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Production of high-quality red wines from native vines through the management of viticultural, technological, aging and packaging variables

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SUMMARY

Chapter 1

Botanical classification, morphology, physiology of grapevine and quality of wine

1.1 Taxonomic classification

Grapevines are grouped in the genus *Vitis*, family Vitaceae that also includes two well-know species, such as Boston Ivy (*Parthenocissus tricuspidata*) and Virginia Creeper (*P. quinquefolia*). The Vitaceae family comprises more than a thousand tropical and subtropical species, subdivided in 15 or 16 genera (Galet, 1988). The genus *Vitis* grows in a temperate-zone and develops indigenously only in the northern hemisphere. It was divided into two subgenera, *Vitis* and *Muscadinia*. *Vitis* (bunch grapes) is the larger of the two subgenera, including all species except *V. rotundifolia* and *V. popenoei*. These latter two species are included in the *Muscadinia* subgenus. The subgenus *Vitis* includes species which are characterized by a shredding bark without protuberant lenticels, a medulla interrupted at nodes by woody tissue, phloem fibers tangentially positioned, branched tendrils, elongated flower clusters, a fruit adhering to the fruit stem at maturity, and a pear-shaped seed having a smooth chalaza and a protuberant beak (Jackson, 2008).

The grapevine species (*Vitis vinifera* L.) is the single *Vitis* species that attained significant economic importance over time, while some other species, i.e. the North American *V. rupestris*, *V. riparia* or *V. berlandieri*, are used as breeding rootstock due to their resistance against grapevine pathogens such as *Phylloxera*, *Oidium* and mildews. Indeed, most of the cultivated varieties, classified as *Vitis vinifera* L. subsp. *vinifera* (or *sativa*), derive from the wild species *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi (Rossetto *et al*., 2002; Sefc *et al*., 2003; Crespan, 2004). This wild grapevine is a heliophilous vine distributed in a wide area from the Western Europe to the Trans-Caucasian zone and around the Mediterranean basin (Arnold *et al*., 1998). Its current distribution is highly fragmented in separated micro-populations or meta-populations, with few individuals, at least in the western part of the Mediterranean Basin. The progressive decline of the wild grape populations may be explained by the anthropogenic pressure on their natural habitats and by the presence of pathogens introduced from North America during the second part of the 19th century (Arnold *et al*., 1998). Indeed, the "*Phylloxera* crisis" that affected the European vineyards had a considerable impact on both cultivated varieties and wild grapes.

The common grapevine is the only common species of the series *Vinifera* and has Eurasian origin. It is also present as spontaneous species. Within the species, there are two subspecies:

- ✓ *Vitis vinifera* subsp. *sativa*, with hermaphrodite plants and bisexual flowers. It is the subspecies that includes the cultivated varieties;
- ✓ *Vitis vinifera* subsp. *sylvestris*, with dioecious plants. It is the spontaneous subspecies, widespread in woodlands and scrub warm temperate regions of the Eurasia. From the agronomic point of view, this subspecies is entirely devoid of interest (http://it.wikipedia.org/wiki/Vitis_vinifera).

1.2 Morphology of grapevine

The shape of a cultivated grapevine is created by pruning and training of the vine into a specific form according to one training system. Over the centuries, several training systems have been developed and modified in the efforts to simplify vine management and offer a favourable growing environment for grape production (Hellman, 2003).

1.2.1 The Root System

The root system of the plant, besides to act as a grip in the ground, serves to absorb water, provide nutrients for its sustenance, produce hormones and retain nutrients, such as sugars, in case of need. The roots of a ripe vine are formed from a woody texture of old roots (Richards, 1983) from which originate young and permanent roots that may develop both horizontally or vertically. These roots are generally multi-branched and could lead to lateral roots. The latter produce fine roots, which have the effect of increasing the amount of land used.

The root system usually extends about 3 feet into the ground, although some roots can spread more in depth (but this depends on the climatic conditions). Distribution of roots is conditioned by factors such as characteristics of the soil, presence of impermeable layers, variety of the vine, rootstock, and agronomic practices. Mycorrhizae, a form of soil fungi, live in a natural, mutually beneficial association with grape roots. Mycorrhizae influence grapevine nutrition and growth and increase the uptake of phosphorus.

a grapevine can be grown on its own roots (roots screws or self-rooted) or can be grafted onto a rootstock. A screw engaged (Figure 1) is divided into two parts, the scion variety (for example, Pinot noir), which produces the fruit, and the variety rootstock (often indicated with a number, for example, 101-14), which provides the root system and the lower part of the trunk. The position on the trunk where the two varieties were grafted and

from which they grow together is called graft union. A successful union requires that the vascular cambiums of stock and scion are in contact with each other, since these are the only tissues having meristematic activity and thus able to produce the new cells that complete the union of the graft. The point of union of the graft often include the production of abundant callus tissue (a tissue wound healing composed of large cells with thin walls that are formed in response to injury), which often make the area a bit bigger than the parts adjacent to the trunk itself. Since the variety of the rootstock and the scion can grow at different rates, the diameter of the trunk can vary above and below the graft union.

Rootstock was born to counter a North American insect, *Viteus vitifolii*, better known as Phylloxera, to which the roots of the *Vitis vinifera L.* (wine grapes "European") were sensitive. The solution was to graft European vines, able to produce quality wines, on foot with vines or its hybrids, resistant to attack of Phylloxera. This method is still of general application. Most rootstock resistant to Phylloxera are native North American species or hybrids of two or more of these species, including *V. riparia*, *V. berlandieri*, and *V. rupestris*. The root system and the depth that can be achieved, as well as other features, varies according to the species and hybrid rootstocks, so the rootstock can influence aspects of the growth of the vine, such as vigor, drought tolerance, efficiency of nutrient absorption, and ability to resist to the attacks.

1.2.2 The Trunk

The *trunk* or stem is permanent and supports the structure of the vegetative and reproductive grapevine. It is mainly vertical, but may have different inclinations depending on the type of farming. The upper part of the trunk is indicated as head and its height is due to the pruning during the initial stages of formation of the young grapevine. The trunk has ramifications are called “shoots” when they are herbaceous, “branches” when they are lignified, and “sarments” when they are detached from the plant after pruning. The branches, in their turn, are constituted by nodes and internodes in number and variable length. The ramifications are located in different positions depending on the system. Some training systems using cords (semi-permanent branches of the trunk) usually were trained horizontally along a trellis wire, with arms placed at regular intervals along their length. Other systems utilize pipes (Figure 1), one-year-old wood that originates from the arms usually located near the head of the screw. The crown is the area of the trunk near the ground, from slightly below to slightly above the ground level.

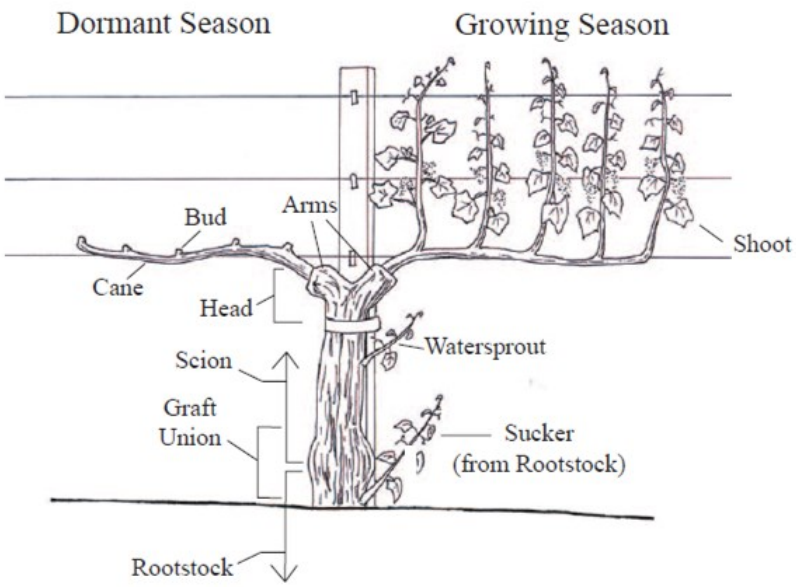


Figure 1 – Grapevine structures and features: grafted vine (source: Hellman, 2003).

1.2.3 Shoots and Canes

Shoot is the basic unit of the growth of the vine and the main key of many agricultural practices. The components of the shoot are illustrated in Figure 2. The main axis of the shoot consists of structural support tissues and conduction tissues for the transport of water, nutrients and products of photosynthesis. Leaves, tendrils, flowers (or fruits), and buds are placed along the shoot in regular patterns. The areas of the shoots are defined as *basal* (closest to its point of origin), *mid-shoot*, and *apex* (tip). The word *canopy* indicates the whole of vine shoots, leaves and fruits; some viticulturists also think that trunk, cordons, and canes are parts of the canopy.

Shoot Tip. A shoot has different points of growth, but normally they extend from the *shoot tip* (growing tip). New leaves and tendrils born from the tip as the shoot grows. The shoot growth rate varies during the season. If the suitable conditions of light, moisture, and nutrients are suitable, the shoots of the grapevine can continue to grow. In fact, they don't block their expansion by forming a terminal bud, as some plants do.

Leaves. The vine leaves are simple, alternate, and with generally segmented distichous edge. They originate from the apical meristem. The shoot gives rise to two or more bracts close-ups (small scale-like leaves) formed at the base of the first true foliage leaf. The leaves are joined to the slightly enlarged surface on the shoot called *node*. The area between two nodes is called *internode*. The distance between the nodes is an indicator of

the rate of shoot growth, so internode length varies along the cane corresponding to changing growth rates during the season.

Leaves are formed by the *blade*, which is the upper part of the leaf responsible of sunlight and CO_2 absorption during photosynthesis, and of the petiole, the stemlike structure that links the leaf to the shoot. The diffusion of CO_2, O_2, and water vapour takes place through the *stomata*, microscopic pores located in the lower part of the leaf blades. Stomata are open in the light and closed in the dark. The petiole has the function of maintaining the orientation of the lamina and to convey nutrients and water to and from the leaf blade.

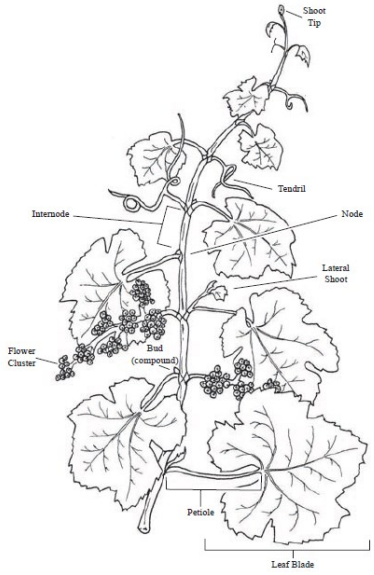


Figure 2 – Principal features of a grapevine shoot prior bloom (source: Hellman, 2003).

Flowers and Fruits. A *fruitfal shoot* usually makes from one to three *flower clusters* (inflorescences) according to the variety. Flower clusters grow opposite to the leaves, typically at the third to sixth nodes from the base of the shoot, depending on the variety. If three flower clusters develop, two develop on adjacent nodes, the next node has none, and the following node has the third flower cluster. The number of flower clusters on the shoot is influenced by grape variety and the conditions in which the plant has undergone during the previous season, as these affect the development of the dormant bud (that produced the primary shoot). A cluster may contain from several to many hundreds individual flowers, depending on variety.

