a central shaft. Shaft rotation draws grape cluster into the stemmer, forces the fruit through the perforations, and expels the stems and leaves out the end. When stemmer-crushers are working optimally, the fruit is separated from the leaves and stems with minimal breakage. Expelling the stems and leaves in as dry a state as possible avoids juice loss and facilitates disposal. Stems and other vine remains may be chopped for subsequent soil incorporation.

4.5 Crushing

Crushing typically follows stemming immediately. Some berries are unavoidably broken in the process and the juice released is highly susceptible to oxidative browning and microbial contamination. Crushing the fruit without delay permits fermentation to commence almost immediately, limits microbial spoilage,and provides better oxidation control. Crushing is accomplished by any of a number of consequences:
The juice is aerated and it is inoculated by yeasts. The fermentation is quicker and the temperature higher;

- Aeration can be harmful. In partially rotted grapes, it can provoke an oxidasic casse;
- All of the juice is fermented; at the time of running-off, the press wine does not contain sugar;
- 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.

Crushing procedures generally preferred involve pressing the fruit against a perforated wall or passing the fruit through a set of rollers. In the former, the berries are broken open, and the juice, pulp, seeds, and skins pass through openings to be collected and pumped to a retaining tank or vat. In the latter process, berries are crushed between a pair or rollers turning in opposite directions. The rollers usually have spiral ribbing or contain grooves with interconnecting profiles to draw the grapes down and through the roller. Spacing between the rollers typically is adjustable to accommodate the variation in berry size found among different cultivars or vintages. Otherwise, contamination with seed oil can eventually lead to the development of rancid odors.
Crushing also can be achieved using centrifugal force.in centrifugal crushers, the fruit is spun against the sides of the crusher. Because they tend to turn the fruit into a pulpy slurry, centrifugal crushers generally are undesiderable. Clarification of the juice is difficult, and seeds may be rupted.
Although grapes are customarily crushed prior to vinification, there are exceptions. The juice to sparkling wine production is typically obtained by pressing whole grape clusters. Special presses extract the juice with a minimum of pigment and tannin extraction. The absence of pigments and tannins is particularly important when white sparkling wines are made from red-skinned grapes. Pressing intact grape clusters is now becoming popular with some table wine producers.
In the production of wines employing carbonic maceration, such as vino novello, it is essential that most of the fruit remain uncrushed, at least at the beginning. Only in intact berries can an internal fermentation occur. This is essential for development of the characteristic fragrance shown by these wines. After a

variable period of autofermentation, berries that have not broken under their own weight are pressed to release their juice. Fermentation is completed by yeast action.

4.6 Filling vats and principal vatting systems

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.
Various type of fermentor exist. They are distinguished by the aeration level supplied to yeasts and the modulation of skin contact. Aeration help to ensure a complete fermentation, and skin contact modulation influences 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 th 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.
Open floating-cap fermentors are used in small-scale installations. They were used in the past because the extended contact with air permitted successful fermentations, even in musts containing high concentrations of sugar. Moreover, temperature increases are less significant. Yet, the inconveniences are undeniable. Alcohol losses can attain and sometimes exceed 0.5%. The risk of oxidasic casse with botryzed grapes is also certain. Additionally, as soon as the active fermentation stops, the pomace cap surface is no longer protected from aerobic germs development. 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, know as punching, can only be carry 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. But this type of fermentor does not permit a long maceration. The tanks must be run off before the carbon dioxide stops begin 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, system have been developed that maintain the cap immersed in the must. 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 ate 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 time to be prolonged, almost as long desired. The tank can be hermetically sealed by a water-filled tank vent or simply closed by placing a cover on the upper part of the tank disappears over time and the protection is no permanent. The tank should therefore be completely filled with wine or 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 system and pumping-over operations with aeration, permitting the dissolution of the necessary oxygen for a successful fermentation.

4.7 Maceration

4.7.1 The role of 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 aroma and precursors, nitrogen compounds, polysaccharides and minerals are also liberated in the must or wine during the 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. Skin 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.
Consequently, the maceration should be modulated and fractionated. Only useful grape constituents should be dissolved – these positively contributing to wine flavor and aroma. The extraction of these desirable substances should be maximal, if not total.
The extraction of pomace constituents during maceration should be modulated according o 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.
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 it is insufficient, tannic red wines rapidly become heavy, coarse and without charm. Short vatting times and limited maceration lessen the occurrence of disagreeable characteristics.
A number of methods are available to the winemaker to adjust extraction levels during maceration. They essentially influence tissue destruction and flavor the dissolution of phenolic compounds. Brutal crushing promotes the extraction of bitter and herbaceous substances. Percolation of must, on the contrary, favors supple and full-bodied tannins. Enzymatic reactions, activated by grape enzymes, are involved in cell wall degradation. They favor the dissolution of their vacuolar contents. Commercial enzymatic preparations have recently been developed to activate these phenomena: they have pectinase, cellulase, hemicellulase and protease activities of diverse origins. These enzymes seem to favor the extraction of skin tannins over skin anthocyanins. They act on the tannins linked to the polysaccharides of the cell wall, giving the enzymatic wine a more full-bodied character than the control wine. Touzani et al 1994 obtained encouraging resuls from an enzymatic pool produced from Botrytis cinerea cultures, not containing laccase. This preparation attacks cell walls and favors the anthocyanin extraction over tannin extraction.
In traditional winemaking, maceration occurs during vatting, 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.

4.7.2 Different type of maceration

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

1. High temperature extraction prior to fermentation used in thermovinification, either followed by normal fermentation, or separated 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 appropriate level of SO_2, as well as by delaying inoculation with active yeasts. A more elaborated form of this technique consists of cooling the grapes around 5°C, by injecting liquid CO_2 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 (Flanzy, 1998).
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.

4.7.3 Principles of maceration

The passage of pomace constituents, particularly phenolic compounds (anthocyanins and tannins), into fermenting juice depends on various elemental factors. The results constitute overall maceration kinetics. The phenomena involved are complex and do not cause a regular increase in extracted substances. In fact, among these various factors, some tend to increase in extracted substances. In fact, among these various factors, some tend to increase phenolic compounds, while others lower concentrations. Moreover, they do not necessarily always act in the same manner on the various constituents of this group. Maceration is controlled by several mechanism:

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 operation that participate in tissue destruction: sulfating, anaerobiosis, ethanol, elevated temperatures, contact time.

2. Diffusion of extracted substances. Dissolution occurs in the pomace, and the impregnating liquid usually 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 fermentor 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 (Ferrè, 1958).
4. Modification of extracted substances (Ribéreau-Gayon, 1973).

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.

4.7.4 The maceration process: grape quality and tannin concentration in wines

4.7.4.1 Grape quality

The grape quality directly influences the consequences of maceration. The quantity and quality of phenolic compounds are directly related to grape varity, terroir, maturity level, disease status, etc.

Proper maturation conditions are essential to the accumulation of phenolic compounds. The climate plays a major role in phenolic compound production, since this requires a considerable amount of energy. Vine culture techniques also affect maturation. Moreover, phenolic compound accumulation is limited in young wines; therefore, relatively old wines are necessary for premium wine production.

Crop yelds also greatly affect the accumulation of tannins and anthocyanins, but this factor must be interpreted carefully. In some cases, the same climatic criteria that favor quality can also favor quantity. In vintage-dependent, temperature vineyards, the best quality years are sometimes also the most abundant. Reciprocally, low-yield vintages do not necessarily produce the best quality grapes. When considering crop yelds, plant density should also take into account: must sugar concentrations have long been known to diminish when per vine production increases. Vineyards with a long traditions of quality choose to maintain their plant density at 10000 vines/ha in poor soils. In this manner, a satisfactory production is assured while respecting grape quality. In richer soils, lower plant densities decrease cultivation costs. As a result, to have the same production per hectare, higher yields per vine are required. In satisfactory climatic conditions, crop volume is more apt to delay maturity, with low plant densities as opposed to high plant densities.

The relationship between vine production and maturation conditions (in particular phenolic compound concnentrations) is difficult and complex to interpret. Practices that increase vine vigor (fertilizing, rootstock, pruning) are known to delay maturation. Phenolic compounds are the first substances affected. When production is excessive, wines rapidly become diluted and lack color. A carefully measured equilibrium between an acceptable selling price and optimal wine quality will determine crop yields and consequently the future of premium red wines. The future of great red wines no doubt depends on maintaining a balance between producing enough wine to achieve reasonable profitability and keeping yields extraordinarily low to produce extremely concentrated wines that find a market at unusually high prices.

Eliminating a fraction of the must can also increase tannin concentrations of dilute grape must. This method increases the ratio of skins and seeds (pomace) to juice. A few hours after filling the fermentor, as soon as the juice and solids can be separated, some of the juice is drawn off (approximately 10-20% of the total juice volume). This operation significantly affects tannin concentrations and color intensity. The method should be used with caution, as excessive concentration of the must can lead to exaggeratedly aggressive tannins. The volume of must to be drawn off depends on skin quality, maturity, the absence of vegetal character and on grape disease status. The drawn-off juice can be used to make rosé wine. It is not advisable to throw away the excess must or rosé wine toavoid pollution. In any case, it constitutes a loss of production, which must be compensated by producing a better quality wine capable of fetching a higher price.

In addition to phenolic compound concentrations, the nature and properties of these substances also play an essential role in the maceration process and it consequences.

The potential dissolution of skin pigment varies, especially according to maturity level. Phenolic maturity corresponds to a maximum accumulation of phenolic compounds in the berry. Cellular maturity is defined with respect to the level of cell wall degradation (Amrani Joutei, 1993). Extraction of phenolic compounds increases with this degradation level defined the anthocyanin extraction coefficient (A_E) as follows (Augustin, 1986):

A_E: (wine anthocyanins/mature grape anthocyanins) x 100.

This coefficient varies from one year to another. Moreover, these values correspond farily well with the maturity level, expressed by the ratio: (sugar concentrations)/(total acidity). The same coefficient varies less for tannins. It does not correspond to juice maturity.
The organoleptical quality of tannins is directly related to maturation conditions. Enologists have defined this quality in terms of "good" tannins and "bad" tannins. The chemical understanding of these phenolic compounds have made it possible to make a better choice of winemaking techniques that optimize the quality of various kind of grapes. A perfect states of phenolic maturity not only supposes a maximum tannin concentrations; it also corresponds to soft, non-aggressive, non-bitter tannins.
Environmental conditions (terroir and climate) and grape variety determine this phenolic maturity. In cool climates, its insufficiently ripe tannins take on characteristic vegetal note. The same flaw can occur in excessively hot climates: the rapid sugar accumulation forces harvesting before the tannins reach their optimum maturity. A harmonious maturation of the various constituents of the grape characterization and great vintages. When conditions permit, grapes should never be harvested before complete phenolic maturity. Harvest dates base on sugar/acid ratios should be delayed, when necessary, so that tannins may soften. To ensure this maturation, several more days are sometimes needed before harvesting.

4.7.4.2 Wine tannin concentration

General red winemaking principals can be improved for the better control of maceration time and intensity.
If grapes have low anthocyanin and tannin concentration, only light red wine should be made. These wines, however, should be fresh and fruity. A limited concentration of grape phenolic compounds nevertheless merits an explanation. It can be a varietal characteristic, which must be taken into account. Vine cultivation conditions, favoring crop yields over quality, can also be responsible. Adapted winemaking techniques are necessary in these cases. Techniques for compensating a phenolic deficiency are palliative and are not a substitute for perfect grape maturity.
Grape rich in phenolic compounds are capable of making premium wines. Tannins play at least as important role in wine aging potential as alcohol or acidity. Their role is at least as important as that of alcohol acidity. However, tannin quality also contributes to aging potential.
The extraction of phenolic compounds should be modulated according to the anticipated aging potential of wine. The tannic aggressiveness of past vintages has contributed to their present quality and extended aging potential, but this line or reasoning is highly debatable. First of all, from the beginning of this century, the fermentations were less pure and the grapes were less healthy, even though crop yields were low and the wine concentrated. As a result, these wines were aggressive when young.
The harshness of the tannins was reinforced by the elevated acidity. Many years were required to soften the tannins. In certain limited cases, great vintage wines resulted. Today, wines are morepleasant to drink at the end of fermentation because of improved winemaking and viticultural techniques. It is possible to judge these wines and evaluate their quality when they are still young. The commercial value of these wines is often established within a few years of their production, whn offered to the market.
Despite the agreeable taste of present-day premium wines immediately following fermentation, they are still capable of long-term aging. Additionally, the number of well-made wines is much higher than in the past. Yet not all wine lend themselves to long-term aging. Terroir and vintage also participate in a wine's aging potential. Vine cultivation conditions leading to high crop yields also limit the proper development of grape constituents. Truly great vintage wines, however, are fruity and enjoyable when young, although they have sufficiently high levels of good-quality tannins to age well for a remarkably long time. Although as pleasant as lighter new wines, these wines are capable of long-term aging. They are made from the grapes that best support extended maceration, resulting in harmonious tannic structure.

Only the best grape varieties grown on the best terroirs produce wines that combine the high tannin content indicative of aging potential with aromatic finesse and complexity. On tasting, these wines are not only superbly concentrated, but also well-balanced and elegant. Winemakers today are aware that excessive tannin extraction tends to mask a wine's fruit and that perfect balance is the sign of a well-made wine.

4.8 Running off and pressing

4.8.1 Choosing the moment for running off

Choosing the optimal vatting time is a complicate decision with many possible solutions. It depends on the type of wine desired, the characteristics preferred (tannin intensity and harmonious structure are not always compatible) and the nature of the grape. This decision also depend on winemaking conditions. For example, only closed fermentors permit extended vatting times. In open fermentors, the must, is full in contact with air, ferments easily, but the risk of bacterial spoilage and alcohol loss make short vatting times necessary.

Premium wines often have vatting times of 2-3 weeks. Extended vatting times are chosen to increase tannin concentrations but, according to analysis, the third week does not significantly increase this concentration. The prolonged vatting time nevertheless has a "maturing effect" on the tannins. This maturation softens the tannins and improves gustative quality of wines. The chemical transformations during this phenomenon are not know precisely, but they can be appreciated by tasting macerating wines between their 8^{th} and 20^{th} day of vatting. The oxidation of tannins is a possible explanation of these transformations. The oxygen introducing during pumping-overs would be responsible for this oxidation. Controlling this phenomenon would be responsible for this oxidation. Controlling this phenomenon would represent a considerable advance in winemaking.

Certain vineyards macerate their wines for only 2-4 days. The wines produces are ordinary. This technique is often used in hot climates. Because short vatting times eliminate the risk of significant bacterial spoilage. Additionally, longer vatting times (and thus greater extraction) risk increasing gustatory flaws to detriment of finesse. In fact, maceration intensity should be established in accordance with grape quality. Maceration is shorter for ordinary varieties and in poor quality terroirs; improved grape varieties in quality viticultural regions allows ectended maceration.

Adjusting the vatting time is a simple method for modifying the maceration and it is therefore one of the most variables character of red winemaking from one region to another. Its duration should be chosen by the winemaker according to grape quality and cannot be generalized: it varies from one vineyard to another, one year to another and one fermentor to another, since grape quality is never homogeneous. This quality depends on the maturity level of the grapes (resulting from vine exposition and age) and their disease state. Winemaking equipment should never be the determining factor for deciding vatting times, but unfortunately too many wineries do not have sufficient tank capacity. Winemakers are therefore sometimes forced to run off wine prematurely, in order to free up tank space. In such cases, vatting times can be too short.

Three types of vatting techniques are summarized below:

- Running off before the end of fermentation. This short vatting time of 3-4 days is generally recommended for average-quality wines coming from hot climates. This method is adopted for producing supple, light, fruity wines for early drinking, but it can also be used to attenuate excessive tannin aggressiveness due to variety or terroir.
- Running off immediately after fermentation, as soon as the wine no longer contains sugar, approximately th 8^{th} day of maceration. In these conditions, a maximum color intensity with a moderate tannin concentration is expected. The gustatory equilibrium of new wines is optimized. Their aromas and fruitiness are not masked by an excessive polyphenol concentration. this vatting method is recommended for premium wines which are able to be rapidly commercialized. The resulting wines are not harsh or astringent and can be drunk relatively young.
- Running off several days after alcoholic fermentation. Vatting times may exceed 2-3 weeks. This method is often used to produce premium wines. The tanning assuring the evolution of the wine are supplied during this extended maceration. After several years, free anthocyanins have all but disappeared. Wine color is essentially due to combinations between anthocyanins and tannins.

4.8.2 Running off

The running-off operation consists of recovering wine which spontaneously flows out of the fermentor by gravity. The wine is then placed in a recipient where alcoholic and malolactic fermentations are completed.

In the traditional, quality-oriented European vineyards,the drawn-off wine wascollected in small wooden barrels. The wooden fermentors were not hermetic enough to protect wine from contact with air. Concrete and stainless steel tanks have been recommended since their development, for wine storage during the completion phase of fermentation. This completion phase precedes barrel aging. The tanks must of course be completely full and perfectly airtight.
When wines are barreled down directly, without blending beforehand, the wine batches may be heterogeneous. Yeast and bacteria participate in these differences and they govern the completion of the fermentations. As a result, wine composition (residual sugar, alcohol and tannin concentration) may be affected. The less the grapes are crushed and the fewer the pumping-over operations, the greater the difference between barrels of wine.

4.8.3 Pressing

Presses of various designs have been used for at least 5000 years. The earliest illustrations appear in ancient Egyptian tombs. Their initial function was simply to separate the juice from the seeds and skins. Only later did technological advances permit presses to become a tool by which winemakers could influence wine attributes.
The first major modern advance in press design involved the use of hydraulic force. It replaced muscle power associated with a massive screw and lever action. Incorporation of a removable bottom permitted easier pomace discharge. Previously, presses had to be dismantled or the pomace shoveled out at the end of each press cycle. Both tasks were unpleasant, time consuming, and laborious.
Increasing the drainage surface area was another momentous development. Not only did it speed juice release, but it also reduced the flow path for fluid escape. By diminishing the force required for juice extraction, higher quality juiceor wine is liberated. Placing the press on its side (horizontally) was another means of increasing surface area for liquid escape. In permitted the length (former height) of the press to be increased without difficulty. The horizontal orientation also permitted a section of the press to be hinged, providing access for both convevient filling and emptying. By suspending the press on heavy gears, the press could both be rotated for pomace crumbling, as well as be inverted for emptying. Crumbling breaks the compacted pomace produced during pressing and helps entrapped juice escape on subsequent pressing. Previously, chains or manual mixing was used to achieve crumbling. This had the disadvantages of both potentially crushing the seeds, and increasing juice clouding, due to the greater release of solids with the juice.
Another significant innovation was the development of the continuous screw press. By permetting uninterrupted operation, such equipment avoids time-consuming filling and emptying cycles. This is especially valuable when large volumes of must or wine need to be pressed in a short period. The principal disadvantage involves an increase in suspended solids, requiring additional fining or clarification.
Because the free- and press.run fractions produced during pressing possess differing physiochemical properties, winemakers can use press design, and how they are used, to influence wine character. Free-run fractions are clearer, possess lower levels of suspended solids, phenolic contents, and flavorants principally derived from the skins. Subsequent press-run fractions contain increasing amounts of suspended solids, anthocyanins, tannins and skin flavorants. Press-run fractions also are more likely to oxidize (possess more polyphenol oxidase), possess lower acidity (higher potassium contents),and have higher concentrations of polysaccharides, gums, and soluble proteins. Most wines are a judicious blending of both free- and the first press-run fractions. Depending on the intentions of the winemaker, and characteristics of the grapes, a portion of the second and possibly third pressing be incorporated.
Views vary considerably on the relative merits of using press-run fractions, and the various types of presses. Until more is known about the dybamics of flavor extraction during pressing,and how to predict their sensory consequences, the choice of press type will depend more on subjective or anecdotal, rather than objective information.

Figure 4.2 compares the operational characteristics of equivalent volumes of wine or juice pressed in vertical, horizontal, and pneumatic presses.

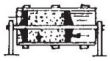
	Vertical	Horizontal	Pneumatic
Press type			
Size of the basket (cm)	113 x 90	215 x 73	215 x 73
Volume (m^3)	0.9	0.9	0.9
Pressure area (m^2)	1	0.42	4.95
Pressure per 1 cm^2 (MPa)	1.25–1.6	1.2	0.6
Pressure over the whole area (MPa)	12,500–16,000	5000	29,700
Pressure per 1 dm of pomace (MPa)	13.9–17.8	5.6	33.0
Average size of the cake (cm) at one half of the original volume	113 x 18	73 x 43	215 x 239 x 3.3
Shape of the cake			
Flowing out of the must (time)	long	short	very short
Time of one pressing (min)	100–120	100–120	50–90
Number of pressings	2	1	1
Total time of pressing (hr)	3 – 4	2	1

Figure 4.2. *Comparison of various type of presses.*

4.9 Fermentation

Fermentation is an energy-releasing form of metabolism in which both the substrate (initial electron donor) and by-product (final electron acceptor) are organic compounds. It differs fundamentally from respiration in not requiring the involvement of molecular oxygen. Although many fermentative pathways exist, S. cerevisiae possess the most common, alcoholic fermentation. In it,ethanol act as the final electron acceptor, whereas glucose is the preferred electron donor (substrate). Although S. cerevisiae possess the ability to respire, it predominantly ferments, even the ability to respire, it predominantly ferments, even in the presence ofoxygen.

Although most organisms are able to ferment sugars, they do so only when oxygen is lacking. This partially results from the toxic action of the usual end products of fermentation, lactic or ethanol. In addition, fermentation is inherently an inefficient mode of energy release.

The two main organisms involved in vinification, Saccharomyces cerevisiae and Oenococcus oeni, are somewhat unusual in selectively employing fermentative metabolism. S. cerevisiae is so adapted to fermentative metabolism that it can generate as many ATP/sec as is normally generated by respiration (Pfeiffer et al., 2001). These properties are partially based on the presence of a highly efficient alcohol dehydrogenase (ADH1), a higher titer of glycolytic enzymes in the cytoplasm, and a mitochondrion that only produces respiratory enzymes in the presence of a preponderance on nonfermentable substrates (Ihmels et al., 2005). In addition, both S. cerevisiae and O. oeni can withstand moderately high ethanol concentrations.

S. cerevisiae also has the properties of osmotolerance, relative insensitivity to high acidity, and acceptance of low oxygen concentrations. Thus, it is preadapted to growing in must and excluding other potential competitors in the must. Once dominating the environment, and in the absence of fermentable substrate, S. cerevisiae can switch to respiring the accumulated alcoholif oxygen is available.

O oeni is less well adapted to growing in grape juice or must than S. cerevisiae. It typically grows slowly in juice, and most commonly in wine, after S cerevisiae has completed alcoholic fermentation. In most habitats,the production of lactic acid by the bacteria lowers the pH of the substrate, excluding competitive bacteria. Lactic acid bacteria are one of the fes acid-tolerant bacterial groups. However, the high acidity of grape juice and wine actually retards or inhibits the growth of most lactic acid bacteria. Thus, in this instance, the conversion of malic acid to lactic acid, a waker acid, has the resul of increasing pH, favoring their growth. Malolactic fermentation also makes excessively acidic wines more acceptable to the human palate, and may improve microbial stability by removing residual fermentable substrates.

Wine is usually batch-fermented. Thus, nutrient is maximalat the beginning of fermentation,and declines progressively thereafter. By the end of fermentation, most sugars have been metabolized, leaving the wine dry.

Batch fermentations generally show a growth pattern consisting of four phases: lag, log, stationary, and decline. Immediately following inoculation, cellsnedd to adjust to the new environment.because some

cells do not acclimate successfully, there is an initial period in which the number of new cells produced approximates the number that die. This is called the lag phase. Once adaptation is complete, most cells begin to multiply at a steady rate,until conditions become unfavorable. Because most microbes are unicellular, the growth curve approximates an exponential equation, and the phase is correspondingly called the exponential or log (logarithmic) phase. During this period, the population of viable cells rapidly increases oits maximum value.
As the nutrient content falls, toxic metabolic byproducts accumulate. Thus, after a period of rapid growth, the rate of cell division (growth) declines and approaches the rate at which cells die (or become metabolically inactive). The culture is now said to have entered the stationary phase. This involves considerable transcriptional modification by the nucleus (Rossignol et al., 2003). As nutrient conditions continue to deteriorate, and the concentration of toxic metabolites keeps increasing, more cells die than divide. At this point, the culture enters a decline phase. Because most viable cells are not replaced, the colony eventually perishes,or become dormant.
Although similar, the population growth pattern displayed by yeast growth in grape juice shows several variations from the norm. typically the lag phase is short or undetectable; the exponential growth phase is relatively short; the stationary phase may be short and commence long before nutrients become limiting; and the decline phase is atypically long and the viable cell population can remain high forseveral moths. As much as 40% of the sugar metabolized to alcohol may occur during the decline phase (Ribéreau-Gayon, 1985).
The brevity or apparent absence of a lag phase in yeast growth may result from the preadapted state of the cells initiating fermentation. Active dry yeast, commonly used for juice or must. Similarly, the indigenous yeast population on grapes requires little enzymatic adaptation to commence rapid cell growth. However, the absence of a prolonged lag period with spontaneous fermentation may be an artifact. Endemic yeast cells are commonly bathed in the juice released from broken grapes during harvesting and may pass through the lag phase before fermentation “officially” begins in the winery. In addition, yeasts growing on berry skin may exist under limited but concentrated nutrient conditions.
Although physiological adjustment to growth in grape juice appears minimal, a lagphase may be observed when conditions are less than optimal. conditions such as low temperature(<10°C),and excessive protection of the juice from oxygen exposure during crushing, may disadvantage yeast cells. In addition, nitrogen deficiency and low juice pH can prolong the lag period. The latter probably results from the enhancement of the antimicrobial effects by any added sulfure dioxide (Ough, 1966a).m High °Brix values or ethanol contents also suppress yest growth and fermentation rate (Ough, 1966b).
During the exponential phase, cells grow and reproduce at the maximal rate permitted by the prevailing conditions. The presence or absence of oxygen does not appear to affect the rate.
As yeast cells enter the stationary phase, there is a change in the enzyme complement, the production of several stress-related proteins, and the accumulation of trehalose and glycerol.
The initiation of the decline phase probably results from increasing membrane dysfunction becoming progressively distruptive to cellular function. Membrane disruption results from the combine effects of ethanol and mid-chain fatty acid toxicity, plus a shortage in sterol precursors.

4.9.1 Biochemistry of alcoholic fermentation

Glucose and fructose are metabolized to ethanol primarily via glycolysis. Although the primary by-product is ethanol, additional yeast metabolites generate the most common aromatic compounds found in wine. Yeast action also may influence the development of the varietal aroma by hydrolyzing nonvolatile aroma precursors, thus potentially liberating aromatic terpenes, phenols, and norisoprenoids (Laffort et al., 1989). In addition, the changing physiochemical conditions produced during fermentation progressively modify yeast metabolism. this is reflect in the various phases of colony growth, related adjustments in the nutrient and energy status of the cells, and the substances released and absorbed throughout fermentation.
During the changing phases of colony growth, yeasts have differing requirements for ATP and reducing power (in form of NADH or NADPH). These energy-carrying chemicals are required to activate cellular functions and maintain an acceptable ionic and redox balance in the cell redox balance refers primarily to the equilibrium between the oxidized and reduced forms of the two major pyridine nucleotides (NAD^+/NADH and $NADP^+$/NADPH).
As shown in Fig. X, glucose and fructose are oxidized to pyruvate, primarily via glycolisis. During the process, electrons are transferred to NAD^+, reducing it to NADH. Pyruvate subsequently may be decarboxylated to acetaldehyde which is reduced to ethanol by the transfer of electrons form NADH. In the process, NADH is reoxidized back to NAD^+.

The release of energy from glucose and fructose, and its storage in ATP and NADH, are inherently much less efficient via fermentation than by respiration. Most of the chemical energy initially associated with glucose and fructose remains bound in the end product, ethanol. Furthermore, the energy trapped in NADH is used to reduce acetaldehyde to ethanol. This process is necessary to maintain an acceptable redox balance. As most cells, yeast contain only a limited supply of NAD^+. In the absence of oxygen, yeast cells are unable to transfer energy stored in NADH to ADP, forming ATP, and reeneratin NAD^+. Under the anaerobic conditions of fermentation, the regeneration of oxidized NAD+ requires the reduction of an inorganic molecule. In most cases, this is acetaldehyde, and the by-product is ethanol. Without the regeneration of NAD+, the fermentation of sugars would quickly cease. The consequence is that alcoholic fermentation generates only about two molecules of ATP per sugar molecule, in contrast to the potential 24-34 ATP produced via respiration. Most of ethanol produced during fermentation escapes from the cell and accumulate in the surrounding juice.
Throughout fermentation, yeast cell adjust physiologically to the changing conditions in the juice/must to prduce adequate levels of ATP, maintain favorable redox and ionic balances, and synthesize necessary metabolic intermediates. Consequently, the concentration of yeast by-products in the juice changes continually during fermentation. Because several of the products are aromatic, for example, acetic acid, acetoin, and succinic acid, their presence can affect bouquet development. The accumulation of acetyl CoA (as a result of the inaction of the Krebs Cycle) may explain the accumulation and release of acetate esters during fermentation. The alcoholysis of acetyl CoA during esterification would release CoA for other metabolic functions. In addition, the formation of other aromatics, notably higher alcohols, reflect the relative availability of amino acids and other nitrogen sources in the juice. Adequate availability also permits amino acids to be used as an energy source,or the release of organic acids, fatty acids, and/or reduced-sulfur compounds.

4.9.2 Must inoculation

In inoculated fermentations, S. cerevisiae is usually added toachieve a population of about 10^5-10^6 cells/mL. with active dry yeasts, this is equivalent to about 0.1-0.2g/L of must (juice). Active dry yeast often contains about 20-30 x 10^9 cells/g.
Before addition, the inoculums is placed in water or dilute juice. Exposure to temperatures between 38 and 40°C for 20 min is generally optimum for rehydration (Kraus et al., 1981). Subsequent cooling to 25°C is followed by a short adaptation period. During this time, cellular metabolism and membrane permeability readjusts to normal.
Commercially available strains of S. cerevisiae possess a wide range of characteristics, suitable for most winemaking situations. These include those that either enhance the release of varietal flavorants, produce an abundance of fruit esters, or are of neutral character. Strains are also available that are notable for their production of low levels of compounds such as acetic acid, hydrogen sulfide, or urea. Others may be selected because of their relative fermentation speed, ability to synthesize or degrade malic or lacticacid, augment the concentration of glycerol, ability to restart stuck fermentation, or known value in producing particular wine styles, notably carbonic maceration, late-harvest, or early- versus late.maturing reds. In addition, there are locally selected strains that are reported to produce regionally distinctive wines.
Despite all this information, the winemaker's choice is still not easy. In only a few instances is intentional inoculation absolutely essential. With thermovinification or pasteurized juice, inoculation is required to achieve rapid initiation of fermentation. In addition, yeast inoculation is necessary to restart "stuck" fermentations and promote fermentation of juice containing significant number of moldy grapes. Finally, inoculation is required to assure the initiation of the second fermentation in sparkling wine production. However, the predominant reason for using specific yeast strains is to avoid the production of undesirable flavors occasionally associated with spontaneous fermentation.

4.9.4 Spontaneous vs. induced fermentation

There has been much discussion over the years concerning the relative merits of spontaneous versus induced fermentation. That various strains of S. cerevisiae supply distinctive sensory attributes is indisputable (Cavazza et al., 1989, Grando et al., 1993). This is particularly important for aromatically neutral cultivars. Nevertheless, strain choice can equally affect the varietal character of aromatically distinctive cultivars, by influencing the liberation of bound grape flavorants. This may be particularly significant with non-Saccharomyces yeasts. Nevertheless, using established strains provides the winemaker with the greatest confidence that fermentation will be rapid and possess relatively predictable flavor and quality characteristics.
In contrast, spontaneous fermentations may accentuate yearly variations in character. It can be part of the uniqueness associated with terroir. However, it also carries the risk of conferring off-odors or other

undesirable traits. Occasionally, but not consistently, spontaneous fermentations generate higher concentrations of volatile acidity than induced fermentations. Spontaneous fermentations also tend to possess noticeable lag periods and, thus, are more susceptible to distruption by killer factors.
Those who favor spontaneous fermentation believe that the indigenous grape flora supplies a desired subtle or regional character, supposedly missing with induced fermentations. Large-scale wineries, where brand-name consistency is essential, cannot take risks with spontaneous fermentation.
An alternative to either spontaneous or standard induced fermentation is inoculation with a mixture of local and commercial yeast strains. The combination appears todiminish individual differences, producing a more uniform distinctive character.

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5. Phenolic compounds in must and wine

5.1 Introduction

Wine contains many phenolic substances, most of which originate in the grape berry. The phenolics have a number of important functions in wine, affecting the tastes of bitterness and astringency, especially in red wine. Second, the color of red wine is caused by phenolics. Third, the phenolics are the key wine preservative and the basis of long aging. Lastly, since phenolics oxidize readily they are component that suffer owing to oxidation and the substance that turns brown in wine when exposed to air. Wine phenolics include the non-flavonoids: hydroxycinnamate, hydroxybenzoates and the stilbenes; plus the flavonoids: flavan-3-ols, the flavonols, and the anthocyanins.

5.2 Definitions

In order to understand phenolic nomenclature, it is helpful to understand the basis of the general terms used.

Simple phenols include those compounds that have a single aromatic ring containing one or more hydroxyl groups, a common example being caffeic acid. Polyphenolic compounds are those that have a multiple phenol rings within the structure, and examples include catechin and ellagic acid. Flavonoids have a very specific three-ring structure. Tannin is a functional term which describes substances that are used to tan hide to leather. Tannin is often used loosely to describe high molecular weight phenolic mixtures. The term condensed tannin refers to mixtures of polymers of flavonoids and hydrolysable tannin refers to gallic- or ellagic-acid-based mixtures, also called gallotannins or ellagitannins, respectively.

5.3 Non flavonoids

Wine phenolics are grouped into two categories, the flavonoids and non-flavonoids. Non-flavonoids are structurally simpler molecules and the main ones are listed below:

1. Hydroxycinnamic acids;
2. Benzoic acids;
3. Hydrolysable tannins;
4. Stilbenes.

5.3.1 Hydroxycinnamates

These are the major phenols in grape juice and the major class of phenolics in white wine. These molecules are also the first to be oxidized and subsequently initiate browning. There are three common hydroxycinnamates in grapes and wine,those based on coumaric acid (mono 4-hydroxy), caffeic acid (catechol substitution) and ferulic acid (guaiacyl substitution) (**Figure 5.1**).

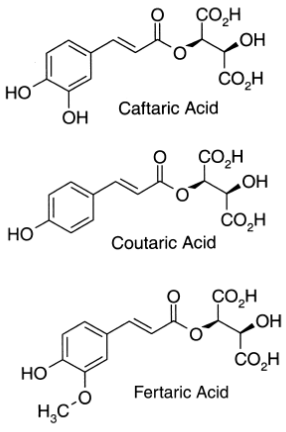


Figure 5.1. *Some of the hydroxycinnamates in wine.*

In grape berries caftaric acid, coutaric acid and fertaric acid are not found. Instead these acids exist as esters of tartaric acid. The levels of these compounds vary in grapes, but caftaric acid is by far the

predominant cinnamate in grapes, averaging about 170 mg/kg in Vitis vinifera grapes, while the p-coutaric and fertaric acids occur at about 20 and about 5 mg/kg, respectively (Ong & Nagel, 1978).
The naturally occurring esters are susceptible to hydrolysis, and this occurs in the aqueous acidic solution of wine, releasing the simple hydroxycinnamic acids which are readily detected in wine of a few weeks old. In addition, the free acid will partially esterify with part of ethanol in wine. The rate of these reactions is variable, depending on the pH of the wine, which typically varies between 3.0 and 3.9 mg/L. Hydrolysis of the caffeic acids esters of tartaric acid can be catalyzed by an enzyme, hydroxycinnamate ester hydrolyze (Somerts et al., 1987). Levels of total hydroxycinnamates in finished wine are typically 60 mg/L in reds.
In terms of wine sensory qualities, the hydroxycinnamates appear to have no perceptible bitterness or astringency ah the levels found in wine (Okamura & Watanabe, 1981).

5.3.2 Benzoic acids

These are a minor component in new wines. Gallic acid appears from the hydrolysis of gallate esters of hydrolysable tannins and condensed tannin after standing for at least a few months. Gallic acid appears to be stable during aging, as it is one phenolic compound readily visible by chromatographic analysis of older red wines. Its levels in red wine average near around 70mg/L (Watwerhouse & Teissedre, 1997).

5.3.3 Hydrolysable tannins

Tannins will be discussed in detail below. In wine hydrolyzable tannins come from oak and levels are are in the range of 250 mg/L after aging two or more years (Quinn & Singleton, 1985). These phenols are composed of gallic acid and ellagic acid esters with glucose or related sugars (**Figure 5.2**). Because of the esters linkage, they are referred to as "hydrolyzable" there are two categories, the gallotannins and the ellagitannins which contain gallic acid or ellagic acid. Hydrolyzable tannins are not found in V. vinifera.

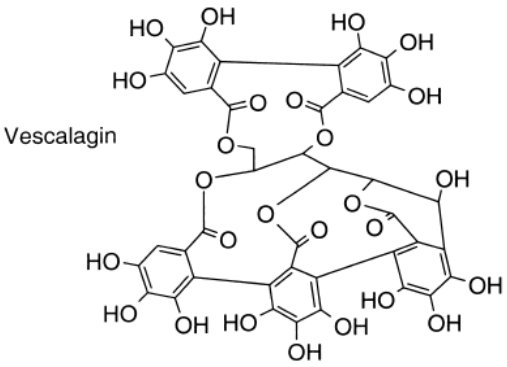


Figure 5.2. *Vescalagin from oak.*

5.3.4 Stilbenes

Stilbenes are another minor class. The principal stilbene in grapes, resveratrol, is produced by vines in response to Botrytis infection and other fungal attacks. The actual anti-fungal compounds are the oligomers of resveratrol called the viniferins. Several forms of resveratrol exist including the cis and the trans isomers as well as the glucosides of both isomers. Resveratrol has been implicated as a wine component that may reduce heart disease or cancer, but bioavailability data has not been reported, so it is difficult to assess physiological significance.

5.4 Flavonoids

The wine flavonoids are all polyphenolic compounds having multiple aromatic rings possessing hydroxyl groups. A specific three-ring system defines flavonoids, there being a central oxygen-containing pyran ring, C ring, of different oxidation states. It is fused to an aromatic ring (A ring) alone one bond and in grapes and wine all have the same hydroxyl substitution groups on ring A, at positions 5 and 7 (**Figure 5.3**). Differences in the oxidation state and substitution on ring C defines the different classes of flavonoids.

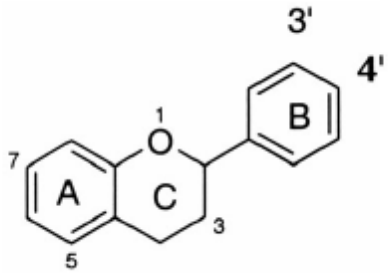


Figure 5.3. *The flavonoid ring system.*

The substitution pattern on ring B defines the member of the class. Normal substitution pattern are a hydroxyl at the 4 position with additional oxygen substitution at 3 and/or 5. Those oxygens can be hydrohyls (phenols) or methoxyls at positions 3 and/or 5. Thus the number of class members is relatively short, however the "free" flavonoid structure can also be substituted further; this gives rise to many additional compounds.
The flavonoids comprise the a majority of the phenolsin red wine and are derived from extraction of the skins and seeds of grapes during the fermentation process. Since red wines are produced by fermentation of the juice's sugar into alcohol, a good solvent for polyphenol extraction, in the presence of the skin and seeds over a period of 4-10 or more days, there is a ample opportunity for extraction of much of the polyphenols into a red wine, and in typical wine making, about half these substance are extracted during the maceration process (Waterhouse, 2002).

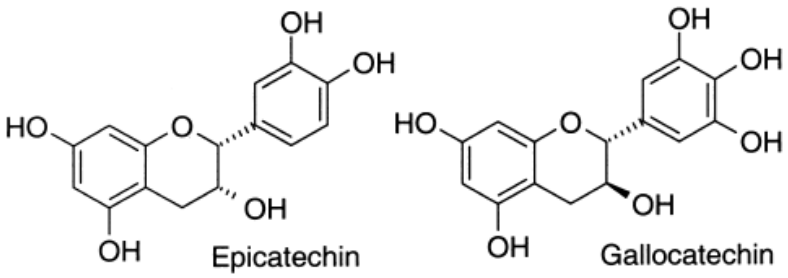
The major classes of wine flavonoids are:

1. Flavanols;
2. Flavonols;
3. Anthocyanins.

5.4.1 Flavanols

Flavanols are the most abundant class of flavonoids in grapes and wine, and in the grape they are found in both the seed and skin. These are often specifically called the flavan-3-ols to identify the location of the alcohol group on the C ring. The flavan-3-ols are most reduced form of the flavonoids. Because positions 2 and 3 on the C ring are saturated, stereoisomers exist (**Figure 5.4**), and two are found in grapes. The trans form is (2R,3S) (+)-catechin and the cis form is (2R,3R) (-)-epicatechin. Both catechin and epicatechin have the 3',4' catechol substitution on the B ring.

Figure 5.4. *Cis and trans form of flavan-3-ols*



The only other B-ring substitution pattern found in wine flavan-3-ols is the 3'4'5' trihydroxy form, appropriately called the gallo-catechins. Some epigallocatechin is found in grape skin, but gallocatechin is

not found in significant amounts. The wine flavan-3-ols are not found as glycosides, typical for the other classes, but instead gallate esters are found, and the gallic acid is esterified at the 3 position of the epi-series only. Epicatechin gallate is a small but significant proportion of the flavan-3-ol pool in grapes in the seeds. Thus, for the simple series of monomeric flavonols, there are four different one found in wine. The levels of total monomeric flavan-3-ols sometimes referred to as the "catechins" (Ritchey & Waterhouse, 1999). The levels of the total monomeric flavan-3-olsin typical red wines is in the range of 40-120 mg/L with the majority usually being catechin. The levels are strongly affected by seed extraction techniques and are higher when extended maceration techniques are used.
The majority of phenolic compounds in red wine is from the condensation of flavan-3-ol units to yield the oligomers (proanthocyanidins) and polymers (condensed tannins). The condensation occurs to form covalent bonds between flavan-3-ol units, the most common linkage being 4→8 and 4→6 positions. On average, epicatechin is the predominant unit in condensed tannins from grapes and wine, catechin is the next most abundant.
The monomeric catechins are bitter and astringent. In the polymer, the bitterness is minimal, but the astringency remains. Over long aging (many years), a disproportionation reaction can occur, perhaps with some oxidation, so the polymers continue to increase in size until they are no longer soluble in wine and form the precipitate common in older red wines.
The distribution of flavanols in grape berries is not the same in all varieties, and in fact has a wide range of differences comparing the seed and skin.