The grape flower is not formed by many petals, which, however, are merged into a green structure defined the *calyptra* (Figure 3) but commonly called *cap*. This contains the reproductive organs and other tissues inside the flower. A flower is formed by a single

pistil (female organ) and five *stamens*, each tipped with an *anther* (male organ). The pistil has a conical shape, with the much larger base of the upper part and the tip (the stigma) slightly flared. The base of the pistil is formed from the ovary that is divided into two internal areas, each of which contains two ovules in which there is a embryonic bag with a single egg. The anthers make yellow pollen grains, which contain the sperm.

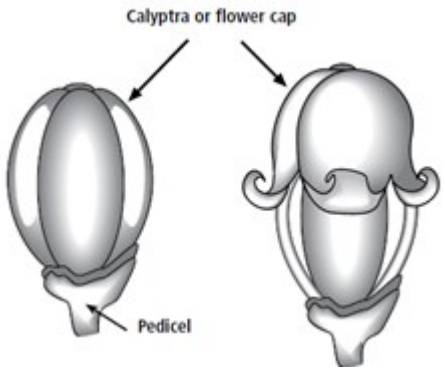


Figure 3 - A grape flower shedding its calyptra (flower cap) during anthesis (source: Dokoozlian, 2000).

The flower can remain open 1-3 weeks depending on weather conditions. This period is named the *bloom*, which happens between the 50th and the 80th days after germination.

When a flower opens, the cap is detached and falls off, thus exposing the pistil and the anthers. The latter issue the pollen falling on the stigma random. This process is called *pollination*. Multiple pollen grains can germinate, each growing a pollen tube down the pistil to the ovary and entering an ovule, where a sperm unites with an egg to form an embryo. This is the *fertilization* and the subsequent growth of fruits is the *fruit set*.

Tendrils. Shoots give rise to tendrils, structures that are responsible for providing support to the shoots during their growth. Flower clusters and tendrils have a common developmental origin (Mullins *et al*., 1992), so occasionally a few flowers develop on the end of a tendril.

Buds. The *bud* is the point of growth that develops just above the point of link between petiole and shoot (leaf axil). The single gem is called *axillary bud* and two buds associated with a leaf defined *lateral bud* and *dormant bud*. The lateral bud is the true axillary bud of the foliage leaf, and the dormant bud forms into the bract axil of the lateral bud.

The lateral bud grows in the current season, but growth may either cease soon after formation of the basal bract or continue, producing a *lateral shoot* (summer lateral) of highly variable length.

The dormant bud appears as a simple structure, being made up of three points of growth, indicated as *primary*, *secondary*, and *tertiary buds*. It is called dormant because it doesn't grow in the same season in which it develops. The three growing points each produce a rudimentary shoot that ultimately will contain *primordia* (organs in their earliest stages of development) of the same basic components of the current season's fully grown shoot: leaves, tendrils, and in some cases flower clusters. In most cases, only the primary bud grows, producing the primary shoot, while the secondary and tertiary bud are as a "backup system" for the grapevines and grows only when the primary bud or young shoot is damaged. Specifically, tertiary bud provide additional backup if the primary and secondary buds are damaged, but usually does not contain flower cluster. If only the primary grows, secondary and tertiary buds remain alive but dormant.

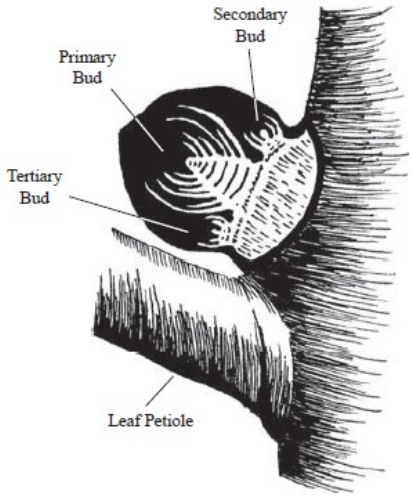


Figure 4 – Cross section of dormant grape bud in leaf axil , showing primary, secondary, and tertiary buds (source: Hellman, 2003).

Suckers and Watersprouts. Shoots may also come from bud placed on old wood, such as cordons and trunks. *Suckers* are shoots that grow in the crown of the trunk. *Watersprout* indicates a shoot deriving from the upper regions of the trunk or cordons. The shoots that grow on old wood develop as axillary buds that have never bloomed. These buds are called *latent buds*, because they can remain dormant indefinitely until an extreme event does not stimulate their development. In general, there are many hidden gems in the "renewal positions" (a term pruning) on the trunk or Cordons.

Canes. Shoot begins transition of midseason, when it begins the *ripen*. Once the leaves fall at the beginning of the dormant season, the mature bud is considered a *cane*. Cane is the main priority in the period of vegetative rest, when the practice of pruning is used to

handle the size and the shape of the vine and to check the amount of potential crop in the next harvest season. Pruning severity is often described in terms of the number of buds retained per vine, or bud count. The "crown" of buds observed at the base of a cane includes the secondary and tertiary growing points of the compound bud that gave rise to the primary shoot, as well as the axillary buds of the shoot's basal bracts (Pratt, 1974). Canes can be pruned to varying lengths, and when they consist of only one to four buds they are referred to as *spurs*, or often as *fruiting spurs* since fruitful shoots arise from spur buds.

1.3 Grape berry growth

Grape flowers are borne on an inflorescence or flower cluster (Figure 5). The rachis is the main axis of the cluster and through the peduncle the flowers are tied to it. The pedicle connects the cluster to the vine, which is prolonged from the bud to the first branch of the rachis. Almost all the most commercially important *Vitis vinifera* grape varieties flowers are hermaphrodite, containing both the male and the female organs (Figure 5). The cup is gamosepalo, underdeveloped, divided into five sepals barely visible. The corolla consists of 5 inconspicuous, greenish, welded petals; at the time of flowering, corolla often opens with petals that unsolder from the base to the apex, and soon falls. The androecium consists of five stamens with dorsifixed anthers with longitudinal dehiscence. In hermaphrodite flowers, the anthers are arranged at the height of the stigma, but are outward-facing, so the pollination of the vine tends to be predominantly cross. The gynoecium consists of a two-roomed ovary containing 4 ovules; the ovary is surmounted by a stylus ending with a bi-lobed stigma. At the base of the flower, there are five scent glands, commonly called nectaries, altohugh they don't produce nectar. These glands are responsible for producing substances that are designed to attract insects, but in *Vitis vinifera* varieties, the substances produced seem to get little attention from insects (Dokoozlian, 2000).

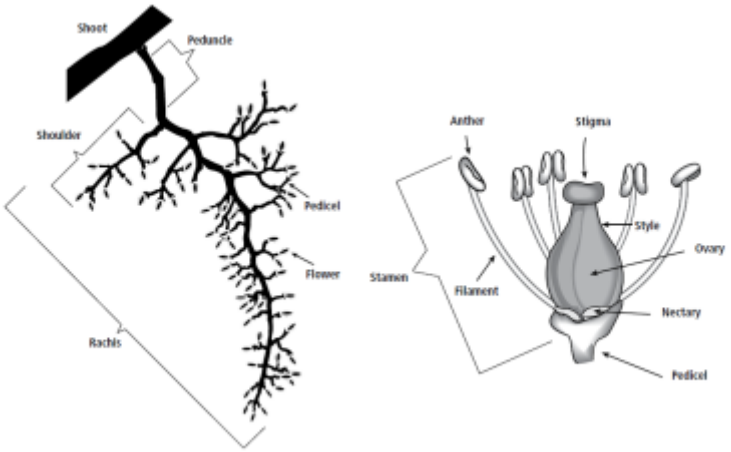


Figure 5 - A typical flower cluster prior to anthesis (to left); a hermaphroditic perfect grape flower (to right) (source: Dokoozlian, 2000).

Anthesis. The calyptra, formed by five fused petals, has the function to protect the flower organs. The process of opening of the flower is called anthesis and consists in the calyptra breaking from the base of the flower with the exhibition of the stamens and pistil. Anthesis lasts 6-8 weeks from the moment in which the shoots born. Its duration varies vary depending on the weather (Dokoozlian, 2000).

Pollination, fruit set and veraison. Pollination is the transfer of pollen from the anther to the stigma. It is autogamous and cleistogamic, thus it takes place inside the ovary. The first visual indication that pollination is occurring is when the cap falls from the flower. The pollen is released from the mature anthers. This is termed cap fall and flowering is generally determined as when 80% cap fall occurs (80% of the caps in a cluster have fallen). The pollen falls onto the stigma, then develops a tube which grows down the style to the ovary to allow the male nucleus to travel to the female nucleus and fertilisation to take place. Fertilisation, which consists in the union of male nuclei from the pollen with the female nuclei in the ovary, generally occurs 2-3 days after pollination. The embryo and berry development begins. The embryo forms the seeds and the ovary becomes the berry (http://it.wikipedia.org/wiki/Vitis_vinifera).

After pollination and subsequent fertilization, the fruit set follows. This phase consists in the transformation of flowers into fruits. Up to 70-80% of the flowers may fail to set. The size of the berry is mainly determined by the number of seeds it contains. The larger fruits are those containing more seeds. A berry may contain up to four seeds, although two or less is usual in the wine grape varieties. Berries with pistils that have not been fertilised

will abscise (break off) from the cluster. This is termed shatter. Normally up to 50% of the potential fruit may shatter, preventing the bunch from becoming too compact. Small seedless berries, which don't enlarge, may remain on the cluster in some varieties. These berries are termed shot berries, resulting in "hen and chickens" or millerandage). In some grape varieties, the fruit can develop without seeds. This is called parthenocarpy. This occurs in Black Corinth variety. Stenospermocarpy occurs in other varieties (eg. Thompson Seedless – a variety used for table grapes, dried fruit, and wine). Fertilisation occurs, but the seeds are small, soft and empty. The berries develop to medium size (http://it.wikipedia.org/wiki/Vitis_vinifera).

After the fruit set phase, the herbaceous ending follows from the first half of July and the first half of August, always in relation to factors of precocity and geographical position. At this stage there is the growth of the berries size and weight, up to the maximum values. The growth of the seeds also occurs, up to the achievement of the final dimensions. At this stage, gradual increases of sugar content (2%) and organic acids (4%) occur in the pulp.

Veraison follows the herbaceous phase. This stage is characterized by the skin colour change. During veraison, biochemical processes and reorganization of tissues also occur. The proper growth has ceased, although an increase in volume of the berries can be observed. The most important phenomena occurring in this phase are the following:

disappearance of chlorophyll. The green colour disappears gradually being replaced by the colour of the other pigments (anthocyanins and carotenoids). The ratio between the various pigments changes among the grape cultivars. As a result, the colour changes from green to yellow in white grapes, and the colour change from green to red or purplish red, in black grapes;

sugar accumulation. This phase occurs as a result of translocation of sugar from other organs (according to some authors from reserves located in the wood, according to others from the leaves);

loss of consistency of the mesocarp (pulp). This is due to the gradual disintegration of the middle lamella, which joins the cell walls of adjacent cells. In grapes juicy pulp is also a drastic reorganization with the breakup of the cell walls (http://it.wikipedia.org/wiki/Vitis_vinifera).

Grape Ripening. The grape ripening is influenced by several factors, including variety, climate, and crop level. The specific number of heat units (frequently expressed as degree-

days [DD]) necessary for berry maturation strongly differs among varieties. Seasonal modifications in degree-day accumulation also affect the rate of ripening. When temperatures are low and degree-day accumulation tardy, ripening is retarded, while when conditions are warm and degree-days accumulate fast, ripening is quicker (Dokoozlian, 2000).

1.4 Grape Composition

Water. Water usually contributes 70 to 80% of berry fresh weight at harvest. Berries transpire large amounts of water during their development. Prior to veraison, most of the water required by the fruit is supplied by the xylem. After veraison, the phloem becomes the primary supplier of water to the berry because of the blocking of the xylem vessels entering the berry. Sugar, minerals, and other compounds entering the fruit during ripening are also supplied by the phloem (Dokoozlian, 2000).

Sugars. Sugar (in the form of sucrose) provides the carbon structure for the synthesis of many compounds, such as organic and amino acids. Sucrose, the predominant sugar transported in the phloem, is made by joining one molecule of glucose with one molecule of fructose. It is synthetized in leaves and, from the initiation of ripening until harvest, it is transported mainly to fruit. Once sucrose reaches the berry it is hydrolized to glucose and fructose, which are present in approximately equal amounts in grape berries at harvest, each ranging from 8 to 12% of fruit fresh weight. During the initial stages of fruit growth, the berry sugar content is quite low, usually around 2% of the berry fresh weight. Sugar concentration starts to increase rapidly at beginning of the onset veraison, and may reach 25% or more of the berry fresh weight by the time of harvest (Dokoozlian, 2000).

Organic acids. Tartaric and malic acids are the main organic acids of the grape berry, making up approximately 90% of the total fruit acidity. Both acids are produced in the berry, along with small amounts of citric acid and several other non-nitrogenous organic acids. The concentrations of tartaric and malic acid, and total acidity progressively increase during the fruit development until just before the beginning of fruit ripening, reaching the highest values near veraison, and decrease during the ripening phase. Tartaric acid is stable after its synthesis since no degradative enzyme has been found in berry. Nevertheless, the concentration of tartaric acid decreases during ripening due to the dilution effect, since berry volume increases while the amount of tartrate per berry remains constant. Instead, malic acid can be respired by several enzymes in the berry to form CO_2 and H_2O. Thus, its

concentration decreases after veraison because of respiration and enzyme degradation as well as dilution. Moreover, the content of free tartaric and malic acids in the berry decrease during ripening due to the formation of acid-salts with potassium and other cations in the berry. Temperature is a key factor controlling berry acid content. During the initial phases of fruit growth, the optimum temperature for acid synthesis ranges between 20 and 25 °C. It is also well recognized that fruit acidity at harvest is negatively correlated with temperature during the ripening period. Generally, grapes ripened at low temperatures have greater total acidity (in particular malic acid) than those ripened at high temperatures. Therefore, grape acidity differs among regions and years, with the higher values in growing seasons and regions characterized by cold temperatures (Dokoozlian, 2000).

Juice pH. The juice pH is relatively constant during the primary stages of berry development, with value around 2.5, then increases gradually as acid anions are formed and the concentration of malic acid in the berry decreases (Dokoozlian, 2000).

Phenolic compounds. Phenolic compounds include tannins, flavonols, and anthocyanins, the latter are responsible for grape colour. These compounds are synthesized in the berry and located especially in the skin, but also in seeds. Red grape varieties contain much greater concentrations of phenolic compounds than white grapes, since the latter does not contain anthocyanins. Phenolics are important constituents of the grape berry because they determine fruit colour and astringency. The oxidation of caftaric acid, the major phenolic compound present in grape pulp and juice, determine the formation of brown pigment (Dokoozlian, 2000).

Nitrogen compounds. In grape berries, nitrogen is mainly present in the form of ammonium ions (NH_4^+) and organic nitrogen compounds (amino acids and proteins), and little amount of nitrate (NO_3). During the initial phases of berry development, ammonium ions represent more than half of the fruit total nitrogen, and amino acids content is rather low. After veraison, the ammonium concentration declines due to the incorporation of the ions into amino acid. The amino acid concentration of the berry may increase from two- to fivefold during ripening, according to the variety and fruit maturity. Arginine and proline are the main amino acids present in most grape varieties (Dokoozlian, 2000).

Inorganic minerals. The inorganic minerals in grape berries include the cations potassium, calcium, and sodium, and the anions phosphate and chloride. They derive from the soil and are transported from roots to fruits via xylem (before veraison) or phloem.

During ripening the concentration of mineral cation increases from two- to threefold (Dokoozlian, 2000).

Aroma and flavour compounds. The type and concentration of aroma and flavour compounds in berries are strongly dependent on grape variety. Monoterpenes are the best-known and best-described flavour compounds of *Vitis vinifera.* They accumulate during the latter stages of ripening and are responsible for the flowery notes (Dokoozlian, 2000).

1.5 Vineyard ecosystem and wine quality

Factors affecting the vineyard cultivation are certainly related to territory and climate, but the "man's hand" blends all the components. This represents what French call *Terroir*. In other words, according to the Fregoni's scheme (1985; Figure 6), it is possible to affirm that the main factors affecting wine quality are:

- ✓ vineyard: choice of the grapevine cultivar (rootstock, clone);
- ✓ land (or territory): latitude, exposure to the sunlight (topography), soil profile, soil structure and composition, zoning according to suitability (mountains and hills are better than plain and areas close to rivers);
- ✓ climate: macro- and micro-climate;
- ✓ human and viticultural factors: pruning, fertilizing, training systems.

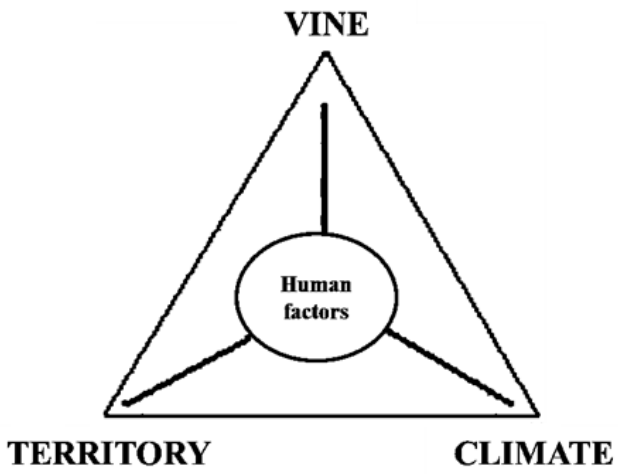


Figure 6 – Fregoni's scheme (source: Fregoni, 1985).

1.5.1 Vineyard

For a quality wine, the choice of vine is necessarily linked to the territory in which wine itself will be produced. In fact, the most successful wines are those produced from grapes that are perfectly integrated into the soil and climate environment.

The current legislature imposes disciplinary, therefore to produce is required the implanting of the varieties authorized and/or recommended by the regulations and especially select the native cultivars. The current trend is to make use of the so-called international varieties, often easier to implant and more consistent in production. Nevertheless, quality Italian varieties are the real point of reference for quality wine production.

The selection of the best clones will lead to a low production, clusters smaller and more compact, and at a higher concentration of substances to be extracted during vinification.

For the vine cuttings propagation, the grafting technique is used. The elements are the rootstock or foot, which represents the portion of the plant provided with radical apparatus, and then the scion, a portion of a branch with one or more gems, which is welded into place. The most known grafting methods are:

- ✓ English double-slit (omega), employed in Central/North Italy;
- ✓ Majorcan (bud), used mainly in Southern and Island Italy and performed directly on the plant (http://www.vinoinrete.it/sommelier/sommelier%20-1-%20viticolturahtm).

For the grapevine selection, it is necessary to conduct a study of the environmental characteristics to identify the best rootstock, which will better adapt to the conditions (such as humidity, drought, heat, cold, wind, and so on). At the same time it will be very important the choice of graft because it proves fruitful and healthy. Around the end of 1800s, *Vitis Labrusca* from America was hiding a serious disease for the vines: *Phylloxera*, a pest that attacked the roots and slowly destroyed the European species. There was an environmental catastrophe and all the vineyards died almost. The attempts to resolve the problem turned out to be a failure after another, until they began to replant vines adopting the technique of grafting European native scion onto a American rootstock, because the phylloxera did not attack the American roots.

Nevertheless it remains some fine local grape varieties such as Nebbiolo and Barbera that have been saved, especially at high altitudes or near the sandy areas where Phylloxera does not proliferate. Currently, Chile is the only country "foot-ex" in the world.

Rootstocks affect vine vigour, as well as influence vine nutritional and hormonal balance. Rootstock selection can also affect the potential wine quality by improving vine healthy status (increasing resistance or tolerance to several pests, diseases and adverse environmental conditions) (Jackson, 2009).

1.5.2 Territory

Latitude. The most of the world's wine producing regions is located between the temperate latitudes of 30° and 50° in both hemispheres. Grapes, although are a temperate-zone plants, can be also cultivated under semitropical conditions. They cannot grow to the cooler parts of the temperate zone, where the seasonal growing time may be too short to allow the fruit to reach maturity or where low winter temperatures (less than -7 °C) may kill the vine or its fruitful buds (http://fnbservicenotes.wordpress.com/2012/07/26/factors-affecting-quality-of-wine/).

Location/Topography. Vines give better grapes in the hills than in the plains. The slope of the ground, called arrangement, provides a higher drainage and increase the impact of the sunray, thus improving vegetative development and fruit ripening. At higher latitude, the slope must ibe higher. In plain are,a the sun exposure is lower as spread over a larger area, spring frosts are more frequent and very dangerous when the plant begins to sprout.

Another important topographic element is the exposure: the vineyards face south have a greater sun exposure and then such provision shall be sought, especially in northern areas.

The local climate could be affected also by the presence of mountains, forests, rivers and lakes that protect the vineyards from cold winds, provide a reservoir of moisture during the hot season and play an important thermoregulatory action (http://www.vinoinrete.it/sommelier/sommelier%20-1-%20viticolturahtm).