5.4.2 Flavonols

Flavonols occur in a wide range of vegetable food source. This class of compounds is always found in a glycoside form in plants including in grape berries where it is found in grape skin. There are only three forms of the simple flavonoid aglycones in grapes, quercetin, myricetin, and keampferol, but since these compounds occur with a diverse combination of glycosidic forms, there are many individual compounds present. The identity of the glycosides has not been established in many different grape varieties, but they have been shown to be mostly the 3-glucosides as well as the 3-glucuronides and small amount of diglycosedes (Cheynier & Rigaud, 1986).

5.4.3 Anthocyanins

Anthocyanins provide the color in red wine and the red and blue colors found in the skins of red or black grapes. The color is based on the fully conjugated 10 electronA-C ring τ system, with some contribution by the B ring as well. If that is disrupted, the color is lost, as when anthocyanins are bleached by bisulfite. The anthocyanins react with the tannins to produce a "stabilized" anthocyanin or pigmented tannin which persist much longer in wine than the initial form, and it is this stabilized color that persists in most red wines more than a few years old.
The term for the simple flavonoid ring system is anthocyanidin. However, anthocyanindin are never found in grapes or wine, except in trace quantities, because they are unstable. There are five basic anthocyanidins in wine: cyanidin, peonidin, delphinidin, petunidin and malvidin, the most abundant in red wines (**Figure 5.5**). "Anthocyanin" implies a glycoside.

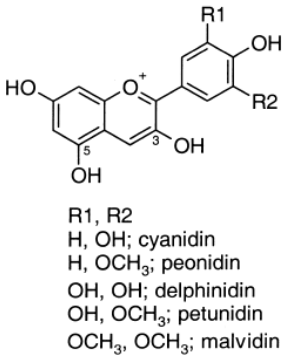


Figure 5.5. *Anthocyanidin structure.*

In wine and similar solutions there are several forms of anthocyanins and the proportions strongly affect the color of a solution containing these substances. The charged C ring is an electrophilic center, and it can react with nucleophiles. Common reactions are with water, a pH-dependent reaction, and with bisulfite. In both cases, the red color is lost as the C-ring conjugation is distrupted. The pKa of the flavylium pseudobase form is 2.7. At low pH (less than 1), all forms are converted to the flavylium form, and this treatment is used to assess total anthocyanin content. In addition, there is a quinine form which has a violet hue, and its pKa is 4.7, so it is present in small amounts at high wine pH values.
Anthocyanins interact with other phenolic compounds is solution to create the effect known as co-pigmentation. This is a transient interaction; no chemical bond are formed. It is a result of the chemical phenomenon called charge transfer complex formation, or τ-τ interactions (Foster, 1969). This occurs when there are two aromatic- ring substance in solution that have very different electron densities.
The overall effect of co-pigmentation is based on two effects. First, the formation of the τ-τ complex causes changes in the spectral properties of the molecules in the flavylium form, in particular increasing absorption and increasing the wavelength of the absorption (Dangles & Brouillard, 1992). But secondly, the stabilization of the flavylium form by the τ-complex shifts the equilibrium to better favor the flavylium, thus boosting the proportion of anthocyanin molecules in red-colored form. So, the magnitude of the co-pigmentation effects is pH dependent because at very low pH values, all the anthocyanin molecules are already in the flavylium form, and at high pH, the flavylium form is not accessible. There is some speculation that the formation of the τ-τ complex enhances the reactions between anthocyanins and the tannins which produce the covalent bonds of pigmented tannins (Mirabel et al., 1999).

5.5 References

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6. Post-fermentation treatments: wine aging

6.1 Introduction

The tendency of wine to improve, or at least change, during aging is one of its most beguiling properties. Regrettably, most wines improve only for a few years before showing irreversible losses in quality. In contrast, red wines produced from varieties such as “Cabernet Sauvignon”, “Shiraz”, “Tempranillo”, “Nebbiolo”, and “Pinot noir”, may continue to improve in flavor and subtlety for decades.

Quality lossis commonly explained as a dissipation of a fresh, fruity bouquet, along with any aroma donated by the grape variety. Wines noted for continued improvement typically show similar aromatic losses, but they gain in aged bouquet. Aging is considered desirable when the development of an aged bouquet, subtle flavor, and smooth texture more than compensate for the fading varietal and fruity character of the wine.

Knowledge of how wines age, and how the effects of aging might be directed is important to all involved or interested wine. At the very least, quality loss can adversely affect the shelf-life of a wine and the financial return to the producer. On the other hand, the prestige connected with long aging-potential adds greatly to the desirability appeal of a wine. It also permits consumers to participate in the process, through the conditions and duration of aging they permit. Because the factors affecting aging are poorly understood, a mystique has built up around vineyards and varieties associated with wines that age well.

Aging is occasionally considered to possess two phases. The first, called maturation, refers to changes that occur between alcoholic fermentation and bottling. Although maturation frequently last from 6 to 24 month, it may continue for decades. During maturation, the wine may undergo malolactic fermentation, be stored in oak cooperage, be racked several times, and be treated to one or more clarification techniques. During racking and clarification, wines may absorb about 40 mL O_2/year, an amount insufficient to give the wine noticeably oxidized character. Only in some fortified wines is obvious oxidation an important component of maturation.

The second phase of aging commences with bottling. Because this stage occur essentially in the absence of oxygen, it has been called reductive aging, this contrasts with oxidative aging, an alternative term for maturation that is used for the aging of some fortified wines.

6.2 Effect of aging

Age-related changes in wine chemistry have long been noted. Initially, these modifications are favorable. They result in the dissipation of the yeasty aspect and spritzy character of newly fermented wines. Subsequently, there is a loss of the fresh fruitiness of the wine. If this is accompanied by the development of an appreciated aged bouquet and smoother mouth-feel, the consequences of aging are highly desirable. To encourage these latter processes, most wine connoisseurs store wine in cool cellars for years to decades. Regrettably, most wines do not age particularly well. Most red wines improve or retain their flavor for little more than 5-10 years.

Nonenzymatic oxidative reactions produce significant sensory changes during aging. This involves the transfer of an electron (or hydrogen atom) from the oxidized compound to oxygen, or another acceptor. In bottled wine, reactions involving molecular oxygen occur slowly , as oxygen diffuses into the bottle via the cork, or between the cork and the neck. Temperature, pH, and the phenolic content significantly affect a wine’s oxidative potential. It is estimated that wine can combine with up to about 6 mg/liter O_2 (saturation at 20°C) within a week or less, depending on the wine’s phenolic content (Singleton and Cilliers, 1995). Others oxidative reactions (not involving molecular oxygen) occur during wine aging, but their influence on wine fragrance and taste are little known. The presence of copper and iron ions are the best known of wine oxidative catalysts. Because the redox potential of wine declines after bottling, reductiove reactions are almost undoubtelly involved in wine aging.

As with other aspects of wine chemistry, determining the significance of changes is more difficult than detecting them. To establish their significance, it is necessary to show that the changes detectably impact sensory perception. Because most chemicals occur at concentrations below their sensory threshold, most changes affect neither wine flavor nor the development of an aged bouquet.

6.2.1 Appearance

One of the most obvious changes during aging is a progressive browning. Red wines may initially deepen in color after fermentation, but intensity slowly fades as the tint takes on a ruby and then a brackish hue. These changes result from a disassociation of self-association and copigment anthocyanin complexes, the

formation of new pigments (pyranoanthocyanins, catechinpyrylium, and canthylium pigments),and the progressive formation of both tannin-tannin and anthocyanin-tannin complexes.
The dynamics of these changes are only beginning to be understood. The significance of polymerization is clear, but little is known about the importance of pigments such as pyranoanthocyanins (Alcade-Eon et al., 2006). Polymerization begins during fermentation, constituting about 25-60% of the anthocyanin content within a year. Polymerization is favored by the peroxidation of dihydroxyphenolics (priamrly o-diphenols) to diquinones (Rose and Pilkington, 1989). These slowly polymerize into colorless dimers, then yellowish dimmers, and finally brownish end products. These changes, originally predicted from a decline in optical density, and a shift in the absorption spectrum, and a shift in the absorption spectrum, have been confirmed by various methods (Peng et al., 2002, Remy et al., 2000), including radioactive isotope analysis (Zimman and Waterhouse, 2004). The degree of polymerization is typically measured as a ratio of optical density measurements taken at 520 and 420 nm. High 540/520 nm values indicate a bright-red color, whereas low values indicate a shift to brackish shades. The color shift results primarily from the accumulation of chromophoric carbonyl compounds.
Although less studied in white wine, color change has been extensively investigated in red wines. The initial purplish color, associated with copigmentation, fades as the complexes dissociate and the freed anthocyanins oxidize or progressively polymerize with tannins. Although small amounts of acetaldehyde, produced following oxygen uptake, enhance the polymerization of anthocyanins and flavonoid tannins, polymerization also occurs directly under anaerobic, acid catalyzed conditions. Because direct polymerization occurs slowly, but continuously throughout the wine, direct polymerization may be the more significant process in bottle aging. Because temperature speeds polymerization, mild heating has been recommended as an alternative to aeration for color stabilization (Somers and Pocock, 1990). Although micro-oxygenation is receiving increased attention,if not carefully controlled, it risks activating dormant acetic acid bacteria, increasing volatile acidity, aggravating potential problems with Brettanomyces, and inducing the precipitation (loss) of polymeric pigments. Red wines are variously considered to benefit from up to about 60 mL O_2 per liter.
Although anthocyanin polymerization is critical to color stabilization, additional mechanisms are involved. One entails a reaction between yeast metabolites, such as pyruvic acid and anthocyanins (Fulcrand, 1998). Subsequent structural rearrangement and dehydration generate a tawny colored product. It is more stable than the original anthocyanin to sulfur dioxide, high temperature, and pH values above 3.5.

6.2.2 Taste and mouth-feel sensations

During aging, residual glucose and fructose may react with other compounds and undergo structural rearrangement. Nevertheless, these reactions do not appear to occur to a degree sufficient to affect perceptible sweetness. In contrast, aging can affect acidity, inducing small but perceptible losses. For example, esterification of acids, such as tartaric acid, removes carboxyl groups involved in the sensation of sourness. Slow deacidification also can result from the isomerization of the natural L- to the D- form of tartaric acid. The racemic mixture is less soluble than the L-form. This is one of the origins of tartrate instability in wine (Edwards et al., 1985).
The most significant gustatory changes during aging affect the bitter and astringent sensation of red wines. The best understood of these reactions is the polymerization of tannins and their subunits with themselves, anthocyanins, proteins, and polysaccharides. Autopolymerization tends to induce a progressive decline in bitterness and astringency, due to chemical reactivity changes, or due to precipitation. However, in the early stages of polymerization, there may be an increase in astringency. This resuls from the greater astringency of medium- versus large-size tannins. The binding of tannins with polysaccharides, peptides or proteins leads to further reduction in bitter, astringent sensation. Nevertheless, condensed tannins may slowly degrade during aging (Vidal et al., 2002), potentially increasing bitterness. Similarly, the breakdown of hydrolyzable tannins (primarily from oak cooperage) may reduce astringency, but enhance bitterness.

6.2.3 Loss of modification of aroma and fermentation bouquet

Esters produced during fermentation generate much of the fresh, fruity, character of young wines. The most significant appear to be esters formed between acetic acid and higher alcohol such as isoamyl and isobutyl acetates. Because yeasts produce and release more of these esters than the equilibrium in wine permits, the esters tend to hydrolyze back to their corresponding acids and alcohols. Thus, the fruity aspect donated by acetate esters tends to fade with time (Gonzalez Viñas et al., 1996). Nevertheless several esters may remain above their detection threshold for several years. Hydrolysis occurs more

slowly at higher pHs and at lower temperatures. Hydrolysis of fruit esters also appears to be diminished in the presence of antioxidants, such as sulfure dioxide and various phenolics.
As second major class of esters is based on ethanol and long-chain saturated fatty acids. The level of these estres may decline, remain stable, or increase during aging. Hydrolysis is delayed by the high ethanol content of wine, but is favored by low pH, high temperatures, and increased molecular weight.
A third group of esters, and eventually the most abundant, forms slowly and nonenzymatically between ethanol and organic acids, such as tartaric, malic , lactic, citric and succinic acids.their formation increases with higher alcohol contents, lower pH values, and it higher temperatures. Most ethyl esters of fixed acids probably play little role in bouquet development, due to their low volatility and nondistinctive odor.
Another group of important flavorants that change during aging are terpenes. These are particularly important to the aroma of "Muscat" and related cultivars. Oxidation of terpenes results in the marked loss of varietal character in these varieties.
The total concentration of monoterpene alcohols falls markedly during aging. This can result in a noticeable lossin floral character. Most of the terpene derivatives have higher perception thresholds than do their monoterpene progenitors. Oxide terpene derivatives also have qualitatively different odors. Terpene-related, heterocyclic oxygen compounds also develop during aging. But their sensory significance is unknown.
Several volatile phenols, such as vinylphenol and vinylguaiacol, are partially converted to nonvolatile ethyoxyethylphenols during aging. At low concentrations, volatile phenols can enhance wine fragrance, but at higher levels they produce phenolic off-flavors. The concentration of important varietal aromatics declines during aging.
In addition to the loss or modification of grape and yeast aromatics, new compounds may be generated. Some of these appear to be the results of oxidation. The best-know of these is acetaldehyde.

6.2.3.1 Origin of a bottle-aged bouquet

Four groups of compounds are known to be involved in the generation of a bottle-aged bouquet. These include constituents liberated by acid or enzymatic hydrolysis of nonvolatile glycosidic conjugates; derived from norisoprenoid precursors and related diterpenes; modified carbohydrates; or generated from reduced-sulfur compounds.
Most flavorants in grapes accumulate as glycosides. This is the basis for the glycosyl-glucose analysis of grape quality. The slow release of terpenes from glycosidic linkages under acidic condition may partially offset some of the aroma loss associated with aging (Simpson and Miller 1983).
The concentration of reduced-sulfur compounds may change during aging. Of these, the most significant is dimethyl sulfide. Its accumulation has occasionally been correlated with the development of a desirable aged bouquet.

6.3 Barrel aging red wines

6.3.1 Role of barrel aging

Traditionally, great red wines are aged in oak barrels from the end of fermentation until bottling. The first motivation in choosing barrels was probably that they were easy for one man to handle and could also be used for shipping. It was not until some time later that their positive effect on wine development, in terms of color, clarity and flavor, came to be appreciated. However, the use of barrels involved a major financial commitment and entailed risks of microbial contamination, as well as the likelihood of communicating organoleptic faults to wine. For these reasons, the practice of aging even high-quality red wines in inert vats became widespread from 1950 until 1960. At that time the elimination of old barrels, responsible for moldy off-flavors, certainly resulted in improved quality. The red wines were perhaps less complex, but cleaner and fruiter.
Over the past few years, a more favorable economic climate has forested a new interest in barrel aging. There is greater awareness of the role played by oak in wine development, and a concern to adapt barrel aging to the quality of each wine. Perfect control of the various parameters and techniques has made it possible to fine-tune the use of wood and its influence on wine quality.
Firstly, clarity is easier to obtain when wine is aged in the barrel rather than in the vat, due to the smaller volume. Clarification is also facilitated by the adsorption phenomena that occur in oak. Furthermore ì, wine in the barrel is more sensitive to outside temperature, so the precipitation of salts, particles and colloidal coloring matter is much more likely to be triggered by winter cold.
Stabilization reactions affecting color, clarity, and colloids, as well as modifications in the phenol structures (softening tannins), also occur in wine during aging, while aroma develop. Barrel aging

promotes these reactions to a much greater extent than large airtight vats, which, being theoretically inert, are considered not to interact with the wine.
The phenol composition of wine is considerably modified by barrel aging, 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 aging, wines have better color than those aged in the vat and this color remains more stable during bottle aging. The flavor is also more attractive, characterized by softer tannins.
Wine also acquires aromatic complexity as a result of odoriferous substances extracted from wood. The oaky aroma must be carefully modulated, to ensure that it blends with the wine's overall structure. Even though producers may wish to give their wines an oak character, this must not be overdone. The barrel's contribution to aroma and flavor may be adjusted by modifying the proportion of wine aged in oak, especially new barrels. Other important factors are the type of oak and the way the barrels are made (degree of toasting),as well as the duration of barrel aging.
Three factors related to this type of ageing are responsible for the wine's development: oxidation-reduction reactions, as well as volatile and non-volatile compounds dissolved from the oak.

6.3.2 Oxidation-reduction

Oxygen in red wines may have various origin. Handling operations, treatments and regular winemaking tasks represent a major proportion (up to 50%), while barrel aging accounts for the remainder. The amount of oxygen absorbed by the wine depends on the origin of the barrels, as well as the type and position of the bung. It is thought that oxygen pass through the wood mainly via gaps between staves. Smaller amounts are admitted through the bunghole. The position of the bung affects the penetration of oxygen into the wine. Wooden bung positioned on the side of the barrel and tight silicon bungs produce a vacuum effect on the order of 120 mbar, which increases the quantity of oxygen dissolved in the wine.
It is, however, diffult to determine the precise quantity of oxygen that penetrates into the wine, as measurements, even in the model solutuions, do not take into account the amounts consumed by ellagitannins in the oak. Dissolved oxygen constantly disappears by oxidizing various components in the wine. Quantities may vary widely, from 100 mg/L to 200 mg/L, values significantly higher than those previously reported, depending on the aging method (in barrel or vat).
The oxidative phenomena involved in barrel aging are not exclusively due to increases in the wine's oxygen content. Ellagitannins from oak are dissolved in wine (castalagin, vescalagin, roburin, atc), and although concentrations are difficult to assess accurately, they may be estimated at around a hundred mg/L. they decrease regularly, due to oxidative phenomena catalyzed by these same substances. Even in the absence of oxygen, ellagic tannins are capable of modifying the tannin structure oa a wine, as well as combinig with anthocyanins and, consequently, stabilizing color.

6.3.3 Non-volatile compounds extracted from oak barrels

In addition to ellagitannins, the oak released a certain number of other compounds, mainly lignins with a high guaiacyl and syringyl content, lignans, and triterpenes. Coumarins are also present in oak. The concentration in wine depends on the type of wood and the way it is seasoned. These compounds may be dissolved in wine in heteroside (scopoline, esculin) and aglycone (scopoletin, esculetin) form.
Another group of molecules extracted from oak, no doubt produced by the transformation of ellagitannins and possibly lignin, contribute to increasing the phenol acid concentration of wine, mainly gallic acid, by a concentration of about 50 mg/L.
In terms of flavor, studies investigating the organoleptic characteristics of these components produced the following findings:

1. Phenol acids (gallic acid) have and acid taste.
2. Coumarins (aglycones) seem acid and have a harsh character. Their glycosides are very bitter.
3. Ellagitannins are astringent as compared to gallotannins, which give a bitter, acidic impression. The benchmark, procyanidin B3, is both astringent and bitter.

Depending on conditions, oak may also release polysaccharides, mainly consisting of hemicelluloses, that contribute to wine flavor.
It is therefore quite understandable that wines aged in oak barrels have different organoleptic characteristics from those aged in the vat.oak has two contradictory effects: it strengthens the impression of harshness due to phenol components it releases, while softening condensed tannin thanks to its heterogeneous polymers. The result depends on the relative intensities of these two phenomena. There is a risk of toughening, depending on the wine's tanninc structure and the characteristics of the barrels. In any event, even if aging in oak barrels increases the phenol content of wine, it is by no means sure that it

increases the overall tannin content, at least in red wines. Analysis has shown that the total phenol index (280 nm) only increases by a few units due to wood tannins, compared to an initial value between 50 and 60.

6.3.4 Volatile compounds extracted from oak barrels

Another fundamental aspect of aging wines in oak concerns the aromatic compounds that are extracted. When these compounds marry perfectly with a wine intrinsic aromas, they make a significant contribution to the richness and complexity of the bouquet, as well as improving the flavor. Great red wines are almost always aged in oak, as barrel aging enhances their quality and finesse. In order to benefit fully from barrel aging, wine must have a certain aromatic finesse and sufficiently complex structure to blend well with the organoleptic input from the oak. Ordinary wines cannot be turned into quality wines by exposing them to oak. Attempts at flavoring wines that do not justify this treatment, resulting in a standardized, "woody" character, should be approached with great caution.

Untreated oak contains a certain number of volatile substances with specific odors. Lactones, in particular β-methyl-γ-octolactone, with four enantiomers, two geometrical isomers and two optical isomers, are produced by the breackdown of complex polymers. The cis(-)isomer has an earthy, rather herbaceous character with hints of coconut, and is 4-5 times more odiferous than the trans (+) isomer. The latter not only smells of coconut, but is also very spicy. Above a certain concentration, excessive amounts of this lactone may have a negative effect on wine aroma, giving it a strong woody or even resinous odor.

Eugenol, with is characteristic odor reminiscent of cloves, is the main volatile phenol. Other volatile phenols are present in relatively insignificant quantities.

Phenol aldehydes are also present, but in relatively small quantities. Vanillin and syringaldehyde have been identified, as well as coniferaldehyde and sinapaldehyde. Vanillin plays an active part in the oaky and vanilla odors that barrels communicate to wine.

Concentrations of trans-2 nonenal vary a great deal from one oak sample to another. Together with trans-2-octanal and 1-decanal, this molecule is responsible for the odor known as "plank smell" that wines may acquire during barrel aging. This unpleasant smell is attributed to unseasoned wood and may be attenuated by toasting the inside of the barrels more intensely.

Oak may also release norisoprenoid compounds into wine. The most important of these is β-ionone.

Oak from different origins is odoriferous to varying degrees. Its characteristic odors are mainly revealed during seasoning and barrel manufacture. Heating forms furanic derivatives, volatile phenols and phenylketones, as well as increasing concentrations of phenol aldehydes and lactones.

6.4 Oak and cooperage

Oak has been used in wine cooperage construction since at least Roman times. Although other types of wood have been used during this period, their use has largely been limited to the construction of large storage cooperage. In Europe, chestnut (*Castanea sativa*) and acacia (*Robinia pseudoacacia*) were employed for this purpose. However. Their use, along with oak,has largely been supplanted by inert materials. Similarly, the former use of wooden barrels as the primary container from transporting wine has been supplanted by the glass bottle. Thus, oak use is now principally restricted to the maturation (and occasionally fermentation of wine). It is particularly popular in the maturation of premium wines. The flavor and occasional slight oxidation provided by in-barrel maturation enhance the character of wines with distinctive varietal aromas.

6.4.1 Oak species and wood properties

Not only does white oak possess the properties required for tight cooperage, but its traditional use has led to an appreciation of (or habituation to) its subtle fragrance. Other woods have either undesirable structural or aromatic characteristics, or have been studied insufficiently to establish their applicability.

Quercus alba, *Q. robur*, and *Q. sessil* are the species most commonly used. *Q. alba* and a series of six related white oak species (*Q. bicolor*, *Q. lyrata*, *Q. macrocarpa*, *Q. muehlenbergii*, *Q. primus*, and *Q. stelata*) constitute the oaks employed in the construction of American oak cooperage. *Q. alba* provides about 45%of the white oak lumber produced in North America. It has the widest distribution of all American white oak species and has the size and structure preferred for select oak lumber.

In Europe, *Q. robur* (*Q. peduncolata*) and *Q. sessil* (*Q. petraea*, *Q. sessiflora*) constitute the primary white oak species employed in cooperage production. Although not genetically isolated, and showing considerable morphologic variability, species identification is generally possible using a combination of features, including leaf and acorn morphology. Differences in chemical composition exist, with *Q. sessil* tending to possess considerably higher levels of extractable aromatic compounds (oak lactones, eugenol and vanillin),but lower concentrations of ellagitannins, ellagic acid and dry extract than *Q. robur*

(Doussot et al., 2000) (**Figure 6.1**). Nevertheless, individual variation is too extensive to permit unequivocal identification based only on chemical analysis. barrel

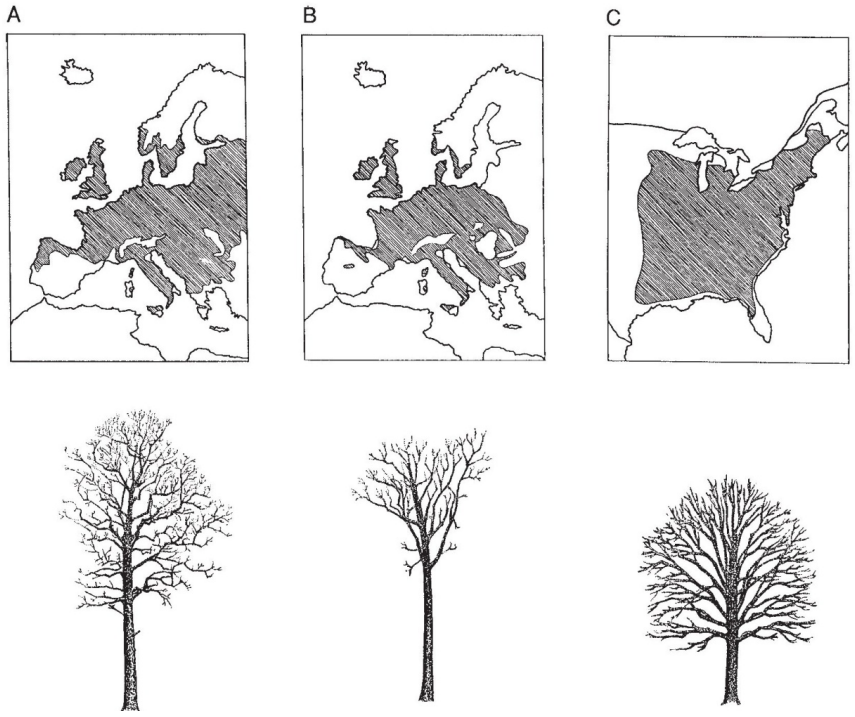

Figure 6 1. *Geographic distribution and winter silhouette of A (Quercus sessilis), B (Q. robur) and C (Q. alba).*

Both species grow throughout much of Europe. Q. robur does better on deep, rich, moist soils, whereas Q. sessilis prefers drier, shallow, hillside soils. Nevertheless, species distribution does not necessarily reflect these ecological preferences. Although *Q. robur* can quickly establish itself in sunlit areas, it is slowly replaced by the more shade-tolerant Q. sessilis. In addition, nonselective acorn collection used in silvicultural plantings, has tended to increase the proportion of Q. robur. It is more productive in acorn production.

Staves produced from different American white oak species are almost indistinguishable to the naked eye. The same is true for the two important white oak species in Europe. They can be differentiated with difficulty, and then only with certainty under the microscope.

In North America, most of the oak used in barrel construction comes from Kentucky, Missouri, Arkansas, and Michigan. There has been little tendency to separate or distinguish oak coming from different states or sites. In contrast, the identification of oak by origin is traditional in Europe. Geographical designation may indicate the wood's country (French, Russian), region (i.e., Slovenian, Limousin), political district (i.e., Vosges, Allier),or forest (i.e., Nevers, Tronçais), origin.

Conditions affecting growth (primarily moisture) also affect wood anatomy and chemistry. Slow growth generally results in the development of less-dense heartwood, due to the higher proportion of large-diameter vessels produced in the spring. In contrast, rapid growth generates wood with higher portion of small vessels (summer wood). This results from growth continuing into the summer months. The major deposition of tannins occurs some 10-15 years after vessel formation, when the sapwood differentiates into heartwood. Because deposition occurs predominantly in large-diameter spring vessels, the growth rate indirectly affects heartwood chemistry. The phenolics not only contribute to significantly to the flavors extracted during in-barrel maturation, but also resist wood rotting.

Due to the higher proportion of large-diameter vessels, slow-grown wood is softer. The lower percentage of cell-wall material in the wood makes it more pliable than oak that grew rapidly. In France, the properties of slowly grown Q. sessilis, found in forests such as Nevers and Allier, are commonly preferred for wine maturation. For brandies, the denser, but less aromatic Q. robur, found in the Limousin region is preferred. The properties and origin of the wood preferred depend largely on the desired balance between varietal and oak attributes in the finished wine.

In addition to growth-rate induced variations, structural and chemical differences occur throughout the tree. More extractable ellagitannins occur in the heartwood at the base of the tree than near its crown, and in heartwood close to the sapwood. This may reflect increased wood close to the sapwood. This may reflect increased phenolic deposition as the tree ages, as well as hydrolysis to ellagic acid and oxidative polymerization to less soluble forms. American white oaks tend to have lowers levels of extractable ellagitannins than its European counterparts. Significant variations in the concentration of oak lactones and vanillin have also been observed across the grain of wood. Nevertheless, variation among individual trees is often more marked than average differences between species.
Wood properties reflect both the climatic conditions prevalent in the region, as well as the silvicultural techniques used tomaintain forest productivity. A classic example is the denser tree spacing used in Tronçais. This is based on the view that thin annual growth rings, associated with slow growth, generates higher quality wood.
Although significant differences exist in the levels of extractable tannins between American and European oaks, the intensity of oak flavor is similar, albeit different in character. Oak species also differ in oak lactone content, and probably is sesquiterpene, hydrocarbon, and fatty acid concentrations. For example, *Q. alba* has often been found to possess the highest oak lactone content, whereas *Q. robur* has the lowest (Masson et al., 1996).
Habituation to the flavor characteristics of locally or readly available supplies oa oak probably explains much of the preferential use of one source over another. Europeans have developed traditional associations with oak derived from particular regions. For example, Spanish vintners customarily prefere American oak cooperage, whereas French producers tend to favor oak derived from their own extensive forests (4.2 million hectares). Intriguingly, the destruction of local oak forests probably explains the nineteenth- century French preference for oak from Russia and Germany, followed by North America, the Austrian Empire, and finally France. Although matching oak flavor with the wine remains subjective, progress in oak chemistry may soon facilitate decision-making. Differences in sensory characteristics often can be recognized by trained panels when identical wines are aged similarly in oak of different origin, seasoning, toasting, or production technique.
Nevertheless, these influences are frequently incredibly difficult to recognize in nonidentical wines. This indicates that oak extractives are simply another component in the interplay of wines. This indicates that oak extractives are simply another component in the interplay of wine flavors-whose sensory input is often difficult to predict.
With all the sources of variation in oak flavor, the only way to achieve some degree of standardization is to use barrels incorporating a randomized selection of staves from a relatively common source (standard practice), blend wine matured in a large selection of barrels, and frequently sample to assess that wines are developing the characteristics desired.

6.4.2 Primacy of oak

The demands placed on wine cooperage require that the wood possess very specific properties. The wood must be straight-grained, that is, possess vessels and fibers running parallel to the length of the trunk, with no undulating growth patterns or vessel intertwining. In addition, the wood should exhibit both strength and resilience. Structurally, the wood must be free of faults that could make the cooperage leaky. The wood also must be free of pronounced or undesirable odors that could taint the wine. In all these aspects, *Q. alba*, *Q. robur*, and *Q. sessilis* excel. The trees also grow large, straight, and tall. This minimizes wood loss during stave production. Furthermore, white oaks combine two relatively unique quality features, large rays and tyloses. With oak's other characteristics, they make oak the wood of choice in constructing tight cooperage.
All trees produce rays, collections of elongated cells positioned radially along the trunk axis. Rays function in conducting water and nutrients between the bark and wood. In oak, the rays are unusually large, being upward of 15-35 cells thick and 100 or more cells high. In cross-section, the rays resemble elongated lenses. Because staves are split (or sawed) along the radius, the broad surface of the stave runs roughly parallel to the rays. The radial plane becomes the inner and outer surfaces of the cooperage. The high proportion od ray tissue in oak (about 28%) and its positioning parallel to the cooperage circumference make rays a major barrier to wine and air diffusion. Wine diffusing into ray cells is deflected along the stave width. Continued lateral flow is limited by nonalignment with the rays in adjacent staves. Wine would have to navigate very tortuous route past five or more large rays to diffuse out through the sides of a barrel. In practice, wine seldom penetrates more than about 6 mm into oaks staves (Singleton, 1974).
Positioning the radial axis of the wood tangential to the barrel circumference has additional benefits in the construction of tight cooperage. The large number of rays permits only minor circumferential swelling.

The swelling (about 4%) is sufficient, however, to help compress the staves together and seal the joints. Positioning the radial plane of the wood outward also directs the axis of greatest wood expansion (its tangential plane) inward. In this alignment, an expansion of about 7% (Peck, 1957) does not influence barrel tightness. The negligible longitudinal expansion of the staves has no effect on barrel tightness or strength.
The high proportion of rays gives oak much of its flexibility and resilience. Otherwise, the staves would be too tough to be easily bent to form the curved sides (bilge) of the barrel without cracking. The bilge permits full barrels, weighing several hundred kilograms, to be easily rolled.
Oak produces especially large-diameter xylem vessels in the spring. These are large enough to be seen with the naked eye. The vessels allow the rapid flow of water and nutrients up the tree early in the season. However, the vessels could also make barrels excessively porous, permitting wine to seep out through the ends of the staves. In white oak, the vessels become tightly plugged as the sapwood differentiates into heartwood. The plugging results from the expansion of surrounding parenchyma cells into the empty vessels. These ingrowths are called tyloses. Tylose production is so extensive that the vessels become essentially impenetrable to the movement of liquids or gases. Only heartwood is used in the construction of tight barrels.
The combined effects of rays, tyloses and the placement of the radial plane of the stave tangential to the circumference severely limit the diffusion of air and wine through the wood. With proper construction and presoaking, oak cooperage is essentially an impervious, airtight container.
As sapwood completes its maturation into heartwood, deposition of phenolics kills any remaining living wood cells. The phenolics (primarily ellagitannins) render the heartwood highly resistant to decay. Because mature heartwood contains only dead cells,the lower moisture content makes the lumber less liable to crack or bend on drying. These features give heartwood the final properties required for superior-quality cooperage wood.

6.4.3 Barrel production

6.4.3.1 Staves

For stave production, trees with diameters between 45 and 60 cm are favored (minimum 100-150 years old). Larger trees tend to be reserved for the production of head staves (headings). After felling, the trees may be cut into sections (bolts) equivalent to the stave length desired. They are then split (or sawed) into quarters. The staves are split out of quarters. In sawing, planks of uniform thickness are cut out, aligning the cuts roughly along the radius (parallel to the rays). In splitting, wedge-shaped planks are removed. Portions too narrow for stave production are removed and discarded. Subsequently, wood is removed from the plank give it a more uniform width. Because splitting follows the plane of vessel elongation, the sections may be somewhat twisted. Any sapwood associated with a stave piece is removed and discarded. Staves may vary slightly in breadth. Staves with a light pinkish coloration (next to the sapwood) are preferred.
Splitting is generally preferred to sawing because it separates the wood along planes of vessel elongation. This cleaves the staves parallel to the rays. Although oak is “straight-grained”, sawing unavoidably cuts across some irregular vessel, increasing surface roughness and potentially enhancing permeability. This is more a problem with European oaks. Their large-diameter spring vessel possess fewer and thinner (more fragile) tyloses than American oak (Chatonnet and Dubordieu, 1998). The consequential greater porosity of European oak may also partially explain why tha staves release more phenolics than do similarly made America oak staves. Sawing across surface vessels is relatively insignificant with American oak. *Q. alba* possesses sufficiently thick, tightly packed tyloses to make even short, severed vessels liquid and gas impermeable.
With splitting, one side of the staves is shaved obliquely to make the sides parallel. This cuts across wood rays, creating a potential point of leakage. With splitting, only about a quarter of a log (including both sap and heart wood) can be converted into stave wood. Heading pieces are cut out similarly, but are removed from shorter lengths of wood,
Stave length, width, and thickness depend on the volume of wine to be held and the rate of wine maturation desired. To accelerate maturation, barrels constructed of thinner (about 2.1 cm), may be preferred. For standard barrels, possessing a capacity of 225 liters, staves and headings are roughly 2.7cm thick. Once cut, the staves and heading pieces are stacked to dry and season. Natural seasoning for about 3 years is traditional (about 1yr/cm thickness). Stacking each stave row at high-angles favors good air circulation, while close spacing of the stack diminishes excessively rapid drying (limiting warping or cracking). The stacks are usually dismantled, the staves randomized, and the piles reconstructed every year. This minimizes variation based on positioning within the piles.

It is generally considered that naturally dried oak gives a more pleasant woody, vanilla-like character, whereas kiln drying produces a more aggressive, green, occasionally resinous aspect (Pontallier, 1982). The latter may result from a reduction in oak lactone, volatile phenol, fatty acid, and norisoprenoid content, and an enhancement in the concentration of furfural and hydroxymethylfurfural (Masson et al., 2000). These effects are magnified if drying occurs at higher temperatures and involves green wood (without prior air drying). Kiln drying normally occurs at between 45 and 60°C. it can rapidly bring newly cut (green) wood down to a desirable moisture content, about 12%. The specific effects of drying method often depend on the species. for example, *Q. sessilis* releases more tannins following kiln drying than does *Q. robur*. Although kiln drying decreases the production or release of oak lactones and eugenol, it can increase the avaibility of trans-methyl octalactone from *Q. sessilis*.
With *Q. alba*, natural seasoning increases susceptibility to pyrolitic breakdown, especially cellulosic constituents. The longer the seasoning, the grater the potential production of compounds such as 5-hydroxymethylfurfural, furfural, and 5.methyl furfural (Hale et al., 1999). The effects of air drying are also noticeably influenced by local climatic conditions. Because of the variation in moisture content of air-dried staves, and the potential for undesirable fungal development, it is now common to combine air drying with kiln drying.
Although fungal attack can reduce wood quality, it may also generate some of the benefits of seasoning. Fungal action has the potential to synthesize aromatic aldehydes and lactones from wood lignins. For example, the wood-rotting fungus, Coriolus versicolor, produces polyphenol oxidases that can degrade lignins, as well as induce phenolic polymerization. Many fungi have been isolated from the outer few millimeters of staves, but it takes almost a year before penetration of the wood becomes microscopically evident (Vivas et al., 1997). The significance, if any, of the frequent isolation of common saprophytic fungi, such as Aureobasidium pullans and Trichoderma spp., has not been established.
Polymerization (and reduced solubility) of ellagitannins is a particularly noticeable consequence of natural seasoning. This undoubtelly affects their extraction by wine. In addition, phenolic oxidation (resulting in the release of peroxide) could favor cellulose hydrolysis (Evans, 1987). Natural seasoning also can produce changes in the concentration of several oak aromatics. Degradation prodicts of lignin, such as eugenol, vanillin, and syringaldehyde, have been variously found to increase or decrease, notably in the outer portions of staves. Typically, however, these changes are much less marked than those associated with stave toasting during barrel production. The concentraction of the isomers of β-methyl-γ-octalactone also varies during seasoning, increasing or decreasing (Sefton, 1993). A more consistent finding, however, is an increase in the proportion of the more aromatic cis isomer of oak lactone. Microbial metabolism could also modify cell-wall constituents. Sugar liberated by cell-wall degradation could increase the furfural content generated during barrel toasting. In addition, the leaching and degradation of phenolic compounds by rain, oxygen, and ultraviolet radiation may be significant. The conversion of the bitter-tasting esculin, to its less-bitter aglycone esculetin, may be another example of how wood character improves with outdoor seasoning.

6.4.3.2 Barrel assembly

In barrel construction, the first step involves checking the staves for knot, cracks, or other structural faults. Once the appropriate number of suitable staves has been assembled, they are dressed. Dressing refers to selective shaving in preparation for raising. The first of the dressing procedure, listing, tapers the broad ends of the staves to give them their basic shape (**Figure 6.2**). The amount of listing required depends on the desired "height" of the barrel, that is, the length of the staves relative to the maximal circumference of the barrel. Subsequently, a small amount of wood may be chisled from the ends (backing) and center (hollowing) to facilitate bending. Hollowing usually reduces the thickness of the central part of the stave from 27 to 24 mm. the staves are now ready for jointing, in which a bevel is planed along the inner edge of the side of each stave. Jointing requires considerable skill because the angle changes along the length of the stave. The bevel depends on the barrel height, being maximal at the center (bilge) and least at the ends (heads). Jointing precision determines the tightness between adjoining staves.
The curved shape of the barrel provides much of its strength. This comes from the engineering principle called the double arch. The sloping sides also provide a point in which the barrel can be pivoted and rolled with comparative ease.
Once dressed, the staves are raised. This involves placing the staves together in an upright circle. Several temporary hoops, including a trussing hoop (runner), help to support the staves. The hoops are forced down and begin to force the staves into the curved barrel outline. At this point, the barrel is inverted and placed approximately 5 cm above an open fire for softening. The inner and outer stave surfaces are frequently sprayed or splashed with water. Alternatively, the staves may be steamed prior to, or instead

of, firing to soften the wood. After sufficient softening, the staves are slowly and periodically pulled together with a windlass. Positioning temporary hoops holds the staves in place until additional heating (about 10-15 min) sets the staves in their curved shape. The firing helps shrink the innermost wood fibers, releasing tension caused by bending. This is termed setting.