Soil. The soil consists of a thin layer tillable, influenced by the cultures of man, and a part below the characteristics of which are given from the geological origin. The soil composition is one of the most important viticulture aspects when planting grape vines. The characteristics of soil are important because it supports the root structure of the vine and influences the drainage levels and amount of minerals and nutrients that the vine is

exposed to. The ideal condition for a vine is an area of thin topsoil and subsoil that sufficiently retains waters but also has a good drainage so that the vine roots don't become overly saturated. The ability of the soil to retain heat and/or reflect it back up to the vine is also an important consideration that affects the ripening of the grape. The composition of the soil has an influence on soil temperature, root penetration, water holding capacity, and vine nutrition. The stony-permeable soil ensures drainage and good maturation of the grapes. In addition, pebbles, less fertile, force plant to sink its roots into the ground and then allow the enrichment of grapes and the wine in mineral extracts. The soil colour is an another essential aspect: dark-coloured soils warm up and favour the ripening of the fruit while clear ones are cooler, slow ripening and then promote wines of higher acidity (http://www.vinoinrete.it/sommelier/sommelier%20-1-%20viticolturahtm). There are several minerals that are vital to the vines and that a good vineyard soil must have. These include: calcium, which helps to neutralize the soil pH levels; iron which is essential for photosynthesis; magnesium, which is an important component of chlorophyll; nitrogen, which is assimilated in the form of nitrates; phosphates. which encourages root development; and potassium, which improves the vine metabolisms and increases it health for the next year's crop. While subsoil always remains geologically true, topsoil composition can be enhanced using manure or fertilizer. Moreover, the degree of acidity of the soil is not overlooked: in Europe the best wines are produced from alkaline and calcareous soils, while in California they are produced from neutral or acidic soils.

1.5.3 Climate

The vine is a very resistant plant and its ability to adapt to various climatic conditions allow a better development of the vine in relation to the production of quality wine.

The average annual temperatures must not be below 10 °C with an average value around 20 °C in summer and -1 °C in winter. The amount of heat is very important since it is preferable a steady ripening that produces fragrant and balanced wines. The key issue is also the winter cold, as it promotes both the maturation of the wood that the death of parasites. Frost is the most feared climatic condition because in late spring it can damage the new shoots, thus reducing the size of the crop. Methods to combat frost include propeller based rind dispersers to disturb air (frost will only occur in still conditions), setting burning stoves (smudge pots) between rows of vines to beat the air, and aspersion-repeatedly spraying vines with water to keep them above 0 °C.

Another important climatic variable is rainfall, because it keeps the soil moist and encourage the ripening of the fruit, especially when concentrated in winter and spring, with cool temperatures. Instead, rains during flowering and at harvest time can create many hazards such as fungal diseases and berries splitting.

Concerning humidity, values around 60-80% are desirable while excessive moisture encourages diseases such as powdery mildew and grey rot.

The other parameter to take into account is the microclimate prevailing in single vineyards or within small regions. Microclimate is determined by the system of pruning, the distance between plants, the distance between rows and the distance of plants from soil. Rise and fall of terrain, location besides water bodies such as lakes or rivers, presence of hills, mountains or forests as wind breakers are other determinants of microclimate (Jackson, 2009). For example, the presence of large bodies of water and mountain ranges can have positive effects on climate and, consequently, on vines. In fact, nearby lakes and rivers can serve as protection for drastic temperature drops at night by releasing the heat stored during the day to warm the vines. Furthermore rivers may raise the ambient temperature of one or two vital degrees.

1.5.4 Training system

Training refers to techniques designed to position the fruit-bearing shoots to optimize both fruit yield and quality, consistent with long-term vine health. There are hundreds of training systems, but few have been studied sufficiently to establish their efficacy. In contrast, several modern training systems, such as the Scott–Henry, Lyre, Smart–Dyson, and Geneva Double Curtain (GDC), have been shown to possess clear advantages in improving both fruit yield and quality (Smart and Robinson, 1991). In addition to increasing vine capacity, fruit health increases (reduced incidence of infection) and cluster location facilitates economic mechanical harvesting. These features both enhance quality and decrease production costs.

The vine's inherent vigour must be restrained on relatively nutrient-poor, dry soils. This has traditionally been achieved by dense vine planting (about 4000 vines/ha) and severe pruning (removal of > 90% of the yearly shoot growth). However, on rich, moist, loamy soil, limited pruning is preferable with wide vine spacing (about 1500 vines/ha). Under these conditions, it is prudent to redirect the increased growth potential of the vine into

greater fruit production, not prune it away. When an appropriate LA/F[1] ratio is established, increased yield and quality can exist concurrently. It is on rich soils that the newer training systems succeed so well. These systems not only achieve a desirable LA/F ratio, but they also provide improved exposure of the grapes to light and air. These features promote ideal berry coloration, flavour development, fruit health, and flower-cluster initiation.

1.5.5 Nutrition and irrigation

Generally, stressing the vines is often viewed as essential to fine wine production. This view probably arose from the reduced-vigour, improved-grape quality association found in some renowned European vineyards. However balancing vegetative and fruit-bearing functions is the real goal. Exposing the vine to extended periods of water or nutrient stress are always detrimental. Equally, supplying nutrients and water in excess is detrimental, as well as wasteful.

In practice, regulating nutrient supply to improve grape quality is difficult. Because the nutrient demands of grapevines are surprisingly small (partially because of the nutrient reserves of the vine's woody parts), deficiency symptoms may not express for several years. Moreover, establishing nutrient availability is still an approximate science.

Irrigation can be employed to control vine development and encourage optimal fruit maturing. Irrigation water can also supply nutrients and disease-control chemicals directly to the roots in specifically measured amounts and at specific times. These possibilities are most applicable in arid and semi-arid conditions, where most of the water supply comes from irrigation (Jackson, 2009).

[1] LA/F ratio: Leaf Area/Fruit weight ratio.

Chapter 2

History, geography, and economics of grape wine derived from red berry

2.1 Wine history

The history of wine dates back over 6000 years BC. According to an ancient Persian fable, wine was the accidental discovery of a princess seeking to end her life with what she thought was a poison (http://rmc.library.cornell.edu/ewga/exhibition/introduction/). Archaeological evidence suggests that the earliest production of grape wine took place in Georgia and Iran. The world's oldest known winery (dated to 3000 BC) was discovered in Armenia (http://en.wikipedia.org/wiki/History_of_wine). The Egyptians were the first to record the process of wine making on stone tablets and on the walls of their tombs but wine was considered a privilege of the upper classes. In the ancient Greece, wine was praised by historians and artists but, like the Egyptians, the ordinary citizens did not consume wine. With the Roman Empire, the production of wine spread throughout Europe and wine became available to the common citizens. The Romans did not drink pure wines but they added flavours such as fermented fish sauce, garlic and onion. During the Dark Ages, wine production was prerogative of monasteries. The Benedictine monks became one of European largest wine producers with vineyards in Champagne, Burgundy, and Bordeaux regions (France), as well as in the Rheingau and Franconia regions (Germany). The wine industry had a brief decline in the 17th Century, while the 19th century was the golden age for wine in many regions, although, around 1863, many French vines suffered from a disease caused by the *Phylloxera* aphid. When American vines were discovered as resistant to *Phylloxera*, it was decided to graft European vines on American rootstocks (http://www.wineinmoderation.eu/en/wine-a-culture-of-moderation/history-of-wine). The resulting products were many hybrid grapes that produced a greater variety of wines. The last century have seen an overall revolution in the wine industry, with the development of a strong scientific background and innovation in equipment and processing (http://www.artmakers.com/wine/history.html).

2.2 Wine geography and economics

Worldwide, the most diffused red grape varieties are in a descending order: Grenache, Carignan, Merlot, Cabernet Sauvignon, Rkatsiteli, Bobal and Tempranillo. Many of these are of European origin. In our continent, the most prevalent grape varieties are: Tempranillo, Merlot, Grenache, Cabernet Sauvignon, Syrah, Bobal, and Sangiovese. Table

1 shows the areas under vines in Europe, divided by grape variety (EU Data, Agricultural Year 2009/2010).

Grape variety	Total	Spain	France	Italy	Others
Tempranillo	213	213	-	-	-
Merlot	165	14	113	24	14
Grenache	155	67	88	-	-
Cabernet S.	101	22	54	16	10
Sirah	93	20	66	7	-
Bobal	76	76	-	-	-
Sangiovese	70	-	-	70	-
Carignan	50	6	44	-	-
Cabernet F.	41	-	36	4	1
Montepulciano	31	-	-	31	-
Pinot Noir	30	-	30	-	-
Gamay	29	-	29	-	-

Table 1 – Most cultivated red grape varieties in EU in thousands of hectares (source: EU Data, 2010).

Italy is a very fragmented reality when compared with neighbouring countries. While in France, Spain and Germany there are varieties that occupy about 25% of the vineyard terroir, in Italy on only 652,000 hectares more than 350 varieties of grapevine are cultivated in different ways in relation to the regional traditions. The most diffused national red grape varieties are: Sangiovese (10%), Montepulciano (4%), Barbera (4%), and Negroamaro (3%). International varieties such as Cabernet Sauvignon, Merlot, and Pinot Noir, and many other regional varieties are also cultivated.

Concerning Apulia region, the vineyard area covers about 85,000 hectares, located for more than 90% in the lowlands and the remainder in the hills. The 2012 production was around 8.3 million of quintals (http://www.inumeridelvino.it/2012/08/puglia-produzione-di-vino-e-superfici-vitate-aggiornamento-2011.htmL), with a yield of about 100 quintals per hectare. The most common forms of farming are the marquee, the guyot and the spurred cordon.

In Apulia, where about 82% of the cultivation of grape involves red grape, 3 areas can be distinguished:

- ✓ North-Bari, including the Capitanata and the province of Barletta-Andria-Trani, with varieties such as Uva di Troia, Montepulciano, and Sangiovese. In this area

half of the vineyards of Apulia is concentrated, with a production of 5 million tons of grapes (60% of regional production) and the highest yields (160 quintals of grape per hectare);

- ✓ Center, including the province of Bari and part of the provinces of Taranto and Brindisi, with red grape varieties such as Primitivo and Aglianico and white grapes;
- ✓ Salento, including the province of Lecce and the remaining parts of the provinces of Taranto and Brindisi, with varieties such as Negroamaro, Primitivo, and Malvasia Nera.

According to preliminary estimates of the OIV (International Organisation of Vine and Wine), in the current year (2014), the world production of wine (excluding juice and musts) would reach 271 Mhl[2] with a decrease of 6% respect to the production of 2013.

France is back in first place among wine producers in the world (46.2 Mhl, up 10% compared to 2013) while Italy recorded a lower production (44.4 Mhl -15%) compared to 2013), and Spain back to a level of medium production (37 Mhl) after a record year (over 45 Mhl in 2013). It should be also mentioned the strong production growth in Germany (9.7 Mhl, + 16%). The decline in production is amplified in Eastern European countries: Bulgaria, Romania, and Slovakia reported significant declines in wine production compared to 2013 (respectively -30, -20 and -16%), due to unfavorable climatic conditions. Only Hungary maintains the level of production of 2013 also in 2014, with 2.7 Mhl.

Elsewhere in the EU, the trends are generally decreasing, as in Croatia (-30% /2013 with a production slightly less than 1 Mhl) or, to a lesser extent, Portugal and Greece, with 5.9 Mhl (-6% /2013) and 2.9 Mhl (-13% /2013), respectively. For the third consecutive year, the United States showed a high production level, and this in spite of the negative effects of the earthquake of August and the adverse climatic conditions occurred in September in California. The 2014 production was of approximately 22.5 Mhl. In the Southern hemisphere, contrasting trends were observed: Chile, after two record years in 2012 and 2013, seeing a significant decline in its production: 2.8 Mhl (-22% /2013), but it is expected to remain stable at 10 Mhl, while in Argentina the wine production grows slightly: 15.2 Mhl (+ 1% /2013). South Africa would reach a wine production of 11.4 Mhl

[2] Mhl: milion of hectoliters.

(+ 4% compared to 2013). In 2014, New Zealand would set a new record, reaching 3.2 Mhl (+ 29% compared to the previous record in 2013: 2.5 Mhl), while in Australia the production of 2014 seems to mark a slight increase respect to that of 2013 (12.6 Mhl compared to 12.3 Mhl).

Concerning, OIV haven't yet complete information on the levels of world wine consumption in 2014, but the available evidences indicate consolidation of the global consumption of around 243 Mhl (http://www.teatronaturale.it/strettamente-tecnico/mondo-enoico/20110-cala-la-produzione-vitivinicola-mondiale-tutta-colpa-dell-europa.htm).

Chapter 3

Red-wine winemaking and aging technologies

3.1 Winemaking technologies

According to Ewing-Mulligan and McCarthy (2005), wines can be classified as red or white wines, sparkling, semi-sparkling or still wines, fortified and sweet wines.

The classic "red vinification" starts with the crushing and destemming of grapes. The derived must remains in contact with skins and seeds for a few or several days (rosé or red wines). The anthocyanins, which are responsible of red wine colour, are extracted from the grape skins during fermentation by the solubilizing action of the produced alcohol. During the maceration/fermentation step, the marc tends to emerge from the must forming the so-called "cap". To submerse this cap in the must can be exerted a mechanical action (pressing), or the must collected from the bottom of the tank can be pumped on the cap by wetting it (pump over), or the emersion of the cap can be avoided by introducing a mesh screen into the tank (submerged cap fermentation).

The colour of a red wine strongly depends on the maceration step length during fermentation since pulps are almost always clear, except for few so called "teinturier" grapes that have coloured pulps.

The main procedures to produce red wines are: addition of co-pigments, enzymes, and tannins; carbonic maceration; co-winemaking; cryomaceration; délestage; prolonged maceration; saignée; thermovinification. These procedures are described below.

3.1.1 Addition of co-pigments, enzymes, and tannins

Co-pigmentation consists in a hydrophobic interaction between free anthocyanins and other phenolics. The co-pigmentation reactions of anthocyanins consist in the conversion from monomeric to polymeric anthocyanins and cause losses of the red colour intensity, changes of tone from purple-red to red, and increase of colour stability (Baranac *et al*., 1997). The most common co-factors for co-pigmentation are flavonols, but also flavanols, hydroxycinnamoyltartaric acids, and hydroxycinnamic acids can react with anthocyanins as co-pigments if present in higher amounts in musts and wines (Liao *et al*., 1992; Eiro *et al*., 2002). To allow co-pigmentation reactions and the contact between anthocyanins and the other phenolic compounds, the addition of co-pigments into must is generally followed by a prolonged pre-fermentative maceration.

Álvarez *et al*. (2009) studied the effects of the pre-fermentative addition of co-pigments and of different winemaking technologies on the phenolic composition of Tempranillo wines after malolactic fermentation. In separate experiments, they added caffeic acid, rutin, (+)-catechin, white grape skin tannin, and white grape seed tannin as co-pigments and compared the traditional vinifcation with the pre-fermentative cold maceration at 6–8°C for 4 days and the pre-fermentative cold soak with dry ice at 0–2°C for 4 days. According to the results, the researchers established that pre-fermentative addition of co-pigments (especially of white grape tannins) increased anthocyanin co-pigmentation reactions and produced wines with a more intense colour, a higher anthocyanin concentration, a higher percentage of tannins polymerised with polysaccharides and a lower astringency. Cold pre-fermentative maceration (in particular when it was performed with solid CO_2) increased extraction of polyphenols, anthocyanin co-pigmentations, and polymerisation reactions between tannins and polysaccharides.

Sacchi *et al*. (2005) reported that the addition of pectolytic enzymes may determine decreases of anthocyanins and increases of polymeric pigments that stabilize wine colour. The level of extraction and the state of pigments polymerization determine red wine colour, structure, and aging potential. An important aspect to take into account is the purity of pectolytic enzymes, in particular the absence of any cinnamoyl esterase activity (in order to avoid concentrations of 4-ethyl-phenol above the sensory threshold in the wine; Gerland, 2003) and of β-glucosidases (to avoid the conversion of anthocyanins to the less stable aglycone forms and thus the colour loss; Sacchi *et al*. 2005).

Amrani Joutei and Glories (1994, 1995) suggested that the extraction of the anthocyanins, (located wholly in the skin cell vacuoles) is due to the action of sulphur dioxide that degrades the proteinaceous fractions of cellular membranes. Pectinolytic enzymes might help the extractability of upper-middle molecular weight tannins attached to the vacuolar membranes and to the polysaccharides of the cell wall, thus enhancing wine structure. The presence of side activities in pectic enzyme formulations, such as cellulases, hemicellulases, and proteases may also allow an enhanced extraction of grape polysaccharides, thus producing positive effects on wine structure (Doco *et al*., 1995). Bucelli *et al*. (2006) performed experiments on Sangiovese grapes to better understand the role of maceration enzymes during the red-winemaking. They investigated: the influence of the composition of four enzymatic preparations (containing different amounts of polygalacturonase, pectinase, galactanase, cellulose, pectinmethylesterase, and acid

protease) on wine colour; the effect of enzymatic treatment during protracted maceration; the evolution of colour and sensory features in the course of wine storage. Authors observed a rise of colour stability, revealed by higher colour intensity and lower hue at 18 months of aging and also enhanced sensory properties, with particular reference to structure, fullness, balance, and aroma complexity. The treatment with enzymes had no effect on the extraction of anthocyanins during fermentation but allowed the extraction of greater amounts of tannins, thus leading to the formation of more stable polymerized pigments. Furthermore, lengthening of the maceration time to 20 days in the absence of enzymes generated wines that were similar to those produced using enzymatic treatments with simply 8 days of contact with skins. Moreno-Pérez *et al*. (2010) evaluated the effectiveness of the application of two macerating enzymes (a pectinase preparation that mainly contain pectin lyase, polygalacturonase, and pectin esterase activities, and a β-galactosidase preparation) on the colour parameters and phenolics during the maceration phase. Authors established that the use of maceration enzymes led to wines richer in anthocyanins and having improved chromatic features and that there were differences among varieties. In the case of Syrah wines, the best results were obtained with the β-galactosidase treatment, whereas for Cabernet-Sauvignon wines the pectinase formulation was to be preferred. Recently, a study of Borazan and Bozan (2013), focused on the effects of pectolytic enzyme addition on the phenolic composition of Okuzgozu red wine during the winemaking and aging phases, demonstrated that, after 6 months of bottle aging, the enzyme-treated wines had lower phenolic acids concentrations and monomeric flavan-3-ol content than the control wines, and no significant differences in terms of extracted free anthocyanins.

The addition of grape condensed tannins (proanthocyanidins) is a suitable technique to improve the anthocyanin stabilization, through the formation of polymeric pigments. In addition, the wood hydrolysable tannins (ellagic tannins and gallotannins) exercised a certain colour protection. Soto Vázquez *et al*. (2010) conducted a study on the evaluation of the addition, during alcoholic fermentation, of pectolytic enzymes (with cinnamyl-esterase, polygalacturonase and pectin lyase activity), procyanidin tannins extracted from white grape pips, ellagitannins derived from oak wood, or not toasted oak chips on phenolic composition and colour features of young red wines, elaborated from Mencía grapes. The authors establish that the main benefit of the addition of enzymes or tannins respect to the traditional winemaking was their greater capacity to favour the extraction of

anthocyanins, as well as to facilitate the co-pigmentation reactions and thus the improvement of colour. Instead, the addition of oak chips did not favour the reactions involved in anthocyanin stabilization. They concluded that the addition of enzymes and tannins seemed to be the most suitable winemaking technologies to obtain red wines for aging, while the addition of oak chips could be used for the production of ready-to-drink red wines.

Baiano *et al*. (2009) observed that the addition of tannins from skin and seeds and ellagitannins determined the increase of the phenolic content and the radical scavenging activity of Primitivo musts and wines than the other technologies (traditional, cryomaceration, saignée, délestage, delayed punching-down, heating of must, and prolonged maceration). Furthermore, after 12-months of aging, the wines obtained through the addition of tannins showed high concentrations of anthocyanins sensitive to SO_2, monomeric anthocyanins, flavonoids, flavans reactive to vanillin, and coumaroylated malvidin and low content of acetylated malvidin. Neves *et al*. (2010) considered the effects of two greatly pure commercial grape seed tannins on the phenolic composition, chromatic characteristics, and antioxidant capacity of two types of red wines made with Castelão/Tinta Miúda (3/2, w/w) varieties by using two different maceration times, which corresponded to wines rich and poor in polyphenols, respectively. The results highlighted that the addition of grape seed tannins comported an increase of colour intensity and antioxidant activity only in wines poor in polyphenols. The authors also found that the addition of tannins after alcoholic fermentation was more effectiveness on phenolic composition than tannins added before it.

Liu *et al*. (2013) investigated the effects of pre-fermentative addition of exogenous condensed or hydrolysable tannins on anthocyanins and colour characteristics of Cabernet Sauvignon wines. The results of this study suggested that the addition of hydrolysable tannins did not produce any influence on wine redness even after 9 months of bottle aging, while condensed tannins exercised a certain protection against pigment degradation and improved wine yellowness and redness since they delayed both degradation of most anthocyanins and decrease of some pyranoanthocyanins. Condensed tannins also protect wine against oxidation or contribute to form co-pigmented anthocyanidins or polymeric pigments.

3.1.2 Carbonic maceration

Carbonic maceration, also referred to as "whole berry fermentation" or "whole grape fermentation", is a vinification procedure in which whole berries or bunches of grapes are held under a carbon dioxide atmosphere and an anerobic fermentation occurs because of various enzymatic reactions inside the grapes, especially glycolytic enzymes (Flanzy, 1935). After a period of one to two weeks, the grapes are pressed and the must is inoculated with yeast to allow the conclusion of the fermentation process. This procedure is typically employed to produce lighter, less tannic, and fruity wines that are destined to be consumed immediately.

Salinas *et al*. (1996) studied the influence of the duration of the anaerobiosis phase on the volatile compounds of wines made from Monastrell grapes by carbonic maceration. They observed that the optimal sensory characteristics were exhibited by wines that had undertaken to a long period of maceration, resulting in a higher ethylic esters and fatty acids contents and a lower alcohol concentration. Etaio *et al*. (2008) reported that wines produced with carbonic maceration and the addition of white grapes (cv. Viura) tended to have higher ethanol, free and total SO_2, red berry aroma and flavour, alcoholic flavour, and acidity than the control wines. Moreover, the wines addition of the white grapes showed lower pH, dry extract, visual colour intensity, purple hue tannins, anthocyanins, and total polyphenol index (due to the dilution effect of lower polyphenol levels in white grape skins), but there were no differences in total acidity and in aroma or flavour attributes.