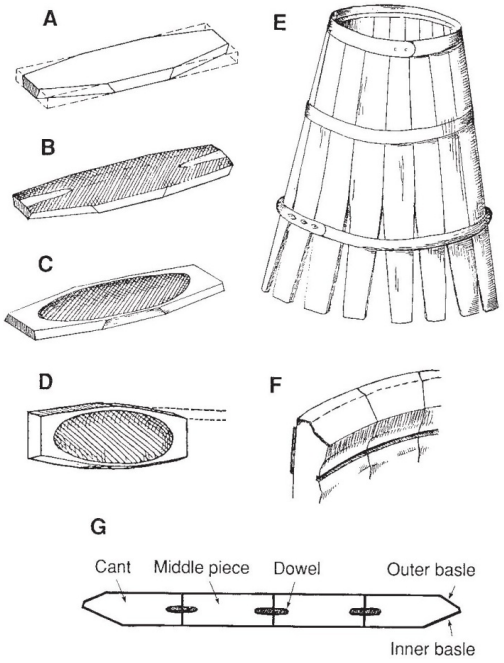

***Figure 6 2**. Stave preparation and barrel construction: A (listing); B (hollowing); C (jointing); D (raising); E (chimed, hoveled); F (crozed stave ends); G (head cross-section).*

Additional heating (termed toasted) is a comparatively recent innovation in barrel production, not being noted in texts prior to the middle of the nineteenth century. Toasting produces sensory changes in the characteristics of the wood. These result primarily from pyrolysis and thermohydrolysis. Heating may be performed directly over the fire or slightly raised off the floor. The top may be closed with a metal cover or left open. Closed firing requires more frequent moistening, but produces more uniform heating of the barrel's inner surfaces (Chatonnet et al., 1991; Matricardi and Waterhouse, 1999). Not only does moistening slow the rate of heating, but it also produces steam that promotes the hydrolytic breakdown of hemicelluloses, lignins, and tannins. The inner-surface temperature of the barrel typically reaches 200°C and above. Carbonization (charring) of the wood begins at about 250°C.
The degree and desirability of pyrolysis depend on the style and characteristics of the wine desired. Light toasting, sufficient to ease stave bending (about 5 min, inner surface temperatures 100° to 150°C), produces few pyrolytic by-products, leaving the wood with a natural woody aspect. Medium toasting (about 15 min, inner surface temperatures >150°C) generate phenolic and furanilic aldehydes.phenolic aldehydes, derived from lignins, donate a vanilla roasted character, whereas most furanilic aldehydes, coming from hemicelluloses, may generate a caramel-like aspect. The degradation of hemicelluloses also produces compounds, such as maltol and 2-hydroxy-3-methyl-cyclopentanone. These generate toasty flavors. Heating also may favor the synthesis of oak lactones from precursors. Medium toasting is generally preferred for Q. sessilis from the forests of central France. Toasting reduces the solubility of oak tannins, particularly useful with European oaks that possesses high levels of soluble ellagitannins. Toasting also activates their degradation, first to ellagic and then gallic acids and subsequently carbonation. Prolonged exposure (about 25 min, inner surface temperatures >200°C) chars the innermost layers of the staves, and destroys or limits the synthesis of phenolic and furanilic aldehydes. These are replaced by volatile phenols, giving the wood a smoky, spicy aspect. Phenolic aldehydes generate guaiacol, 4-methyl guaiacol, and dimethoxy-2,6-phenol, whereas furanilic aldehydes give rise to eugenol and 4-vinylguaiacol. In addition, volatile compounds, such as vanillin and syringaldehyde are progressively destroyed. Heavy toasting also limits the release of phenolic components. Heavy toasting is often preferred for Q. sessilis obtained from southwestern France. With heavy toasting, small fractures (6µm), up to several millimeters long, appear in the wood. These fissures can penetrate upwards of 600-

700 μm into the wood. These facilitate extraction of constituents from areas less affected by heat. Charring, desirable for Bourbon maturation, is avoided in the production of wine cooperage. Charcoal on the inner surfaces can deodorize red wines, as well as remove desirable flavorants.
Aromatic aldehydes, furfurals, furans, oxygen heterocycles,pyrazines, pyridines, and pyrans are among the many pyrolytic compounds derived from tannins and hemicelluloses. In contrast, volatile phenolics such as guaiacol, 4-methyl guaiacol, vanillin, syringaldehyde, and coniferaldehyde are lignin decomposition products. Toasting also degrades several unsaturated aldehydes, notably (E)-2-nonenal, the primary ingredient of the sawdust off-odor occasionally found in wine matured in new oak cooperage.
Although barrel manufactures attempt to maintain uniformity in the level of toasting, there are no industry-wide standards for what light, medium, or heavy toasting means. Consequently, there can be considerable variation among manufacturers (Chatonnet et al., 1993). Significant variation is also detected among barrels assembled by the same producer from a common source of randomly selected staves. The traditional method of toasting over a brassier, burning oak chips, is difficult to control. As well, different portions of the barrel may be exposed to marked temperature variations. Although this increases the incidence of blistering, blisters do no increase the risk of microbial contamination (Vivas, 2001).
The exterior of the fire generates temperatures that can vary from 460 to 600°C, whereas the interior can vary from 850 to 1000°C, the wood itself generally does not go much above 200°C until the later stages of toasting. One technique to reduce the disparity involves a prolonged exposure to a small fire. This produces greater heat penetration, but without the production of dark-colored pyrolytic breakdown products. Another potential solution involves the use of several, linear, infrared-heat-generators.
After setting, and any toasting, the cooper puts a bevel on the inner surface of the stave ends. This is followed by chiming, preparing the ends for positioning the headpieces. The first task involves planning the ends of the staves (the chime). Shaving the inner edge produces the bevel. Cutting a concave groove slightly below the chime produces the howel. A deeper cut into the howel (the croze) produces the slot into which the headings fit.
The outer surface of the barrel is planed to give it a smooth surface, whereas the inner surface is left rough. The rough inner surface aids wine clarification by providing increased surface area for the deposition of suspended particular matter.
Next, a bung hole is bored and enlarged with a special auger to receive a tapered wooden, rubber, or plastic peg. A tap hole may also be bored near the end of the central head-stave.
In temporary hoops were employed during raising, they are replaced with permanent hoops. For 225-liter barrels, this usually consists of two chime hoops, located just below the heads of the barrel; two bilge hoops, positioned one-third of the way in form the ends; and a set of quarter hoops, placed approximately one-fourth of the way in from the heads. So positioned, the hoops limit the wear on the staves during rolling. At this point, the heading pieces are selected and prepared.
The head consists of several heading pieces, typically between the heading are straight, not beveled. In addition, the heading pieces (constituting about 25% of the barrel surface) receive no toasting. Short dowels inserted between each heading piece keep them in alignment. Caulking with river reshes, called flags, may be used to prevent leakage.
The circular shape of the head is now sawed, in preparation for cutting the head. Cutting refers to shaving two bevels, called basles, on the upper and lower surfaces of head stave-ends.
The bottom head is inserted first. Removal of the bottom head hoop allows the head to be forced into the croze. After repositioning the head hoop, the barrel is inverted to remove the opposite head hoop. A heading vice may be screwed into the barrel. The head is pulled up into its groove with the vice. Alternatively, a piece of iron forced in a joint between two staves levers the head into position. The stave alignment of the two heads is positioned perpendicular to one another. This limits the pressure that develops during swelling from acting in the same direction, thus minimizing leakage.
He final task involves hammering the hoops tight. This forces the staves together and closes most cracks. After soaking for about 24h in water, a well-made barrel becomes leak-proof (Shulz, 2004).
Larger-volume barrels, ovals, and vats are made in essentially the same manner. The primary differences, other than overall size, are stave thickness and degree of curvature. The last affects the need for heating. Typically, large cooperage is not toasted.

6.5 Oxygen uptake

Slight oxidation is commonly viewed as an important consequence of maturation in oak. Wine placed in well-made barrels, bunged tightly, and rotated, so that wine covers the bung, are generally well protected from oxygen exposure (Moooutonet, 1998). Wine exposure to oxygen occurs principally as a consequence of periodic procedures such as racking. Air does not usually diffuse into tight barrels in

significant quantities. Ribéreau-Gayon (1931) estimated oxygen ingress at about 2-5mL/liter/yr in tightly bunged, full barrels. This increased to about 15-20 mL/liter/yr in barrels with typical ullage. The difference appears to relate to slower ingress via staves in contact with wine. Partial drying of the wood above an ullage increases penetration. Singleton argues that most of the oxygen that enters likely reacts with phenolic constituents (notablyellagitannins), before traversing the stave. The water and alcohol lost through the surfaces of the barrels are only slowly replaced, resulting in the generation of a partial vaccum. Atmospheric pressure compresses the upper edges of the barrel, frequently resulting in a stabilization of the vacuum within within 5-15 days.barrelsmay differ markedly in tightness. The negative pressures observed in barreled wine can vary considerably. This may explain some of the variation in barrel-to-barrel maturation rate. Depending on barrel tightness, temperature, and relative humidity, a barrel may lose from 4 to 10 liters of wine per year. A mathematical model wine loss may help design air circulation system in cellars to minimize such losses (Ruiz de Adana et al., 2005).
Evaporative losses tend to be more marked in barrels left their bungs upright. Despite this, the practice makes their bungs upright. Despite this, the practice makes topping and sampling considerably easier. Coincidentally, both procedures increase exposure to oxygen. During normal racking, topping, and sampling, oxygen uptake has been variously estimated at between 15 and 40mL O_2/liter/year. Oxygen access is undoubtedly higher when glass or loosely fitting bungs are used. Under such conditions, the oxygen content in the ullage may remain between 5 and 9% (Moutounet et al., 1998).
Racking can result in up to 6mL O_2/liter being absorbed per racking. In red wine, the absorbed axygen is consumed within approximately 6 days at 30°C (Singleton et al., 1987)- equivalent to 15-20 days at cellar temperature. Most of the oxygen is presumably consumed in the oxidation of o-diphenols. Smaller amounts may be in volved in the oxidation of ethanol, sulfite, ascorbic acid, ferrous ions, and various other organic constituents.
The slight uptake of oxygen during racking and other processes is generally viewed as desirable, especially for red wines. It promotes the polymerization of anthocyanins with tannins and, therefore, color stability. In addition, hydrogen sulfide formed in , and released by the lees, is oxidized (Pfeifer et al., 1995). This is especially valuable for white wines. The action of yeasts in the lees also speed oxygen consumption, protecting the wine from excessive oxidation. Ellagitannins and residual sulfur dioxide provide additional antioxidant protection.
The ullage that develops over wine as liquid escapes through the wood is not a source of spoilage. Its oxygen content is usually low to undetectable. Thus, filling the ullage space (topping) is necessary only if air enters the barrel. Only with prolonged aging, as in brandy, is drying (and shrinkage) of the staves over the ullage likely tobe sufficient to generate cracks between (or in) the staves.

6.6 Advantages and disadvantages of oak cooperage

For premium wines, fermentation and/or aging in oak is often desirable. The expense and effort are justified by the addition flavor complexity obtained. This is especially true for red wines. Exposure time is crucial. Short duration tends to extract a higher proportion of woody flavors, with the more appreciated aspects being extracted only with extended contact. The level of toasting and the proportion of new-to-use barrels can also significantly influence the oak character procured. For wines of neutral or delicate flavor, exposure to oak is neither cost effective nor necessary beneficial.
Oak barrels are both costly to purchase and maintain, and new barrels need conditioning prior to use. The tartrates and tannins that accumulate on the inside of the barrel during wine maturation are both difficult and unpleasant to remove. When not containing wine, barrels must be protected from drying and microbial contamination. Off-flavors produced by bacteria and fungi rowing on internal surfaces can subsequently taint wine. Examples are corky off-odors (Amon et al., 1987), vinegary taints (predominantly from the metabolism of acetic acid bacteria), and manure or stable notes (due to the enzymatic reduction of vinylphenols to ethyl phenols by Brettanomyces spp.).
Because the rate of maturation varies from barrel to barrel, frequent and time-consuming barrel sampling is required to follow the progress of the wine. Racking is more labor intensive and inefficient than its automate d equivalent in large cooperage. In addition, considerable economic losses can result fromwine evaporation from barrel surfaces. Up to 2-5% of the volume may be lost per year in this way. Volume loss is especially marked at warm temperatures. Depending on the relative humidity of the cellars, wine may either increase or decrease in alcoholic strength. High relative humidity suppresses water evaporation, but has no influence on alcohol loss. Consequently, the alcoholic strength of wine decreases in humid cellars. Under dry conditions, water evaporates more rapidly than ethanol, resulting in an increase in alcoholic strength. In addition to water and ethanol, small amounts of acetaldehyde, acetal, acetic acid, and ethyl acetate are lost by evaporation from barrel surfaces. In contrast, less volatile and nonvolatile compounds accumulate as a result of the concentrating effect of water and ethanol loss. Relative humidity also

influences the types and amounts of phenols extracted. Low relative humidity decreases total phenolic uptake, but increases vanillin synthesis (Hasuo et al., 1983).
Another sources of wine loss, associated with in-barrel maturation, results from absorption by staves. It is estimated that new 225-liter barrels absorb between 5 and 6 liters of wine.

6.7 Alternative source of oak flavor: the oak chips

The addition of oak chips or shaving to wine has been investigated as an economical alternative to barrel aging. Not only does it save by delaying or avoiding the purchase of new barrels, but it can also reduce costs associated with topping and lost wine volume. Values commonly suggested are about 10g/liter/year for white wine, with more than twice that for red wines. With small oak chips (≤1 mm diameter), about 90% of the extractives are removed within 1 week. The perception of some aromatic constituents may take longer to appear, partially because they are formed slowly during wine maturation. The sensory effects obtained from aging on oak chips may differ from those obtained during barrel aging. This may arise from the absence of heat-induced hydrolysis of oak constituents (if the chips are not toasted), reduced oxygen exposure (due to the absence of barrel racking), or differences in microbial modification of oak compounds during seasoning of the wood or during wine maturation. In addition, the surface area-to-volume contact is grater, as is the exposure of the wine to the less-permeable summer wood. Increased surface are also greatly facilitates the uptake of ellagitannins, most of which are hydrolyzed on the inner surfaces of barrel staves during toasting (Swan et al., 1997). Combining chips with different levels of toasting ideally should reproduce the diversity of extractives obtained from oak barrels (the slow penetration of heat during toasting generating a progressive range of chemical changes across the stave).
When used, chips are usually added during fermentation. This promotes the early precipitation of the extra tannins and phenols, extracted due to the extensive wine/chip contact. In addition, oxygen present in the wood structure in rapidly consumed during fermentation. In the chips are added after fermentation, oxygen uptake can be minimized if the oak is presoaked for a few hours.
Although chips can be added directly to wine, it creates a removal problem. Chips can also clog drains, pump and filters. This usually involves inserting a series of oak slats (battens) or tubes in a stainless steel holder. The inserts are often derived from used barrels, coming from outer portions of staves unaffected by wine. The sections are usually split in two , essentially doubling the contact surface area. Oak inserts may be exposed to infrared radiation to generate a desired toast level.
The sensory generated from various oak treatments may be less complex than those derived from staves. This may be due to the thinness of the slats-thin inserts not possessing the diversity of aromatics found in thicker barrel staves. Conversely, this can be used to advantage, permitting the winemaker to more precisely select the oak attributes (defined by the level of toasting) desired in the wine. Potential problems with oxygen uptake from the wood can be largely avoided by insertion during fermentation or presoaking.
If only oak flavors are desired, an even simpler procedure is the addition of an oak extract. Powered oak "flour" is another alternative.
Previously, European winemakers were prohibited by law from using any of these barrel alternatives. This restriction has been removed, at least for some categories of wine.

6.8 References

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7. C-Glycosidic Ellagitannins and their influence on wine chemistry

7.1 Introduction

The pharmacological activities of tannins described in medicinal books before the recent achievements on ellagitannins chemistry were mostly those of gallotannins and condensed tannins of poor chemical uniformity. The gallotannins extracted from Chinese or Turkish gall, sometimes called tannic acid, are variable mixture of polygallates of carbonydrates. They cause irradiation on skin and mucous membranes, although they have been utilized in some traditional medicinal applications, and are technically defined on the basis of their general capacity to bind to proteins and nitrogen basic compounds such as alkaloids. The condensed tannins, mixtures of oligomeric and polymeric flavonols (e.g. catechins), are chemically more unstable and heterogeneous, thus confirming old concept of tannins. They were mainly used for leathering and staining, although some plants containing them have been used as traditional medicines. Phlorotannins are highly unstable oligomers of phloroglucinol produced by algate that have never been isolated without being first converted into their methyl or acetylderivatives, and as such they constitute a third but rather peculiar group of tannins.
As for ellagitannins, although some members of this class of hydrolyzable tannins were obtained early on, it is the isolation and structural determination of over 500 pure compounds since 1975 from various plants, many of which used in tradictional medicines, that brought remarkable changes in the definition of concept of "tannins" (Okuda, 2005; Quideau & Feldeman, 1996).

7.2 Characteristic structural features and natural occurrence

The C-glycosidic ellagitannins constitute a subclass of hydrolyzable tannins. Today, over 500 members of this family of gallic acid-derived polyphenolic natural products have been isolated from various plants and fully characterized (Khanbabaee & van Ree, 2001). Among this myriad of gallic acid metabolites, the C-glycosidc ellagitannins present the structural particularity of having a highly characteristic C-C linkage between the carbon-1 atom of an open chain glucose core and the carbon-2' atom of a galloyl-derived unit esterified to the 2-position of the glucose core. This C-1 linked galloyl-derived unit is either part of a terarylic nonahydroxyterphenoyl (NHTP) unit (also known as a flavogalloyl group) that is attached via three ester bonds to the 2-, 3- and 5 positions of the glucose core, as exemplified in the structures of vescalagin and castalagin, or part of a biarylic variant bridging the 2- and 3- position of the glucose core, as exemplified in the structures of stachyurin and casuarinin. The latter biarylic unit is commonly referred to as HHDP for hexahydroxydiphnoyl, but acronym HHBP is also used for hexahydroxybiohenoyl to signify more explicitly the biarylic nature of this ellagitannin unit type (Quideau et al., 2004) (**Figure 7.1**).

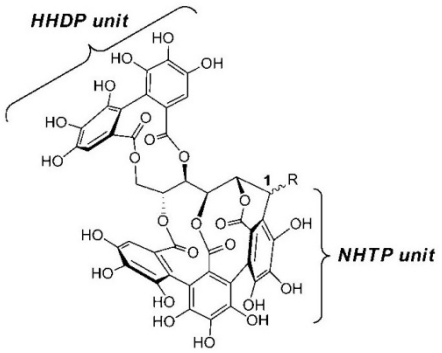

Figure 7.1. *Typical example of a monomeric C-glycosidic ellagitannin. R1: β-OH, vesclagin; R1: α-OH, castalagin.*

Ellagitannins of the C-glycosidic type have been found in various plant species from the *Hamamelidaceae*, *Fagaceae*, *Betulaceae*, *Casuarinaceae*, *Juglandaceae* and *Rhoipteleaceae* families in the *Hamamelidae* subclass, from the *Rosaceae*, *Combretaceae*, *Lythraceae*, *Melastomataceae*, *Myrtaceae*, *Punicaceae*, *Trapaeae* and *Elaeagnaceae* families in the *Rosidae* subclass, and from the *Theaceae* and *Stachyuraceae* families in the *Dilleniidae* subclass (Okuda et al., 1993; 2000).

7.3 Oak and chestnut C-glycosidic ellagitannins

Vescalagin and its C-1 epimer castalagin are the first C-glycosidic ellagitannins that have benn investigated after their isolation thirty years ago from *Castanea* (chestnut) and *Quercus* (oak) woody secies of the *Fagaceae* family by Mayer and co-workers (Mayer et al., 1967; 1969; 1971). Their structures, as well as those of their 2,3-HHBP-bearing analogues, stachyurin and casuarinin were fully determined much later after revision of their respective configuration at C-1 by the Nishioka's group. The combined amount of these two epimers in the heartwood of oak species such as *Quercus petraea* and *Quercus robur* has been evaluated to vary from ca. 3 to 57 mg/g dry wood, depending on the species, age and from sampling position in the tree (Mosedale et al., 2001; Vivas et al., 1996; Viriot et al., 2004). The heartwood of chestnut species such as *Castanea sativa* can even contain up to 63 mg of castalagin and vescalagin per gram of dry wood. Six other NHTP-containing C-glycosidic ellagitannins were later isolated from fagaceous *Quercus* and *Castanea* hardwood species, for example the dimers roburins A and D, and the lyxose/xylose-bearing monomers grandinin and roburin E and dimmers roburins B and C. Among these eight typical NHTP-containing C-glycosidic ellagitannins, vescalagin and castalagin largely predominate in the fagaceous woody species containing them, representing for example between 40% and about 60% by weight of this group of ellagitannins in *Quercus petreae* and *robur* heartwood (Masson et al., 1996)s.

Many additional C-glycosidic ellagitannins, including monomers, oligomers and complex tannins, have been identified over the years from species belonging to the selection of plant families mentioned above.

7.4 Complex C-glycosidic ellagitannins

The C-glycosidic ellagitannin subclass also encompasses so-called complex tannins, which are structural hybrids composed, in their simplest variations, of a C-glycosidic ellagitannin moiety derived for example from the monomers vescalagin or stachyurin and a flavan-3-ol unit such as catechin or epicatechin. In these complex tannins, both parts are connected via a C-C linkage between the carbon-1 center of the open-chain glucose core of the ellagitannin moiety and either the carbon-8 or the carbon-6 center of the ring-A of the flavan-3-ol unit. Depending on the nature of each miety, the regiochemistry of attachment to each other, and the type of bond connectivities through which each miety can lead to oligomeric variants, complex tannins further contribute to the ellagitannin structural diversity. Their natural occurrence appears to be limited to plant species of the families *Fagaceae*, *Combretaceae*, *Myrtaceae*, *Theaceae* and *Melastomataceae* (Yoshida et al., 1992). Typical examples of these flavonoid/ellagitannin hydrids are the catechin/stachyurin-based regioisomeric stenophyllanins A and B isolated from *Quercus stenophylla* MAKINO (Nonaka et al., 1990), the catechin/vescalagin based regioisomeric acutissimins A and B isolated from *Quercus* and *Castanea* species, the catechin/vescalagin-based procyanidino-ellagitannin mongolicanin, which features the catechin dimer procyanidin B-3 as its flavan-3-ol derived unit and which was isolated from the bark of Quercus *mongolica* var. grosseserrata, and the catechin/vescalagin-based anogeissinin, which features two vescalagin-derived moieties connected to the C-6 and C-8 centers of catechin and which was isolated from the bark of Anogeissus acuminate var. lanceolata, a combretaceous plant species largely distributed in South East Asia (Lin et al., 1991).

The camelliatannins A and B and malabathrin A are examples of C-glycosidic flavano-ellagitannins featuring epicatechin-type flavanol moieties connected to the C-1 center of the open-chain glucose core of either stachyurin or its 5-O-desgalloylated variant, desgalloylstachyurin.

The inherent chemical reactivity of flavano-ellagitannins renders them sensitive to additional structural transformations upon oxidations. Mongolicains A and B are two examples illustrating the consequences of such a rather unique propensity of flavanol-bearing ellagitannins to participate in further oxidation-driven reactions. These two complex tannins, first isolated (and characterized) from five *Quercus* and one Castanopsis species, feature a spiro-linked dihydrofuran-cyclopentenon motif. The formation of this motif can conceivably derive from a ring contraction of the C-1 linked O-2-galloyl part of a former NHTP unit with concomitant decarboxylation and nucleophilic attack of one of the phenolic hydroxyl groups of the A-ring of a C-1 linked catechin moiety. Hence, mongolicains A and B can be considered as direct metabolites of acutissimins A and B, respectively. This proposal is further supported by the fact that these four substances invariably co-exist in their fagaceous plant sources. The initiation step of the conversion of acutissimins into mongolicains would be the dehydrogenation of the pyrogallol motif of the NHTP C-1 linked galloul unit into an α-hydroxy-ortho-quinone/cyclohexenetrione system.

7.5 Vescalagin *versus* castalagin

Vescalgin and castalagin, despite their quasi-identical structure. Express drastic differences in chemical reactivity, as well as in biological activity (Quideau et al., 2005). These differences are really striking when one considers that the only structural difference between these two epimers of relatively high

molecular mass (934 Da) is the orientation of their small OH group at C-1. When Quideau et al. started to study they chemistry, they realized that condensation reactions under mild acid-catalyzed nucleophilic substitution conditions were possible only with vescalagin but not with castalagin. Moreover, the more reactive vescalagin participates in nucleophilic substitution reactions with full retention of configuration at C-1. Starting from vescalagin, the condensation products obtained exclusively display a β-orientation of the newly formed bond at C-1, like in all of the related C-glycosidic ellagitannins isolated (Viriot et al., 1994; Vivas et al., 2004).

7.5.1 Refractory chemical behavior of castalagin

The first chemical reaction investigated from vescalagin/castalagin was their condensation with (+)-catechin in order to hemisynthesize the acutissimins A/B. All attempts to form these flavano-ellagitannins using castalagin instead of vescalagin under similar conditions (1,5% (v/v) TFA/THF, 60°C, 5h) were unsuccessful.

Molecular-mechanisms calculations performed using Macromodel (MM3* force field) indicated that the minimum-energy conformer of castalagin (501.6 kJ/mol) was slightly more stable than that of vescalagin (504.5 kJ/mol) (Quideau et al., 2004). Admittedly, this energy difference of 2.9 kJ is rather slimand the reactivity differences observed between vescalagin and castalagin cannot solely rely upon this figure. A closer examination of the minimum energy conformations reveals that the β-oriented OH-1 group of vescalagin is exo-located relatively to the most crowded face of the molecule, whereas the α-oriented OH-1 group in castalagin is endo-positioned. This endo-positioning of the α-orinted OH-1 group in castalagin renders it more available to participate in an intramolecular (stabilizing)hydrogen-bond between its oxygen atom and the hydrogen atom of the phenolic OH-3' group of the galloyl-derived I-ring of the NHTP unit. This H-bond of 2.21 Å with an O-1…H-O-3' angle of 146° may be invocked to suggest that the basicity of the O-1atom in castalagin is consequently lower than that of the same oxygen atom in vescalagin, hence rendering it less prone to protonation under mild acidic conditions. Furthermore, departure of a protonated OH-1 group may be energetically favored from vescalagin, since this OH group in rather axially oriented on the six-membered ring lactone defined by the double connection of the galloyl-derived NHTP I-ring to the O-2 and C-1 positions of the open-chain glucose core (**Figure 7.2**). In castalagin, this OH-1 group adopts a less energetically demanding equatorial orientation thus also contributing to the lower overall strain energy of castalagin versus vescalagin (Yoshida et al., 1991; Haslam, 1998).

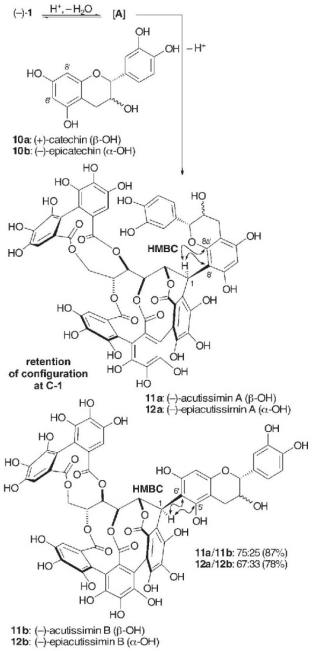

Figure 7.2. *Acid-catalyzed formation of acutissimin and epiacutissimin from vescalagin and (+)-catechin and (-)-epicatechin, respectively.*

Interestingly, vescalagin is almost systematically found in lower concentration than castalagin in plant extracts. This intriguing observation could be a consequence of the lack of chemical reactivity at C-1 of castalagin. Thus, on the sole basis of this chemical reactivity difference between vescalagin and castalagin, one can argue that vescalagin is the preferred precursor of the in vivo formation of C-glycvosidic ellagitannin olimgomers and C-1 conjugates such as the flavano-ellagitannins and the lyxose/xylose-bearing conjugates. For example, considering the structures of the C-glycosidic ellagitannins typically found in significant amounts in fagaceous wood species, grandinin and the roburins A-E would be, all six of them, derived from a nucleophilic substitution at C-1 of vescalagin. Overall, the construction of these six compounds would require nine equivalents of vescalagin for only one equivalent of castalagin, which would be involved in the formation of ruburin D via a nucleophilic attack of its 4,6-HHBP group onto the C-1 position of a vescalagin unit.

7.6 Hemisynthesis of flavano-ellagitannins: the acutissimin

The acutissimins A and B, two regioisomeric flavano-ellagitannins that have been isolated from the bark and/or leaves of various oak and chestnut species (Fagaceae) are examples of complex tannins that can derived from an acid-catalyzed nucleophilic substitution between vesclagin and (+)-catechin. The flavanol unit, (+)-catechin, ends up connected to the C-1 center of vesclagin via either its nucleophilic C-8 center, leading to the acutissimin A, or its C-6 center, leading to acutissimin B.

Fagaceous woody species are generally very resistant to pathogens and herbivores. In particular, *Quercus acutissima*, from which the acutissimin A and B are first isolated, is usually pest-free and disease-free. Of particular curiosity is the fact that this robust oak species, native to Japan, has been selected as an ornamental tree in North America urban areas where air pollution, poor damage and/or drought are common. Plant extracts containing acutissimins are used worldwide in folk medicines to treat diseases including gastritis and gastric ulcer, diharrhea, various infiammations (oral, genital and anal mucosa), and are used as a tonic and antitussive medicines. Kashiwada and co workers (Kashiwada et al., 1993) reported the extremely potent in vitro activity of acutissimin A against human DNA topoisomerase II.

Complex tannins are present in the bark of oak species commonly used to make barrels, such as *Quercus robur* and *Quercus petraea* (König et al., 1994), and in which fine wines are aged. However, the bark is removed from oak tree logs, and the heartwood part from which the staves are cut to manufacture barrels does not contain any acutissimin. Nevertheless, the heartwood of the aforementioned oak species used for cooperage is a rich source of C-glycosidic ellagitannins, and notably of vescalagin. Even though the long-term seasoning, or drying, of wood and the various pyrolityc heating stages involved in the construction of the cask (Puech, 1998) considerably diminish the quantity of vescalagin and its congeners, a non-negligible portion resists thse drastic conditions (Dussot et al., 2002). During aging in oak barrels, the hydroalcoholic and slightly acidic (pH about 3.4) wine solution enables the solid-liquid extraction of vescalagin and its congeners. Once in the wine solution, these ellagitannins are slowly but continuously transformed through possible physical complexations with polysacaccharides and proteins and through carious chemical reactions such as oxidation, hydrolysis, polymerization and condensation events. Analyses of wines aged for 12 or 18 months in oak barrels have indicated amounts of vesclagin comprised between 0 and 7 mg/L. So, vescalagin, the C-glycosidic ellagitannin part of the acutissimins, gets extracted from the oak into the wine solution, which does contain significant amounts of the other part of the hybrid structure of these complex tannins

Quideau et al performed the hemisyntheses of acutissimins A/B at the laboratory in an acidic organic medium (15% (v/v) TFA/THF)at 60°C over a period of 7 h. The mechanistic description of these hemisyntheses follows a classical S_N1-type nucleophilic substitution pathway. The protonation of the OH-1 group of vescalagin, under the acid-catalyzed conditions used, leds to the formation of stable benzylic cation, which is then trapped by the nucleophilic flavan-3-ol counterpart. Starting from vescalagin, these nucleophilic substitutions proceed with full retention of configuration at C-1. These hemisyntheses constitute an in vitro mimicry of the non enzymatic yet diastereoselective formation of acutissimin flavano-ellagitannins.

7.7 Oxidative conversion of acutissimin A into mongolicain A

Most investigations on the fate of wine phenolics upon oxygenation at the various stages of the wine making process have concerned phenolic acids, anthocyanins, flavanols and their proanthocyanidic oligomers. Very few studies have addresses what happens to oak ellagitannins in this context at the molecular level, and the information glaned from the literature is rather contradictory. Some authors concluded that oak ellagitannins play a major role as oxidation regulator in wine, quickly absorbing dissolved oxygen and facilitationg the hydroperoxidation of some wine components (vivas & Glories, 1996) (ethanol into athanal),whereas others concluded that the oxidation of ellagitannins, like vescalagin,

is a very slow process (Moutounet et al., 1992). One possible explanation to this apparent contradiction is that the galloyl-derived units of oak C-glycosidic ellagitannins are engaged in fast inter- and/or intramolecular oxido-reductive processes during which their pyrogallol moieties are reversibly converted into semiquinone free radical and/or ortho-quinones through one- and/or two electron transfers.
Support for this speculative interpretation can be drawn from a molecular level observation made in our laboratory. In an aqueous solution left under air at 60°C, pure acutissimin A was converted into mongolicain A, which could be isolated by semi-preparative HPLC in a yield of 22%. Interestingly, it is worth recalling here that mongolicain A is thought to be naturally derived from the osidation of acutissiminA, as both molecules usually co-exist in their plant sources.
The construction of the characteristic spiro-linked dihydrofuran-cyclopentenone motif of mongolicain A is the result of a multi-step oxidative process starting with dehydrogenation of the NHTP galloyl-derived I-ring of the vescalagin part of acutissimin A. in the presence of oxygen, autoxidation can mediate this dehydrogenation into the α-hydroxy-ortho-quinone **A**, with concomitant formation of hydrogen peroxide.
The phenolic 7-OH group of the A-ring of the catechin-derived part of the molecule would the attack this ortho-quinone in a 1,6-addition to deliver intermediate **B**. Addition of water to its 1,2-diketone **B'** tautomer could give rise to hydrate **C** that can then undergo a ring contraction via a benzylic acid-type rearrangement to furnish a carboxylic acid **D**, which could tautomerize into the β-keto carboxylic acid **D'**. Decarboxylation of its ketone tautomer can lead to a enediol **E** which would require a final dehydrogenative oxidation into the cyclopentane-1,2-diketone **F** to lead the thermodynamically more stable cyclopentenone unit of mongolicain A (**Figure 7.3**).

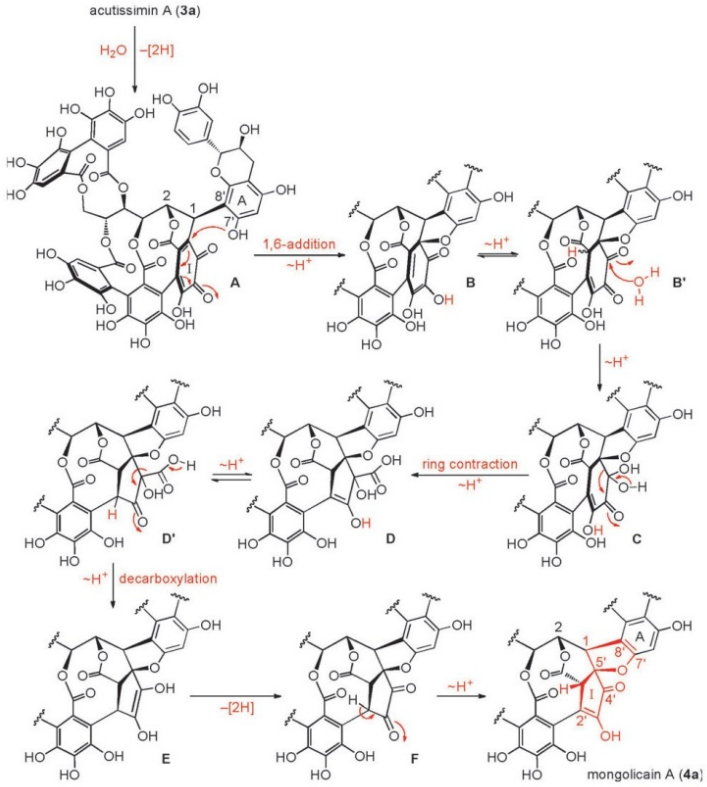

Figure 7.3. *Mechanism of the conversion of acutissimin A into mongolicain A.*

This complex succession of events constitutes a plausible but admittedly putative mechanistic description of the generation of mongolicain A from acutissimin A under autoxidation conditions, and one might wonder why the gallolyl-derived I-ring of acutissimin A is the only pyrogallol unit thus succumbing to dehydrogenative oxidation.
In fact, the other four pyrogallol units are probably also to some extent, but reversibly, converted into ortho-quinones. As alluded to above, all of the pyrogallol rings of a C-glycosidic ellagitannin such as vescalagin would follow the same behavior under wine oxygenation conditions in oak barrels. However,

in the case of a flavano-ellagitannin such as acutissimin A, the ortho-quinone derived from pyrogallol I-ring is the only one that can be irreversibly trapped by the proximal flavanol unit with its 7-OH group well-positioned to engage in such an intamolecular reaction.

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8. Economical and legal aspect of the use of oak chips in wine

The use of barrels to make wine has not changed much since the time Caesar fought the Gauls, the people who invented them. But things are changing fast for the European barrel industry, which is seeing its sales decline. Part of the drop is due to the financial crisis. But an important part of the loss in sales stems from the increasing use of alternative materials, a trend which dates more than a decade, but which has been reinforced by the recent legal liberalization of alternative materials use in Europe.
In fact, one major cost associated with producing wine is the cost of the barrel in which it is fermented and/or aged. A brand new American oak barrel in 2011 costs roughly $ 400, while a brand new French oak barrel in 2001 costs roughly $900 (though some can be more expensive, depending upon toasting and other customized options). One way wineries are cutting costs when it comes to barrel choice, is using toasted oak chips in wine that is fermenting/aging in stainless steel tanks, which costs hundred less than the oak barrel option.
It is said that this combination of steel tanks and oak chips produces the same result at the end of the winemaking process. If the goal is produce a lower price point wine that still retains quality oak barrel fermented characteristics, then the use of oak chips in a stainless steel tank may be very intriguing.
Up until 1993, in the United States, the use of oak chips in wine was illegal. The use of oak chips is also not highly advertised by winemakers or wineries, because they carry with it the stigma that the wine is of poorer quality. What consumers feel about the use of oak chips in wine is relatively unknow. It is know that customers generally choose their wines based on the type of wine, price point, the quality certification, the grape variety, and the brand.
Fermenting and aging wine in oak barrels gives certain sensory characteristics that are pleasing to many consumers. Basically, during the aging process in an oak barrel, many compounds are extracted from the wood that adds complexity to the wine. Small amounts of oxygen are also entering the wine through the pores in the wood, which react with the phenolic components of the wine to increase stability and also aroma and mouthfeel complexity. This process is often slow, thus delaying the release of the wine to the customers. If a winery is interested in producing wine with complex oak character but would like a faster way to get it out to the public, then an alternative such as adding wood chips in a stainless steel tankmay be the solution.
The use of oak chips first started in the production of spirits when they would use wood fragments instead of barrels for aging. Once the wood chips are added to the spirits or wine, the oak-phenols and other volatile compounds that give “oaky” complexity to the wine is absorbed in the same manner it was when in the oak barrel. The only difference, however, is that no oxygen enters the wine in the stainless steel tanks, so as to mimic the same process that occurs in the traditional oak barrels. When it’s all said and done, this entire process cost much less and goes to completion much faster than the traditional oak barrel aging technique.
In October 11th 2006 the European Commission has promulgates the new decree and therefore also in Europe the use of wood chips in the production of wines is permitted. Regulation N. 1507/2006 of October 11th, 2006, provides for the use of oak chips in the production of wines, obligating producers who want to use this technique to write it in the labels. Doing so, it is ensured the right of choice of consumers, although relying on the honesty of producers and the hope there are rules and procedures in order to avoid frauds. It is on the honesty of the producer on must trust because this decree obliges they to write, in a special record and documents, the possible use of wood chips in the production.
At the beginning, the orientation of Italy was to permit the use of wood chips for the production of table wines only, whereas it was not provided for the use in wines belonging to superior categories (IGT, DOC and DOCG). In November 2nd, 2006 has been approved by the Italian Minister of Agricultural Politics Mr. Paolo De Castro the reception of the new European Regulation and in which it is found, besides wines tables, in Italy will be permitted the use of wood chips in Typical Geographic Indication (IGT) wines as well. The decree forbids the use of wood chips in all VQPRD (Quality Wines Produced in Determined Region), that is in wines belonging to the Determinazione d’origine Controllata (Denomination of Controlled Origin, DOC) and Denominazione d’Origine Controllata e Garantita (Denomination of Controlled and Guaranteed Origin, DOCG). A choice that represents the safeguarding of quality wines of quality wines, as well as the fulfillment of the requests some producers asked for their right of choice, in order to equally compete with the producers of the countries of the New World where this technique is permitted.
The European Regulation allows the possibility of using wood chips in the production in order to pass to the wine some components contained in oak wood. The type of wood allowed for the production of wines must exclusively come from trees belonging to the *Quercus species*, in their natural condition or heated in

the defined ways light, medium or strong, without having undergone burning processes, not even in the surface, must no show any coal like aspect or being friable to the touch and must not release substances in concentration such to cause risks for the health and any other treatment must be reported in a record. The size of at least 95% of wood chips used for the production of wine must be greater than 2 millimeters. The Regulation is aware of the fact wood chips, as they give the wine organoleptic qualities similar to the ones aged in oak barrels, make difficult for the consumer to realize what method has been used for a specific wine.

Despite the fact they understand the use of wood chips allow the production of wines at a lower cost, the Regulation understand this practice can cause confusion among consumers and it is adequate to adopt measures in order to ensure clearness and information about the type of wine. In the labels of wines produced with this technique must be stated the origin and the botanical species of oaks from which the chips are being obtained, the intensity of heating and keeping practices. It is forbidden to write in the label any term usually destined for wines fermented or aged in cask, such as “fermented in cask” or “aged in barrique”. This Regulation give satisfaction both to consumers and producers, while ensuring the right of choice of both. It is then granted the choice of the producer who wants to make use of wood chips, but only in some categories of wine, obligating they to clearness towards consumers who, at that point, are granted the right of choice.

9. Effect of size and toasting degree of oak chips on the polyphenols and on the ellagitannin content and on the acutissimin formation in wine model solution

Oak wood has always been a material of choice for the construction of barrel and for the production of aok chips used in wine aging (Doussot et al., 2002). *Quercus robur* and *Quercus petraea* are the two species predominant in Europe. The oak barrel being not only a container, but also a way of wine cooperaging, the chemical composition of oak wood appears to be very important. In fact, knowledge of the chemical composition of oak wood is the first step in predicting which components should be expected in the final product after wine aging (De Simón et al., 2006).
From an enological point of view, the main extractable oak wood components are the monomer ellagitannins, castalagin, roburin E, vescalagin, and grandinin, and low molecular weight phenolic compounds such as ellagic and gallic acids, besides lignin constituents, especially vanillin. Several studies reported that the isomers vescalagin and castalagin are the most two abundant ellagitannins isolated in oak wood (*Q. petraea, Q. robur*) and the chestnut (*Castanea sativa*). In the last decades, the dimer ellagitannins and pentosylated forms have been described (Penhoat et al., 1991a, 1991b), they are roburins A, B, C, D, E and grandinin. These structures appear to be constituted of a linear glycosidic chain in which the OH is esterified by the carboxylic functions of the hexahydroxydiphenic and nonahydroxytriphenic groups (Vivas et al, 1995). The presence of the hydroxyl groups in *ortho* positions at the periphery of the ellagitannins structure enables these compounds to take part in oxidation reactions, acting as consumers of oxygen and causing, among other reactions, the transformation of ethanol into acetaldehyde (Vivas and Glories, 1996). In particular, vescalagin is able to react with (epi)catechins via C-C linkage between the carbon-1 atom of the vescalagin moiety and either carbon-8 or -6 of the A ring of the (epi)catechin to form acutissimin A or B, respectively (Quideau et al., 2005). These compounds have been reported to possess interesting biological properties (Quideau et al. 2005)
The main compounds susceptible to migration from oak wood to wine are the aldehydes such as vanillin and syringaldehyde, and volatile phenolic aldehydes such as vanillin and syringaldehyde, and volatile phenols such as eugenol, guaiacol, and ethyl- and vinylphenols. Phenolic aldehydes such as vanillin contribute to olfactory characteristics of wine with notes of vanilla, coffee, black chocolate, and smoke. Finally, several pigments were recently found to produced from reaction between catechin and cinnamic aldehydes, such as coniferyl or sinapyl, extracted from oak wood (Chatonnet. et al., 1992, Spillman et al., 1997).
The geographical origin of wood, the silvicultural treatments and the cooperage operations such as seasoning and toasting are factors that can affect ellagitannins content and their proportions (Cadahía et al., 2001; Doussot et al., 2002; Fernández de Simón et al., 1999; Fernández de Simón et al., 2003; Masson et al., 1995). Among them, toasting has been pointed out as one of the most important factors affecting the ellagitannins contents of wood (Fernández de Simón et al., 2010). Moreover toasting significantly modifies the volatile composition of oak wood and consequently the aromas of aged wines (Alañon et al., 2010; Caldeira et al., 2006).
The influence of toasting on the extraction of phenolics from the oak wood from Spanish and Chestnut heartwood (Cadahía et al., 2001; Doussout et al., 2002; Fernández de Simón et al., 2003; Hale et al., 1999) is well established in literature. Some of the works focused in the phenolic composition also studied the ellagitannin composition depending on the toasting degree, but they only determined the ellagitannin levels by the quantification of ellagic acid released during acidic hydrolysis. (Chira & Teissedre, 2013). There are few studies focusing on the importance of the size of the pieces of oak wood. Moreover, they are devoted to the volatile composition (Fernández de Simón et al., 2010). To our knowledge there are no studies that have monitored the individual extraction of polyphenols and ellagitannins depending on the size of oak chips and on their toasting levels and, taking into account the differences on their reactivity and activity (Jourdes et al., 2008, Quideau et al., 2005), it is important to know the effect of toasting on the different ellagitannins.
On the other hand, the formation of flavano-ellagitannin has been studied both in model media solution (Quideau et al., 2005) and in wine (Jourdes et al., 2011), but there are no studies that try to correlate the formation of these compounds with the toasting degree of the oak or with the flavanol concentrations. This could be important considering the properties described for these derivative ellagitannins (Quideau et al., 2005).
Thus, the aim of this work was to study how the size (2 and 8 mm) and the toasting degree (light, medium and high) of oak chips affect the *C*-glycosidic ellagitannins composition of model wine solutions where

these chips are added. Furthermore, the effect of the presence of (+)-catechin (50 mg/L and 200 mg/L) in the solution media on ellagitannins extraction and on acutissimin A and B formation was also investigated.

9.1.References

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10. Materials and methods

10.1 Chemicals

Vescalagin was isolated from commercial chestnut tannin provided by Laffort (Laffort oenologie, Bordeaux, France). Castalagin, roburin E and grandinin were isolated from non-toasted *Q. petraea* oak chips. Acutissimin A and B were obtained by hemisynthesis from vescalagin as it was described by Quideau and co-workers (Quideau et al., 2005) and purified by semi-preparative HPLC.
Others available reference compounds were obtained from commercial sources: gallic acid, ferulic acid, caffeic acid, sinapic acid, ellagic acid, hydroxymethylfurfural (HMF), furfural (F), syringaldehyde, coniferyladehyde, sinapaldehyde, (+)-catechin and (-)-gallocatechin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All of the solvents used were of analytical grade and were purchased from Prolabo (BHD) VWR International (Briare, France). The ultrapure water was obtained from a Direct-Q water purification system equipped with a Millipak 40 (0.22 μm) filter unit (Millipore, Billerica, MA, USA).

10.2 Oak chips

The oak chips added to model wine solutions were provided from A&B Group (Brescia, Italy) and were obtained from naturally seasoned (24 months) *Quercus petraea* oak. Two different sizes (Length × width × height = 4 × 2 × 1 mm (2 mm), and L × w × h = 4 × 8 × 1 mm (8 mm)) and three different levels of toasting for each size (light, L; medium, M; and high, F) were used.

10.3 Preparation of the model wine solutions

Model wine solutions (MWS) consisted of 12 mL/100mL ethanol in ultrapure water, 3.8 g/L of tartaric acid and 0.6 g/l of potassium chloride (adjusted at pH 3.3 using NaOH 1N). Oak chips (4g/L) were added in the model wine solutions with different concentrations of (+)-catechin: 0 mg/L (reference sample), 50 mg/L and 200 mg/L. Two sizes of oak chips were used in the study (2 mm and 8 mm), and different toasting level for each size (light, L; medium, M; and high, F) were used. In total, eighteen different model wine solutions were prepared (see **Table 10.1** for code descriptions). The sampling started the day after the oak chips (and the (+)-catechin in non-reference samples) were added (day 1) and it was prolonged for 35 days. Samples were taken weekly. Each sample was analyzed in triplicate by HPLC-ESI-MS/MS-multiple reaction monitoring.

Table 10.1. *Characteristics of the model solutions analyzed: code, (+)-catechin content (mg/L) and toasting degree and size of oak chips used.*

Sample code	(+)-catechin content (mg/L)	Oak chips toasting degree	Oak chips size
0F2L	0	Ligh	2
0F2M	0	Medium	2
0F2F	0	High	2
50F2L	50	Ligh	2
50F2M	50	Medium	2
50F2F	50	High	2
200F2L	200	Ligh	2
200F2M	200	Medium	2
200F2F	200	High	2
0F8L	0	Ligh	8
0F8M	0	Medium	8
0F8F	0	High	8
50F8L	50	Ligh	8
50F8M	50	Medium	8
50F8F	50	High	8
200F8L	200	Ligh	8
200F8M	200	Medium	8
200F8F	200	High	8

10.4 Purification of the ellagitannins

For the extraction of vescalagin, 3 g of the commercial chestnut tannin was dissolved in 150 mL of acidified water (AcOH 2.5mL in 100mL of ultrapure water). The solution was cleaned three times with diethyl ether and three times with ethyl acetate in order to remove other oak wood compounds which might interfere in further isolation steps. Traces of organic solvents were removed under vacuum and the extract was diluted in 750 mL of acidified water. Castalagin, grandinin and roburin E were extracted from oak chips following the procedure described by Garcia-Estévez and co-workers (García-Estévez et al., 2010). The purification of ellagitannins was performed using an Agilent Technologies 1260 Infinity LC Purification system (Agilent Technologies, Palo Alto, CA) controlled by ChemStation software (Agilent Technologies, Palo Alto, CA). The purification was performed using a 21.2 mm × 150 mm, 5 µm Agilent Prep C-18 column (Agilent Technologies, Palo Alto, CA). Mobile phases were solvent A: acidified water (AcOH 2.5mL in 100mL of ultrapure water) and B: methanol. The following gradient was used: (0-14 min, 0% B; 14-22 min; 0-65% B; 22-27 min, 65-0% B). Detection was carried out at 250 nm, 280 nm and 360 nm. The final purities of the isolated ellagitannins (higher than 95%) were determined from the results of the HPLC-DAD-MS analyses (García-Estévez et al., 2010).

10.5 Purification of acutissimins A and B

Purification of acutissimins A and B was performed in the same equipment as ellagitannins employing the same column and mobile phases. Nevertheless, the gradient used was different: (0-10 min, 0% B; 10-30 min; 0-30% B; 30-40 min, 30-60%; 40-45 min, 60-75% B; 45-50 min, 75-0% B). Detection was performed at 250 nm, 280 nm and 360 nm. Final purity of each acutissimin was higher than 95%.

10.6 Global valuations

In model wine solutions total phenols were determined by the Folin-Ciocalteau assay (Singleton & Rossi, 1965) with ellagic acid as standard.

10.7 HPLC/DAD quantification of oak phenols

The analyses were carried out using an Agilent 1100 series liquid chromatography system equipped with a diode array detector (DAD) and managed by a Chemstation for LC system (Agilent Technologies, Palo Alto, CA). The column was a 150 mm x 4,6 mm i.d. x 0,5 µm, Zorbax eclipse ODS C18. The HPLC profiles were monitored at 280, 230, 375 and 520 nm, and the UV/Vis spectra were recorded from 190 to 650 nm. 20 µL of the sample was filtered and then directly injected. The mobile phase was composed of solvent A (H_2O/TFA (999:1)) and solvent B (acetonitrile/TFA (999:1)) and the elution conditions were as follows: flow rate 1 mL/min; gradient profile, 0-10% B in 0-5 min, 10-60% B in 5-25 min, 60-65% B in 25-30 min, 65-100% B in 30-35 min. Quantification was carried out by the external standard method. The concentration of each substances was measured by comparing it with calibrations made with the pure compound analyzed under the same conditions and linear regression coefficient between 0,9990 and 0,9999 were obtained.

10.8 Sample preparation for LC-ESI-MS/MS analysis

2mL of samples were evaporated under reduce pressure and re-dissolved in acidified water (AcOH 2.5mL in 100mL of ultrapure water) to a final volume of 2 mL. (-)-gallocatechin was used as internal standard in a concentration of 15 mg/L.

10.9 LC-ESI-MS/MS quantification of ellagitannins and acutissimins A and B

Vescalagin, castalagin, roburin E and grandinin were quantified by using the previously validated HPLC-ESI-MS/MS-multiple reaction monitoring method (García-Estévez et al., 2010; García-Estevéz et al., 2012). HPLC analyses were performed in a Hewlett-Packard 1100 series liquid chromatograph (Agilent Technologies, Palo Alto, CA). MS detection was performed in an API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) equipped with an ESI source and a triple-quadrupole linear ion trap mass analyzer that was controlled by Analyst 5.1 software (Applied Biosystems, Darmstadt, Germany). Zero grade air served as nebulizer gas (50 psi) and turbo gas for solvent drying (400 °C, 60 psi). Nitrogen served as curtain (20 psi) and collision gas (high). Both quadrupoles were set at unit resolution. The ion spray voltage was set at −4500 V in the negative mode. Optimization of the conditions was carried out automatically by direct infusion of acutissimin A. Settings used were as follows: declustering potential (DP), −100 V; entrance potential (EP), −10 V; collision energy (CE), −38 V; collision cell exit potential (CXP), −28 V. Acutissimin A and acutissimin B were quantified by using a calibration curve built with data supplied by mass spectrometry using the signal obtained for the transition (each parent ion- daughter

ion pair) 1215/917 corresponding to the acutissimin A, corrected with the signal obtained for the transition corresponding to the internal standard (-)-gallocatechin (305/249). Detection (LOD) and quantification (LOQ) limits for acutissimin were calculated following the procedure described by García-Estévez and co-workers (García-Estévez et al., 2012) for ellagitannins. LOQ was 0.02 mg/L and LOQ was 0.09 mg/L.

10.10 Statistical analysis

All determinations and experiments were performer in triplicate, and the results are the average value of three determinations. Using data from chemical analyses, one way analysis of variance (ANOVA) was performed to compare the long-macerated wines. Statistical significance was attributed to *p* values of <0.05. The post-hoc Tukey test was applied for pairwise comparison. In addition, a principal component analysis (PCA) was carried out. Statistical analysis was performed using XLSTAT 2006, version 2006.6 (Addinsoft, Paris,France).

10.11 References

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11. Result and discussion

11.1 Global evaluation

Figures 11.1, **11.2** and **11.3** illustrates the total phenols concentration for model wine solutions (MWSs) that contained the smallest oak chips (2 mm) at different toasting degree (low, medium and high) and three concentrations of (+)-catechin: 0mg/L (reference sample) 50 mg/L and 200 mg/L . In all MWSs the optical density at 700 nm increased during artificial aging (35 days). Contact time between hydroalcoholic solution and oak chips significantly influenced the optical density for all MWSs analyzed.

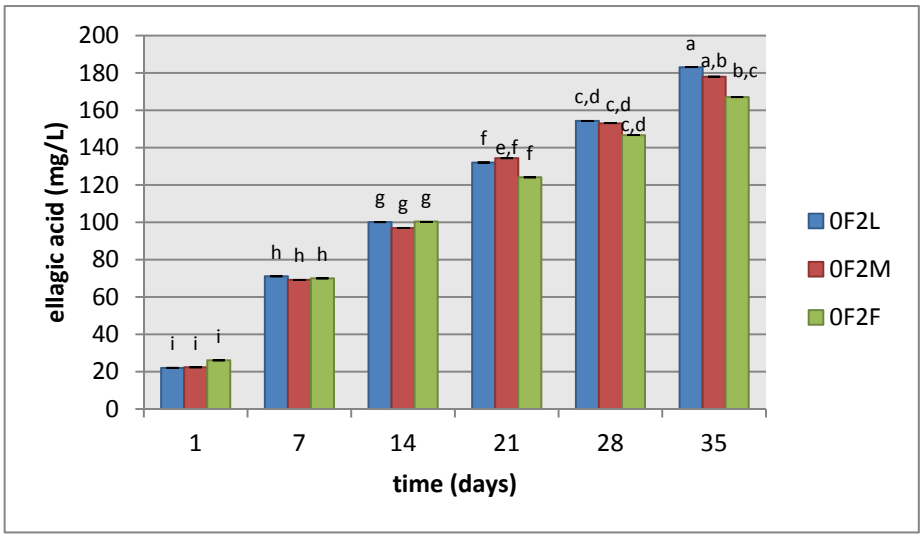

Figure 11.1. *Concentration of the total phenols (mg ellagic acid/L) in reference model wine solutions during accelerated aging with 2 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences (p > 0.05; n=3).*

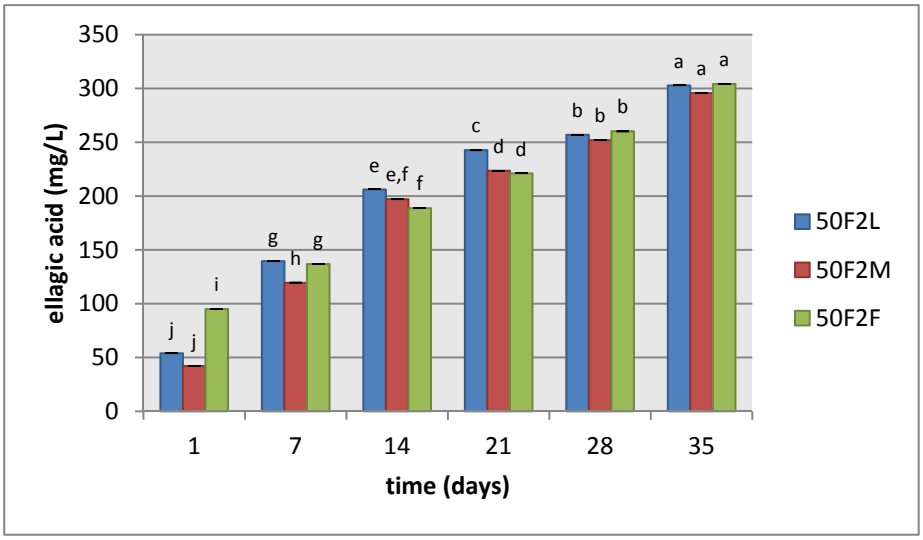

Figure 11.2. *Concentration of the total phenols (mg ellagic acid/L) in model wine solutions containing 50 mg/L of (+)-catechin during accelerated aging with 2 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences (p > 0.05; n=3).*

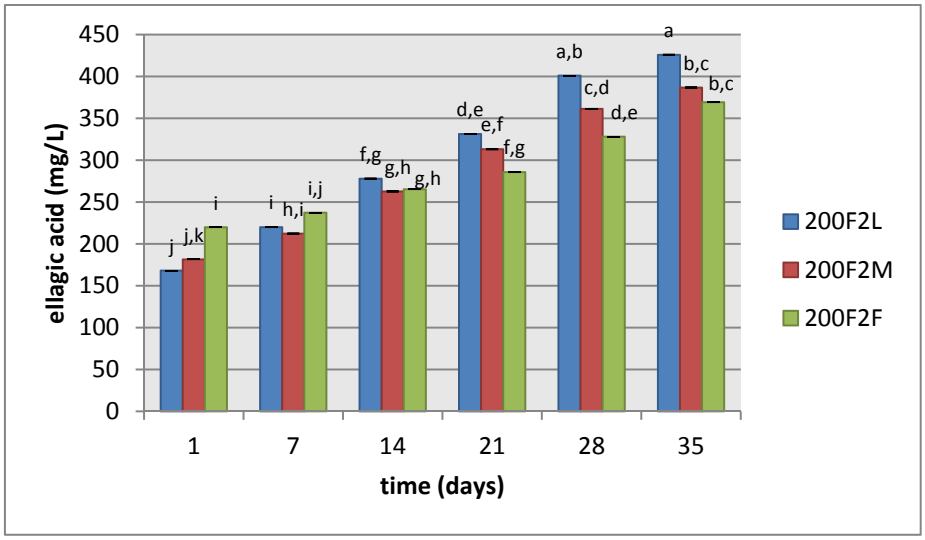

Figure 11.3. *Concentration of the total phenols (mg ellagic acid/L) in model wine solutions containing 200 mg/L of (+)-catechin during accelerated aging with 2 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences (p > 0.05; n=3).*

Except for reference WMSs, oak chips toasting degree significantly affected the optical density at 700 nm. However, in other studies (Cadahía et al., 2001, Matricardi & Waterhouse., 1999) it was reported that the Folin-Ciocalteau index is not an indicator of the amount of phenolic thermal breakdown because this wavelength is strongly influenced by the Maillard reaction products induced by the heat in the wood (Chatonnet & Dubordieu 1989). In fact, in MWSs containing high and medium toasted oak chips, this value includes oxidized ellagitannins, low molecular weight polyphenols, and furanic aldehydes that strongly affects the specificity of the reaction with phenolic compounds in the case of the toasted wood. For this reason, authors suggest that Folin-Ciocalteau index must be taken into account only to compare oak wood of different origin (e.g. Spanish species with French and America ones) and not thermal degradation measurement.

Figures 11.4, **11.5** and **11.6** illustrates the total phenol concentration for MWSs that contained the highest oak chips (8 mm) at different toasting degree (low, medium and high) and three concentrations of (+)-catechin: 0 mg/L (WMSs used as reference) 50 mg/L and 200 mg/L.

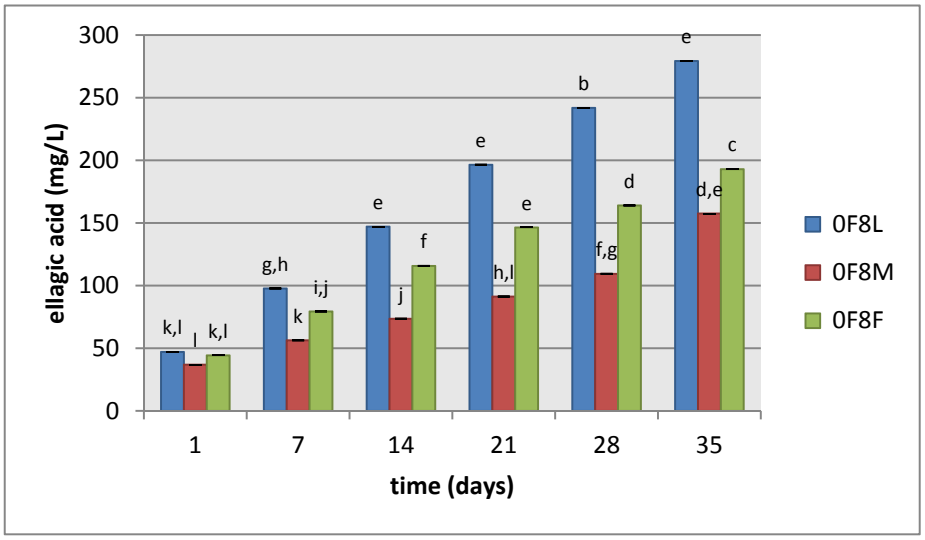

Figure 11.4. *Concentration of the total phenols (mg ellagic acid/L) in reference model wine solutions during accelerated aging with 8 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences (p > 0.05; n=3).*

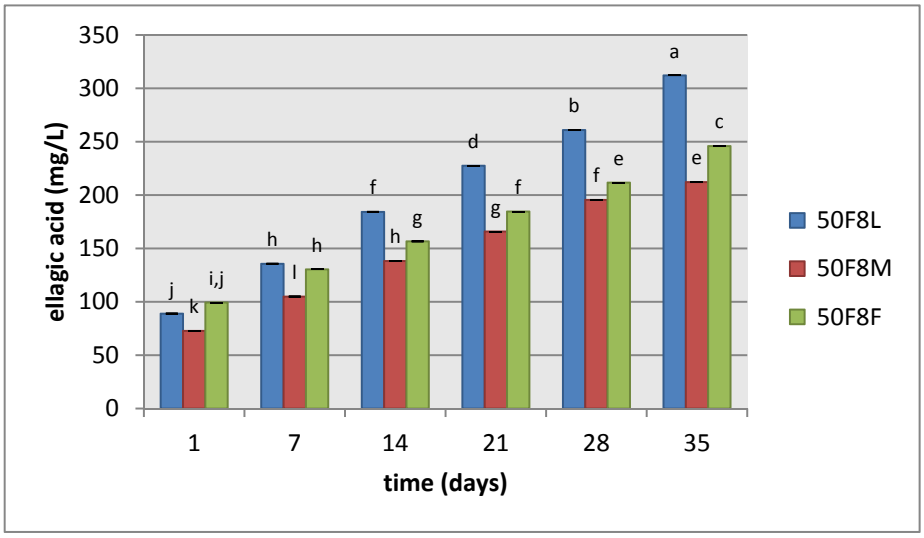

Figure 11.5. *Concentration of the total phenols (mg ellagic acid/L) in model wine solutions containing 50 mg/L of (+)-catechin during accelerated aging with 8 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences (p > 0.05; n=3).*

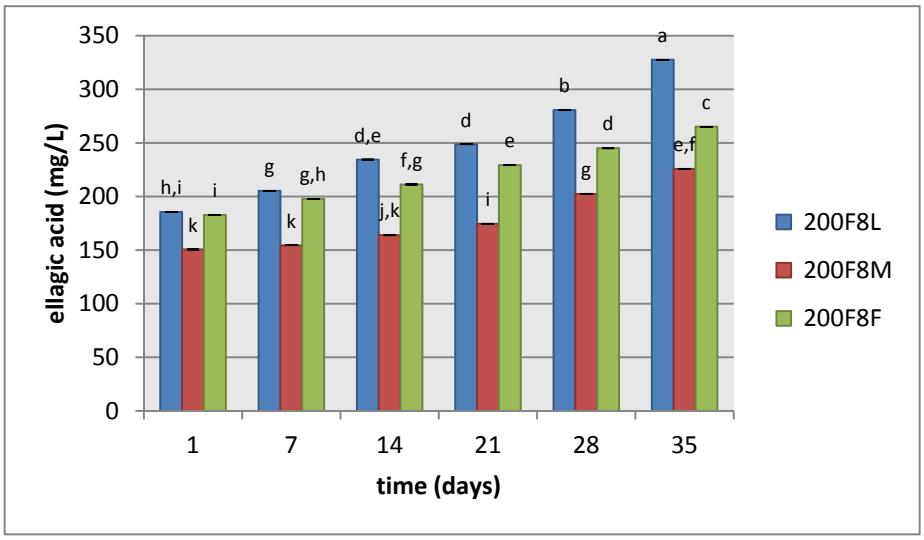

Figure 11.6. *Concentration of the total phenols (mg ellagic acid/L) in model wine solutions containing 200 mg/L of (+)-catechin during accelerated aging with 8 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences (p > 0.05; n=3).*

Also for these WMSs, Folin-Ciocalteau index is significantly affected by the time of contact and by the toasting degree of oak chips, even for reference samples (0F8L, 0F8M and 0F8F). The reasons were similar to that previously described.

Oak chips dimension significantly affected the Folin-Ciocalteau values (data not reported). The comparison between the Folin-Ciocalteau value observed in MWSs with smaller or larger oak chips with the same toasting level revealed that hydroalcoholic media in contact to the smaller oak chips presented the highest Folin-Ciocalteau values. Probably, greater surface of 2 mm oak chips favored the dissolution of phenolic substances in MWSs.

11.2 Low molecular weight polyphenols

Figure 11.7 illustrates the HPLC/DAD chromatograms of the phenolic compounds from the references MWSs (0 mg/L (+)-catechin) in contact with 2 mm oak chips and subjected to 35 days of artificial aging

while the results of the chemical analysis are showed in **Tables 11.1.a-b-c**. The three chromatograms shows qualitative and quantitative differences related to the toasting degree of the oak chips used.

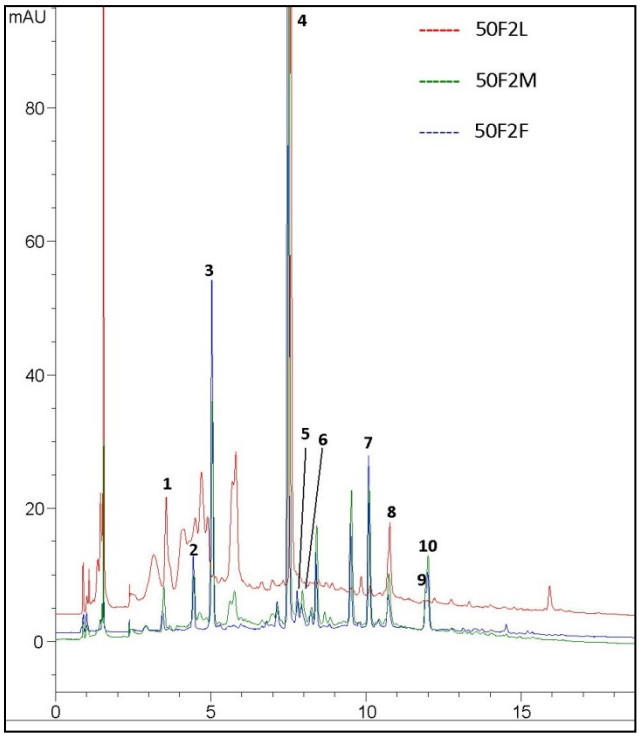

Figure 11.7. *HPLC-DAD chromatograms of wine model solution aged with 2 mm light (red), medium(green) and high (blue) toasted oak chips, recorded at 280 nm. Peak identification: 1, gallic acid; 2, hydroxymethylfurfural; 3, furfural; 4, catechin; 5, ferulic acid; 6, caffeic acid; 7, syringaldehyde; 8, ellagic acid; 9, coniferyl aldehyde; 10, sinapaldehyde.*

Different compounds have been separated but only 10 phenolic compounds (gallic acid, ferulic acid, caffeic acid, sinapic acid, ellagic acid, hydroxymethylfurfural (HMF), furfural (F), syringaldehyde, coniferyl aldehyde and sinapaldehyde) have been identified by comparing their retention time and UV and mass spectra with those of the standard.

All the molecules identified showed a positive trend in reference MWSs during artificial aging, and contact time between the hydroalcoholic media and the oak chips significantly affected the polyphenols conten. The results showed that toasting level of oak chips caused severe modification in phenolic composition of MWSs aged with them. Several studied (Matricardi & Waterhouse, 1999; Jordao et al., 2007; Michel et al., 2013; Alañón et al., 2011) reported that seasoned wood is characterized by its richness in hydrolyzable tannins, which are of the gallotannin and ellagitannin type. Toasting causes the degradation of hydrolyzable tannins, as well as the decrease in total phenols.

Among the phenolic acids, the gallic acid and the ellagic acid were the most abundant. The average concentration of gallic acid was of 4.58 mg/L in 0F2L solution and of 1.50 mg/L and 0.77 mg/L in 0F2M and 0F2F solutions, respectively. The results were in accordance with Sanz et al., 2010, that reported that the content of gallic acid was higher in seasoned oak chips respect to medium and high toasted oak chips. Gallic acid is extremely sensitive to heat treatment that its content systematically and quickly decreases in the wood with the duration of toasting (Chatonnet et al., 1989).

In other studies (Hale et al., 1999; Canas et al., 2000) it was observed that ellagic acid content increased in toasted oak and chestnut wood, while in this study it did not seem to be dependent on the toasting degree of the oak chips. In fact, only in 0F2M MWSs average content of ellagic acid (8.86 mg/L) was greater than 0F2L MWSs (7.06 mg/L), and 0F2F MWSs (6.62 mg/L). This is possibly due to the fact that ellagic acid is so poorly extracted by maceration that its content sometimes seems not increase (Chatonnet et al., 1989).

Table 11.1. a-b-c. *Mean and standard deviation for low molecular polyphenols in the reference model wine solutions with the three different types of oak chips (2 mm) used (L, light toasted; M, medium toasted, F, high toasted). The error bars show the ±SD values. Values with the same letter indicate no statistically differences among the different type of chips studied.*

A – light toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	$1.48^{d,e,f}$	± 0.26	$2.79^{b,c}$	± 0.28	3.65^{b}	± 0.17	3.44^{b}	± 0.27	7.81^{a}	± 0.52	8.30^{a}	± 0.35
Ferulic acid	$n.d.^{f}$	-	$n.d^{f}$	-	$n.d^{f}$	-	$n.d^{f}$	-	$n.d^{f}$	-	$n.d^{f}$	-
Caffeic acid	$n.d^{d}.$	-	0.05^{d}	± 0.01	0.11^{d}	± 0.01	$0.17^{c,d}$	± 0.03	$0.23^{b,c,d}$	± 0.07	$0.24^{b,c,d}$	± 0.02
Sinapic acid	$0.43^{a,b,c}$	± 0.01	0.27^{c}	0.00	$0.32^{b,c}$	± 0.00	$0.41^{a,b,c}$	± 0.02	$0.56^{a,b,c}$	± 0.15	$0.70^{a,b,c}$	± 0.28
Ellagic acid	1.53^{g}	± 0.01	1.53^{g}	±0.14	$2.91^{f,g}$	± 0.50	$2.85^{f,g}$	± 0.38	$15.70^{a,b}$	± 3.39	17.82^{a}	± 2.89
Σ phenolic acids	**3.44^{g}**	**± 0.27**	**$4.64^{f,g}$**	**± 0.43**	**$7.00^{e,f,g}$**	**± 0.68**	**$6.87^{e,f,g}$**	**± 0.12**	**24.30^{a}**	**± 3.70**	**27.06^{a}**	**± 3.51**
HMF	$n.d^{f}$	-	$n.d^{f}$	-	$n.d^{f}$	-	$n.d^{f}$	-	$n.d^{f}$	-	$n.d^{f}$	-
F	$n.d^{e}$	-	$n.d^{e}$	-	$n.d^{e}$	-	$n.d^{e}$	-	$n.d^{e}$	-	$n.d^{e}$	-
Σ furfurals	$n.d^{f}$	-	$n.d^{f}$	-	$n.d^{f}$	-	$n.d^{f}$	-	$n.d^{f}$	-	$n.d^{f}$	-
Syringaldehyde	$n.d^{j}$	-	$n.d^{j}$	-	$n.d^{j}$	-	$n.d^{j}$	-	$n.d^{j}$	-	$n.d^{j}$	-
Coniferyl aldehyde	$n.d^{i}$	-	0.06^{i}	± 0.00	0.08^{i}	± 0.01	0.09^{i}	± 0.01	0.10^{i}	± 0.00	0.11^{i}	± 0.00
Sinapaldehyde	0.15^{l}	± 0.02	0.30^{l}	± 0.02	0.31^{l}	± 0.01	0.30^{l}	± 0.04	0.42^{l}	± 0.01	0.42^{l}	± 0.00
Σ oak aldehydes	**0.15^{i}**	**± 0.02**	**0.37^{i}**	**± 0.03**	**0.39^{i}**	**0.00**	**0.39^{i}**	**± 0.05**	**0.52^{i}**	**± 0.01**	**0.54^{i}**	**± 0.00**

B – medium toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	$0.99^{e,f,g,h}$	± 0.08	$0.84^{f,g,h}$	± 0.44	$1.29^{e,f,g}$	± 0.05	$1.77^{d,e}$	± 0.20	$1.86^{d,e}$	± 0.00	$2.24^{c,d}$	± 0.06
Ferulic acid	0.41^{d}	± 0.11	0.40^{d}	± 0.03	0.34^{d}	± 0.07	$0.71^{b,c}$	± 0.00	0.39^{d}	± 0.06	0.33^{d}	± 0.07
Caffeic acid	$0.29^{b,c,d}$	± 0.00	$0.23^{b,c,d}$	± 0.02	$0.48^{a,b,c}$	± 0.23	$0.33^{a,b,c,d}$	± 0.00	$0.29^{b,c,d}$	± 0.07	$0.56^{a,b}$	± 0.07
Sinapic acid	$0.48^{a,b,c}$	± 0.05	$0.32^{b,c}$	± 0.00	$0.51^{a,b,c}$	± 0.05	0.87^{a}	± 0.19	$0.58^{a,b,c}$	± 0.14	$0.75^{a,b}$	± 0.07
Ellagic acid	$3.21^{f,g}$	± 0.62	$5.17^{e,f,g}$	± 0.40	$6.84^{d,e,f}$	± 0.51	$11.09^{b,c,d}$	± 1.33	$12.58^{b,c}$	± 0.14	$14.25^{a,b}$	± 0.22
Σ phenolic acids	**$5.39^{f,g}$**	**± 0.87**	**$6.97^{e,f,g}$**	**± 0.88**	**$9.47^{d,e,f}$**	**± 0.35**	**$14.78^{b,c,d}$**	**± 1.33**	**$15.71^{b,c}$**	**± 0.12**	**18.12^{b}**	**± 0.22**
HMF	$0.24^{d,e,f}$	± 0.09	$0.08^{e,f}$	± 0.01	$0.32^{c,d,e}$	± 0.03	0.56^{b}	± 0.11	0.96^{a}	± 0.00	1.11^{a}	± 0.03
F	$1.18^{c,d}$	± 0.14	0.90^{d}	± 0.01	1.32c,d	± 0.02	$1.59^{b,c,d}$	± 0.02	$1.69^{bc,d}$	± 0.00	$1.78^{b,c,d}$	± 0.03
Σ furfurals	**$1.42^{d,e}$**	**± 0.23**	**$0.98^{e,f}$**	**± 0.03**	**$1.64^{c,d,e}$**	**± 0.06**	**$2.14^{b,c,d}$**	**± 0.13**	**$2.65^{a,b,c}$**	**± 0.01**	**$2.90^{a,b}$**	**±0.07**
Syringaldehyde	2.08^{i}	0.00	1.92^{i}	± 0.04	3.70^{gh}	± 0.05	3.80^{g}	± 0.00	4.07^{f}	± 0.03	5.24^{d}	± 0.04
Coniferyl aldehyde	1.48^{f}	± 0.05	$1.08^{g,h}$	± 0.03	2.10^{e}	± 0.11	2.76^{e}	± 0.01	$2.49^{c,d}$	± 0.02	3.74^{b}	± 0.36
Sinapaldehyde	4.87^{j}	± 0.04	5.52^{i}	± 0.06	11.58^{d}	± 0.12	11.28^{d}	± 0.01	12.37^{c}	± 0.06	15.26^{b}	± 0.13
Σ oak aldehydes	**8.44^{g}**	**0.00**	**8.52^{g}**	**± 0.07**	**17.38^{d}**	**± 0.27**	**17.84^{d}**	**± 0.02**	**18.93^{c}**	**± 0.07**	**24.25^{b}**	**± 0.19**

C – high toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	0.29^{h}	± 0.02	$0.40^{g,h}$	± 0.18	$0.51^{g,h}$	± 0.03	$0.54^{g,h}$	± 0.18	$1.09^{e,f,g,h}$	± 0.13	$1.78^{d,e}$	± 0.01
Ferulic acid	0.42^{d}	± 0.10	$0.54^{c,d}$	± 0.03	$0.72^{b,c}$	± 0.01	$0.78^{a,b}$	± 0.03	0.49^{d}	± 0.11	0.95^{a}	± 0.05
Caffeic acid	$0.29^{b,c,d}$	± 0.02	$0.48^{a,b,c}$	± 0.08	$0.53^{a,b}$	± 0.00	$0.58^{a,b}$	± 0.08	0.66^{a}	± 0.21	$0.57^{a,b}$	± 0.12
Sinapic acid	$0.38^{b,c}$	± 0.03	$0.40^{a,b,c}$	± 0.17	$0.50^{a,b,c}$	± 0.03	$0.70^{a,b,c}$	± 0.17	$0.52^{a,b,c}$	± 0.22	$0.66^{a,b,c}$	± 0.00
Ellagic acid	$3.83^{e,f,g}$	± 0.90	$3.98^{e,f,g}$	± 0.01	$6.16^{e,f,g}$	± 0.91	$5.87^{e,f,g}$	± 0.01	$5.76^{e,f,g}$	± 0.11	$8.13^{c,d,e}$	± 0.23
Σ phenolic acids	**$5.20^{f,g}$**	**± 0.92**	**$5.79^{f,g}$**	**± 0.03**	**$8.42^{e,f,g}$**	**± 0.83**	**$8.47^{e,f,g}$**	**± 0.03**	**$8.52^{e,f,g}$**	**± 0.14**	**$12.09^{c,d,e}$**	**± 0.18**
HMF	$0.32^{b,c,d}$	± 0.00	$0.39^{b,c,d}$	± 0.02	$0.39^{b,c,d}$	± 0.00	$0.41^{b,c,d}$	± 0.02	$0.41^{b,c,d}$	± 0.20	$0.52^{b,c}$	± 0.01
F	$2.02^{b,c}$	± 0.01	$2.29^{a,b}$	± 0.01	$2.23^{a,b}$	± 0.03	$2.35^{a,b}$	± 0.01	$2.29^{a,b}$	± 0.92	2.96^{a}	± 0.14
Σ furfurals	**$2.34^{b,c,d}$**	**± 0.01**	**$2.68^{a,b,c}$**	**± 0.01**	**$2.61^{a,b,c}$**	**± 0.03**	**$2.76^{a,b}$**	**± 0.01**	**$2.70^{a,b,c}$**	**± 1.12**	**3.48^{a}**	**± 0.15**
Syringaldehyde	2.58^{h}	± 0.06	$5.34^{c,d}$	± 0.00	5.46^{c}	± 0.02	6.06^{b}	± 0.00	7.62^{e}	± 0.01	7.82^{a}	± 0.03
Coniferyl aldehyde	0.82^{h}	± 0.01	1.50^{f}	± 0.07	1.56^{f}	± 0.07	$2.23^{d,c}$	± 0.07	$2.47^{f,g}$	± 0.03	4.57^{a}	± 0.06
Sinapaldehyde	4.05^{k}	± 0.08	8.00^{h}	± 0.05	9.33^{f}	± 0.13	10.90^{e}	± 0.05	11.66^{g}	± 0.01	13.02^{b}	± 0.04
Σ oak aldehydes	**7.44^{h}**	**± 0.01**	**14.84^{f}**	**± 0.12**	**16.35^{e}**	**± 0.08**	**19.19^{c}**	**± 0.12**	**21.76^{f}**	**± 0.04**	**25.40^{a}**	**± 0.13**

Higher content of furanic derivatives was found in 0F2M and 0F2F MWSs (2.90 mg/L and 3.48 mg/L after 35 days, respectively). The thermal degradation of wood polysaccharides originates the formation of furanic derivatives, namely furfurals and hydroxymethylfurfural. HMF proceeds from hexoses, that are the main constituents of cellulose, and F derives from pentoses, the main constituent of hemicelluloses (Caldeira et al., 2006). The hemicelluloses are the most thermosensitive wood polymer.
Regarding oak aldehydes, in MWSs containing light toasted oak chips only sinapaldehyde at low concentration was found (0.42 mg/L after 35 days) while in MWSs containing medium and toasted oak chips syringaldehyde, coniferylaldehyde and synapaldehyde were detected in high concentration (**Figure 11.8**). Toasting produced lignin degradation that led to the formation of low molecular phenolic compounds such as hydroxycinnamic aldehydes (Martinez et al., 1996).

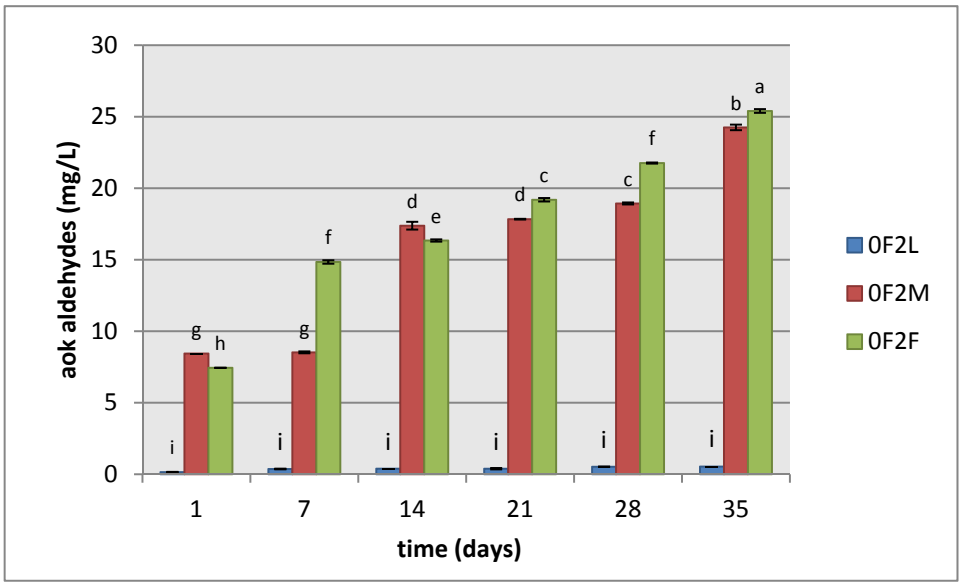

Figure 11.8. *Concentration of the total oak aldehydes (mg/L) in reference model wine solutions during accelerated aging with 2 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences (p > 0.05; n=3).*

A study based on principal component analysis (PCA) was applied (**Figure 11.9**). This provides a evaluation of the discriminating efficiency of the selected features and also a visualization of the sample trends (Martin et al., 2001).
The two first principal components (F1 and F2) were extracted explaining 95,36% of the total information. F_1 explained 68.05% and F_2 explained 27.30% of the total variance. Descriptor with more contribution to F_1 were ferulic acid, caffeic acid, HMF, F, Σ furfurals, the aldehydes and Σ oak aldehydes while F_2 was constituted of gallic acid, sinapic acid, ellagic acid and Σ phenolic acid. A fair separation of the MWSs aged with light chips and medium or high toasted chips can be seen on the plot. Solutions containing light toasted oak chips appeared at negative values of F1 whereas solutions containing medium and toasted oak chips are located at positive values of this axis, except samples 0F2M1, 0F2M2 and 0F2F1 which appear at negative values but very close to zero.

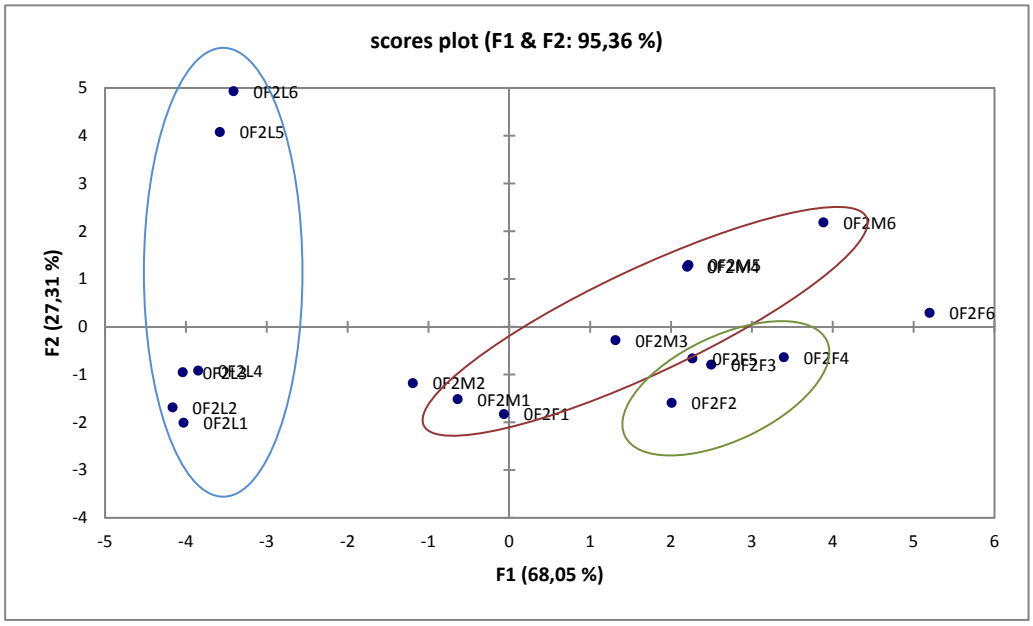

Figure 11.9. *Distribution of the reference model wine solutions studied in the plane defined by canonical functions 1 and 2 according to the type of oak chips (2 mm) used (L, light toasted; M, medium toasted, F, high toasted) and time.*

Presence of 50 mg/L and 200 mg/L of (+)-catechin (**Tables 11.2. a-b-c** for 50F2L, 50F2M and 50F2F, respectively and **Tables 11.3. a-b-c** for 200F2L, 200F2M and 200F2F, respectively) did not affected the concentration of phenolic compounds (statistical analysis not showed) while the oak chips dimension significantly affected the content of the detected molecules in MWSs (statistical analysis data not shown).