3.1.3 Co-winemaking

Each grape variety has its specific phenolic composition and aroma profile. Some grape varieties can be rich in determined compounds, while other grapes may be poor. The content of these compounds may be changed through the winemaking process. An example is represented by the "coupages", a technique consisting in an initial production of mono-varietal wines, which are later mixed. However, the simultaneous maceration and fermentation of different grape varieties could benefit from additional molecules provided by the other varieties (Gómez García-Carpintero *et al*., 2010; Gómez Gallego *et al*., 2012), which results in a more complex formation than in mono-varietal wines (Lorenzo *et al*., 2008a; 2008b). This complementary effect can be realized with the co-winemaking of different grape varieties that involves both co-maceration and co-fermentation steps. In

fact, during maceration and just at the beginning of fermentation, interactions between phenolic compounds, stabilization reactions, and aroma extraction occur. This means that better results can be attained if blending occurs at the vinification step. Nevertheless, if this procedure is inadequately performed, it may have worsening effects on wine quality because of anthocyanin dilution or pigment adsorption to the pulps and skins (Diago-Santamaría and Boulton, 2003). Generally co-winemaking is not employed due to the different ripening epochs of grapes, even though this problem could be partially resolved with the grape cooling.

Gómez García-Carpintero *et al*. (2010) examined the influence of co-winemaking on the sensory profile of wines obtained from several minority red grape varieties cultivaed in La Mancha region (Spain). Co-winemaking red wines were obtained by blending (a) Cencibel (50%) and Bobal (50%); (b) Cencibel (50%) and Moravía Agria (50%); (c) the three grape varieties Cencibel (33%), Bobal (33%), Moravía Agria (33%). Authors concluded that mono-varietal Cencibel and Bobal wines presented similar olfactory profiles, while wines obtained from the blending of the two varieties showed more complexity and a general greater intensity of attributes than the mono-varietal wines. The sensory profile of the wines obtained by co-winemaking of the three grape varieties was the most complex of all the co-winemaking wines.

Gómez Gallego *et al*. (2012) applied co-winemaking to produced red wines by pre-fermentative blend of grapes (1:1, w/w) from Cencibel (Tempranillo) and the minor varieties Bobal, Moravia Agria, Moravia Dulce, Tortosí, and Rojal. A three variety blend (1:1:1, w/w/w) of Cencibel, Bobal, and Moravia Agria grapes was also produced. The best results were obtained using Bobal, Moravia Agria, and Moravia Dulce varieties for co-winemaking, especially in terms of colour characteristics. All co-winemaking wines significantly increased the total resveratrol content as compared to the Cencibel reference wine.

Lorenzo *et al*. (2008a) studied the co-winemaking of Monastrell wines with Cabernet Sauvignon or Merlot has been studied in terms of odour activity value (OAV) of volatiles in young and aged (in French oak barrels or in the bottle) wines. The co-winemaking wines were richer in fruity and sweetness attributes than the mono-varietal ones. The best proportion was 60:40 in the case of young or aged in barrels Merlot and in the case of the bottled Cabernet Sauvignon.

3.1.4 Cryomaceration

Cryomaceration (or cold soak) is one of the most important techniques used in wine-making. In the past years, the pre-fermentative cold maceration was mainly used in the production of white wines, and only recently it has been employed for red grapes (Parenti *et al*., 2006). According to this technology, the mashed grapes are submitted to a rapid cooling (down to about 5 °C) and this temperature, maintained for several hours or days before fermentation, allows to increase the extraction of the compounds contained in the grape skins, such as phenolics and primary aroma compounds (Carillo *et al*., 2011). If the pre-fermentation skin contact is performed with dry ice, freezing increases the volume of the intracellular liquids thus damaging the cell membranes and allowing an easy leaving of the aromatic and phenolic compounds (Couasnon, 1999). Carillo *et al*. (2011) reported that wines produced by cryomaceration show higher aroma intensity, stability to oxidation, and stability of taste properties than wines prepared by classical maceration. The cryomaceration process can be performed in different ways. One of these needs the use of large-capacity refrigerator groups, which involve a high amount of energy consumption and very expensive plant costs. Besides, they very often damage the product due to friction generated in the tubes during the passage of the mashed grapes (Ribèreau-Gayon *et al*., 2000). A more suitable process is based on the use of cryogenic gas (CO_2 or N_2) directly injected into the line, which during evaporation, absorb heat and cool the product (Carillo *et al*., 2011).

Parenti *et al*. (2006) evaluated the effects of different cryogenic agents (i.e., solid state carbon dioxide and liquid nitrogen) and temperatures (from -5 to +5 °C) on Sangiovese wines. According to their results, wines submitted to pre-fermentative cold maceration showed a better quality profile than the control. Moreover, liquid nitrogen showed a higher extraction of phenolics than solid carbon dioxide and, for the latter, wine quality increased as the cold maceration temperature decreased.

Radeka *et al*. (2012) assessed the influence of pre-fermentative cryomaceration on the aromatic profile of rosé and red wines from Croatian aromatic cv. Muskat ruza porecki (*Vitis vinifera* L.). They found higher concentrations of free and bound varietal aroma compounds in wines obtained by maceration at room temperature with respect to the cryomacerated wines. From a sensory point of view, longer maceration treatments improved odour and overall wine quality, together with the intensity and recognisability of

varietal Muscat aroma, while short-term cryomaceration was the favourite technique for the production of light rosé wines with pronounced Muscat aroma and low phenolic content.

The effects of a cold pre-fermentative maceration and other technologies (traditional maceration and addition of pectolytic enzymes) on the extraction of colour and phenolic compounds during red wine making of Tannat, Syrah, and Merlot wines were examined by González-Neves *et al*. (2013). They observed that Tannat and Merlot wines elaborated in presence of skins at low temperature (10 to 15ºC) for five days before fermentation showed higher colour intensity and total polyphenol, anthocyanin, and proanthocyanidin contents.

Marais (2003) assessed the effects on of low-temperature skin contact (1, 2, and 4 days at 10 and 15 °C) before fermentation of Pinotage grapes on wine composition and quality. Polyphenol content slightly increased with the increase of the contact time. The best wine quality was obtained by a pre-fermentative maceration at 10 °C for 4 days, while the worse results were obtained at 15 °C.

A study on the extraction of phenolics from berry skins and seeds of Cabernet Sauvignon grapes during a red vinification and the influence of cold soak were examined by Koyama *et al*. (2007). According to their results, anthocyanin, flavonol, and epigallocatechin units of proanthocyanidins mainly contained in berry skins were extracted during the early stage of maceration, whereas gallic acid, flavan-3-ol monomers, and epicatechin-gallate units of proanthocyanidins contained in seeds were gradually extracted. Cold soak reduced the extraction of phenolics from the seeds.

Gambacorta *et al*. (2011) assessed the effects of cryomaceration (24 h at 5 °C using dry ice) on the phenolic composition of Aglianico, Montepulciano, Nero di Troia, and Sangiovese wines. They observed that cryomaceration led to a decrease of anthocyanins (about 15%) in all cultivars. The extraction of phenolic compounds from grapes was found to be strongly dependent on the grape variety rather than on the applied winemaking technique.

3.1.5 Délestage

This maceration technology, also called "rack and return", improves the interchange between liquid (must) and solid (skins, seeds, remains of leaves and stalks, etc.) phase by draining the fermentation tanks of liquid while the juice is aerated and, after some hours, the liquid is slightly pumped over or returned to the marc. In fact, the production of CO_2 bubbles during the must fermentation determines the upping of the solid parts of the must and the pressing of the marc toward the surface and its simultaneous pushing back down by the force of gravity. As a consequence, a hard cap forms and only partially releases its elements to the juice. Délestage allows a replacement of oversaturated juice with some less concentrated juice, which would produce an ideal extraction from the pomace. Moreover, this procedure is designed to help oxygenation while minimizing mechanical grinding of the marc and to increase the formation of acethaldehyde that induces the formation of more stable pigments, thus stabilizing wine colour.

In a study of 2008, Zoecklein *et al*. compared Merlot and Cabernet Sauvignon wines obtained by délestage (involving partial seed expulsion) with wines produced by manual cap punching (Merlot), and by mechanical punch-down (pigeage) systems (Cabernet Sauvignon). According to their results, fermentation determined a decrease of the percentage of colour due to free anthocyanins and an increase of the percentage of colour from polymeric pigments for all the assessed procedures. In particular, délestage wines generally exhibited a higher percentage of colour due to large polymeric pigments than either manual cap-punched or pigeage wines. Total 3-*O*-monoglucosides were in greater concentration in the manual cap-punched Merlot wines, while similar concentrations were detected both in the Cabernet Sauvignon wines produced by pigeage and délestage.

Baiano *et al*. (2009) observed that Primitivo wines obtained by délestage technique showed the highest concentrations of total anthocyanins and of coumaroylated forms of anthocyanins if compared to other maceration technologies (traditional, saignée, delayed punching-down, addition of tannins, heating of must-wine, cryomaceration, and prolonged maceration).

3.1.6 Prolonged maceration

This practice prolongs skin contact after the must has fermented to dryness. In this way, the extraction of anthocyanins, flavan-3-ols, and tannins from grape skins and seeds is prolonged beyond the period of alcoholic fermentation.

Extraction of these compounds greatly influences the sensory characteristics and the aging aptitude of wines. Oenologists can extend the duration of maceration in two ways: by postponing the must inoculation with yeasts or by delaying the pressing of the skins after the conclusion of the alcoholic fermentation (Heartherbell *et al*., 1996; Watson *et al*., 1997; Boulton *et al*., 1998). The first procedure allows to extract free anthocyanins that in wine can polymerize increase colour stability (Auw *et al*., 1996), while the second one allows to extract alcohol soluble compounds such as high molecular weight astringent and bitter seed tannins (Cerpa-Calderón and Kennedy, 2008). A study of Budic-Letoc *et al*. (2003), focused on the influence of different maceration techniques and aging on proanthocyanidins and anthocyanins of red wine cv. Babic reported that prolonged maceration increased total phenols, vanillin index and proanthocyanidins, as well as decreased the anthocyanins concentration in young wine.

In another study of Budic-Letoc *et al*. (2008), the effects of maceration conditions on the phenolic composition of red wine Plavac mali were studied. The authors observed that protracted skin contact time significantly improved the content of low molecular weight proanthocyanidins and decreased the concentration of anthocyanins. Moreover, weaker colour intensity and the highest proanthocyanidins/anthocyanidins ratio wines were detected after 14-month of aging in the wine made by prolonged skin maceration time.

According to Baiano *et al*. (2009), the extended maceration was the worst winemaking technology among those assayed for the processing of Primitivo grapes (traditional, délestage, saignée, delayed punching-down, addition of grape seed tannins, addition of ellagic-skin-seed tannins, heating of must-wine, cryo-maceration, and prolonged maceration) in terms of antioxidant content.

Recently, Casassa *et al*. (2013) evaluated if there may be a synergistic effect of ethanol and prolonged skin contact on the extraction of certain phenolics that may negatively impact wine sensory properties. The authors established that differences by 1.2% v/v in the ethanol content did not affect tannin and anthocyanin extraction, colour, tannin degree of

polymerisation, polymeric pigment formation, and recovery of anthocyanins and tannins in the pomace after maceration. In addition, they observed that the tannin content of wines obtained from extended maceration technique mainly derived from seed tannins, whereas control wines had a balanced amount of seed and skin tannins. The anthocyanin concentration was lower in wines made by extended maceration.