Table 11.2. a-b-c. *Mean and standard deviation for low molecular polyphenols in the model wine solutions containing 50 mg/L of (+)-catechin and the three different types of oak chips (2 mm) used (L, light toasted; M, medium toasted, F, high toasted). The error bars show the ±SD values. Values with the same letter indicate no statistically differences among the different type of chips studied.*

A – light toasted oak chips

	Time (Days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	$1.86^{e,f}$	± 0.21	2.86^{d}	± 0.18	3.01^{d}	± 0.22	$3.28^{c,d}$	± 0.04	7.46^{b}	± 0.29	9.21^{a}	± 0.06
Ferulic acid	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-
Caffeic acid	$n.d.^{g}$	-	$0.08^{f,g}$	± 0.00	$0.15^{e,f,g}$	± 0.00	$0.29^{e,f}$	± 0.02	$0.30^{e,f}$	± 0.02	$0.32^{e,f}$	± 0.16
Sinapic acid	$0.42^{d,c}$	± 0.07	$0.47^{b,c,d,e}$	± 0.01	$0.43^{c,d,e}$	± 0.01	$0.43^{c,d,e}$	± 0.01	$0.82^{a,b}$	± 0.25	$0.67^{a,b,c,d,e}$	± 0.05
Ellagic acid	1.43^{j}	± 0.11	$7.61^{e,f,g,h}$	± 0.59	$9.45^{c,d,e,f,}$	± 0.58	$10.39^{c,d,e}$	± 0.30	$13.19^{b,c,}$	± 4.00	19.43^{a}	± 0.49
Σ phenolic acids	**3.71^{h}**	**± 0.16**	**$11.03^{d,e,f}$**	**± 0.41**	**$13.04^{d,e}$**	**± 0.36**	**14.38^{d}**	**± 0.36**	**21.76^{b}**	**± 4.51**	**29.63^{a}**	**± 1.44**
HMF	n.d.	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-
F	$n.d.^{j}$	-	$n.d.^{j}$	-	$n.d.^{j}$	-	$n.d.^{j}$	-	$n.d.^{j}$	-	$n.d.^{j}$	-
Σ furfurals	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-
Syringaldehyde	$n.d.^{l}$	-	$n.d.^{l}$	-	$n.d.^{l}$	-	$n.d.^{l}$	-	$n.d.^{l}$	-	$n.d.^{l}$	-
Coniferyl aldehyde	$n.d.^{j}$	-	0.10^{j}	± 0.00	0.07^{j}	± 0.00	0.08^{j}	± 0.00	0.12^{j}	± 0.01	0.11^{j}	± 0.00
Sinapaldehyde	0.19^{j}	± 0.01	0.27^{j}	± 0.01	0.33^{j}	± 0.01	0.38^{j}	±0.01	0.37^{j}	± 0.01	0.47^{j}	± 0.00
Σ oak aldehydes	**0.19^{i}**	**± 0.01**	**0.37^{i}**	**± 0.01**	**0.40^{i}**	**± 0.01**	**0.46^{i}**	**± 0.01**	**0.49^{i}**	**± 0.02**	**0.58^{i}**	**± 0.00**

B – medium toasted oak chips

	Time (Days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	0.60^{i}	± 0.02	1.09^{h}	± 0.04	$1.34^{g,h}$	± 0.07	2.26^{g}	± 0.07	3.05^{e}	± 0.05	3.66^{c}	± 0.04
Ferulic acid	$0.40^{f,g}$	± 0.03	$0.53^{e,f}$	± 0.03	$0.41^{e,f,g}$	± 0.03	$0.60^{c,d}$	± 0.00	0.19^{h}	± 0.06	0.19^{h}	± 0.03
Caffeic acid	$0.24^{e,f,g}$	± 0.01	$0.30^{e,f}$	± 0.02	$0.61^{a,b,c}$	± 0.16	$0.34^{d,e,f}$	± 0.01	$0.38^{c,d,e}$	± 0.09	$0.42^{b,c,d,e}$	± 0.02
Sinapic acid	0.41^{e}	± 0.06	$0.60^{a,b,c,d,e}$	± 0.03	$0.56^{a,b,c,d,e}$	± 0.07	$0.55^{a,b,c,d,e}$	± 0.04	$0.72^{a,b,c,d,e}$	± 0.08	0.83^{a}	± 0.00
Ellagic acid	$2.90^{i,j}$	± 0.57	$6.12^{e,f,g,h,i}$	± 0.82	$8.47^{d,e,f,g}$	± 0.09	$16.33^{a,b}$	± 0.20	$16.20^{a,b}$	± 0.20	17.65^{a}	± 0.44
Σ phenolic acids	**$4.54^{g,h}$**	**± 0.46**	**$8.63^{e,f,g}$**	**± 0.74**	**$11.40^{d,e,f}$**	**± 0.04**	**$20.08^{b,c}$**	**± 0.15**	**20.54^{b}**	**± 0.19**	**22.76^{b}**	**± 0.44**
HMF	0.14^{h}	± 0.02	0.34^{g}	± 0.00	0.45^{e}	± 0.01	0.72^{c}	± 0.02	0.83^{b}	± 0.01	1.05^{a}	± 0.00
F	1.05^{i}	± 0.01	1.35^{h}	± 0.04	$1.41^{g,h}$	± 0.00	$1.72^{e,f}$	± 0.08	$1.54^{f,g}$	± 0.02	$1.82^{d,e}$	± 0.00
Σ furfurals	**1.20^{h}**	**± 0.02**	**1.68^{g}**	**± 0.04**	**1.85^{g}**	**± 0.01**	**$2.44^{d,e}$**	**± 0.06**	**$2.37^{d,e}$**	**± 0.02**	**$2.87^{d,e}$**	**± 0.01**
Syringaldehyde	1.78^{k}	± 0.04	$3.92^{h,i}$	± 0.12	3.84^{i}	± 0.06	4.31^{g}	± 0.04	4.12^{h}	± 0.03	5.38^{e}	± 0.03
Coniferyl aldehyde	0.96^{h}	± 0.09	2.46^{d}	± 0.03	2.94^{c}	± 0.04	3.11^{c}	± 0.03	3.32^{b}	± 0.08	2.97^{c}	± 0.09
Sinapaldehyde	4.09^{i}	± 0.06	$10.85^{e,f}$	± 0.04	10.74^{f}	± 0.08	12.88^{b}	± 0.32	$11.26^{d,c}$	± 0.05	16.16^{a}	± 0.31
Σ oak aldehydes	**6.82^{h}**	**± 0.07**	**17.23^{l}**	**± 0.11**	**17.52^{l}**	**± 0.11**	**20.30^{b}**	**± 0.40**	**18.70^{d}**	**± 0.07**	**24.51^{a}**	**± 0.19**

C – high toasted oak chips

	Time (Days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	0.27^{i}	± 0.05	0.37^{i}	± 0.04	0.50i	± 0.05	0.45^{i}	± 0.14	0.76^{h}	± 0.15	1.39^{g}	± 0.05
Ferulic acid	0.36^{g}	± 0.01	$0.55^{d,e}$	± 0.06	0.73^{c}	± 0.03	0.91^{b}	± 0.03	0.91^{b}	± 0.06	1.16^{a}	± 0.10
Caffeic acid	$0.28^{e,f}$	± 0.01	$0.28^{e,f}$	± 0.01	$0.33^{e,f}$	± 0.06	$0.61^{b,c,d}$	± 0.08	0.69^{b}	± 0.10	1.02^{a}	± 0.00
Sinapic acid	0.39^{e}	± 0.02	$0.67^{a,,c,d,e}$	± 0.05	$0.67^{a.b,c,d,e}$	± 0.10	$0.77^{a,b,c,d}$	± 0.03	$0.78^{a,b,c}$	± 0.12	$0.63^{a,b,c,d,e}$	± 0.15
Ellagic acid	$3.28^{h,i,j}$	± 0.20	$3.98^{h,i,j}$	± 0.65	$4.78^{g,h,i}$	± 0.04	$5.98^{e,f,g}$	± 0.38	$12.61^{c,d}$	± 0.08	$15.23^{b,c}$	± 0.16
Σ phenolic acids	**$4.58^{g,h}$**	**± 0.26**	**$5.85^{g,h}$**	**± 0.82**	**$7.01^{f,g,h}$**	**± 0.21**	**$8.71^{e,f,g}$**	**± 0.51**	**$15.7^{c,d}$**	**± 0.07**	**$19.43^{b,c}$**	**± 0.47**
HMF	0.33^{g}	± 0.00	0.39^{f}	± 0.01	0.41^{f}	± 0.00	0.38^{f}	± 0.02	0.58^{d}	± 0.00	0.82^{b}	± 0.01
F	$1.92^{c,d}$	± 0.00	2.14^{b}	± 0.00	2.21^{b}	± 0.01	$2.10^{b,c}$	± 0.04	2.49^{a}	± 0.16	2.66^{a}	± 0.06
Σ furfurals	**2.25^{f}**	**± 0.00**	**$2.53^{d,e}$**	**± 0.01**	**2.62^{d}**	**± 0.01**	**$2.48^{d,e}$**	**± 0.06**	**3.07^{b}**	**± 0.16**	**3.48^{a}**	**± 0.05**
Syringaldehyde	2.69^{j}	± 0.02	4.84^{f}	± 0.03	5.75^{d}	± 0.04	6.06^{c}	± 0.11	6.40^{b}	± 0.01	7.51^{a}	± 0.05
Coniferyl aldehyde	0.49^{i}	± 0.01	1.69^{g}	± 0.02	2.23^{e}	± 0.01	1.63^{g}	± 0.06	2.03^{f}	± 0.03	4.06^{a}	± 0.09
Sinapaldehyde	4.71^{h}	± 0.09	8.23^{g}	± 0.18	10.34^{f}	± 0.10	$11.77^{c,d}$	± 0.14	12.22^{c}	± 0.03	13.15^{b}	± 0.10
Σ oak aldehydes	**7.89^{g}**	**± 0.08**	**14.75^{f}**	**± 0.23**	**18.32^{d}**	**± 0.15**	**19.47^{c}**	**± 0.20**	**20.65^{b}**	**± 0.00**	**24.73^{a}**	**± 0.24**

Table 11.3. a-b-c. *Mean and standard deviation for low molecular polyphenols in the model wine solutions containing 200 mg/L of (+)-catechin and the three different types of oak chips (2 mm) used (L, light toasted; M, medium toasted, F, high toasted). The error bars show the ±SD values. Values with the same letter indicate no statistically differences among the different type of chips studied*

A – light toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	$1.12^{g,h}$	± 0.03	2.80^{d}	± 0.03	3.31^{c}	± 0.00	3.52^{c}	± 0.10	7.18^{b}	± 0.13	8.05^{a}	± 0.16
Ferulic acid	n.d^{d}.	-	n.d.^{d}	-	n.d.^{d}	-	n.d.^{d}	-	n.d.^{d}	-	n.d.^{d}	-
Caffeic acid	n.d.^{d}	-	0.08^{d}	± 0.01	$0.17^{d,e}$	± 0.03	0.11^{e}	± 0.02	$0.21^{d,e}$	± 0.07	$0.23^{d,e}$	± 0.02
Sinapic acid	$0.50^{c,d}$	± 0.01	$0.55^{c,d}$	± 0.08	0.40^{d}	± 0.07	0.45^{d}	± 0.08	$0.55^{c,d}$	± 0.21	$0.66^{b,c,d}$	± 0.06
Ellagic acid	1.43^{k}	± 0.01	$8.74^{e,f,g}$	± 0.54	$10.89^{c,d,e}$	± 1.11	$11.65^{c,d}$	± 1.57	19.99^{b}	± 0.57	23.78^{a}	± 0.85
Σ phenolic acids	**3.04^{l}**	**± 0.03**	**$12.18^{e,f,g}$**	**± 0.58**	**$14.76^{d,e}$**	**± 1.21**	**15.72^{d}**	**± 1.61**	**27.94^{b}**	**± 0.84**	**32.71^{a}**	**± 0.73**
HMF	n.d.^{g}	-	n.d.^{g}	-	n.d.^{g}	-	n.d.^{g}	-	n.d.^{g}	-	n.d.^{g}	-
F	n.d.^{f}	-	n.d.^{f}	-	n.d.^{f}	-	n.d.^{f}	-	n.d.^{f}	-	n.d.^{f}	-
Σ furfurals	n.d.^{i}	-	n.d.^{i}	-	n.d.^{i}	-	n.d.^{i}	-	n.d.^{i}	-	n.d.^{i}	-
Syringaldehyde	n.d.^{g}	-	n.d.^{g}	-	n.d.^{g}	-	n.d.^{g}	-	n.d.^{g}	-	n.d.^{g}	-
Coniferyl aldehyde	n.d.^{i}	-	0.06^{i}	± 0.01	n.d.^{i}	-	0.10^{i}	± 0.00	0.07^{i}	0.00	0.08^{i}	± 0.02
Sinapaldehyde	0.14^{i}	± 0.01	0.23^{i}	± 0.01	0.23^{i}	± 0.01	0.20^{i}	± 0.00	0.25^{i}	± 0.01	0.32^{i}	± 0.04
Σ oak aldehydes	**0.14^{g}**	**± 0.01**	**0.30^{g}**	**±0.03**	**0.23^{g}**	**± 0.01**	**0.30^{g}**	**± 0.00**	**0.32^{g}**	**± 0.01**	**0.40^{g}**	**± 0.02**

B – medium toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	1.01^{h}	± 0.16	$1.09^{g,h}$	± 0.08	$1.43^{f,g}$	± 0.05	1.70^{f}	± 0.18	1.74^{f}	± 0.03	2.37^{e}	± 0.15
Ferulic acid	$0.53^{b,c}$	± 0.04	$0.43^{c,d}$	± 0.03	$0.55^{b,c}$	± 0.02	$0.33^{c,d}$	± 0.43	$0.42^{c,d}$	± 0.03	$0.56^{b,c}$	± 0.07
Caffeic acid	$0.58^{a,b,c,d}$	± 0.01	$0.68^{a,b,c}$	± 0.01	$0.75^{a,b}$	± 0.01	$0.28^{c,d,e}$	± 0.31	0.87^{a}	± 0.15	0.86^{a}	± 0.06
Sinapic acid	$0.59^{c,d}$	± 0.06	$0.64^{b,c,d}$	± 0.01	$0.55^{c,d}$	± 0.06	$0.60^{c,d}$	± 0.15	$0.78^{b,c,d}$	± 0.02	$1.07^{a,b}$	± 0.05
Ellagic acid	$5.14^{i,j}$	± 0.60	$5.75^{h,i,j}$	± 0.51	$9.25^{e,f}$	± 0.45	12.89^{d}	± 0.03	18.88^{c}	± 0.18	$20.74^{b,c}$	± 0.48
Σ phenolic acids	**$7.84^{i,j,k}$**	**± 0.33**	**$8.60^{h,i,j}$**	**± 0.60**	**$12.52^{e,f}$**	**± 0.46**	**15.79^{d}**	**± 0.39**	**22.69^{c}**	**± 0.01**	**$25.61^{b,c}$**	**± 0.81**
HMF	$0.27^{e,f}$	± 0.02	0.24^{f}	± 0.01	$0.36^{d,e}$	± 0.04	$0.42^{c,d}$	± 0.01	1.06^{b}	± 0.00	1.34^{a}	± 0.11
F	$1.88^{a,b,c}$	± 0.10	1.19^{e}	± 0.02	1.22^{e}	± 0.01	$1.35^{d,e}$	± 0.04	1.52d	± 0.01	1.74^{c}	± 0.03
Σ furfurals	**$2.16^{c,d,e}$**	**± 0.12**	**1.42^{h}**	**± 0.04**	**$1.58^{g,h}$**	**± 0.05**	**$1.77^{f,g}$**	**± 0.05**	**2.57^{b}**	**± 0.00**	**3.08^{a}**	**± 0.14**
Syringaldehyde	$3.10^{e,f}$	± 0.02	$3.44^{d,e,f}$	± 0.05	$3.89^{c,d,e,f}$	± 0.03	$4.95^{b,c,d}$	± 1.70	$4.04^{c,d,e}$	± 0.01	$4.80^{b,c,d}$	± 0.02
Coniferyl aldehyde	$2.13^{d,e}$	± 0.03	$1.92^{a,f}$	± 0.08	3.64^{a}	± 0.06	2.35^{c}	± 0.01	$2.01^{e,f}$	± 0.00	1.12^{g}	± 0.09
Sinapaldehyde	6.26^{g}	± 0.02	$8.29^{f,}$	± 0.10	$9.85^{d,e}$	± 0.08	11.21^{c}	± 0.10	12.35^{b}	± 0.01	16.02^{a}	± 0.26
Σ oak aldehydes	**11.48^{e}**	**± 0.03**	**13.65^{d}**	**± 0.03**	**$17.38^{b,c}$**	**± 0.11**	**$18.51^{b,c}$**	**± 1.79**	**$18.41^{b,c}$**	**± 0.00**	**21.93^{a}**	**± 0.37**

C – high toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	0.25^{i}	± 0.04	0.39^{i}	± 0.05	0.38^{i}	± 0.01	0.33^{i}	± 0.02	0.50^{i}	±0.13	1.00^{i}	± 0.05
Ferulic acid	$0.42^{c,d}$	± 0.02	$0.44^{c,d}$	± 0.06	$0.74^{a,b,c}$	± 0.14	$0.69^{b,c}$	± 0.18	$0.99^{a,b}$	±0.17	1.21^{a}	± 0.02
Caffeic acid	$0.34^{b,c,d,e}$	± 0.04	$0.22^{d,e}$	± 0.00	$0.40^{b,c,d,e}$	± 0.02	$0.40^{b,c,d,e}$	± 0.27	$0.73^{a,b,c}$	± 0.13	$0.79^{a,b}$	± 0.07
Sinapic acid	0.39^{d}	± 0.02	$0.67^{b,c,d}$	± 0.04	$0.91^{a,b,c}$	± 0.24	$0.70^{b,c,d}$	± 0.00	$0.63^{b,c,d}$	± 0.17	1.30^{a}	± 0.18
Ellagic acid	$3.67^{j,k}$	± 0.31	$4.54^{j,k}$	± 0.08	$6.86^{g,h,i}$	± 0.86	$7.10^{g,h,i}$	± 0.61	$8.28^{f,g,h}$	± 0.06	$10.18^{d,e,f}$	± 0.23
Σ phenolic acids	**$5.08^{k,j}$**	**± 0.27**	**$6.26^{k,j}$**	**± 0.23**	**$9.29^{g,h,i}$**	**± 1.00**	**$9.22^{h,i}$**	**± 1.07**	**$11.14^{f,g,h}$**	**± 0.33**	**$14.48^{d,e}$**	**± 0.06**
HMF	0.08^{g}	± 0.00	$0.32^{d,e,f}$	± 0.00	$0.30^{d,ef}$	± 0.00	$0.28^{e,f}$	± 0.03	$0.34^{d,e,f}$	± 0.02	0.54^{c}	± 0.01
F	$1.90^{a,b,c}$	± 0.01	2.05^{a}	± 0.00	$1.85^{a,b,c}$	± 0.09	$1.75^{b,c}$	± 0.15	$1.97^{a,b}$	± 0.09	2.05^{a}	± 0.03
Σ furfurals	**$1.98^{e,f}$**	**± 0.02**	**$2.37^{b,c}$**	**± 0.00**	**$2.15^{c,d,e}$**	**± 0.10**	**$2.03^{d,e,f}$**	**± 0.19**	**$2.30^{b,c,d}$**	**± 0.11**	**2.58^{b}**	**± 0.04**
Syringaldehyde	2.32^{f}	± 0.04	$4.54^{b,c,d,e}$	± 0.04	$5.45^{a,b,c}$	± 0.06	$5.94^{a,b}$	± 0.11	$5.88^{a,b}$	± 0.02	6.79^{a}	± 0.03
Coniferyl aldehyde	0.49^{h}	± 0.01	1.19^{g}	± 0.00	1.88^{f}	± 0.03	$2.33^{c,d}$	± 0.09	$2.34^{c,d}$	± 0.03	3.41^{b}	± 0.16
Sinapaldehyde	4.09^{h}	± 0.03	8.29^{f}	± 0.05	$9.27^{d,e}$	± 0.12	10.20^{d}	± 0.02	10.95^{c}	± 0.01	12.42^{b}	± 0.71
Σ oak aldehydes	**6.90^{f}**	**± 0.03**	**14.01^{d}**	**± 0.01**	**16.59^{c}**	**± 0.09**	**$18.46^{b,c}$**	**± 0.04**	**19.17^{b}**	**± 0.01**	**22.61^{a}**	**± 0.91**

In MWSs containing highest oak chips (8 mm) gallic acid was more abundant in MWSs containing light toasted oak chips (**Tables 11.4. a-b-c**). The average content of gallic acid in 0F8L, 0F8M and 0F8F solutions was of 4.84 mg/L, 2.84 mg/L and 1.21 mg/L, respectively. In MWSs with medium and high toasted oak chips furanic derivatives and oak aldehydes were more concentrated than in MWSs with light toasted oak chips (**Figure 11.10**).
Results of PCA analysis (**Figure 11.11**) of MWSs containing the highest oak chips (8 mm) revealed that solutions containing light toasted oak chips appeared at negative values of F1 whereas solutions containing medium and toasted oak chips are located at positive values of this axis, except samples 0F8M1, 0F8M2 and 0F8F1 which appear at negative values but very close to zero, that was similar to previous results. The two first principal components (F1 and F2) were extracted explaining 73.96% of the total information. F_1 explained 53.97% and F_2 explained 19.99% of the total variance. Descriptor with more contribution to F_1 were ferulic acid, sinapic acid, ellagic acid, F, Σ furfurals, the aldehydes and Σ oak aldehydes while F_2 was constituted of gallic acid, caffeic acid and Σ phenolic acid.
Even for MWSs where has been added 50 mg/L and 200 mg/L of (+)-catechin (**Tables 11.5. a-b-c** for 50F8L, 50F8M and 50F8F, respectively and **Tables 11.6. a-b-c** for 200F8L, 200F8M and 200F8F, respectively) no significant differences were observed respect to the reference samples (data not shown).

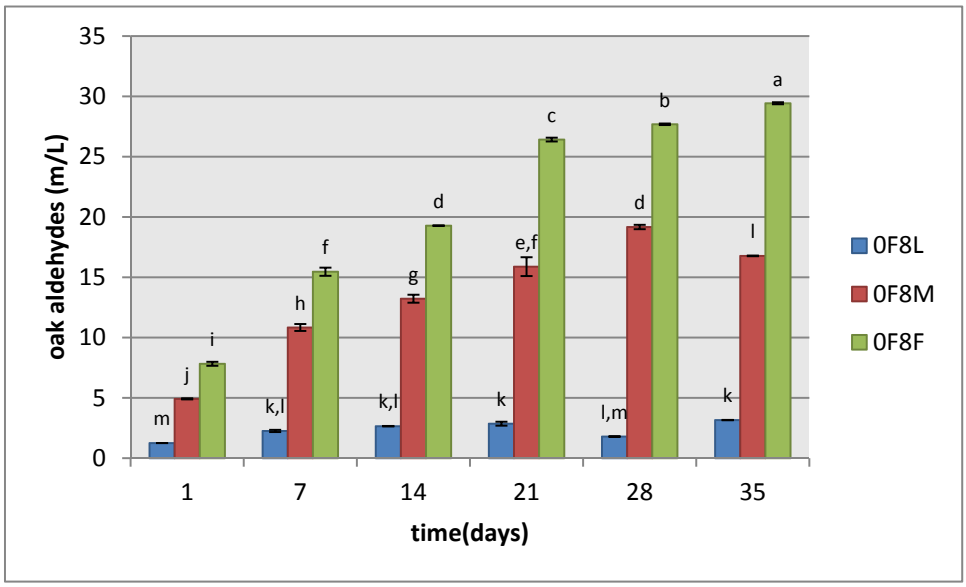

Figure 11.10. *Concentration of the total oak aldehydes (mg/L) in reference model wine solutions during accelerated aging with 8 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences (p > 0.05; n=3).*

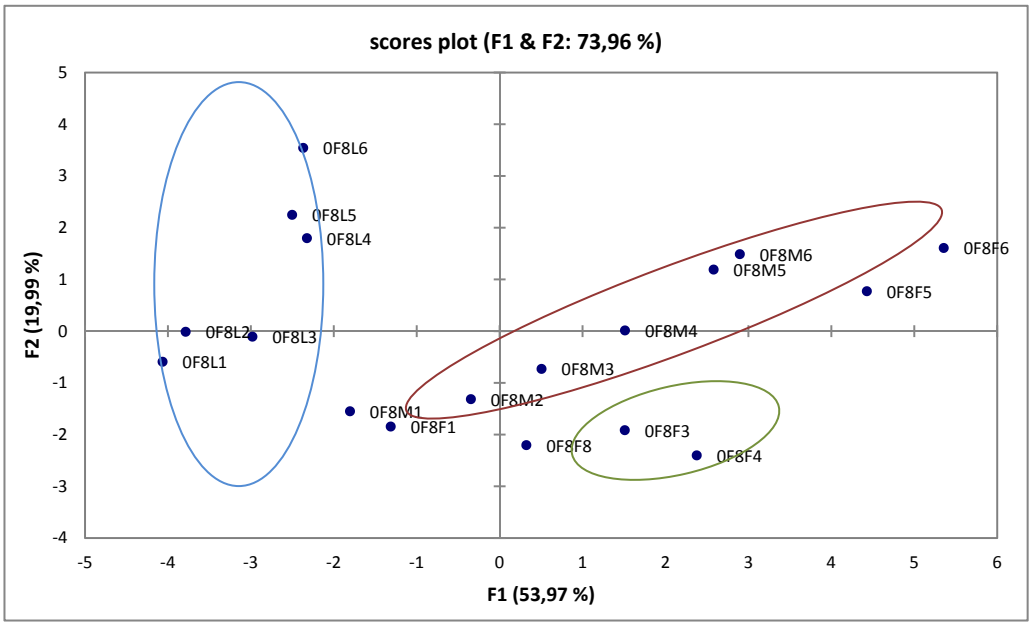

Figure 11.11. *Distribution of the reference model wine solutions studied in the plane defined by canonical functions 1 and 2 according to the type of oak chips (8 mm) used (L, light toasted; M, medium toasted, F, high toasted) and time.*

***Table 11.4. a-b-c**. Mean and standard deviation for low molecular polyphenols in the reference model wine solutions with the three different types of oak chips (8 mm) used (L, light toasted; M, medium toasted, F, high toasted). The error bars show the ±SD values. Values with the same letter indicate no statistically differences among the different type of chips studied.*

A –light toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	$2.67^{d,e,f}$	± 0.11	$3.43^{c,d}$	± 0.04	$3.68^{c,d}$	± 0.32	5.69^{b}	± 0.61	$6.55^{a,b}$	± 0.05	7.01^{a}	± 0.07
Ferulic acid	$n.d.^{g}$	-	$n.d.^{g}$	-	$n.d.^{g}$	-	$n.d.^{g}$	-	$n.d.^{g}$	-	$n.d.^{g}$	-
Caffeic acid	0.08^{f}	± 0.00	0.13^{f}	± 0.00	$0.32^{d,e,f}$	0.00	$0.88^{b,c}$	± 0.11	$0.61^{c,d}$	± 0.01	$1.06^{a,b}$	± 0.03
Sinapic acid	$0.45^{c,d}$	± 0.06	$0.46^{c,d}$	± 0.04	$0.50^{c,d}$	± 0.03	$0.53^{c,d}$	± 0.08	$0.50^{c,d}$	± 0.10	$0.68^{b,c}$	± 0.01
Ellagic acid	0.83^{i}	± 0.02	3.40^{h}	± 0.20	3.59^{h}	± 0.47	8.68^{f}	± 0.24	$12.54^{d,e}$	± 0.20	13.99^{d}	± 0.38
Σ phenolic acids	**4.03^{k}**	**± 0.07**	**$7.41^{h,i,j}$**	**± 0.20**	**$8.09^{g,h}$**	**± 0.75**	**15.77^{e}**	**± 1.04**	**20.20^{d}**	**± 0.13**	**22.73^{c}**	**± 0.48**
HMF	$n.d.^{g}$	-	$n.d.^{g}$	-	$n.d.^{g}$	-	$n.d.^{g}$	-	$n.d.^{g}$	-	$n.d.^{g}$	-
F	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-
Σ furfurals	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-
Syringaldehyde	$n.d.^{k}$	-	$n.d.^{k}$	-	$n.d.^{k}$	-	$n.d.^{k}$	-	$n.d.^{k}$	-	$n.d.^{k}$	-
Coniferyl aldehyde	0.26^{j}	± 0.01	$0.32^{i,j}$	0.00	$0.69^{h,i}$	± 0.09	$0.95^{g,h}$	± 0.03	$0.35^{i,j}$	± 0.00	$0.83^{g,h}$	± 0.01
Sinapaldehyde	1.00^{l}	± 0.02	$1.94^{j,k}$	± 0.10	$1.96^{j,k}$	± 0.07	$1.91^{j,k}$	± 0.13	$1.44^{j,k}$	± 0.02	$2.33^{i,j}$	± 0.02
Σ oak aldehydes	**1.25^{m}**	**± 0.00**	**$2.26^{k,l}$**	**± 0.09**	**$2.65^{k,l}$**	**± 0.02**	**2.86^{k}**	**± 0.16**	**1.80^{l}**	**± 0.02**	**3.16^{k}**	**± 0.01**

B – medium toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	$1.09^{h,i,j}$	± 0.05	$1.93^{f,g,h}$	± 0.00	$2.36^{e,f,g}$	± 0.13	$3.28^{c,d,e}$	± 0.00	4.19^{c}	± 0.71	4.17^{c}	± 0.12
Ferulic acid	$0.11^{f,g}$	± 0.01	$0.28^{e,f,g}$	± 0.01	$0.34^{e,f}$	± 0.05	$0.47^{d,e}$	± 0.24	$0.12^{f,g}$	± 0.05	$0. 10^{g}$	± 0.04
Caffeic acid	$0.17^{e,f}$	± 0.04	$0.36^{d,e,f}$	± 0.01	$0.52^{c,d,e}$	± 0.05	$0.56^{c,d}$	± 0.36	$0.51^{c,d,e}$	± 0.04	$0.37^{d,e,f}$	± 0.02
Sinapic acid	0.30^{d}	± 0.02	$0.53^{b,c}$	± 0.01	$0.45^{c,d}$	± 0.05	$0.40^{c,d}$	± 0.09	$0.53^{c,d}$	± 0.02	$0.64^{b,e}$	± 0.08
Ellagic acid	3.05^{h}	± 0.03	2.93^{h}	± 0.24	6.56^{g}	± 0.32	11.64^{e}	± 0.12	18.30^{c}	± 0.85	21.36^{b}	± 0.15
Σ phenolic acids	**$4.72^{j,k}$**	**± 0.03**	**$6.02^{i,j}$**	**± 0.28**	**10.22^{f}**	**± 0.60**	**16.34^{e}**	**± 0.34**	**23.66^{c}**	**± 0.26**	**26.56^{b}**	**± 0.03**
HMF	0.32^{e}	± 0.05	0.50^{d}	± 0.07	0.71^{c}	± 0.07	1.02^{b}	± 0.08	1.19^{a}	± 0.01	1.29^{a}	± 0.01
F	1.50^{f}	± 0.16	1.85^{e}	± 0.05	1.95^{de}	± 0.02	2.28^{c}	± 0.04	3.09^{b}	± 0.06	3.64^{a}	± 0.04
Σ furfurals	**1.82^{f}**	**± 0.21**	**2.35^{e}**	**± 0.12**	**2.66^{d}**	**± 0.10**	**3.30^{c}**	**± 0.03**	**4.28^{b}**	**± 0.05**	**4.94^{a}**	**± 0.03**
Syringaldehyde	0.73^{j}	± 0.01	1.46^{i}	± 0.02	1.82^{h}	± 0.04	2.11^{g}	± 0.03	2.85^{f}	± 0.00	2.70^{f}	± 0.02
Coniferyl aldehyde	1.16^{g}	± 0.09	2.20^{d}	± 0.16	$2.72^{d,e}$	± 0.25	$2.75^{d,e}$	± 0.17	2.64^{e}	± 0.06	$3.19^{b,c}$	± 0.03
Sinapaldehyde	3.02^{i}	± 0.13	7.18^{g}	± 0.14	8.69^{f}	± 0.13	11.02^{e}	± 0.57	13.68^{d}	± 0.12	10.88^{e}	± 0.01
Σ oak aldehydes	**4.92^{j}**	**± 0.05**	**10.84^{h}**	**± 0.29**	**13.22^{g}**	**± 0.33**	**$15.88^{e,f}$**	**± 0.78**	**19.17^{d}**	**± 0.18**	**16.77^{e}**	**± 0.03**

C – high toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	0.40^{j}	± 0.01	$0.81^{i,j}$	± 0.01	$1.14^{h,i,j}$	± 0.27	$1.03^{h,i,j}$	± 0.05	$1.57^{g,h,i}$	± 0.27	$2.30^{e,f,g}$	± 0.14
Ferulic acid	$0.39^{d,e,f}$	± 0.12	$0.82^{b,c}$	± 0.00	$0.99^{a,b}$	± 0.01	1.20^{c}	± 0.02	$0.67^{c,d}$	± 0.12	$0.92^{a,b,c}$	± 0.02
Caffeic acid	$0.30^{d,e,f}$	± 0.05	$0.30^{d,e,f}$	± 0.04	$0.35^{d,e,f}$	± 0.00	$0.38^{d,e,f}$	± 0.02	$1.09^{a,b}$	± 0.06	1.36^{a}	± 0.06
Sinapic acid	$0.41^{c,d}$	± 0.08	$0.64^{c,d}$	± 0.02	0.94^{b}	± 0.03	$0.54^{c,d}$	± 0.12	1.58^{a}	± 0.22	1.88^{a}	± 0.08
Ellagic acid	3.72^{h}	± 0.22	3.95^{h}	± 0.01	6.52^{h}	± 0.81	$7.79^{f,g}$	± 0.03	19.63^{c}	± 0.32	23.14^{a}	± 0.59
Σ phenolic acids	**$5.22^{j,k}$**	**± 0.04**	**$6.53^{h,i}$**	**± 0.05**	**$9.95^{f,g}$**	**± 1.12**	**10.94^{f}**	**± 0.14**	**24.53^{c}**	**± 0.01**	**29.61^{a}**	**± 0.56**
HMF	$0.11^{f,g}$	± 0.01	0.18^{f}	± 0.01	0.18^{f}	± 0.01	$0.08^{f,g}$	± 0.01	0.17^{f}	± 0.05	$0.21^{e,f}$	± 0.01
F	1.15^{g}	± 0.04	$1.35^{f,g}$	± 0.03	$1.40^{f,g}$	± 0.04	1.46^{f}	± 0.01	$2.11^{c,d}$	± 0.12	2.26^{c}	± 0.13
Σ furfurals	**1.26^{g}**	**± 0.04**	**$1.54^{f,g}$**	**± 0.02**	**1.58^{f}**	**±0.04**	**$1.54^{f,g}$**	**± 0.02**	**2.29^{e}**	**± 0.07**	**$2.47^{d,e}$**	**± 0.15**
Syringaldehyde	2.22^{g}	± 0.03	4.16^{e}	± 0.16	4.87^{d}	± 0.03	6.64^{b}	± 0.01	5.84^{c}	± 0.02	7.33^{a}	± 0.03
Coniferyl aldehyde	1.13^{g}	± 0.00	1.89^{f}	± 0.04	3.05^{c}	± 0.03	$3.43^{b,c}$	± 0.17	4.15^{a}	± 0.02	3.46^{b}	± 0.02
Sinapaldehyde	4.48^{h}	± 0.14	9.41^{f}	± 0.21	11.36^{e}	± 0.08	16.35^{c}	± 0.31	17.70^{b}	± 0.07	18.64^{a}	± 0.03
Σ oak aldehydes	**7.83^{i}**	**± 0.17**	**15.46^{f}**	**± 0.34**	**19.28^{d}**	**± 0.03**	**26.42^{c}**	**± 0.16**	**27.69^{b}**	**± 0.06**	**29.43^{a}**	**± 0.08**

Table 11.5. a-b-c. *Mean and standard deviation for low molecular polyphenols in the model wine solutions containing 50 mg/L of (+)-catechin and the three different types of oak chips (8 mm) used (L, light toasted; M, medium toasted, F, high toasted). The error bars show the ±SD values. Values with the same letter indicate no statistically differences among the different type of chips studied*

A – light toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	4.77^{e}	± 0.20	6.33^{d}	± 0.34	$6.83^{c,d}$	± 0.13	8.57^{a}	± 0.35	$7.32^{b,c}$	± 0.01	7.83^{b}	± 0.17
Ferulic acid	$n.d.^{f}$	-	$n.d.^{f}$	-	$n.d.^{f}$	-	$n.d.^{f}$	-	$n.d.^{f}$	-	$n.d.^{f}$	-
Caffeic acid	0.10^{i}	± 0.00	$0.17^{h,i}$	± 0.01	$0.29^{e,f,g,h}$	± 0.05	$0.40^{d,e,f,g}$	± 0.07	$0.46^{d,e,f}$	± 0.01	$0.41^{d,e,f,g}$	± 0.02
Sinapic acid	0.35^{g}	± 0.04	$0.41^{f,g}$	± 0.01	$0.44^{e,f,g}$	± 0.03	$0.51^{d,e,f,g}$	± 0.07	$0.58^{d,e,f,g}$	± 0.03	$0.43^{f,g}$	± 0.00
Ellagic acid	2.60^{h}	± 0.05	3.57^{h}	± 0.46	$4.50^{f,g,h}$	± 0.07	10.42^{e}	± 0.52	$13.78^{c,d}$	± 1.24	$14.66^{b,c}$	± 1.01
Σ phenolic acids	**$7.82^{i,j}$**	**± 0.22**	**$10.48^{g,h}$**	**± 0.81**	**$12.06^{f,g}$**	**± 0.21**	**19.90^{d}**	**± 0.86**	**$22.14^{b,c,d}$**	**± 1.28**	**$23.32^{a,b,c}$**	**± 0.86**
HMF	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-
F	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-
Σ furfurals	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-
Syringaldehyde	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-	$n.d.^{i}$	-
Coniferyl aldehyde	0.36^{i}	± 0.03	0.16^{i}	± 0.01	0.83^{g}	± 0.04	0.87^{g}	± 0.12	0.57^{h}	± 0.02	0.78^{g}	± 0.01
Sinapaldehyde	1.00^{k}	± 0.03	$1.92^{i,j}$	± 0.22	$1.71^{i,j}$	± 0.04	$1.73^{i,j}$	± 0.06	$1.35^{j,k}$	± 0.02	2.30^{i}	± 0.04
Σ oak aldehydes	**1.35^{l}**	**± 0.06**	**2.08^{k}**	**± 0.23**	**$2.54^{j,k}$**	**± 0.08**	**$2.60^{j,k}$**	**± 0.17**	**$1.92^{k,l}$**	**± 0.00**	**3.08^{j}**	**± 0.03**

B –medium toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	1.11^{k}	± 0.04	$1.82^{h,i}$	± 0.00	2.01^{h}	± 0.26	2.81^{g}	± 0.01	$3.17^{f,g}$	± 0.03	3.47^{f}	± 0.07
Ferulic acid	0.12^{f}	± 0.04	$0.28^{d,e,f}$	± 0.00	$0.46^{d,e}$	± 0.02	$0.22^{e,f}$	± 0.02	$0.28^{d,e,f}$	± 0.26	0.09^{f}	± 0.00
Caffeic acid	0.37^{g}	± 0.01	$0.37^{d,e,f,g,h}$	± 0.00	$0.58^{c,d}$	± 0.04	$0.37^{d,e,f,g,h}$	± 0.08	$0.49^{d,e}$	± 0.00	$0.41^{d,e,f,g}$	± 0.03
Sinapic acid	0.30^{g}	± 0.00	$0.50^{d,e,f,g}$	± 0.05	0.77^{d}	± 0.00	$0.43^{f,g}$	± 0.02	$0.53^{d,e,f,g}$	± 0.02	$0.73^{d,e,f}$	± 0.13
Ellagic acid	2.41^{h}	± 0.47	3.47^{h}	± 0.11	6.34^{f}	± 0.45	10.05^{e}	± 0.42	10.05^{e}	± 0.42	$11.60^{d,e}$	± 0.14
Σ phenolic acids	**4.31^{k}**	**± 0.38**	**$6.44^{j,k}$**	**± 0.07**	**$10.17^{g,h,i}$**	**± 0.77**	**$13.88^{e,f}$**	**± 0.45**	**$14.51^{e,f}$**	**± 0.68**	**16.29^{e}**	**± 0.04**
HMF	$0.32^{e,f}$	± 0.03	$0.45^{c,d}$	± 0.03	0.47^{c}	± 0.07	0.67^{b}	± 0.01	0.68^{b}	± 0.01	0.82^{a}	± 0.01
F	$1.40^{d,e}$	± 0.02	$1.52^{c,d}$	± 0.05	$1.64^{b,c}$	± 0.09	1.72^{b}	± 0.00	1.74^{b}	± 0.01	1.92^{a}	± 0.04
Σ furfurals	**$1.73^{e,f}$**	**± 0.01**	**$1.96^{c,d}$**	**± 0.08**	**2.12^{c}**	**± 0.16**	**2.39^{b}**	**± 0.01**	**2.41^{b}**	**± 0.02**	**2.74^{a}**	**± 0.02**
Syringaldehyde	0.67^{h}	± 0.01	1.45^{g}	± 0.02	$1.70^{f,g}$	± 0.02	$1.89^{e,f}$	± 0.03	2.36^{d}	± 0.01	2.31^{d}	± 0.03
Coniferyl aldehyde	0.85^{g}	± 0.07	2.30^{f}	± 0.04	3.42^{e}	± 0.05	2.31^{f}	± 0.03	3.42^{e}	± 0.06	4.05^{c}	± 0.06
Sinapaldehyde	3.05^{h}	± 0.07	6.27^{g}	± 0.05	7.61^{f}	± 0.24	9.31^{e}	± 0.02	11.89^{c}	± 0.52	$11.25^{c,d}$	± 0.04
Σ oak aldehydes	**4.57^{i}**	**± 0.01**	**10.02^{h}**	**± 0.06**	**12.72^{g}**	**± 0.27**	**13.51^{g}**	**± 0.03**	**$17.67^{d,e}$**	**± 0.48**	**17.61^{e}**	**± 0.13**

C – high toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	0.44^{l}	± 0.10	$0.77^{k,l}$	± 0.03	$1.18^{j,k}$	± 0.15	$0.91^{k,l}$	± 0.00	$1.37^{i,j,k}$	± 0.08	$1.78^{h,i,j}$	± 0.14
Ferulic acid	$0.48^{d,e}$	± 0.01	$0.82^{b,c}$	± 0.09	0.95^{b}	± 0.03	1.70^{a}	± 0.17	$0.45^{d,e}$	± 0.06	$0.54^{c,d}$	± 0.07
Caffeic acid	$0.24^{g,h,i}$	± 0.02	$0.25^{f,g,h,i}$	± 0.01	$0.33^{e,f,g,h}$	± 0.02	0.72^{c}	± 0.14	1.58^{b}	± 0.11	1.94^{a}	± 0.07
Sinapic acid	$0.40^{f,g}$	± 0.01	$0.77^{d,e}$	± 0.03	0.82^{d}	± 0.06	3.24^{b}	± 0.01	3.78^{a}	± 0.30	1.35^{c}	± 0.01
Ellagic acid	2.51^{h}	± 0.36	$3.82^{g,h}$	± 0.20	$6.10^{f,g}$	± 0.79	$14.57^{b,c}$	± 0.11	16.67^{b}	± 0.86	19.26^{a}	± 1.10
Σ phenolic acids	**4.08^{k}**	**± 0.22**	**$6.44^{j,k}$**	**± 0.24**	**$9.38^{h,i}$**	**± 0.95**	**$21.14^{c,d}$**	**± 0.18**	**$23.85^{a,b}$**	**± 0.32**	**24.87^{a}**	**± 0.84**
HMF	$0.09^{h,i}$	± 0.01	$0.13^{g,h}$	± 0.01	$0.17^{g,h}$	± 0.02	$0.36^{d,e}$	± 0.07	$0.39^{c,d,e}$	± 0.02	$0.22^{f,g}$	± 0.03
F	1.04^{g}	± 0.03	1.25^{f}	± 0.03	$1.34^{e,f}$	± 0.05	$1.40^{d,e,f}$	± 0.02	1.75^{b}	± 0.09	1.69^{b}	± 0.00
Σ furfurals	**1.13^{h}**	**± 0.04**	**1.38^{g}**	**± 0.04**	**$1.51^{f,g}$**	**± 0.07**	**$1.75^{d,e}$**	**± 0.05**	**2.13^{c}**	**± 0.11**	**$1.91^{c,d,e}$**	**± 0.03**
Syringaldehyde	$2.10^{d,e}$	± 0.03	3.61^{c}	± 0.36	4.80^{b}	± 0.00	4.82^{b}	± 0.02	6.73^{a}	± 0.02	7.07^{a}	± 0.02
Coniferyl aldehyde	$0.72^{g,h}$	± 0.04	2.29^{f}	± 0.03	3.74^{d}	± 0.01	4.01^{c}	± 0.02	4.51^{b}	± 0.09	5.51^{a}	± 0.04
Sinapaldehyde	$1.23^{j,k}$	± 0.00	9.95^{e}	± 0.07	9.91^{e}	± 0.08	10.95^{d}	± 0.33	15.44^{b}	± 0.06	16.19^{a}	± 0.21
Σ oak aldehydes	**4.05^{i}**	**± 0.01**	**15.85^{f}**	**± 0.41**	**18.45^{d}**	**± 0.07**	**19.78^{c}**	**± 0.28**	**26.69^{b}**	**± 0.00**	**28.78^{a}**	**± 0.26**

Table 11.6. a-b-c. Mean and standard deviation for low molecular polyphenols in the model wine solutions containing 50 mg/L of (+)-catechin and the three different types of oak chips (8 mm) used (L, light toasted; M, medium toasted, F, high toasted). The error bars show the ±SD values. Values with the same letter indicate no statistically differences among the different type of chips studied.