3.1.7 Saignée

The Saignée (French for "bleed") method is the practice of removing ("bleeding off") some of the juice from the must in order to concentrate phenolics, colour and flavour of red wines (Ritchie, 2010). In fact, when the juice is released from a crushed red grape, it contains little amounts of aroma compounds, anthocyanins, and other phenolics because they are initially contained in the skins. After about 30-60 minutes, the skins start to release these compounds thus, to concentrate must, saignée should be performed as soon as possible after crushing. Since must also contains organic acids and yeast assimilable nitrogen, thus a nitrogen addition or a correction of acidity could be needed (Ritchie, 2010). The percentage of juice removed is in the range 5-30% depending on the raison of the saignée. In some cases, this technique should be applied with attention. In fact, saignée could also concentrate undesirable vegetal flavours responsible for bitterness and astringency, especially when the must concentration is made to counterbalance the impossibility for grapes to reach the optimum maturity.

Wines produced by saignée are characterized by higher tannin and polymeric pigment contents (Zamora *et al*., 1994) and by slightly higher colour intensity and phenolic content (Gerbaux, 1993). Baiano *et al*. (2009) compared the effects of saignée to those of other winemaking procedures on the phenolic content and antioxidant activity of Primitivo musts and wines. They concluded that saignée was recommended to increase the radical scavenging activity of Primitivo wines, even if the wines obtained by the application of other procedures (for example, the addition of tannins) showed higher antioxidant contents also after 1 year of storage.

3.1.8 Thermovinification

This technology consists in heating grapes and juice to near-boiling temperatures (generally from 60 to 70 °C) for a short time before fermentation. It is used to improve the extraction of colour and tannins from grapes relatively deficient in one or the other or as a sanitizing agent to reduce the *Brettanomyces* contamination. The effects of heating deal with its aptitude to break down the cell structures. The heat treatment of the crushed-destemmed grapes determines a damage on the hypodermal cell membranes, thus favouring the anthocyanins extraction, and denatures polyphenol oxidase, thus preventing browning. After the heat treatment, must is flash-cooled, decanted, and clarified before fermentation.

Stella *et al*. (1991) compared thermovinification and traditional fermentation for three Italian varieties: Negroamaro, Primitivo, and Ibrido P. They highlighted that thermovinification increased anthocyanin content in all the wines and determined decreases catechin and total polyphenols for Primitivo and Ibrido P wines.

Chiaramonti *et al*. (1999) observed that Niellucciu wines produced by classic maceration and thermo-maceration showed similar anthocyanin contents, but the structure of the pigments was different. Whereas the classic maceration gave a wine richer in anthocyanins in the flavylium cation form, the thermo-maceration determined an extraction of anthocyanins in the quinonic, carbinol bases, and chalcone forms.

Atanackovic *et al*. (2012) studied the influence of winemaking technologies and cultivars on resveratrol content, total phenolic content, and antioxidant potential of red wines made from different grape cultivars (Merlot, Cabernet Sauvignon, Pinot Noir and Prokupac). Authors observed that thermovinification increased the total phenolic content and antioxidant potential of all wines. In addition, Fretté *et al*. (2012) compared the effects of thermovinification (70 °C) to those a traditional pre-treatment (30 °C) on the content of phenolic compounds in Rondo wines. Compared to traditional pre-treatment, thermovinification increased the content of anthocyanins, catechin, and resveratrol by 62%, 69% and 260%, respectively.

3.2 Aging technologies

Wine aging is an important process to produce high-quality wines. Traditionally, wines are aged in oak barrel aging systems. However, due to the disadvantages of the traditional aging technology, such as lengthy time needed, high cost, etc., innovative aging technologies have been developed. These technologies involve aging wines using wood fragments, application of micro-oxygenation, aging on lees, and aging in amphora. Moreover, wine bottling can be regarded as the second phase of wine aging and it is essential for most wines.

3.2.1 Wine aging in oak barrel

The aging in barrel is one of the most common methods in winemaking process, and oak wood has been used to construct wine barrels for over 2000 years (Jackson, 2008). The main oak species used are *Quercus alba* from North America and *Q. robur* (also known as *Q. peduncolata*) and *Q. sessilis* (also known as *Q. petrae* or *Q. sessiflora*) from France. Oak barrel can benefit wines in two ways. On one hand, astringency-related phenolic compounds and oak-responsible aromatic compounds are transferred to wine during aging. On the other hand, atmospheric oxygen that permeates through the barrel wall allows certain compounds to be oxidized gently, which results in a reduction of astringency and changes in colour (Bozalongo *et al*., 2007).

There are several studies about wine aging in oak barrel and several conclusions could be obtained from them. First, oak species and their geographical origins play an important role in defining oak compositional differences and oxygen diffusion rates. For instance, the concentration of *cis*-lactone, which is one of the most sensorially important compounds, is higher in American oak than in French oak (Sauvageot and Feuillat, 1999). Moreover, wines aged in American oak barrels have a higher *cis*/*trans*-lactone ratio than those aged in French oak barrels (Gómez-Plaza *et al*., 2004; Garde-Cerdán and Ancín-Azpilicueta, 2006a). On the other hand, the natural oxygen permeation rate is lower for American oaks than for French oaks, probably due to the higher porosity of French woods (Nevares and Álamo, 2008).

Second, wood seasoning and toasting in cooperage is required to produce high-quality barrels suitable for wine aging. Seasoning not only decreases the high percentage of

humidity in wood to make it balance with the ambient humidity, but also promotes the wood maturation by reducing bitterness and astringency and increasing aromatic properties (Simón *et al*., 2010). Toasting is used for wood bending during barrel assembly and encourages the pyrolysis of lignin, tannins, and hemicellulose. There are three different toasting intensities: light, medium, and heavy. Light toasting produces few pyrolytic by-products, which results in less aromatic compounds but more tannins. Medium toasting produces many phenolic and furanilic aldehydes which provides woods with vanillin and roasted characters. Heavy toasting destroys or limits the synthesis of phenolic and furanilic aldehydes, and simultaneously generates volatile phenols that donate a smoky and spicy character (Jackson, 2008).

Barrel age is another crucial factor tobe considered in the barrel choice. Several oak-related volatile compounds extracted from oak become gradually exhausted with barrel reuse. Thus, the initial extraction rate of these compounds in new barrels is higher than that in used barrels, and more compounds related to toasting can be extracted from new barrels (Gómez-Plaza *et al*., 2004). Therefore, for long-term aging (12-15 months), the concentrations of most of the oak-related volatile compounds (furanic aldehydes and oak lactones) in wines aged in American oak barrel (*Quercus alba*) can be similar to those obtained by aging in new barrels and in once-used barrels (Garde-Cerdán and Ancín-Azpilicueta, 2006b). Although the extraction rate of oak-related compounds in new barrels is higher than that in used barrels, the preservation effect of free anthocyanins against oxidation enhances when wines are aged in used barrels (Gambuti *et al*., 2010). This is probably because used barrels release lower contents of reactive compounds, such as ellagitannins, low molecular weight phenols, and hydrolysable tannins than the new ones (Barrera-Garcia *et al*., 2007; Gambuti *et al*., 2010). In this way, in used barrels, the reactions between free anthocyanins and wood released components are slowed. Furthermore, the lower permeability of the used barrel to oxygen also contributes to the preservation effect of free anthocyanins (Ribèreau-Gayon *et al*., 2006). As barrels get older, they might be contaminated by yeasts such as *Brettanomyces* and *Dekkera*, which has been found 8 mm deep within the wood barrel staves (Suarez *et al*., 2007). Taking into account the cost of barrels and the negative effects of old barrels, the frequency of reuse of oak barrels should depend on economics and intensity of barrel character desired.

Besides the characteristics of barrel, the extractive capacity of wine is closely correlated with the wine composition. Sulfur dioxide can act as a solvent of phenols and encourage

the extraction of these compounds, while classic oenological parameters, such as titratable acidity, pH, and ethanol, can directly affect the ethanolysis of wood components. However, the crystals of potassium hydrogen tartrate, which is formed from potassium and tartaric acids, can impede the contact of wines and woods. In some cases, they may even obstruct the wood pores and slow down the diffusion rate of wood components (Ortega-Heras *et al*., 2007). Therefore, it is necessary to take into account both characteristics of barrels and wine composition to decide the length of the in barrel-aging.

Due to the high cost of purchasing and maintaining of oak barrels and the limited supply of current oak resources, new materials have been considered as alternatives to American and French oaks to construct barrels for aging. Among them, acacia, cherry, chestnut, mulberry, and Spanish oak show interesting potentials for replacing the American and French oaks (Kozlovic *et al*., 2010; Rosso *et al*., 2009; Caldeira *et al*., 2006; Gambuti *et al*., 2010; Símon *et al*., 2003a, 2003b; Símon *et al*., 2008). Although these woods could be regarded as suitable for barrel production, their influences on the wine quality must be elucidated.

3.2.2 Wine aging using wood fragments

One of the alternative aging systems involves the addition of wood fragments, such as oak chips and oak staves, into wines. Up to now, the addition of wood chips to wines has been applied in the last decades to provide them an oak flavour in Australia, United States, South Africa, and South America. Since October 2006, the European Union also approved the use of pieces of oak wood during winemaking, but wines treated in this way should be labeled (Bautista-Ortín *et al*., 2008).

Morales *et al.* (2004) found that when the addition of oak chip was at a dose of 2% w/v, the wine vinegar aged using toasted oak chips for 15 days extracted vanillin 20-fold more than that aged in oak barrels for 180 days. The evolution of phenolic compounds of low molecular weight in a red wine, including benzoic and cinnamic acids and aldehydes, was analysed by Sanza *et al*. (2004). During the aging period of 12 months, the wines in barrels showed a slower evolution than those aged with oak chips. The extraction rate of several compounds from the hydroalcoholysis of oak wood but also the decreasing rate of some compounds that took part in condensation and browning process were higher in oak chip-treated wines than in the barrel-aged ones. Most studies conclude that the use of wood fragments can enhance the extraction rate of wood-related volatile compounds and

accelerate the aging process. On one hand, the small size of wood fragments allows wines to be adsorbed quickly whereas only the inner surface is soaked in barrel aging system. In this case, wines can penetrate and soak wood fragments totally, which makes the diffusion of wood-related volatiles from woods to wines easier. On the other hand, the entire surface of wood fragments is usable rather than only 40% of barrel surface (Stutz *et al*., 1999). Therefore, the extraction rate of oak-related compounds increases in the aging system in the presence of wood fragments and the length of the contact time can be reduced.