A – light toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	$3.44^{e,f}$	± 0.11	$4.09^{d,e}$	± 0.09	4.79^{d}	± 0.15	6.78^{c}	± 0.16	8.09^{b}	± 0.76	9.03^{a}	± 0.08
Ferulic acid	$n.d.^{f}$	-	$n.d.^{f}$	-	$n.d.^{f}$	-	$n.d.^{f}$	-	$n.d.^{f}$	-	$n.d.^{f}$	-
Caffeic acid	0.11^{i}	± 0.01	$0.17^{h,i}$	± 0.01	$0.20^{h,i}$	± 0.02	$0.43^{e,f,g}$	± 0.06	$0.67^{c,d}$	± 0.02	$0.60^{c,d}$	± 0.00
Sinapic acid	$0.39^{h,i}$	± 0.06	$0.61^{f,g,h,i}$	± 0.03	$0.48^{g,h,i}$	± 0.06	$0.44^{g,h,i}$	± 0.01	$0.52^{g,hi}$	± 0.00	$0.51^{g,h,i}$	± 0.00
Ellagic acid	$2.90^{h,i}$	± 0.41	$3.95^{g,h,i}$	± 0.01	$5.68^{f,g}$	± 0.81	11.23^{d}	± 0.14	16.53^{b}	± 0.10	17.68^{b}	± 0.49
Σ phenolic acids	**6.84^{k}**	**± 0.58**	**$8.82^{i,j}$**	**± 0.06**	**$11.15^{g,h}$**	**± 0.62**	**$18.88^{d,e}$**	**± 0.02**	**25.81^{c}**	**± 0.88**	**27.82^{b}**	**± 0.57**
HMF	$n.d.^{e}$	-	$n.d.^{e}$	-	$n.d.^{e}$	-	$n.d.^{e}$	-	$n.d.^{e}$	-	$n.d.^{e}$	-
F	$n.d.^{d}$	-	$n.d.^{d}$	-	$n.d.^{d}$	-	$n.d.^{d}$	-	$n.d.^{d}$	-	$n.d.^{d}$	-
Σ furfurals	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-	$n.d.^{h}$	-
Syringaldehyde	$n.d.^{k}$	-	$n.d.^{k}$	-	$n.d.^{k}$	-	$n.d.^{k}$	-	$n.d.^{k}$	-	$n.d.^{k}$	-
Coniferyl aldehyde	0.31^{i}	± 0.06	$0.42^{h,i}$	± 0.03	$0.89^{f,g,h,i}$	± 0.02	$0.84^{f,g,h,i}$	± 0.05	$0.59^{g,h,i}$	± 0.10	$0.91^{f,g,h}$	± 0.01
Sinapaldehyde	1.04^{g}	± 0.01	1.60^{e}	± 0.07	1.50^{e}	± 0.06	1.49^{d}	± 0.03	2.04^{c}	±0.00	2.50^{c}	± 0.08
Σ oak aldehydes	**1.36^{n}**	**± 0.04**	**$2.02^{m,n}$**	**± 0.04**	**2.39^{m}**	**± 0.04**	**2.33^{m}**	**± 0.08**	**2.63^{m}**	**± 0.10**	**3.41^{l}**	**± 0.09**

B – medium toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	$1.16^{j,k,l}$	± 0.01	$1.67^{h,i,j}$	± 0.08	$1.91^{h,i,j}$	± 0.09	$2.06^{g,h,i}$	± 0.05	$2.75^{f,g}$	± 0.09	3.23^{f}	± 0.03
Ferulic acid	$0.34^{d,e}$	± 0.00	0.28^{e}	± 0.02	$0.44^{d,e}$	± 0.00	0.29^{e}	± 0.14	$0.42^{d,e}$	± 0.14	$0.33^{d,e}$	± 0.13
Caffeic acid	$0.49^{e,f}$	± 0.03	$0.33^{f,g,h}$	± 0.02	$0.50^{d,e}$	± 0.04	$0.46^{e,f,g}$	± 0.01	$0.45^{e,f,g}$	± 0.05	$0.47^{e,f}$	± 0.05
Sinapic acid	0.33^{i}	± 0.01	$0.61^{f,g,h,i}$	± 0.02	$0.89^{d,e}$	± 0.04	$0.65^{e,f,g,h}$	± 0.16	$0.63^{e,f,g,h}$	± 0.02	$0.82^{d,e,f}$	± 0.09
Ellagic acid	2.54^{i}	± 0.11	$3.51^{h,i}$	± 0.35	6.12^{f}	± 0.09	12.14^{d}	± 0.29	14.47^{c}	± 1.03	$15.96^{b,c}$	± 0.65
Σ phenolic acids	**4.85^{l}**	**± 0.09**	**$6.39^{k,l}$**	**± 0.28**	**$9.87^{h,i}$**	**± 0.19**	**15.59^{f}**	**± 0.26**	**18.72^{e}**	**± 1.19**	**20.80^{d}**	**± 0.45**
HMF	0.44^{c}	± 0.00	0.40^{c}	± 0.02	0.47^{c}	± 0.11	0.67^{d}	± 0.01	$0.75^{a,b}$	± 0.03	0.82^{a}	± 0.01
F	1.25^{c}	± 0.03	1.14^{c}	± 0.01	1.21^{c}	± 0.18	1.25^{c}	± 0.02	1.50^{b}	± 0.02	1.73^{a}	± 0.02
Σ furfurals	**$1.69^{d,e}$**	**± 0.03**	**$1.54^{e,f}$**	**± 0.04**	**$1.68^{d,e}$**	**± 0.29**	**$1.92^{c,d}$**	**± 0.03**	**$2.25^{a,b}$**	**± 0.04**	**2.55^{a}**	**± 0.02**
Syringaldehyde	0.67^{j}	± 0.02	1.34^{i}	± 0.01	1.81^{h}	±0.01	2.06^{g}	± 0.02	$1.83^{g,h}$	± 0.01	$2.11^{f,g}$	± 0.04
Coniferyl aldehyde	$0.84^{f,g,h,i}$	± 0.03	$1.16^{f,g}$	± 0.03	2.85^{c}	± 0.10	$2.24^{d,e}$	± 0.51	2.07^{e}	± 0.29	$2.70^{c,d}$	± 0.03
Sinapaldehyde	3.12^{i}	± 0.04	$6.09^{h,i}$	± 0.04	$6.92^{h,i}$	± 0.13	8.56^{i}	± 1.02	$9.71^{h,i}$	± 0.12	$10.28^{g,h}$	± 0.14
Σ oak aldehydes	**4.63^{k}**	**± 0.03**	**8.59^{i}**	**± 0.08**	**11.58^{h}**	**± 0.24**	**12.86^{g}**	**± 0.49**	**13.62^{f}**	**± 0.40**	**15.09^{e}**	**± 0.13**

C – high toasted oak chips

	Time (days)											
Compound	**1**		**7**		**14**		**21**		**28**		**35**	
Gallic acid	0.58^{l}	± 0.03	$0.79^{k,l}$	± 0.04	$1.27^{j,k,l}$	± 0.01	$1.41^{j,k,l}$	± 0.01	$1.91^{h,i,j}$	± 0.01	$2.31^{g,h}$	± 0.15
Ferulic acid	$0.58^{c,d}$	± 0.05	0.78^{c}	± 0.01	1.11^{b}	± 0.10	1.83^{a}	± 0.00	1.32^{b}	± 0.10	1.59^{a}	± 0.05
Caffeic acid	$0.28^{g,h,i}$	± 0.07	$0.18^{h,i}$	± 0.02	$0.46^{e,f}$	± 0.11	0.71^{c}	± 0.01	1.54^{b}	± 0.03	1.97^{a}	± 0.07
Sinapic acid	$0.51^{g,h,i}$	± 0.06	$0.68^{e,f,g}$	± 0.02	0.99^{d}	± 0.13	2.13^{b}	± 0.05	2.60^{e}	± 0.01	1.57^{c}	± 0.14
Ellagic acid	$3.76^{h,i}$	± 0.24	$4.69^{f,g,h}$	± 0.39	8.96^{e}	± 0.44	11.34^{d}	± 0.20	24.59^{a}	± 0.65	26.20^{a}	± 0.12
Σ phenolic acids	$5.71^{k,l}$	± 0.03	$7.13^{j,k}$	± 0.30	12.79^{g}	± 0.09	$17.42^{e,f}$	± 0.23	31.95^{a}	± 0.70	33.65^{a}	± 0.29
HMF	$0.10^{d,e}$	± 0.01	$0.10^{d,e}$	± 0.02	0.16^{d}	± 0.00	0.43^{c}	± 0.02	0.44^{c}	± 0.02	0.17^{d}	±0.03
F	1.08^{c}	± 0.06	1.11^{c}	±0.03	1.15^{c}	± 0.04	1.23^{c}	± 0.01	$1.60^{a,b}$	± 0.02	1.21^{c}	± 0.05
Σ furfurals	1.18^{g}	± 0.07	1.20^{g}	± 0.05	$1.31^{f,g}$	± 0.04	$1.66^{d,e}$	± 0.03	$2.04^{b,c}$	± 0.03	$1.39^{e,f,g}$	± 0.02
Syringaldehyde	2.16^{f}	± 0.03	3.63^{e}	± 0.06	4.56^{d}	± 0.02	4.82^{c}	± 0.03	5.86^{b}	± 0.01	6.18^{a}	± 0.04
Coniferyl aldehyde	1.24^{f}	± 0.04	2.06^{e}	± 0.03	3.64^{b}	± 0.00	3.58^{b}	± 0.00	4.10^{b}	± 0.06	5.80^{a}	± 0.06
Sinapaldehyde	4.28^{f}	± 0.08	8.25^{d}	± 0.05	9.94^{c}	± 0.01	11.40^{b}	± 0.05	14.34^{a}	± 0.02	13.61^{a}	± 0.03
Σ oak aldehydes	7.68^{l}	± 0.15	13.94^{j}	± 0.08	18.15^{d}	± 0.03	19.81^{c}	± 0.01	24.30^{b}	± 0.10	25.59^{a}	± 0.12

11.3 Ellagitannins

Analysis of the MWSs by HPLC HPLC-ESI-MS/MS revealed the presence of several ellagitannins (**Figure 11.12**), identified according to their retention times and *m/z* ratio (**Figure 11.13 a-b-c-d**) compared to standards.

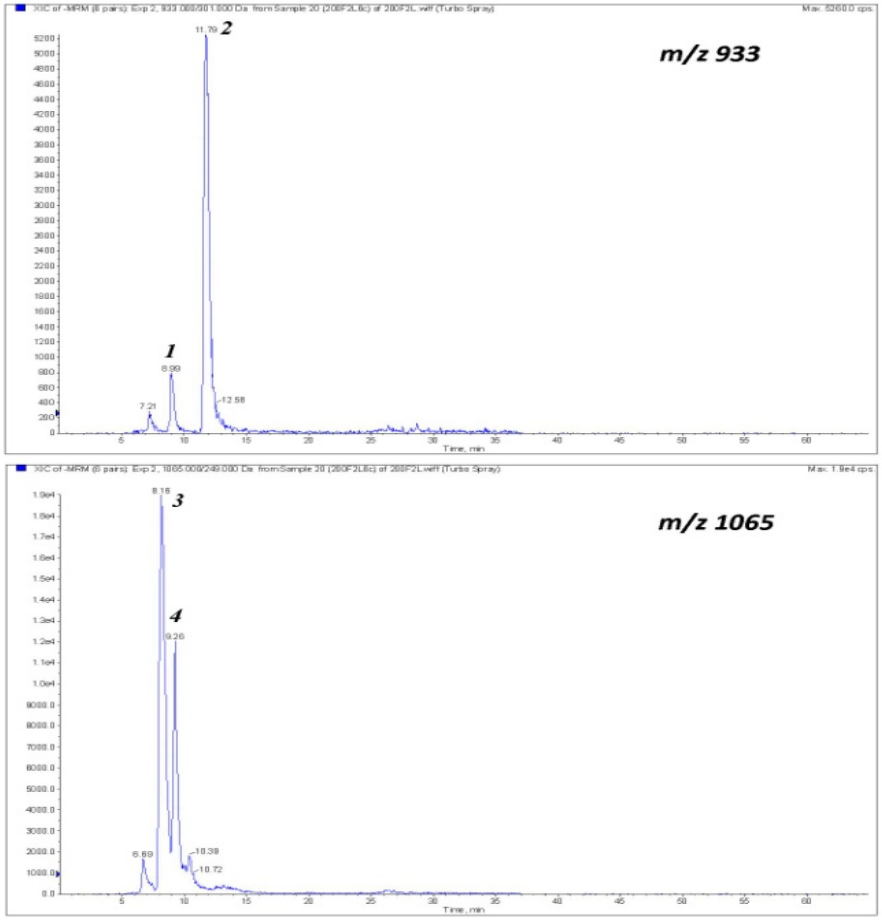

Figure 11.12. *EIC (extracted ion current chromatogram) of the signals at m/z 933 and m/z 1065 in reference model wine solution aged for 35 days with light toasted oak chips. (1), vescalagin; (2), castalagin; (3), grandinin; (4), roburin E.*

In accordance with mass spectra data in **Table 11.7**, vescalagin and castalagin showed a deprotonated molecule at *m/z* 933 and also gave ions at *m/z* 631 and 301 due to the loss of ellagic acid residue. The difference between their MS was provided by the ions at *m/z* 915 and 613, corresponding to $[M\text{-}H\text{-}H_2O]^-$ and $[M\text{-}H\text{-ellagic acid-}H_2O]^-$. This type of water loss in ESI-MS is characteristics of vescalagin-type ellagitannins with the C-1 OH group of the glucose at the R_1 position and not as R_2, as in castalagin-type ellagitannins (Quideau et al., 2005, Moilanen & Salminen, 2008). The quasimolecular ion of grandinin and roburin E was *m/z* 1065 and both gave an intense ion at *m/z* 301 $[\text{ellagic acid H}]^-$

The results of the ellagitannin content in reference MWSs (0F2L, 0F2M and 0F2F) containing smallest oak chips (2 mm) are showed in **Figure 11.14**. Significant differences in the total contents of *C*-glycosidic ellagitannins were found for the toasting levels light (L), medium (M) and high (F). This is in accordance with other studies (Cadahía et al., 2001), which described that toasting implies a decrease in ellagitannin contents. It is also reported that this decrease would contribute to diminish the sensation of bitterness and astringency of wines (Chira & Tesseidre, 2013). In the model wine solutions that contained the smallest oak chips (2 mm), the highest levels of ellagitannins were detected in that in contact with light-toasted chips (0F2L). In this case, the total concentration of *C*-glycosidic ellagitannins increased to

reach maximum value of 41.76 mg/L after 7 days, and then ellagitannins content decreased over time to reach the value of 25.78 mg/L after 35 days. In samples that contained medium-toasted oak chips (0F2M), the maximum concentration of total ellagitannins (18.89 mg/L) was reached after 21 days.

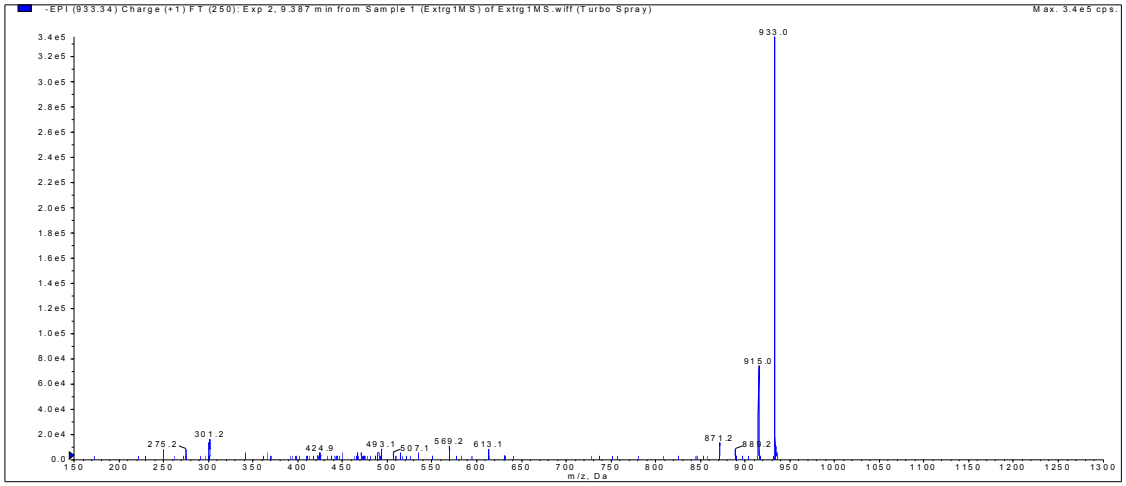

***Figure 11.13.a**. Mass spectra of the vescalagin.*

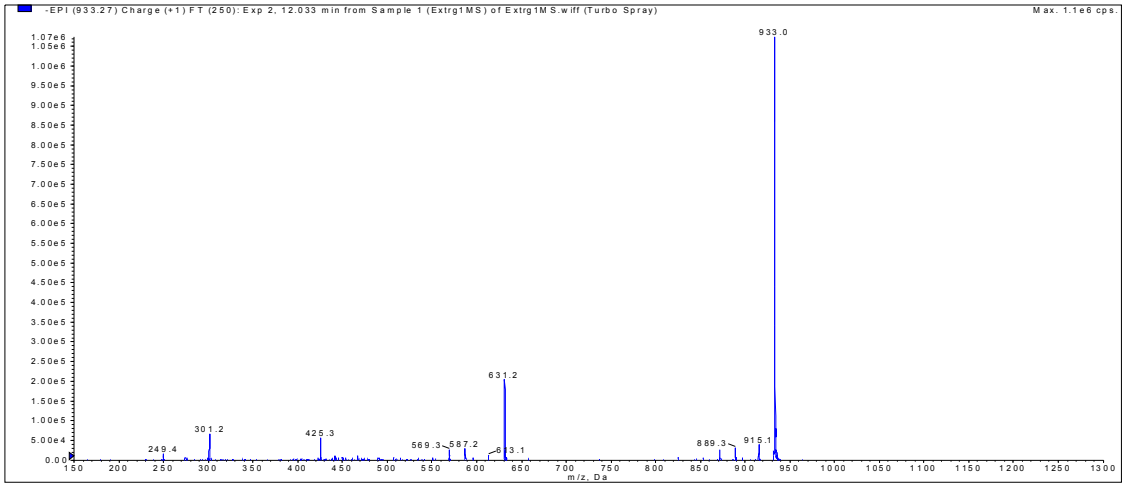

***Figure 11.13.b.** Mass spectra of the castalagin.*

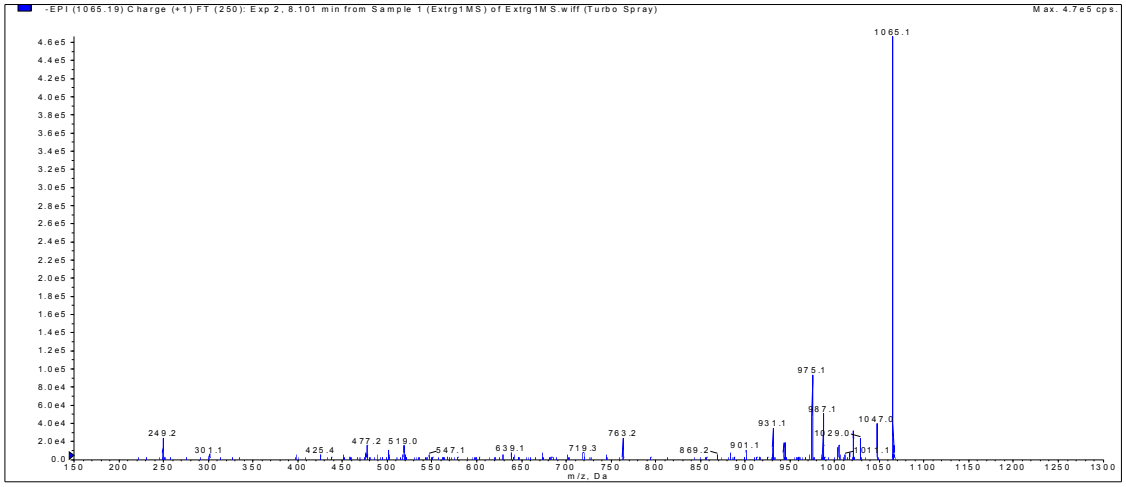

***Figure 11.13.c.** Mass spectra of the grandinin.*

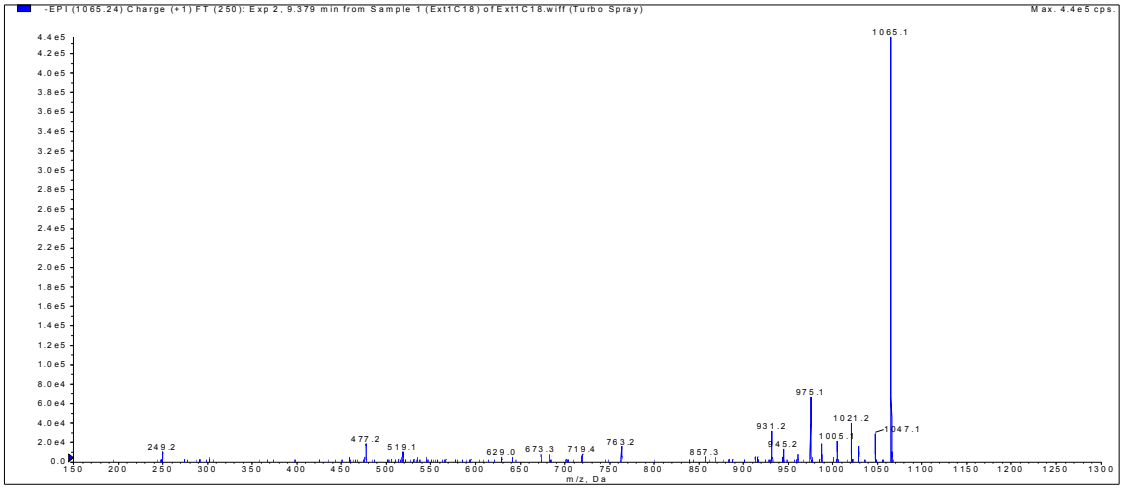

Figure 11.13.d. *Mass spectra of the roburin E.*

Table 11.7. *Spectrometric data of C-glycosidic ellagitannins and acutissi,ins A and B.*

Compound	**Negative ions *m/z***
Vescalagin	933 [M-H]$^-$; 915 [M-H-H_2O]$^-$; 631 [M-H-ellagic acid]$^-$; 613 [M-H-ellagic acid-H_2O]$^-$; 301 [ellagic acid-H]$^-$
Castalagin	933 [M-H]$^-$; 915 [M-H-H_2O]$^-$; 631 [M-H-ellagic acid]$^-$; 301 [ellagic acid-H]$^-$
Roburin E	1065 [M-H]$^-$; 915 [vescalagin-H2O-H]$^-$; 301 [ellagic acid-H]$^-$
Grandinin	1065 [M-H]$^-$; 301 [ellagic acid-H]$^-$
Acutissimin A/B	1205 [M-H]$^-$; 915 [vescalagin-H2O-H]$^-$; 613 [M-H-ellagic acid-H_2O]$^-$; 301 [ellagic acid-H]$^-$

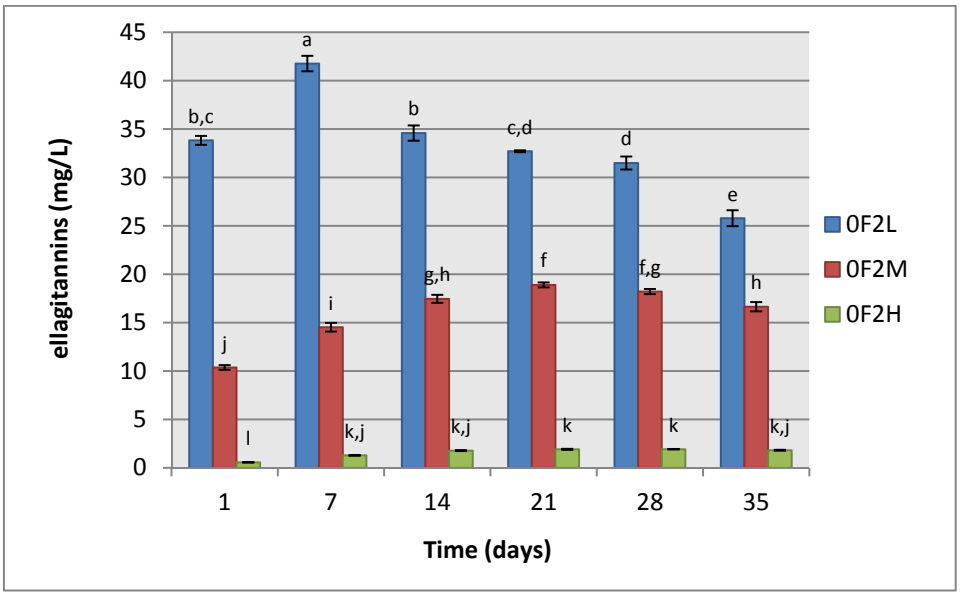

Figure 11.14. *Concentration of the total ellagitannins (mg/L) in reference model wine solutions during accelerated aging with 2 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences (p > 0.05; n=3).*

After that, a decrease in the ellagitannin content was also observed. The lowest content of *C*-glycosidic ellagitannins was detected in MWSs in contact with high-toasted oak chips (0F2F), and the maximum

concentration in that sample (1.92 mg/L) was reached later (after 28 days) than in the other MWSs studied. In all samples, castalagin was the *C*-glycosidic ellagitannin most representative (**Figure 11.15**) representing percentages of 49.99%, 55.83% and 67.88% in 0F2L, 0F2M and 0F2F samples, respectively, followed by vescalagin (26.14%, 36.88% and 30.85% in 0F2L, 0F2M and 0F2F, respectively) Roburin E represented 4.98%, 1.99% and 1.26% in 0F2L, 0F2M and 0F2H samples, respectively whereas grandinin was only detected in 0F2L and 0F2M samples although in higher percentages than roburin E (18.88% and 5.30%, respectively)

Regarding the MWSs containing the largest oak chips (8 mm) it was observed that in those in contact with light-toasted chips (0F8L) the total concentration of *C*-glycosidic ellagitannins did not increase regularly and two maximum contents (29.64 mg/L after 7 days and of 30.16 mg/L after 35 days) were observed (**Figure 11.16**). In samples 0F8M total ellagitannins content increased regularly up to 21 days reaching at this point the maximum concentration (15.85 mg/L). In MWS in contact with high-toasted oak chips (0F8F) the maximum concentration of 8.32mg/L was detected after 28 days . In the case of MWS in contact with the largest oak chips (samples 0F8L, 0F8M and 0F8F) castalagin was also the most abundant ellagitannin (**Figure 11.17**), representing 51.80%, 64.66% and 63.77%, respectively, followed by vescalagin (34.92%, 29.96% and 32.78% in 0F8L, 0F8M and 0F8F, respectively), grandinin (10.57%, 3.81% and 2.35% in 0F8L, 0F8M and 0F8F, respectively) and roburin E (2.69%, 1.54% and 1.08% in 0F8L, 0F8M and 0F8F, respectively).

So, in the case of the MWSs with the smallest chips (2 mm), higher differences between L and M samples in the elagitannin content were observed in the first sampling points than in the last ones. Toasting process provokes not only a decrease in the ellagitannin levels of oak wood, but also important changes in the oak structure (Hale et al., 1999). These changes imply the cracks of the cell walls of the oak wood, making easier the permeation of the hydroalcoholic solution through the wood and the extraction of ellagitannins from the deeper layers of the oak wood. Thus, in the case of the ligh-toasted oak chips, the ellagitannins from the most superficial layers can be easily extracted making possible to reach high levels of ellagitannin earlier. However, the extraction from the deeper layer is more limited and at the end of the study or probably the ellagitannin evolution rate (oxidation, hydrolysis, etc.) may be faster than the extraction rate. In the case of the medium-toasted oak chips, the extraction from deeper layers could compensate the lower contents from the outermost ones (Jourdes et al., 2011). All this may explain why the differences on the ellagitannin content between the MWS in contact with L chips and those in contact with M chips were reduced at the end of the study. This could not be observed for the MWS in contact with the high-toasted oak chips since, due to their small size, toasting have probably affected the whole chip and even the ellagitannin from the deepest layers could have been degraded by toasting. As a result, a limited extraction of ellagitannins from these oak chips is achieved and, thereby, the amounts of ellagitannin determined in samples 0F2F were very low during all the study. Thus, the size of oak chips can be crucial depending on the toasting degree, and high temperatures or long times of toasting should not be suitable when the oak chip size is small.

Comparing the samples containing oak chips with the same toasting level and different size, it can be observed that for L and M toasting levels, the highest content of ellagitannins was observed for the MWS in contact with the smallest oak chips. However, for the toasting level F, the highest concentrations were found in the MWSs in contact with the largest oak chips. This can be explained since the higher size can make that toasting could not harm the deeper layers of wood making then possible the extraction of the ellagitannins from these layers.

The evolution of the ellagitannin content was different depending on the oak chip size. While the total content of ellagitannins increased regularly during 35 days in 0F2L MWSs, in the solution in contact with larger oak chips (0F8L samples) the total content of *C*-glycosidic ellagitannins increased during 7 days and then the concentration slightly decrease and other maximum value was observed after 35 days . It is possible that for 2 mm oak chips the hydroalcoholic solution rapidly permeated in the middle of the oak chips so the extraction of the ellagitannins was exhaustive. On the contrary, in the case of the 8 mm oak chips the extraction of ellagitannins present on the surface occurred during the first 7 days while in the subsequent days, hydroalcoholic solutions deeply infiltrated the primary and the secondary xylem vessels of the wood structure. So another maximum concentration was observed. Therefore, the dimension of oak chips affected the extraction of the ellagitannins: a greater total surface area of chips favored the extraction of *C*-glycosidic ellagitannins mainly during the first period of contact with the MWSs, whereas the extraction from the largest oak chips involved not only the surface layers, but also the deepest ones resulting in more homogeneous ellagitannin levels during all the study.

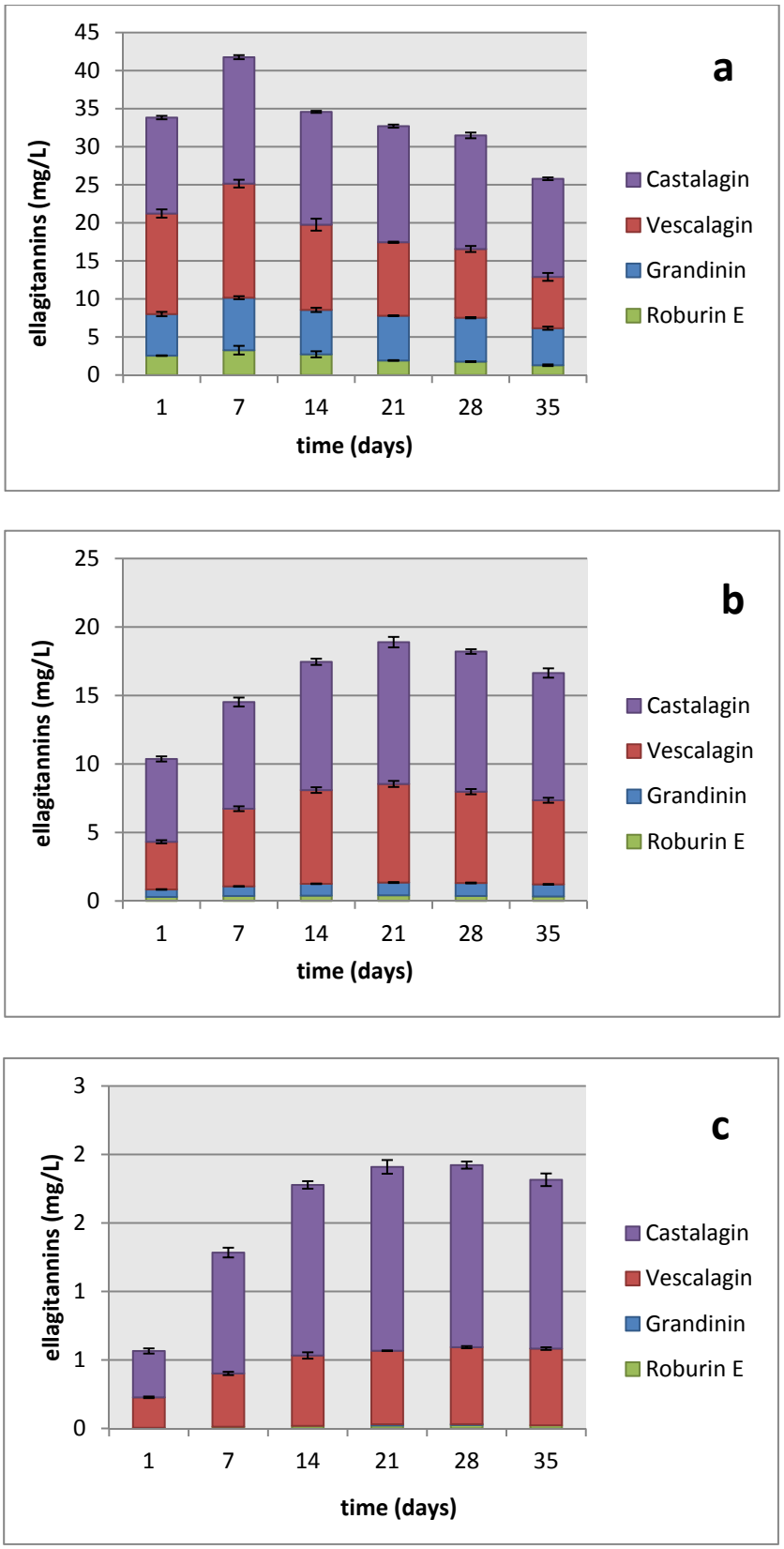

Figure 11.15. *Quantitative evaluation of individual C-glycosidic ellagitannins (mg/L) in model wine solutions in contact with 2 mm oak chips - (a), medium (b) and high (c). The error bars show the ±SD values.*

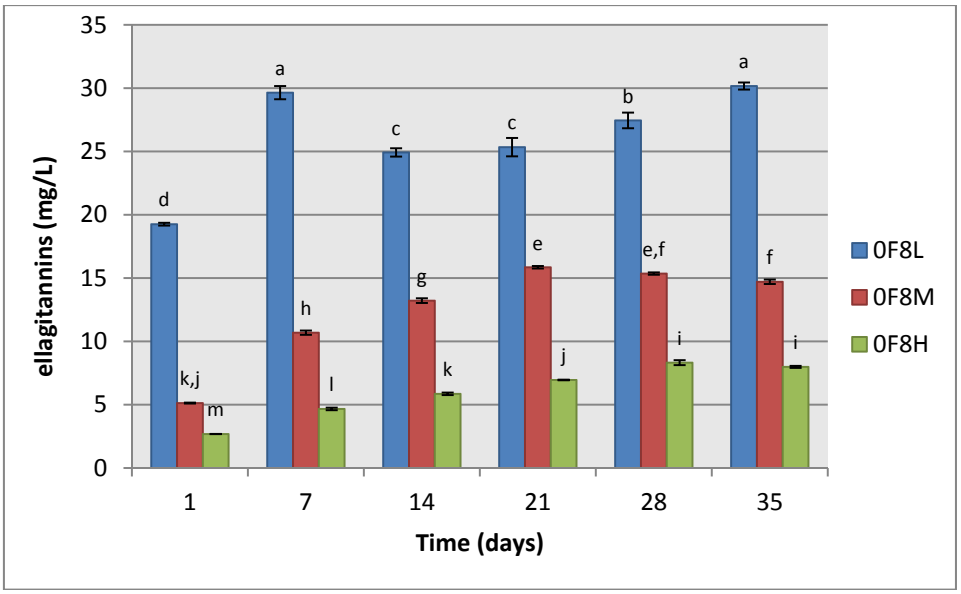

Figure 11.16. *Concentration of the total ellagitannins (mg/L) in reference model wine solutions during accelerated aging with 8 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences ($p > 0.05$; $n=3$).*

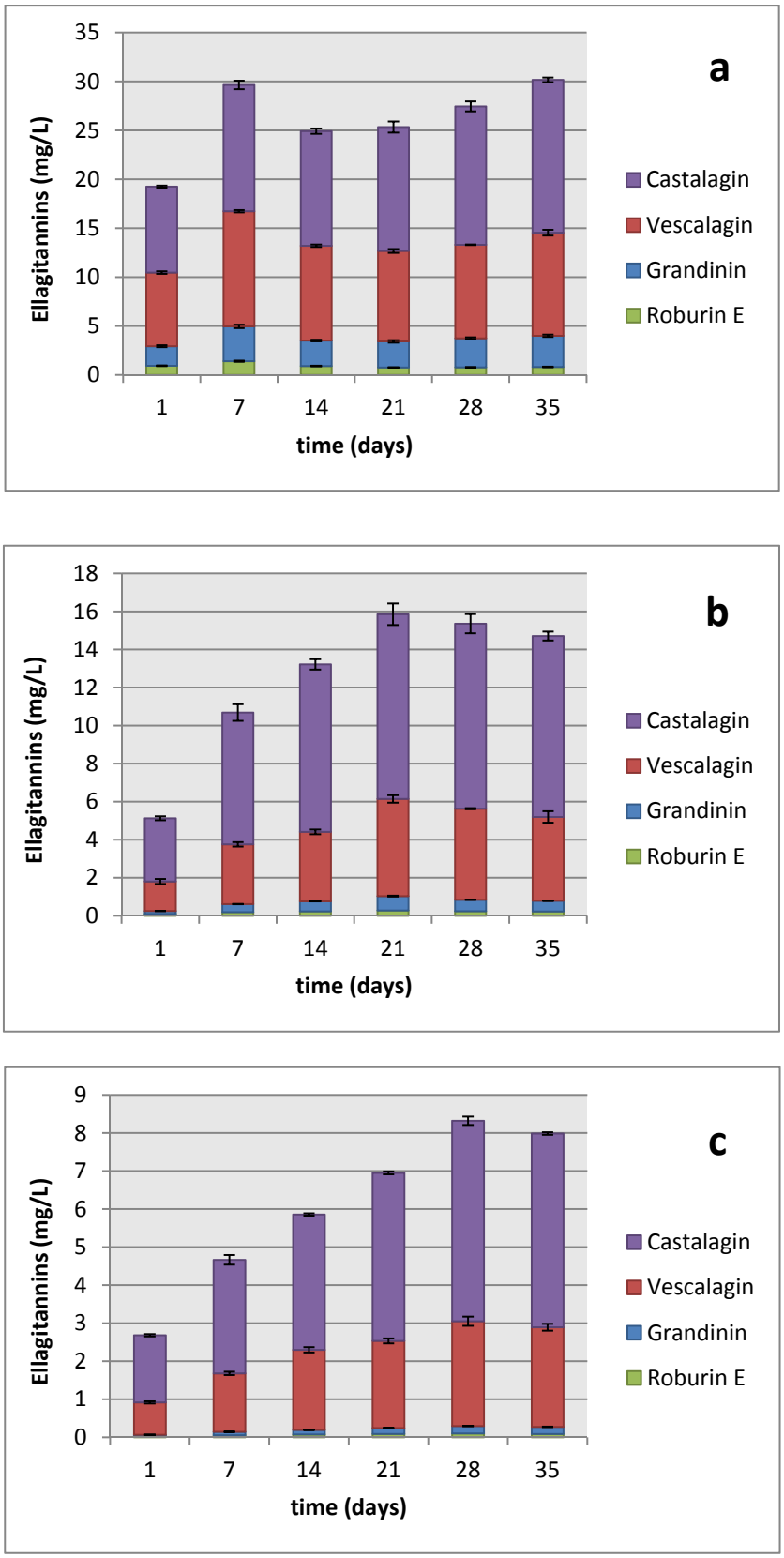

Figure 11.17. *Quantitative evaluation of individual C-glycosidic ellagitannins (mg/L) in model wine solutions in contact with 8 mm oak chips - light (a), medium (b) and high (c). The error bars show the ±SD values.*

Regarding the individual composition, it can be seen that castalagin, which is the major ellagitannin in the oak wood, is also the major ellagitannin in the MWS, followed by vescalagin. Castalagin also seemed to be the most stable ellagitannin since its percentage increased at the end of the study in all the MWS studied, due to a higher rate of disappearance of the other ellagitannins. This is in agreement with other studies pointing out that castalagin is more stable and less reactive than other ellagitannins such as vescalagin (Jourdes et al., 2008; Quideau et al., 2010).
In relation to the proportions of ellagitannins in the solutions, they are conditioned by the level of toasting. Grandinin and roburin E were the ellagitannins most susceptible to heat treatments respect to other monomeric ellagitannins. Previous studies carried out in our laboratory (data not shown) suggested lower stability of grandinin and roburin E in relation to vescalagin and castalagin, in view of their faster decrease during wine aging. Moreover, the percentages represented by grandinin and roburin E are higher in the MWSs in contact with the smallest oak chips than in the corresponding MWSs in contact with the highest oak chips, excepting for the high-toasted ones for the reasons explained above. The lower percentages of grandinin and roburin E in the MWSs in contact with the largest oak chips may be related to a lower extractability of these ellagitannins in relation to castalagin and vescalagin. This has also been observed in previous studies carried out in our laboratory (data not shown) but further studies are necessary to confirm their lower extractability. In addition, the lower surface-to-volume ratio of the 8 mm oak chips might contribute to the lower extraction of grandinin and roburin E in the MWS in contact with these chips.
In the samples containing 50 mg/L and 200 mg/L of (+)-catechin the contents and the evolution of ellagitannins are similar to those observed in the corresponding MWSs without catechin, and no significant differences were found as a consequence of the presence of catechin in the media (data not shown).

11.3 (+)-catechin

In **Figure 11.18** the evolution of (+) catechin in MWSs containing 50 mg/L and light, medium and high toasted oak chips during rapid aging (35 days) was showed. As can be seen, (+)-catechin decrease during aging, particularly in 0F2F solution. After 35 days, the content of the flavanol in 0F2L and 0F2M solutions was of 44.81 mg/L and 38.76 mg/L, while in 0F2F MWS was of 38.12 mg/L.
In MWSs containing the highest concentration of (+)-catechin (200 mg/L) and 2 mm oak chips, after 35 days (+)-catechin content was of 187.52 mg/L, 167.09 mg/L and 163.68 mg/L in 200F2L, 200F2M and 200F2F solutions, respectively (**Figure 11.19**).
In the case of the MWSs containing 50 mg/L of (+)-catechin and 8 mm oak chips, the lowest content of the flavanol was found in 50F8L solution (29.17 mg/L after 35 days), while in 50F8M and 50F8F solutions it was of 30.19 mg/L and 29.26 mg/L, respectively (**Figure 11.20**).
In MWSs with 200 mg/L of (+)-catechin and 8 mm oak chips, after 35 days was reached a concentration of 187.52 mg/L, 167.09mg/L and 163.68 mg/L in 200F8L, 200F8Mand 200F8F solutions, respectively (**Figure 11.21**).
Polymerization and condensation of the (+)-catechin increase during rapid aging was in agreement with the decrease of monomeric flavanol observed, and recently the formation of new red/orange catechinpyrylium-derived pigment has been shown from the direct reaction between (+)-catechin and sinapaldehyde. Analogous compounds can be expected to form through the reaction of oak-derived aldehydes with one or several units of (+)-catechin (Sousa et al., 2005). Moreover, the C-glycosidic ellagitannins vescalagin that dissolved in the slightly acidic solution can react with nucleophilic entities, such as (+)-catechin, to generate the flavano-ellagitannins acutissimins A and B. The possible reactions described above explain the decrease of (+)-catechin in MWSs.

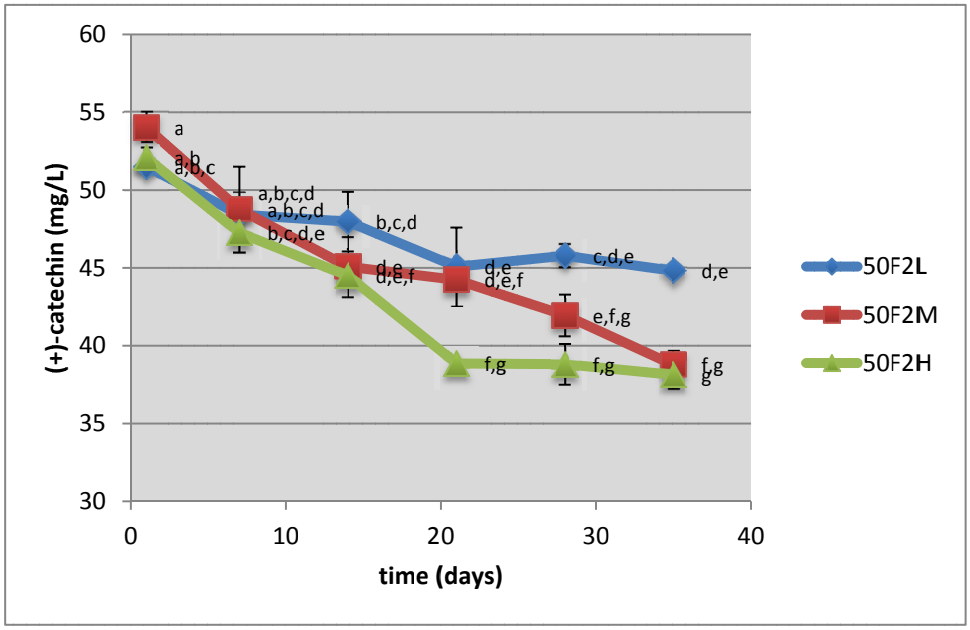

Figure 11.18. *Concentration of the (+)-catechin in model wine solutions containing 50 mg/L of (+)-catechin. during accelerated aging with 2 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences (p > 0.05; n=3).*

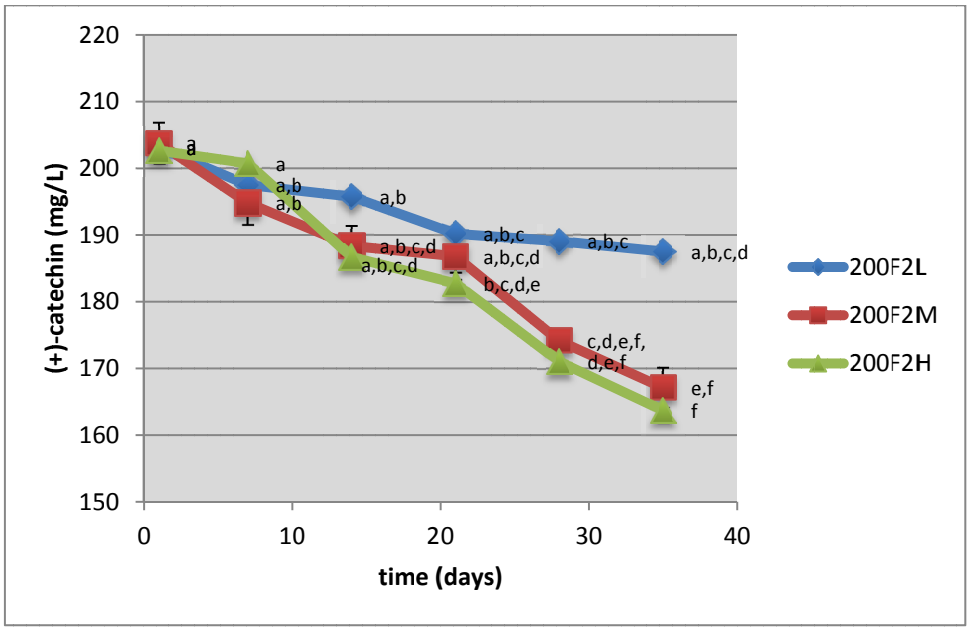

Figure 11.19. *Concentration of the (+)-catechin in model wine solutions containing 200 mg/L of (+)-catechin. during accelerated aging with 2 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences (p > 0.05; n=3).*

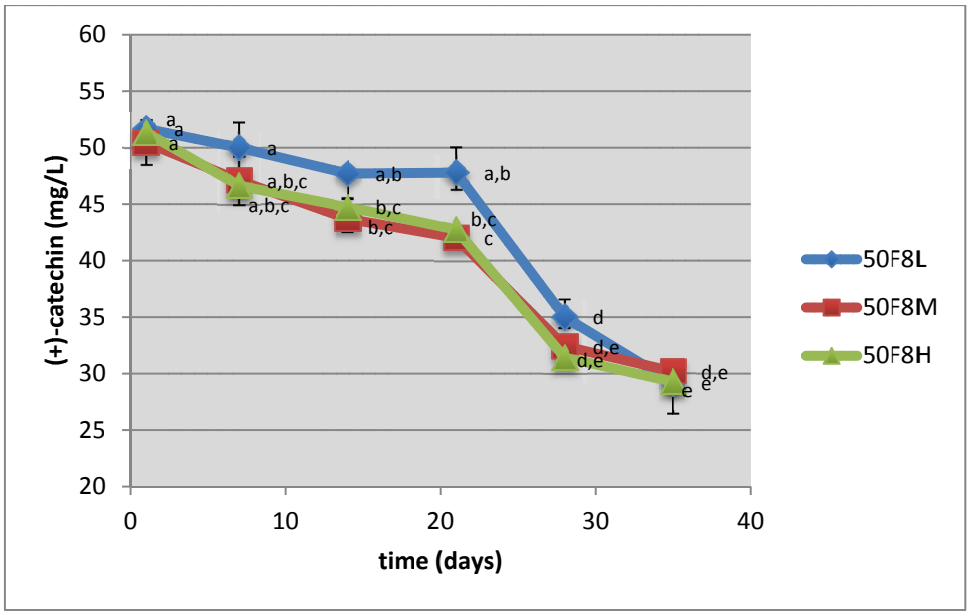

Figure 11.20. *Concentration of the (+)-catechin in model wine solutions containing 50 mg/L of (+)-catechin. during accelerated aging with 8 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences ($p > 0.05$; $n=3$).*

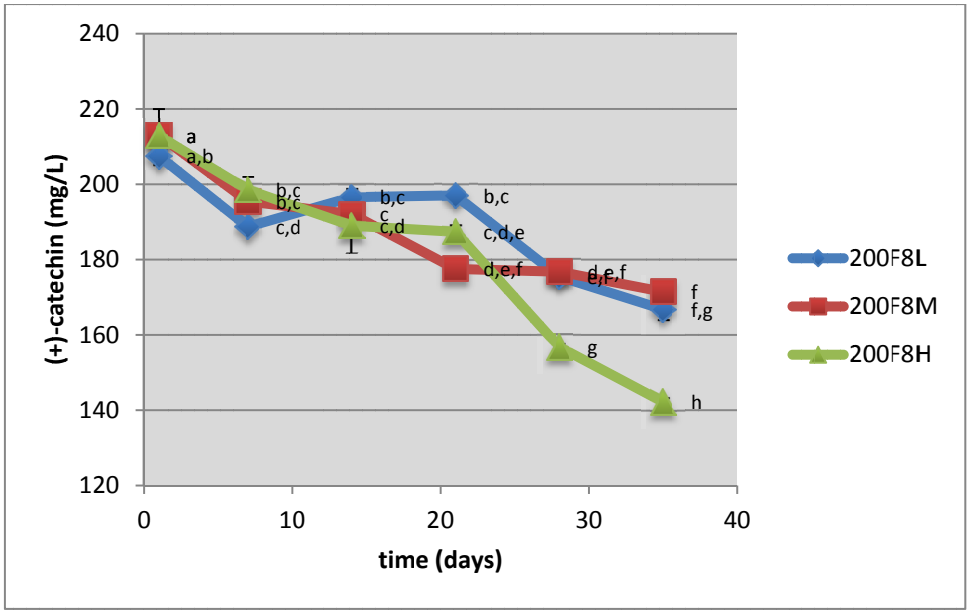

Figure 11.21. *Concentration of the (+)-catechin in model wine solutions containing 200 mg/L of (+)-catechin. during accelerated aging with 8 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences ($p > 0.05$; $n=3$).*

11.4 Acutissimins A and B

In MWSs containing (+)-catechin, the formation of flavano-ellagitannins could be monitored. Acutissimins A and B showed a deprotonated molecule at *m/z* 1205 and also gave characteristic fragments at *m/z* 915 (loss of the substituent at C-1 of the vescalagin-derived core structure), 613 (loss of the 4,6-hexahydroxybiphenoyl unit from the latter fragment), and 301 (loss of ellagic acid) (**Figure 11.22 a-b**).

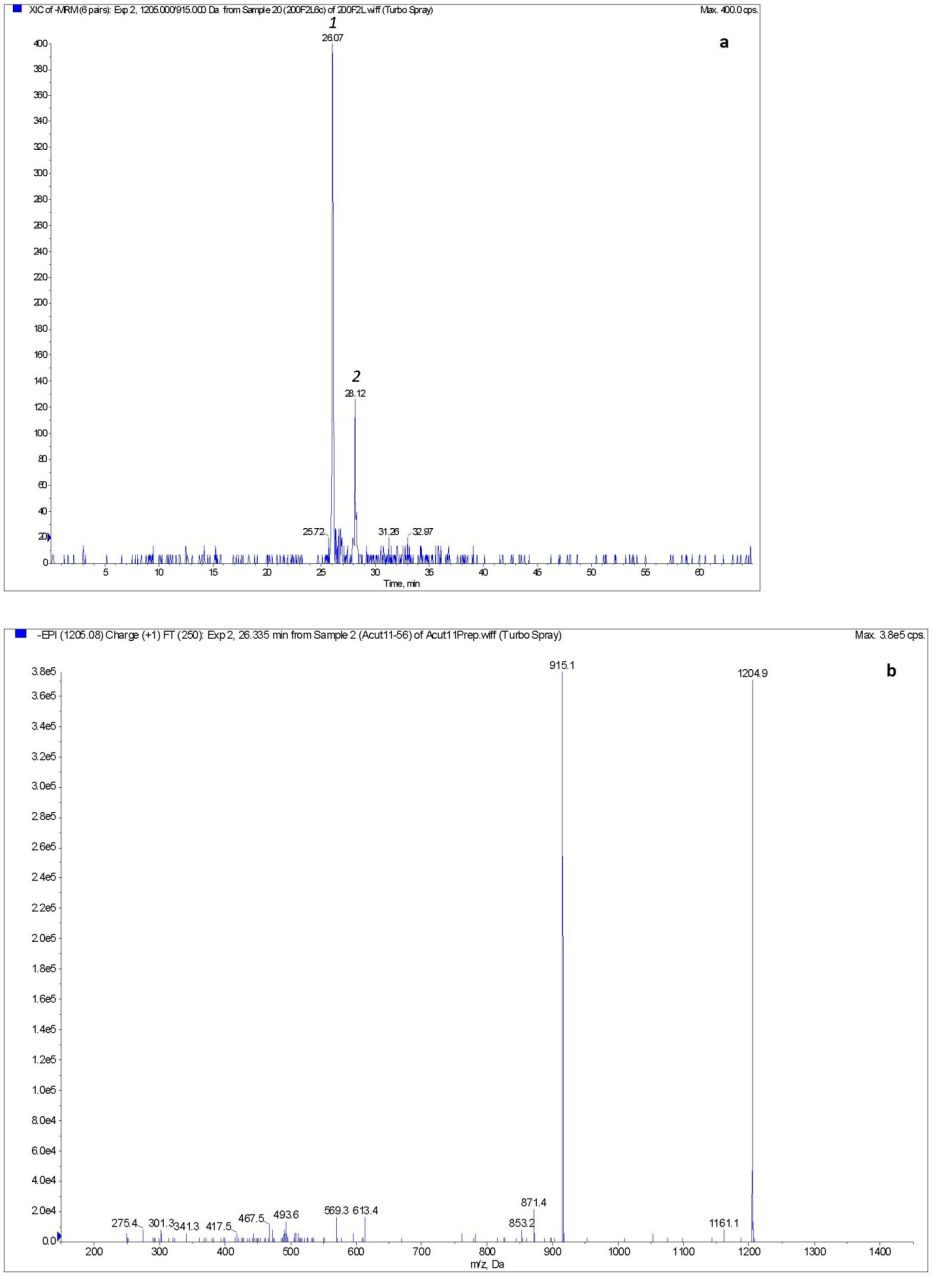

Figure 11.22. *EIC (extracted ion current chromatogram) of the signals at m/z 1205 in model wine solution containing 200 m/L of (+)-catechin and light toasted oak chips. (1), acutissimin A; (2), acutissimin B (a). Mass spectra of the acutissimins A and B (b).*

A gradual formation of acutissimins A (**Table 11.8**) and B (**Table 11.9**) was observed. The highest amounts of acutissimin A and B were detected in the MWSs containing 200 mg/L of (+)-catechin that were in contact with the light-toasted oak chips (samples 200F2L and 200F8L).

Table 11.8. *Concentration of acutissimin A (mg/L) in model wine solutions containing (+)-catechin during accelerated aging with oak chips. Different lower case letters indicate significant differences (p>0.05, n=3). .n.d. not detected.*

Sample	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
50F2L	$0.17 \pm 0.001^{a.b.c.d}$	0.46 ± 0.01^{e}	0.53 ± 0.01^{f}	$0.62 \pm 0.01^{g.h}$	$0.74 \pm 0.02^{i.j}$	$0.82 \pm 0.02^{k.l}$
50F2M	n.d.	n.d.	$0.11 \pm 0.002^{a.m}$	$0.19 \pm 0.01^{b.c.d.n}$	$0.26 \pm 0.003^{p.q.o}$	$0.33 \pm 0.02^{o.r.s}$
50F8L	n.d.	$0.21 \pm 0.01^{c.d.n.p}$	$0.29 \pm 0.01^{o.q.r}$	$0.39 \pm 0.02^{e.s.t}$	$0.57 \pm 0.02^{f.g}$	$0.66 \pm 0.03^{h.u}$
50F8M	n.d.	n.d.	0.09 ± 0.03^{m}	$0.14 \pm 0.01^{a.b.m}$	$0.16 \pm 0.01^{a.b.c.m}$	$0.19 \pm 0.002^{b.c.d.n}$
200F2L	$0.34 \pm 0.01^{r.s}$	$0.78 \pm 0.004^{i.k}$	0.87 ± 0.03^{l}	1.19 ± 0.06^{v}	$1.25 \pm 0.03^{v.w}$	1.28 ± 0.05^{w}
200F2M	n.d.	$0.18 \pm 0.01^{a.b.c.d.n}$	$0.23 \pm 0.01^{d.n.p.q}$	$0.36 \pm 0.02^{r.s.t}$	$0.42 \pm 0.02^{e.t}$	0.55 ± 0.03^{f}
200F8L	$0.15 \pm 0.01^{a.b.c.m}$	$0.55 \pm 0.02^{f.g}$	$0.70 \pm 0.02^{j.u}$	1.00 ± 0.03^{x}	$1.03 \pm 0.04^{x.y}$	1.10 ± 0.04^{y}
200F8M	n.d.	$0.16 \pm 0.01^{a.b.c}$	$0.19 \pm 0.01^{b.c.d.n}$	$0.25 \pm 0.01^{n.p.q}$	$0.29 \pm 0.01^{o.q.r}$	$0.42 \pm 0.02^{e.t}$
200F8F	n.d.	n.d.	$0.15 \pm 0.00^{a.b.c.m}$	$0.21 \pm 0.01^{c.d.n.p}$	$0.25 \pm 0.01^{n.p.q}$	$0.38 \pm 0.01^{s.t}$

Table 11.9. *Concentration of acutissimin B (mg/L) in model wine solutions containing (+)-catechin during accelerated aging with oak chips. Different lower case letters indicate significant differences (p>0.05, n=3). .n.d. not detected.*

	Concentration of acutissimin B (mg/L)					
Sample	**Day 1**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**
50F2L	n.d.	$0.13 \pm 0.00^{a,b}$	$0.16 \pm 0.01^{c,d,e,f}$	$0.19 \pm 0.01^{g,h,i,j}$	$0.22 \pm 0.01^{k,l}$	$0.23 \pm 0.00^{k,l}$
50F2M	n.d.	n.d.	n.d.	n.d.	$0.11 \pm 0.00^{a.m.n}$	$0.13 \pm 0.01^{a,b}$
50F8L	n.d.	n.d.	$0.12 \pm 0.01^{a,m}$	$0.15 \pm 0.01^{b,c,d,e}$	$0.18 \pm 0.04^{f,g,h,i}$	0.22 ± 0.00^{o}
50F8M	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
200F2L	$0.13 \pm 0.00^{a,b}$	$0.22 \pm 0.01^{k,l}$	0.26 ± 0.00^{p}	0.28 ± 0.02^{p}	0.32 ± 0.00^{q}	0.28 ± 0.01^{p}
200F2M	n.d.	n.d.	$0.10 \pm 0.01^{m,n}$	$0.13 \pm 0.00^{a,b}$	$0.19 \pm 0.01^{h,i,j,o}$	$0.24 \pm 0.01^{k,l}$
200F8L	n.d.	$0.17 \pm 0.01^{e,f,g,h}$	$0.21 \pm 0.01^{i,j,o,k}$	$0.21 \pm 0.01^{j,o,k}$	0.24 ± 0.01^{l}	$0.22 \pm 0.01^{k,l}$
200F8M	n.d.	n.d.	0.09 ± 0.01^{n}	$0.12 \pm 0.01^{a,m}$	$0.15 \pm 0.01^{b,c,d}$	$0.17 \pm 0.01^{d,e,f,g}$
200F8F	n.d.	n.d.	n.d.	$0.09 \pm 0.01^{m,n}$	$0.11 \pm 0.01^{a,m}$	$0.14 \pm 0.00^{a,b,c}$

In these samples, the highest concentration of acutissimin A (1.28 mg/L and 1.10 mg/L, respectively) was reached after 35 days and that of acutissimin B after 28 days (0.32 mg/L and 0.24 mg/L, respectively for samples 200F2L and 200F8L). In the MWSs containing 50 mg/L of (+)-catechin, acutissimins A and B were also detected although in lower levels. The highest levels were also found in the samples that were in contact with light-toasted oak chips (samples 50F2L and 50F8L) and were also reached after 35 days (0.82 mg/L and 0.66 mg/L, respectively, for acutissimin A and 0.23 mg/L and 0.22 mg/L for acutisimin B). In relation to the MWSs containing light-toasted oak chips, the MWSs containing medium-toasted oak chips showed much lower contents of acutissimin A and B and among them, the contents were always lower in the MWSs in contact with the largest oak chips. In the MWSs containing high-toasted oak chips, acutissimins A and B were not detected except for the set samples 200F8H where the content after 35 days was 0.37 mg/L and 0.13 mg/L, respectively.
The amount of (+)-catechin significantly affected the ellagitannins derivatives content, although, as it is said before, it did not affect the total ellagitannins content. It was also observed that toasting degree significantly affected acutissimins A and B concentrations since it affects the ellagitannin contents. Comparing the set of samples added with the same content of (+)-catechin and with oak chips of the same size but with different toasting degree, it was observed that the quantity of the two acutissimins was lower in solutions that contained medium-toasted oak chips as a consequence of the lower contents of vescalagin. In the MWSs in contact with light-toasted oak chips and containing 200 mg/L of (+)-catechin the formation of acutissimins was important at the earlier sampling points whereas in the last sampling points their levels were slightly stabilized, probably as a consequence of a less important formation rate or a higher evolution rate (oxidation, degradation, etc.) at the end of the study. However, in the MWSs in contact with the medium-toasted chips the formation of these derivative ellagitannins was more important at the end of the study, in accordance with the evolution of the ellagitannin levels. Acutissimin A and B were not detected in MWSs containing the high-toasted oak chips except for the set samples containing 200 mg/L of (+)-catechin and 8mm oak chips, where the vescalagin content was enough to allow the formation of these derivative ellagitannins at the end of the study. Thus, the toasting level of the oak chips may determine the time when the maximum levels of acutissimins are reached.
The oak chip size also affected the levels of acutissimins, since it affects the ellagitannin concentration in the media. In the MWSs with the same concentration of (+)-catechin and in contact with oak chips with the same toasting degree but with different size, the highest levels of acutissimins were always determined in the MWS in contact with the smallest chips. This can be explained by the higher levels of vescalagin in these MWSs.
The amounts of acutissimin A were much higher than the amounts of acutissimin B in accordance with the reactivity described for vescalagin and (+)-catechin (Quideau et al., 2005). Furthermore, the formation of the A-isomer was faster than of the B-isomer, since the former could be detected and quantified at earlier sampling points.

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12. Conclusion

The first topic explain the evolution of the oak phenols and of C-glycosidic ellagitannins concentration during rapid aging of oak chips in wine model solutions. Moreover, the formation of C-glycosidic ellagitannins derivatives was monitored. Effect of different features of oak chips (dimension and toasting degree) and of (+)-catechin content was observed. There are no study that try to correlate the formation of these compounds with the toasting degree of the oak or with the flavanol concentration.

During rapid aging of oak chips, different oak phenols were extracted by the hydroaloholic media. Gallic acid, ferulic acid, caffeic acid, sinapic acid, ellagic acid, HMF, F, syringaldehyde, coniferyl aldehyde and sinapaldehyde were the molecules identified and quantified. The highest content of furanic derivatives and oak aldehydes were found in set samples in contact with medium and toasted oak chips while highest content of gallic acid was found in samples containing light toasted oak chips. The greater surface (2 mm oak chips) favored the extraction of oak phenols in hydroalcoholic solutions.

Also the levels of total ellagitannins was significantly affected by the toasting degree of the oak chips. The highest contents of total ellagitannins were found in the set of samples in contact with light-toasted oak chips, and the maximum concentration was reached earlier for the solutions in contact with these chips. Grandinin and roburin E were the ellagitannins most susceptible to heat treatments. Thus, toasting could also determined the qualitative composition of ellagitannins. The dimension of oak chips also significantly affected the total content of ellagitannins. Generally a greater surface area favored the extraction of *C*-glycosidic ellagitannins and the highest contents were found in the set of samples in contact with the smallest oak chips. However, high-toasting levels can affect the whole chip if its size was too small degrading most of the ellagitannins. Thus, the size of oak chips can be crucial depending on the toasting degree, and high temperatures or long times of toasting should not be used when the oak chip size is small. Moreover, the size also affected the evolution of the ellagitannin contents and in the case of the largest oak chips with a light level of toasting a second maximum in the levels of ellagitannins could be observed as a consequence of the extraction of the ellagitannis from the deeper layers of the oak chip. Hence, taking into account the size and the toasting degree of the oak chips the levels and evolution of ellagitannin content could be modulated.

The concentration of (+)-catechin did not significantly affect the total concentration of oak phenols and of ellagitannins in the solutions. The formation of acutissimins A and B depends on both the ellagitannin and the (+)-catechin levels. The toasting degree and size of oak chips affected the formation of acutissimins since these factors affected the moment when the maximum ellagitannin concentration was reached. Thus, in the solutions in contact with light-toasted oak chips, the formation of acutissimins was more important in the earlier steps. Moreover, the formation of acutissimins was lower when larger oak chips were used.

13. Effect of size and toasting degree on the ellagitannin content and on acutissimin formation in "*Aglianico di Taurasi*" wine

Wine aging using oak barrels is a traditional practice to which quality wines are subjected. Nowadays, wine aging in stainless steel tanks with oak chips is an alternative practice to the use of oak barrels since one major cost associated with producing wine is the cost of the barrel in which it is fermented and or/aged. One way wineries are cutting costs, is using toasted oak chips in wine that is fermenting/aging in stainless steel tanks, which costs hundred less than the oak barrel option. It is reported that the combination of steel tanks and oak chips produces the same result at the end of the winemaking process (Jourdes et al., 2011),then the use of oak chips in a stainless steel tank may be very interesting.
Before 2006 in the wine producing emerging countries (Australia, New Zeland, California, Chile), the addition of wood chips in wine was already being permitted. As a result, these countries have conquered the mass market with cheap wines that are qualitatively poor. To improve the competitiveness of EU wine market, in 2006 the addition of oak chips in the wine was authorized in the EU countries by the Regulation (CE) No. 1507/2006. The traditional methods of winemaking are very strictly regulated, and the use of oak chips were approved provided that the production method be clearly labeled on the bottle of wine so that the consumer knows exactly how that wine eas made.
During aging in barrel, wine undergoes a series of transformations leading to important changes in aroma, color, taste, and astringency (Glabasnia & Hofmann, 2006; Puech et al., 1999; Sciancalepore, 2006; Vivas & Glories, 1996). This is due in part to a progressive extraction of wood compounds such as aldehydes, phenolic acids and *C*-glycosidic ellagitannins (Fernández de Simón et al., 2006). Among the *C*-glycosidic ellagitannins, castalagin and vescalagin are the two monomeric ellagitannins that largely predominate in oak wood representing 40%-60% of the ellagitannins, although lyxose/xylose derivatives (grandinin and roburin E) are also present in smaller percentages (Fernández de Simón et al., 1999 Hervé du Penhoat et al., 1991; Jourdes, et al., 2008; Masson et al., 1995). The C-glycosidic ellagitannin display the structural particularity of having a highly characteristic C-C linkage between the carbon-1 of their open chain glucose core and the carbon-2 of the O-2 galloyl unit of their 2,3,5-nonahydroxyterphenoyl unit (Jourdes et al., 2011). Their chemical structure enables these compounds to take part in oxidation reactions, acting as consumers of oxygen and causing, among other reactions, the transformation of ethanol into acetaldehyde (Vivas and Glories, 1996). The acetaldehyde can, in turn, be involved in polymerization reactions between flavanols and between flavanols and anthocyanins (Es-Safi et al., 1999; Francia-Aricha et al., 1997, Timberlake and Bridle, 1976; Vivas and Glories, 1996), affecting wine astringency and colour, respectively. Moreover, ellagitannins can react directly with other wine constituents. In particular, vescalagin is able to react with (epi)catechins via C-C linkage between the carbon-1 atom of the vescalagin moeity and either carbon-8 or -6 of the A ring of the (epi)catechin to form acutissimin A or B, respectively (Quideau et al., 2005). These compounds had interesting biological properties, Quideau et al. 2005 reported that acutissimin A is an inhibitor of human DNA topoisomerase II. Furthermore, ellagitannins could directly modify wine color because they can react with anthocyanins leading to anthocyano-ellagitannin hybrid pigments that show purple hues in accordance with the bathochromic shift observed in the absorption band of their visible spectra (Chassaing et al., 2010; Quideau et al., 2005). In literature, it has also been reported that ellagitannins can contribute to increase the astringency and the bitterness of wine aged in oak barrels or with oak chips due to their ability to precipitate salivary proteins (Glabasnia & Hofmann, 2006).
The quantification of ellagitannins and of flavano-ellagitannins derivatives has been studied only in French wines (Saucier et al., 2006; Jourdes et al., 2011; Jourdes et al., 2010) but there are no study that quantificate these compounds in Italian red wines.
The aim of this study was to monitor the extraction kinetics of the C-glycosidic ellagitannin and the formation of acutissimins A and B in an "*Aglianico di Taurasi*" red wine rapid aged in oak chips to compare the influence of oak chips toasting degree (light, medium and high) and dimension (2 mm and 8 mm). Moreover, ellagitannins and derivative C-glycosidic ellagitannin composition of red wines aged in oak barrel was investigated with the aim to identificate a marker useful to discriminate wines aged with oak chips or in oak barrel.

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14. Materials and methods

14.1 Chemicals

Vescalagin was isolated from commercial chestnut tannin provided by Laffort (Laffort oenologie, Bordeaux, France). Castalagin, roburin E and grandinin were isolated from non-toasted *Q. petraea* oak chips (See *Section 10.4*). Acutissimin A and B were obtained by hemisynthesis from vescalagin as it is described by Quideau and co-workers (Quideau et al., 2005) and purified by semi-preparative HPLC (See *Section 10.5*).

Others available reference compounds were obtained from commercial sources: (+)-catechin and (-)-gallocatechin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All of the solvents used were of analytical grade and were purchased from Prolabo (BHD) VWR International (Briare, France). The ultrapure water was obtained from a Direct-Q water purification system equipped with a Millipak 40 (0.22 μm) filter unit (Millipore, Billerica, MA, USA).

14.2 Oak chips

The oak chips added to model wine solutions were provided from A&B Group (Brescia, Italy) and were obtained from naturally seasoned (24 months) *Quercus petraea* oak. Two different sizes (Length × width × height = 4 × 2 × 1 mm (2 mm), and L × w × h = 4 × 8 × 1 mm (8 mm)) and three different levels of toasting for each size (light, L; medium, M; and high, H) were used.

14.3 Wine sampling

"Aglianico di Taurasi" red wine was kindly provided from Eng. Vito Napolitano. The grapes (10q) were subjected to the experimental vinification process that took place in Taurasi (Avellino, Campania, Italy) (41°00'11.94''N; 14°58'.82''E).

Soon after harvest, the grape were subjected to stemmer-crushing. No potassium metabisulphite and no autochtonous starter were added but spontaneous fermentation was carried out during 2012 vintage. Alcoholic fermentation was conducted for 8 days at 25 ± 1°C. After the tumultuous phase of alcoholic fermentation, the wine without solid parts was kept in 50 hL stainless steel tanks for aging at 16 ± 1°C. After 5 months of aging without contact with the wood, were added 4g/L of oak chips, different for size (2 mm and 8 mm) and toasting degree (light, medium and toasted) (see **Table 14.1** for code descriptions). The sampling started the day after the oak chips were added (day 1) and it was prolonged for 35 days.

Table 14.1. *Characteristics of "Aglianico di Taurasi" wine code: oak chips dimension (mm) and toasting degree.*

Sample code	Oak chips toasting degree	Oak chips size
F2L	Ligh	2
F2M	Medium	2
F2F	High	2
F8L	Light	8
F8M	Medium	8
F8F	High	8

Samples were taken weekly. Each sample was analyzed by HPLC-ESI/MS mode.

Bottles of 750 mL of wine were purchased in a local market (A-C). Sample A: Cannonau di Sardegna D.O.C. (aged at least 6 months in oak or chestnut barrels); Sample B: Monepulciano d'Abruzzo D.O.C. (aged at least 9 months in wood barrels); Sample C: Salice Salentino D.O.C. (aged at least 6 months in wood barrels).

14.4 Global valuations

Total phenols were determined by the Folin-Ciocalteau assay (Singleton & Rossi, 1965) with gallic acid as standard. The pH, alcoholic content, total titratable acidity, volatile acidity were determined in accordance with the official methods described in the Commission Regulation 2776/90 (1990).

14.5 Wine sample preparation for LC-ESI/MS analysis

4mL of wine were evaporated under reduce pressure and re-dissolved in acidified water (AcOH 2.5mL in 100mL of ultrapure water) to a final volume of 2 mL. (-)-gallocatechin was used as internal standard in a concentration of 15 mg/L.

14.6 LC-ESI/MS quantification of ellagitannins and acutissimins A and B

Vescalagin, castalagin, roburin E and grandinin were quantified by using the previously validated HPLC-ESI-MS/MS-multiple reaction monitoring method (García-Estévez et al., 2010; García-Estevéz et al., 2012) with some modification due to the instrument. HPLC analyses were performed in a Hewlett-Packard 1100 series liquid chromatograph (Agilent Technologies, Palo Alto, USA). MS detection was performed in an Agilent LC-MSD VL system (Agilent Technologies, Palo Alto, USA) operating in negative ion mode. The MS spectra were acquired in a negative ion mode. N_2 was used as both drying gas, with a flow rate of 13 L/min, and as nebulizer gas with a pressure of 50 psig. The drying gas temperature was set at 350°C and the capillary voltage was set to 3000 V and the fragmentation voltage to 80 eV. The data was collected in scan ion mode in the range of 250-1500 *m/z*, and in singular ion mode. (+)-catechin, ellagitannins, and acutissimins A and B were quantified by using a calibration curve built with data supplied by mass spectrometry using the parent $[M-H]^-$ ions 289 for (+)-catechin, 933 for vescalagin and castalagin, 1065 for grandinin and Roburin E and 1205 for acutissimin A and B. The signals obtained were corrected with the signal obtained for the $[M-H]^-$ 305 ions obtained for the internal standard (-)-gallocatechin. Detection (LOD) and quantification (LOQ) limits for acutissimin were calculated following the procedure described by García-Estévez and co-workers (García-Estévez et al., 2012) for ellagitannins. LOQ was 0.02 mg/L and LOQ was 0.09 mg/L.

14.7 Statistical analysis

All determinations and experiments were performer in triplicate, and the results are the average value of three determinations. Using data from chemical analyses, one way analysis of variance (ANOVA) was performed to compare the long-macerated wines. Statistical significance was attributed to *p* values of <0.05. Statistical analysis was performed using XLSTAT 2006, version 2006.6 (Addinsoft, Paris,France).

14.8 References

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15. Results and discussion

15.1 Conventional parameters

The conventional parameters of "*Aglianico di Taurasi*" wine and of commercial wine used in the experimentation are listed in **Table 15.1**. All the determinations were in accordance with law (Reg 2776/90).

Table 15.1. *Wine chemical parameters*

	Aglianico di Taurasi		**A**		**B**		**C**	
Alcohol (% v/v)	12.70	± 0.05	12.00	± 0.05	12.00	± 0.05	12.00	± 0.03
pH	3.50	± 0.00	3.55	± 0.00	3.51	± 0.00	3.65	± 0.00
TTA	6.15	± 0.37	4.99	± 0.05	5.55	± 0.13	4.88	± 0.11
VA	0.79	± 0.06	1.07	± 0.22	0.74	± 0.10	0.78	± 0.06
TP	1569.00	± 12.72	1897.86	± 12.09	1577.86	± 13.15	1804.26	± 17.34
Free SO_2 (mg/L)	-	-	14.88	± 0.23	14.10	± 0.25	30.81	± 3.24
Total SO_2 (mg/L)	-	-	80.32	± 0.00	71.38	± 6.34	108.57	± 1.35

TTA, total titrable acidity (tartaric acid g/L); VA, volatileacidity (acetic acid g/L); TP, total phenols (gallic acid mg/L)

Total phenolic content (**Figure 15.1**) ranging from 1520 to 1707 mg/L for F2L wine set samples (WSSs), from 1407 to 1632 mg/L for F2M WSSs and from 1420 to 1838 mg/L for F2F WSSs. In wine aged with the smallest oak chips, the highest average concentration of TP was presented in that in contact with light toasted oak chips (1629 mg of gallic acid /L), followed by wine in contact with medium toasted oak chips (1495 mg/L) and high toasted oak chips (1473 mg/L). A two way ANOVA analysis indicated significant differences in the TP content in function of both, time and oak chips toasting degree. During rapid aging, all WSSs in contact with different toasted degree oak chips presented a not regular TP evolution trend. As reported by Monagas et al. 2005, the method used for the determination of TP is based on the oxidation of the hydroxyl groups of phenols in basic media by the Folin-Ciocalteau reagent. Changes observed in TP during wine aging was possible due to the transformation of phenolic compounds in other forms that possess slightly different chemical properties and reactivities towards the Folin-Ciocalteau reagent.
Figure 15.2 illustrates the total phenol concentration for WSSs that contained the highest oak chips (8 mm) at different toasting degree (low, medium and high). Also in this case significant differences in the TP content in function of both, time and oak chips toasting degree, were showed. Total phenolic concentration ranging from 1213 to 1728 mg/L for F8L WSSs, from 1430 to 1655 mg/L for F8M WSSs and from 1444 to 1891 mg/L for F8F WSSs. The highest average concentration of TP was showed in wine in contact with high toasted oak chips (1532 mg/L), followed by wine in contact with medium toasted oak chips and light toasted oak chips (1545 mg/L and 1532 mg/L, respectively).
As previously demonstred for MWSs, TP is not an indicator of the amount of phenolic thermal breakdown, but might be useful to compare oak wood of different origin.

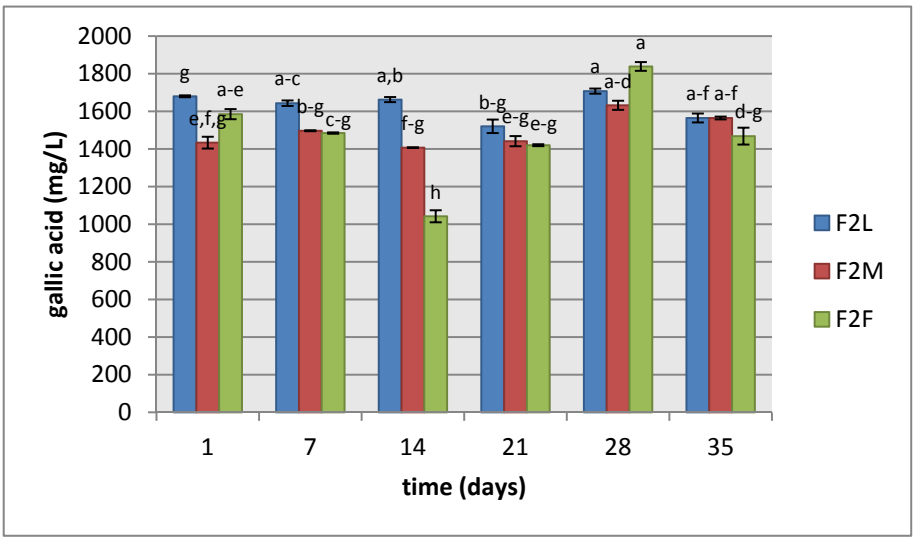

Figure 15.1. *Concentration of the total phenols (mg gallic acid/L) in "Aglianico di Taurasi" wine during accelerated aging with 2 mm oak chips. (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences (p > 0.05; n=3)*

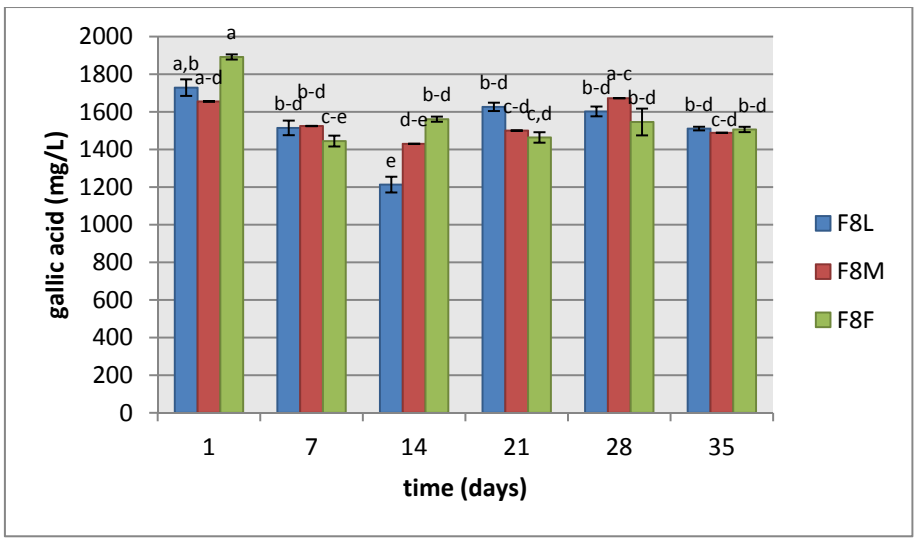

Figure 15.2. *Concentration of the total phenols (mg gallic acid/L) in "Aglianico di Taurasi" wine during accelerated aging with 8 mm oak chips. (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences (p > 0.05; n=3).*

15.2 Ellagitannins

Figure 15.3 presents the chromatogram obtained from wine aged for 35 days with the smallest oak chips (2 mm) with light toasting degree.

The impact of oak chips toasting degree and size on the C-glycosidic ellagitannins extraction during "*Aglianico di Taurasi*" rapid aging was evaluated. As previously observed for wine model solutions, in wine were detected the main C-glycosidic ellagitannins vescalagin, castalagin, roburin E and grandinin. They were identified according to the respective retention times and parent $[M-H]^-$ ions that were *m/z* 933 for both vescalagin and castalagin and *m/z* 1065 for both grandinin and roburin E (**Figure 15.4**).

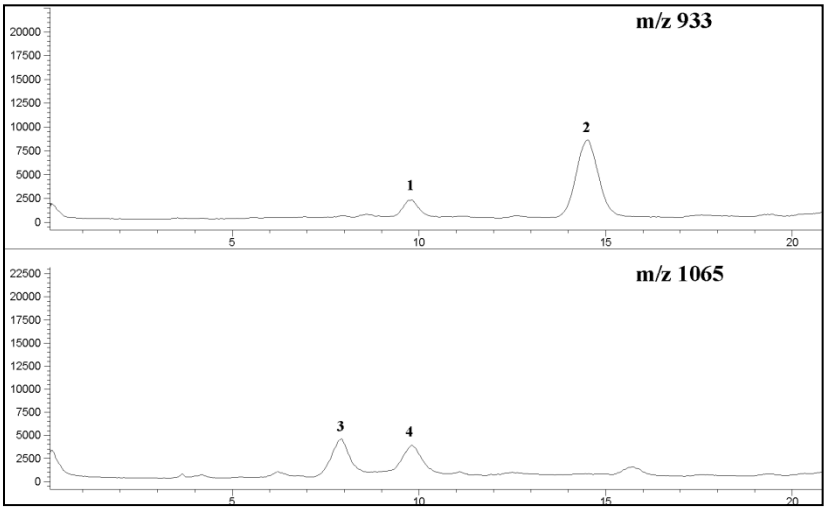

Figure 15.3. *EIC (extracted ion current chromatogram) of the signals at m/z 933 and m/z 1065 in reference model wine solution aged for 35 days with light toasted oak chips. (1), vescalagin; (2), castalagin; (3), grandinin; (4), roburin E obtained with an Agilent LC-MSD VL system.*

The results of the ellagitannins content in WSSs (F2L, F2M and F2F) containing smallest oak chips (2mm) are showed in **Figure 15.5**. Significant differences in the average content of ellagitannins were found for the toasting level light (L), medium (M) and high (F).This is in accordance with those previously reported (see *Section 11.3*), which described that MWSs containing high toasted oak chips had the lowest ellagitannin content. In the "*Aglianico di Taurasi*" wine that contained the smallest oak chips (2 mm), the highest level of ellagitannins was detected in that in contact with light-toasted oak chips (F2L). The total concentration of C-glycosidic ellagitannins increased to reach maximum value of 44.48 mg/L after 7 days, and then ellagitanins content decreased over time to reach the value of 25.57 mg/L after 35 days. In wine containing medium toasted oak chips (F2M), the maximum concentration was observed after 21 days (8.64 mg/L). After that a decrease in the ellagitannin content was also observed. In wine aged with high toasted oak chips, only castalagin was detected. Maximum concentration in that sample was reached after 35 days (4.35 mg/L).

F2L WSSs was composed of 61% of castalagin, 22% of grandinin, 9% of roburin E and 8% of vescalagin (**Figure 15.6**), while F2M WSSs was composed of 63% of castalagin, 30% of roburin E, 6% of grandinin and 1% of vescalagin.

Regarding the WSSs containing the largest oak chips (8 mm) (**Figure 15.7**) it was observed that in those in contact with light toasted oak chips (F8L), the total concentration of C-glycosidic ellagitannins increase regularly up to 14 days reaching the maximum concentration (26.12 mg/L). In wine aged with medium toasted oak chips the C-glycosidic ellagitannins concentration reached two maximum contents (25.87 mg/L after 14 days and 18.57 mg/L after 28 days). In WSSs that contained high toasted oak chips only vescalagin and castalagin were detected and the maximum concentration of 6,58 mg/L was reached after 21 days.

F8L WSS was composed of 72% of castalagin, 13% of grandinin, 8% of roburin E and 7% of vescalagin, while F8M WSS was composed of 79% of castalagin, 9% of both roburin E and grandinin and 7% of vescalagin. F8F WSS was composed of 96% of castalagin and 4% of vescalagin (**Figure 15.8**).

As previously observed (see *Section 11.3*), significant differences in the C-glycosidic ellagitannin content between wines aged with light, medium and high toasting degree oak chips were observed. Differences were expected since the ellagitannins undergo thermolytic degradation during the toasting process

(Mosedale et al., 1999; Doussot et al., 2002). Moreover, the ellagitannin evolution rate was faster than the extraction of C-glycosidic ellagitannins from the oak chips.

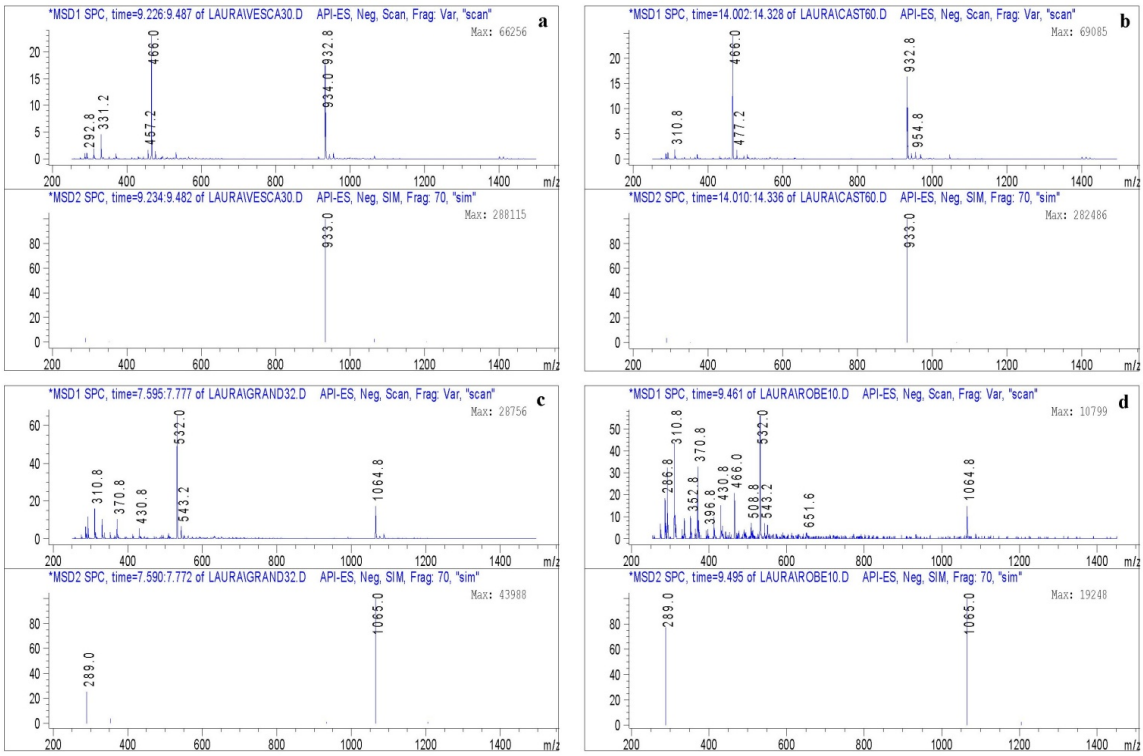

Figure 15.4. *Mass spectra of the ellagitannins: vescalagin (a), castalagin (b), grandinin (c) and roburin E (d) obtained with an Agilent LC-MSD VL system.*

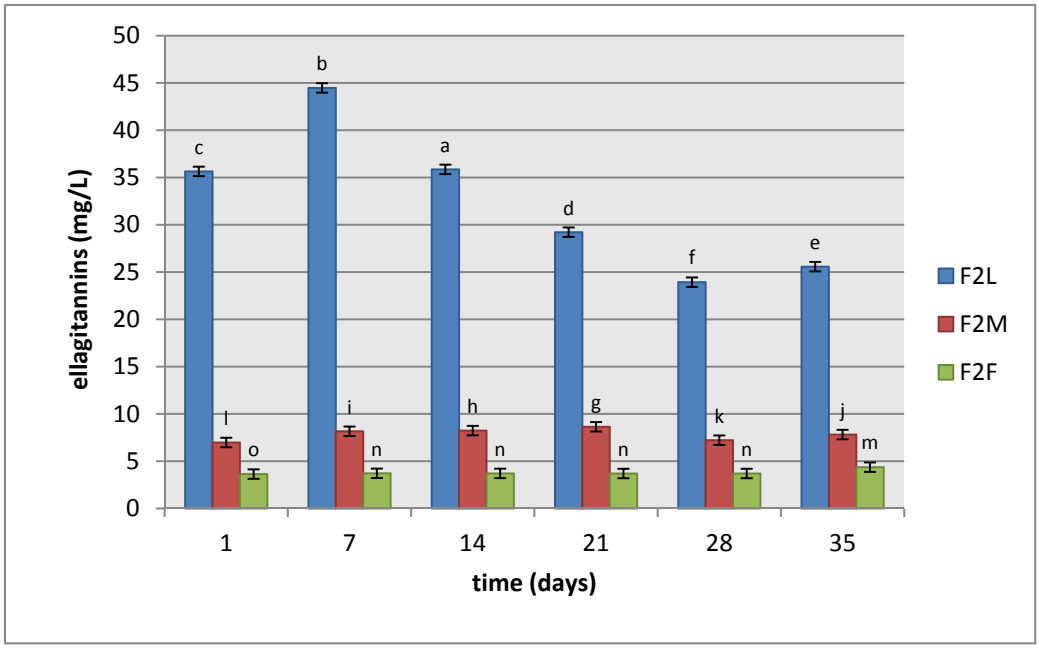

Figure 15.5. *Concentration of the total ellagitannins (mg/L) in "Aglianico di Taurasi" wine during accelerated aging with 2 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences ($p > 0.05$; n=3).*

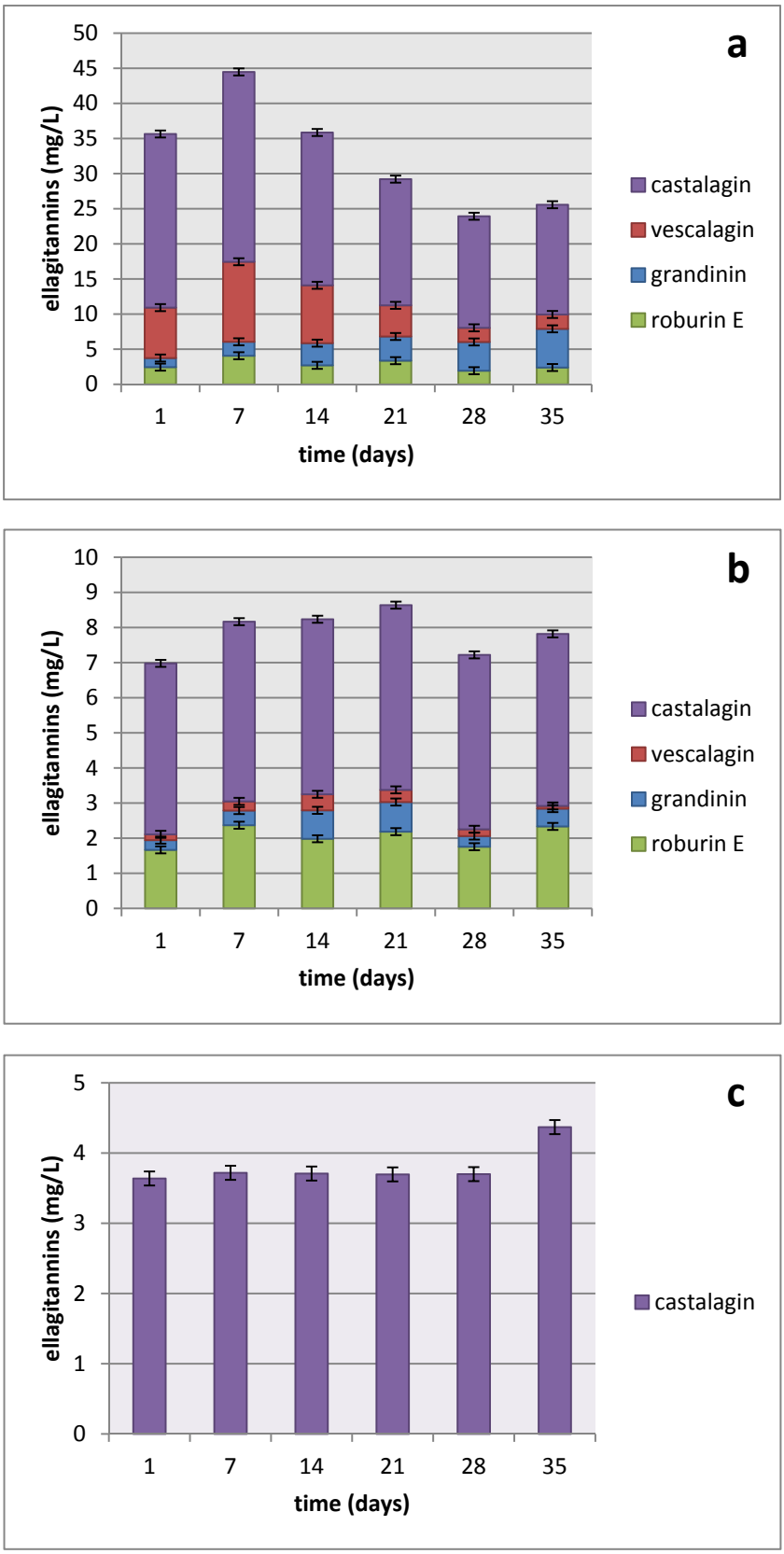

Figure 15.6. *Quantitative evaluation of individual C-glycosidic ellagitannins (mg/L) in "Aglianico di Taurasi" wine in contact with 2 mm oak chips - light (a), medium (b) and high (c). The error bars show the ±SD values.*

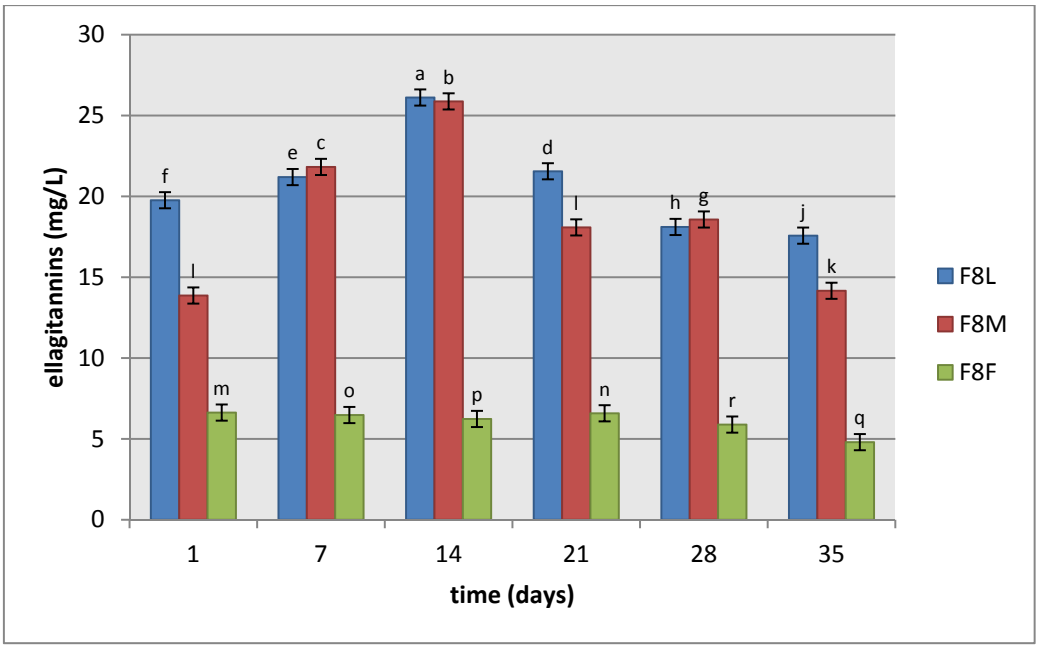

Figure 15.7. *Concentration of the total ellagitannins (mg/L) in "Aglianico di Taurasi" wine during accelerated aging with 8 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences ($p > 0.05$; n=3).*

Comparing the samples containing oak chips with medium and high toasting level and different size, the highest content of ellagitannins was observed for samples in contact with the largest oak chips. Such a difference is probably the result of a weaker thermolytic degradation of the ellagitannins located inside the larger oak chips during the toasting process. Moreover, the ellagitannins located in the wood of the larger oak chips are still available for extraction, since, as mentioned in *Section 11.3*, the wine can infiltrate the primary and secondary xylem vessels in order to penetrate the entire oak chips (Jourdes et al., 2011). Differently, comparing the samples containing oak chips with light toasting level, it was observed that the solutions in contact with the smallest oak chips, the ellagitannins were more abundant than in the solution in contact with the highest oak chips. In this solution (F2L), a greater surface area favored the extraction of C-glycosidic ellagitannins.

In relation to the proportions of the C-glycosidic ellagitannins in the solutions, castalagin was the most abundant ellagitannin, instead, vescalagin was the less abundant. This results could be a consequence of the lack of chemical reactivity at C-1 of castalagin. At the contrary, the vescalagin is the preferred precursor of the formation of C-glycosidic ellagitannin oligomers and C-1 coniugates such as flavano-ellagitannins and the lyxose/xylose-bearing conjugates (Quideau et al., 2009). Moreover, the transformation of ellagitannins in the wood when they are in contact with wine to form hemiketal or ketal derivatives may largely explain their reduced levels or absence in wine (Puech et al., 1999; Puech & Fulcrand, 2000).

Regarding the commercial wine, only in B and C ellagitannins were detected. In particular, B contained only 4.37 mg/L of castalagin, while C contained 0.25 mg/L of vescalagin and 7.71 mg/L of castalagin. In **Figure 15.9**, total ellagitannin content in "*Aglianico di Taurasi*" wine after 35 days of contact with oak chips, and in commercial wine was shown. It can be seen that there were statistical differences among the samples, but this is not allowed to discriminate the wine aged in oak barrel from the wine aged with oak chips. Same lower case letters were observed between samples F2H6 and B, and samples F2M6 and C.

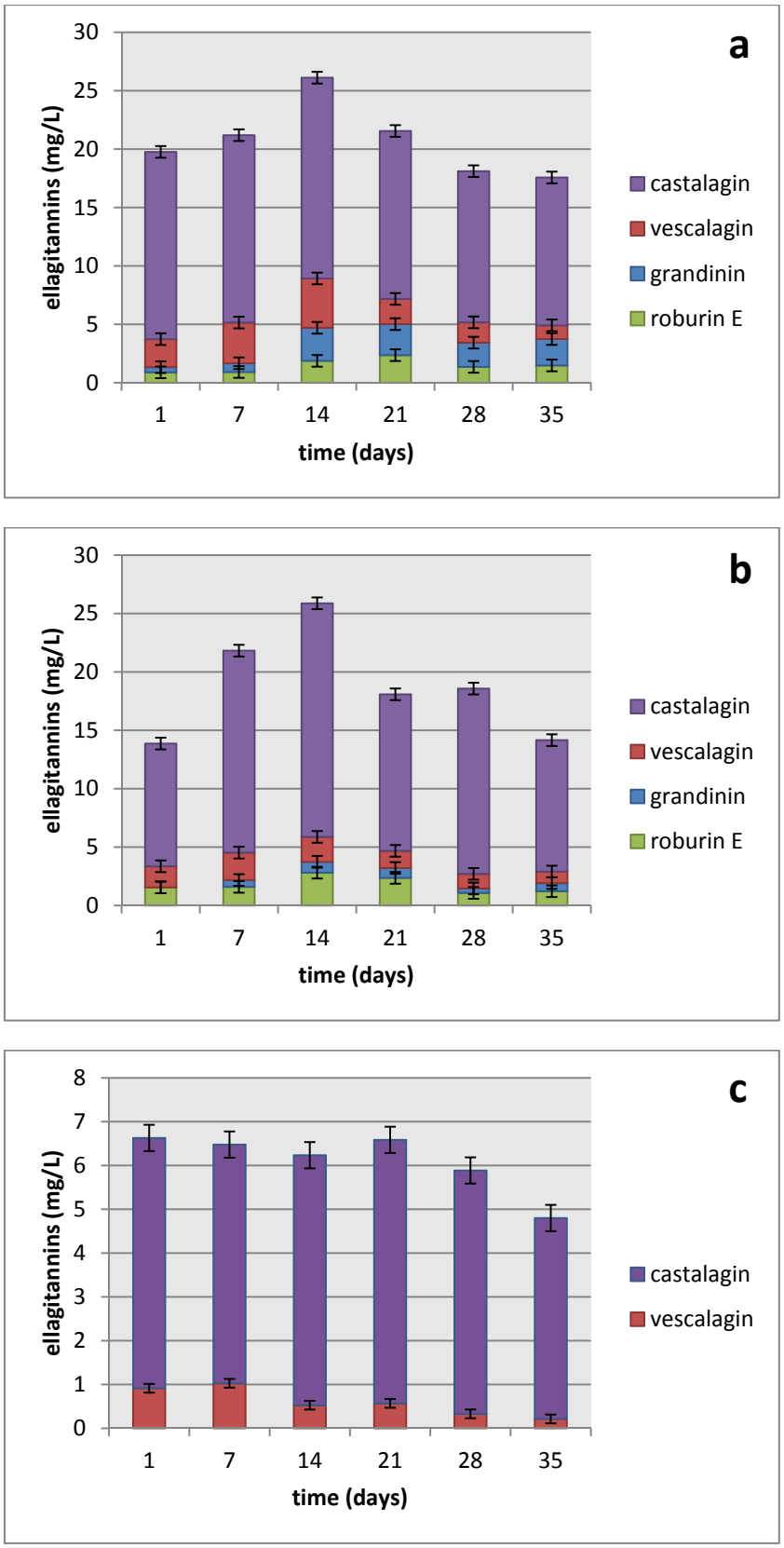

Figure 15.8. *Quantitative evaluation of individual C-glycosidic ellagitannins (mg/L) in "Aglianico di Taurasi" wine in contact with 8 mm oak chips - light (a), medium (b) and high (c). The error bars show the ±SD values.*

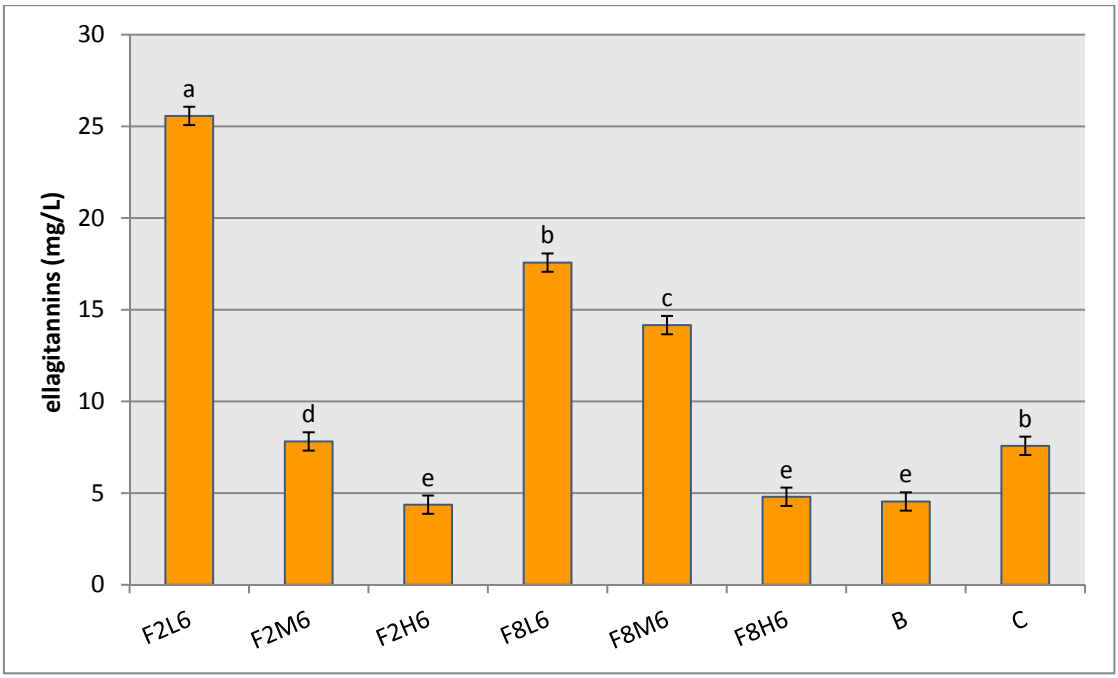

Figure 15.9. *Comparation of the total ellagitannins content between the "Aglianico di Taurasi" wine containing the different types of oak chips (2 mm and 8 mm) (L, light toasted; M, medium toasted, F, high toasted) and the commercial wine. The error bars show the ±SD values. Values with the same letter indicate no statistically differences among the different type of chips studied.*

15.3 (+)-catechin

In **Figure 15.10** the evolution of (+)-catechin in "*Aglianico di Taurasi*" wine containing light, medium and toasted 2 mm oak chips during rapid aging (35 days) was showed. As previously observed (see *Section 11.3*), (+)-catechin decreased during aging. The initial content of (+)-catechin in wine was of 35.08 mg/L while after 35 days the content of the flavanol in F2L, F2M and F2F was of 26.36 mg/L, 28.25 mg/L and 28.24 mg/L, respectively. The percentage of decrease of the (+)-catechin was of 28.47%, 19.46% and 19.51% in F2L, F2M and F2F wines, respectively.

In "*Aglianico di Taurasi*" wine in contact with 8 mm oak chips (**Figure 15.11**), after 35 days the content of (+)-catechin was of 26.19 mg/L, 24.94 mg/L and 25.64 mg/L in F8L, F8M and F8F, respectively.

The percentage of decrease of the (+)-catechin was of 25.35%, 28.91% and 26.92% in F8L, F8M and F8F wines, respectively.

A two way ANOVA analysis indicated significant differences in the (+)-catechin content in function of both, time and oak chips toasting degree.

Previous studies have described similar decreases in (+)-catechin, and dimeric derivatives, which depend on the characteristics of oak wood barrels (Fernández de Simón et al., 2003; Barrera-García et al., 2007). Pérez-Margariño & González-Sanjosé, 2004 reported that this decrease in free flavanols take place in conjunction with an increase in the levels of polymerized derivatives and of anthocyanin derivatives or "new pigments", that contribute to the maintenance of wine colour intensity. In these and other polymerization and copigmentation processes the wood plays an important role, since physical characteristics like grain, porosity, or permeability, and polyphenolic composition (Cadahía et al., 2009).

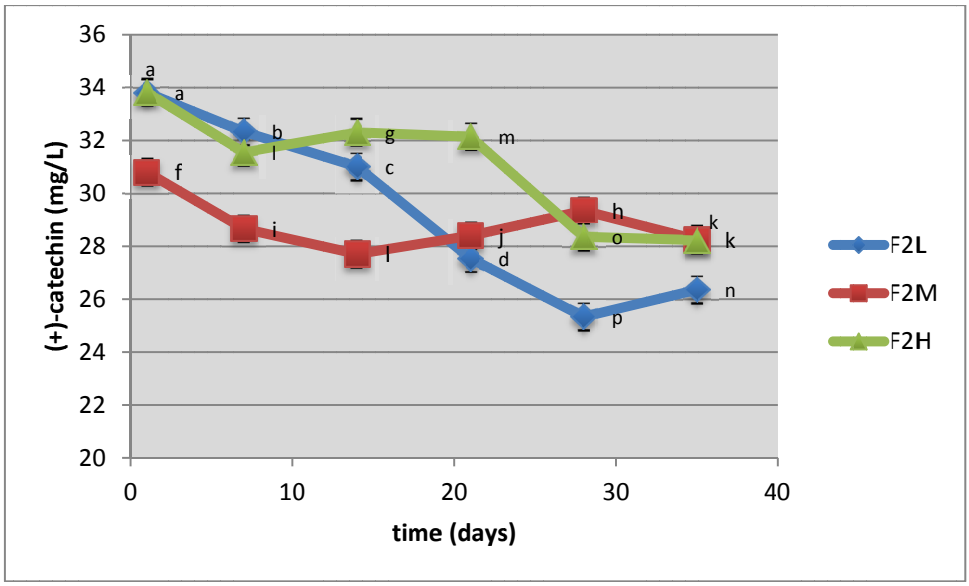

Figure 15.10. *Concentration of the (+)-catechin (mg/L) in "Aglianico di Taurasi" wine during accelerated aging with 2 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences ($p > 0.05$; $n=3$).*

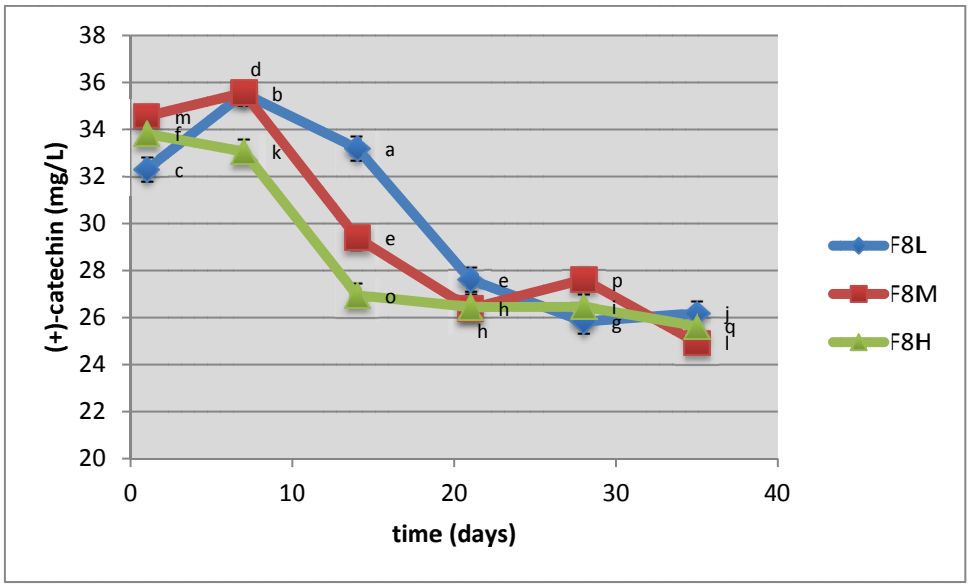

Figure 15.11. *Concentration of the (+)-catechin (mg/L) in "Aglianico di Taurasi" wine during accelerated aging with 8 mm oak chips (L, light; M, medium; F, high toasted level). The error bars show the ±SD values. Different lower case letters indicate significant differences ($p > 0.05$; $n=3$).*

15.4 Acutissimins A and B

While in MWSs increase in acutissimins A and B was observed during aging with oak chips (see *Section 11.4*), in wine the molecules were not detected. The results obtained were in accordance with previously literature (Jourdes et al., 2011) that reported only 0.15 mg/l of acutissimin A in a wine aged at least 120 days with light toasted oak chips while in the same wine aged for 30 days acutissimin A was under detection limit (**Table 15.2**). Instead, 0.40 mg/L and 0.28 mg/L of acutissimins A and B, respectively, in a 18 Month oak-aged 2001 Bordeaux red wine were reported(Saucier et al., 2006). In these studies, only vescalagin was quantified while (+)-catechin content was not reported.

The reaction between castalagin and vescalagin is just one among several other processes leading to the transformation of native C-glycosidic ellagitannins extracted from oak made cask or chips by wine solution.

Table 15.2. *Content of vescalagin, of acutissimin A and of (+)-catechin in wine aged 30 days and 120 days with oak chips and in wine aged 8 months and 18 months in oak barrel.*

Molecule	Wine aged 30 days with light toasted oak chips*	Wine aged 120 days with light toasted oak chips*	Wine aged 8 months in new French barrel*	Wine aged 18 months in ? oak barrel†
Vescalagin (mg/L)	4.00	1.02	7.00	2.20
Acutissimin A (mg/L)	< LOD	0.15	0.37	0.40
(+)-catechin (mg/L)	?	?	?	?

* Jourdes M., Michel J., Saucier C, Quideau S., Teissedre PL (2011). Identification, amounts and kinetics of extraction of C-glucosidic ellagitannins during wine aging in oak barrels or in stainless steel tanks with oak chips. Analytical and Bioanalytical Chemistry 500: 1531-1539.

† Saucier C, Guerra C, Pianet I, Laguerre M, Glories Y (1997) (+)-Catechin-acetaldehyde condensation products in relation to wine ageing. Phytochemistry 46: 229-234.

Other investigations need to be done before assess that the acutissimins A anb B could be useful to discriminate wine aged in oak barrel or with oak chips. In fact, it is important to keep in mind that wine is a complex multi-component reaction system, which slowly but continuously evolves under mildly acidic and oxidative conditions. As far as the acutissimins are concenrned, they are further transformed in wine in mongolicain A or B, but they will continue to form as long as grape flavan-3-ols and oak vescalagin ere present in wine solution. Furthermore, red wine is particularly rich in various components derived from grape (skin and seeds) and its fermented juice, and contains a broad panel of nucleophilic species, which may compete in the trapping of the benzylic cation intermediate derived from vescalagin during its condensation reaction at C-1. Examples of such nucleophiles are numerous other flavonoids (e.g., from procyanidins to proanthocyanidins, and anthocyanins) and, more generally, a large library of all kinds of phenols, alcohols, amines, carboxylic acids, enolizable carbonyl compounds, and thiols (Es-Safi et al., 2000; Nonier et al., 2008; Nonier et al., 2007; Sousa et al., 2005), including macromolecules such as proteins and polysaccharides that feature some of these functional groups (Fulcrand et al., 1996; Saucier et al., 1997). Furthermore, not only vescalagin, but any C-glycosidic ellagitannins displaying a free hydroxyl group at C-1, such as roburin A can act like “nucleophile sponge” if present in wine.

15.5 References

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16. Conclusion

Wine aging using oak barrels is a expensive practice to which some wines are subjected. One way winaries are cutting costs, is using toasted oak chips, which costs hundred less than the oak barrel. The combination of steel tanks and oak chips produces the same result at the end of the winemaking process. The addition of oak chips in wine is a practice that appears first in the wine producing emergent countries and became authorized in the EU community in 2006 ((CE No. 1507/2006). Nevertheless, the traditional methods of winemaking wine are very strictly regulated.

The study showed the evolution of C-glycosidic ellagitannins and of acutissimins A and B concentration during "*Aglianico di Taurasi*" wine aging (35 days) with different oak chips. Moreover, tried to compare the content of ellagitannins in wines aged in oak barrels or with oak chips.

The concentration of the ellagitannins in aged red wine was significantly affected by the oak chips toasting degree. The C-glycosidic ellagitannins was lower in red wine aged in contact with the oak chips with the F toasting level, while in wine in contact with light toasted oak chips the highest content of ellagitannins was found. Therefore, light toasted oak chips was characterized by its richness in hydrolyzable tannins, but toasting conditions provoked important modification in the ellagitannins composition.

Furthermore, the impact of oak chips size on the C-glycosidic ellagitannins was estimated. It was observed that in "*Aglianico di Taurasi*" wine aged with medium and high toasted oak chips, the highest content was found for samples in contact with the largest oak chips and this can be explained since the higher size can make that toasting could not harm the deeper layers of wood making possible the extraction of ellagitannins. The maximum concentration of the C-glycosidic ellagitannins was reached in red wine aged with 2 mm light toasted oak chips, that was significantly higher than in all the samples. In commercial wines only vescalagin and castalagin were detected but their presence did not allow to discriminate wines aged in oak barrels or with oak chips.

A decrease in (+)-catechin was observed, which depend on both, oak chips dimension and oak chips toasting degree. The decrease in free flavanols take place in conjunction with an increase in the levels of polymerized derivatives. Average decrease in (+)-catechin content was of 21.28% for wine aged with 2 mm oak chips, and of 27.06% for wine aged with 8 mm oak chips.

As observed in other studies, in "*Aglianico di Taurasi*" wine aged with oak chips acutissimins A and B were not detected. However, the presence of the molecule in a wine could indicate that aging in barrique occur. It is important to take into account that wine is a hydroalcoholic heterogeneous media that contain different molecules that might participate in several reaction with ellagitannins and flavanols, reducing the possibility that the nucleophilic substitution between vescalagin and (+)-catechin occur. Many wood phenols, as phenolic and furanic aldehydes, serve as intermediates in the reaction between flavanols and anthocyanins, while diverse condensation products have been described as resulting from the direct reaction of ellagitannins with proteins, proanthocyanidins, antochyanins of wine. This may explain the absence of the acutissimins A and B in the samples.

Appendix

List of contribution

Posters

a. In vino analytica scientia symposium, 2-5 luglio 2013, Università di Reims (Francia), titolo del contributo: *effect of the size and toasting degree of oak chips on the extraction of C-glycosidic ellagitannins in model wine solutions*;

b. Chimalsi 2012 – IX Congresso Italiano di Chimica degli Alimenti; 3-7 Giugno 2012, Ischia (NA), titolo del contributo: *influence of catechin concentration and oak chips (Quercus alba) on acutissimin formation in model solutions and red wine*;

c. XVII Workshop on the Development in the Italian PhD Research on Food Science Technology and Biotechnology; 19-21 Settembre 2012, Cesena (FC), titolo del contributo: *evolution of Southern red wine composition and kinetics of acutissimins and oaklins formation during accelerated aging with oak chips or oak barrels*.

Journals

a. Naviglio D., Le Grottaglie L., Vitulano M., Manzo N., Romano R., *In Press*, *Caratterizzazione mediante GC/MS di oli essenziali per uso alimentare*. Industrie alimentari.

b. Francesca N., Romano R., Sannino C., Le Grottaglie L., Settanni L., Moschetti G. 2014. *Evolution of microbiological and chemical parameters during red wine making with extended post-fermentation maceration*. International Journal of Food Microbiology, 171, 84-93.

c. Romano R., Giordano A., Le Grottaglie L., Manzo N., Paduano A., Sacchi R., Santini A. 2013. *Volatile compounds in intermittent frying by gas chromatography and nuclear magnetic resonance*. European journal of lipid science and technology, 115, 764-773.

d. Romano R., Manzo N., Le Grottaglie L., Giordano A., Romano A., Masi P., 2013. *Comparison of the frying performance of high oleic oils subjected to discontinuous and prolonged thermal treatment*. Journal of the American oil chemists, 90, 965-975.

e. Romano R., Le Grottaglie L., Manzo N., Arena M., Lombardo G. L., Blaiotta G. 2013. *Caratterizzazione della componente organica volatile del vino Catalanesca del Monte Somma IGT*. Industrie delle bevande, n. 247, 11-18.

f. Romano R., Le Grottaglie L., Manzo N., Giordano A., Vitiello S., Santini A., 2012. *Confronto delle performance di frittura degli oli di oliva, palma bi-frazionato e girasole*. Progess in Nutrition, Vol. 14, N. 3, 199-218.

g. Romano R., Giordano A., Vitiello S., Le Grottaglie L., Spagna Musso S., 2012. *Comparision of the frying performance of olive oil and palm superolein*. Journal of Food Science , Vol. 77, Nr. 5.

h. Romano R., Giordano A., Le Grottaglie L., Guastaferro D., Spagna Musso S., 2012. *Applicazione della gas-cromatografia ad alta risoluzione (HRGC) allo studio della composizione acidica e trigliceridica del latte di asina*. Progress in nutrition, 14 n. 1, 50-57

Conference proceedings

a. Raffaele R., Daniele N., Le Grottaglie L., Giordano A., Visconti A., Ritieni A., 2012. *Fatty acid composition of Arabica and Robusta coffee cultivars as a discriminative factor*. Proc. Int. Conference "Emirates Journal of Food Agricolture – Supplementary Issue" (United Arab Emirates University, eds.) , Volume 24, p. 11;

b. Romano R., Le Grottaglie L., Giordano A., Naviglio D., Santini A., Spagna Musso S., 2012. *Influence of catechin concentration and oak chips (Quercus alba) on acutissimin formation in model solutions and red wine*. Proc. Int. Conference "Emirates Journal of Food Agricolture – Supplementary Issue" (United Arab Emirates University, eds.) , Volume 24, p. 134;

c. Romano R., Giordano A., Le Grottaglie L., Spagna Musso S., 2012. *Frying performance of high oleic sunflower oil subjected to prolonged and discontinuous deep frying*. Proc. Int. Conference "Emirates Journal of Food Agricolture – Supplementary Issue" (United Arab Emirates University, eds.) , Volume 24, p. 134;

d. Romano R., Francesca N., Le Grottaglie L., Spagna Musso S., 2012. *Effect of post-ferementation estende maceration on phenolic compounds extraction of "Aglianico di Taurasi" wine*. Proc. Int.

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e. Romano R., Giordano A., Le Grottaglie L., Spagna Musso S., 2011. *Applicazione della gas-cromatografia ad alta risoluzione (HRGC) allo studio della composizione acidica e trigliceridica del latte di asina*. Proc. Int. Conference “Ricerche e innovazioni nell’industria alimentare” (Chiriotti Editore), Volume X, p 216;

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Books

Moschetti G., Francesca N., Sannino C., Settanni L., Romano R., Le Grottaglie L., 2013. *Lavoro sperimentale: fasi per l’ottenimento di uno starter autoctono per il vino Fiano di Avellino D.O.C.G.*. In “I lieviti del vino Fiano di Avellino D.O.C.G.: la tipicità attraverso le biotecnologie” (Imago Editrice s.r.l.) pp. 63-91, Regione Campania – Assessorato all’agricoltura. ISBN : 978-88-95230-20-7

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