However, it should be taken into account that the alternative aging system in the presence of wood fragments cannot replace the traditional aging system completely, especially for long-term aging. For example, in the study of the evolution of aromatic compounds in Monastrell red wines aged both in oak chip systems and American oak barrels, it was found that chips released aromatic compounds into wine rapidly in the first three months of aging, and significant quantities of *cis*- and *trans*-oak lactones and vanillin were detected (Bautista-Ortín *et al*., 2008). However, in the following six months, the concentration of these compounds remained constant or decreased, while the wines aged in new and used barrels continued to extract aromatic compounds for a long time. The overall quality of wine aged in new barrels was also better than that of wines aged with chips. In a recent study, the phenolic compositions and sensory characteristics between red wines aged in oak barrels and those aged with oak chips were compared (Ortega-Heras *et al*., 2010). The results indicated that, although wines aged with chips for 30 days had phenolic content and colour characteristics similar to those of the wines aged in barrels for three months, chip-treated wines created a more astringent mouth-feel sensation and had more grassy and vegetal odours than barrel-aged ones. Furthermore, the evolution of the redox potential, which reflects the oxidation-reduction reactions during wine aging, is also different in the two aging systems (Álamo *et al*., 2006). The differences of basic oenological parameters and phenolic compositions between wines treated with wood fragments and those aged in barrels could augment as the wood contact time increases (Álamo *et al*., 2008). From an organoleptic point of view, the use of wood fragments can be regarded as a good alternative to barrels for producing short-term aged wines with satisfied quality. However, in some cases, the sensory quality of wines aged with wood fragments cannot be as good as those long-term aged wines in new barrels. On the other hand, during bottling, barrel-aged wines also behave differently from those aged with wood fragments (Álamo *et al*., 2008). In this sense, a quicker loss of anthocyanins was found during the bottling of red

wines treated with wood fragments that those aged in barrels (Sanza and Domínguez, 2006).

The characteristics of wood fragments, including size, prior treatment, and geographic origin, play an important role in the quality of final wine. The shape and size of wood fragments are various. The shapes of wood include "oak powder", "cubes" or "oak beans", "granulates" that are granulated pieces, "pencil shavings", "dominoes" with the shape of domino counters, etc. (Álamo *et al*., 2008). The traditional wood fragments widely esed are oak chips, staves, tablets, and segments. The size of wood fragments is closely related to the extraction kinetics of wood-related volatile compounds. Arapitsas *et al*. (2004) found that guaiacol extraction rate in the wine treated with oak chips of big size (3.4 x 2 x 1 cm) was higher than in wine treated with chips of small size (1 x 1 x 0.1 cm). Bautista-Ortín *et al*. (2008) studied the effect of size of oak chips on aromatic compounds and found that, the highest concentrations of *cis*-oak lactones and vanillin were detected in the wine in contact with chips in the format of cubes (10 x 6 x 4 mm) than in the format of shavings (8 x 3 x 1 mm), or powder (< 3 x < 1 x < 1 mm) (contact surface area being equal). Simón *et al*. (2010), using oak wood of two sizes (staves, 100 x 8 x 1 cm; chips, 1 x 0.5 cm, approximately) for the age of red wine, found that chips transferred oak-related compounds like furanic aldehydes more rapidly than staves. Concerning the evolutions of both oak-related and no-oak- related compounds, these compounds in the chip-aged wines became stable after 90-day of aging, while stave-aged wines evolved slowly throughout the whole aging period (180 days). As can be seen, since the size of staves is bigger than that of chips, the extraction rate of wood-related compounds in stave-aged wines is slower than that in chip-aged wines. Big size of wood can slow wine permeation in wood and produce a concentration gradient between wine and wood. Due to the great stock of volatile compounds in toasted staves, the extraction can last a long period. On the other hand, when chips are used, the bigger size doesn't seem to affect the extraction rate, negatively. Although the small size can promote the toasting effect of chips and more volatile compounds related to wood toasting could be produced, losses of these compounds due to evaporation may happen if the size is less than 5 mm (Bautista-Ortín *et al*., 2008).

Toasting is a conventional pretreatment of wood fragments. It can increase the amounts of aromatic compounds, including furfural, 5-methylfurfural, eugenol, vanillin, guaiacol, and its derivatives in wood (Sarni *et al*., 1990). Its effect on whisky lactone depends on species and origin of wood chips (Cadahía *et al*., 2003). Different toasting pre-treatments of wood

fragments can affect the final wine quality, and their influence on wine chemical compositions and sensory characteristics are even greater than the type of wood used (Guchu *et al*., 2006). By comparing the wine vinegars aged in presence of American oak chips submitted to two different prior treatments (toasted at 180 °C, or boiled and toasted at 180 °C), Morales *et al*. (2004) found that wine vinegars aged in presence of only toasted oak chips showed more significant increases of the oak lactone concentration. The *cis/trans* isomer ratio was also different: about 5 for wine vinegars aged in presence of toasted chips and 2 for those aged in presence of boiled and toasted chips. The toasting level of wood fragment is related to the production of wood-related volatile compounds and Bozalongo *et al*. (2007) highlithed that medium and heavy toasting can produce more contents of 5-hydroxymethylfurfural, 5-methylfurfural, furfural, vanillin, 4-methylguaiacol, guaiacol, and syringol than light toasting. According to Koussissi *et al*. (2009), during aging, wines treated with medium-toasted oak chips extracted the highest amounts of furfural and *cis*-oak lactone followed by those aged in presence of heavily and lightly toasted chips. The effect of toasting level of wood fragments on sensory characters of wine is also significant. Heavy toasting gave wines higher wood-related properties, but also made wines more astringent and bitter. Therefore, by controlling the toasting level of wood fragments, the desired sensory characters of wine can be obtained.

Besides conventional pretreatments of wood fragments, the microfungal treatment of wood fragments also shows interesting potentials in increasing the concentrations of some volatile components during wine aging process. Petruzzi *et al.* (2010) reported that the use of oak chips inoculated with *Penicillium purpurogenum* could significantly increase the concentrations of oak-related compounds such as furfural, furfuryl alcohol, guaiacol, 2-phenylethanol, and syringol in red wine during a 17-day aging period. This phenomenon is probably related to the degradation of lignocelluloses and the metabolism of fungi (Dhouib *et al*., 2005). However, due to the possible production of toxins by fungi, the feasibility of the microfungal treatment remains to be demonstrated. Therefore, a selection of suitable strains and toxicity tests should be carried out before its application.

Another factor that should be considered during wood fragment selection is the botanical characters and the geographical provenances of wood. Besides the size and pre-treatments of wood fragments, the contents of aromatic compounds extracted by wines also depend on the species of wood and their origins. For example, the Chardonnay wine treated with Hungarian oak chips (*Quercus petrae*) extracted trace quantities of oak lactones whereas

the same wine treated with American oak chips (*Quercus alba*) extracted significant quantities during a 25-day aging period (Guchu *et al*., 2006). In wine-brandy, higher levels of ethyl 2-methylpropanoate, ethyl butyrate, and ethyl octanoate and lower levels of butanoic acid, *cis*-*β*-methyl-*γ*-octalactone, and syringol were detected in the brandies aged with French oak staves and tablets (*Quercus robur* L.) compared with those aged with Portuguese chestnut wood (*Castanea sativa* Mill.) (Caldeira *et al*., 2010). A red wine macerated in contact with Spanish chips (*Quercus pyrenaica* and *Quercus petrae*) was richer in furanic aldehydes and eugenol whereas those macerated with American chips (*Quercus alba*) contained higher concentrations of *cis*-whiskey-lactone, vanillin, and methyl vanillate (Rodríguez-Bencomo *et al*., 2009). Therefore, wood fragment should be selected with extreme care by considering the whole characteristics of wood.

In the presence of wood fragments, the aging time can be reduced due to the high extraction rate of aromatic compounds. However, colour evolution commonly requires a longer maturation time and a small amount of oxygen to promote the necessary chemical reactions. However, atmospheric oxygen cannot diffuse through stainless steel tanks. Thus the chromatic characteristics and colour stabilization of wines treated with wood fragments may be not similar to those of barrel-aged wines. To solve this problem, caramel colouring additive E-150, which is authorized in the European Union, can be added to wines during aging. By using this additive, Monedero *et al*. (2000) found that dry *oloroso* wines aged with oak shaving had the similar colour characters of commercial wines. Another solution is to introduce small, controllable amounts of oxygen into wines during aging, which is called micro-oxygenation.

3.2.3 Wine aging using micro-oxygenation

Micro-oxygenation is a common winemaking practice for red wine, consisting of the continuous addition of small amounts of oxygen into wine in order to improve its colour, aroma, texture and conservation. Micro-oxygenation was formally developed in France in the mid of 1990s in order to replicate barrel conditions for wine matured in large stainless steel and cement vessels (Ducournau and Laplace, 1995; Lemaire, 1995) although, nowadays, it has also become a powerful tool to produce colour-stable wines and it may reduce the length of production process. In terms of sensory qualities, the micro-oxygenation process increases fruity and spicy flavours, enhances the stability of red tones and decreases herbaceous aromas and astringency (Gómez-Plaza and Cano-López, 2011;

Cejudo-Bastante *et al*., 2011a). Chemically, this technique increases volatile compounds such as acetaldehyde, vanillin and syringaldehyde, and the non-volatile products of reaction between anthocyanins and flavanols, pyruvic acid, acetaldehyde and vinylphenols. Furthermore, it has been proved that micro-oxygenation influences the concentration of different secondary metabolites in wine (Cejudo-Bastante *et al*., 2011a; Gómez-Plaza and Cano-López, 2011; Waterhouse and Laurie, 2006). Anthocyanins are responsible for the purple-red colour of young wines but they are unstable and participate in reactions during fermentation and maturation to form complex pigments. Several mechanisms have been known for the formation of these new pigments, which stabilize wine colour since they partly resist discolouration by SO_2 and provide better colour stability at wine pH (Mateus *et al*., 2002): a) direct reactions between anthocyanin and flavanols (Fulcrand *et al*., 1996; Remy *et al*., 2000); b) reactions between anthocyanins and flavanols involving acetaldehyde, to give a compound with an ethyl bond, that can be protonated to form a coloured compound (Atanasova *et al*., 2002; Saucier *et al*., 1997); c) formation of pyranoanthocyanins through the reaction between anthocyanins, acetaldehyde, and other compounds, such as pyruvic acid, vinylphenols and vinylflavanols (Fulcrand *et al*., 1996; 1997; Romero and Bakker, 2000a; 2000b). The best results can be obtained in the first 2 months of aging (Parish *et al*., 2000). Micro-oxygenation can be applied at any moment during vinification and maturation but, if colour stabilization is the main goal, a distinction must be made between the influence of this procedure before and after malolactic fermentation. The differences highlighted are partially related to differences in the concentration of SO_2 and mainly attributed to the free anthocyanin concentration. Micro-oxygenation seems to be much more effective in improving wine structure when applied before malolactic fermentation when tannins and anthocyanins are still mostly in simple monomeric form (Gómez-Plaza and Cano-López, 2011). High oxygen levels, instead, reduces wine aromatic complexity and promote wine oxidation. Another advantage of micro-oxygenation is that treatment with low doses of oxygen speeds up tannin polymerization resulting in softer tannins, decreased astringency and vegetative and herbaceous flavours of wine (Parish *et al*., 2000; Paul, 2002; Blackburn, 2004), so to obtain an overall mouthfeel improvement.

During aging, micro-oxygenation is generally carried out in presence of wood fragments to simulate the traditional barrel aging system. Cejudo-Bastante *et al*. (2011a) investigated the effects of micro-oxygenation and oak chip treatments on colour-related phenolics,

volatile composition and sensory characteristics of Petit Verdot wines. Micro-oxygenation treatment promoted the stabilization of red wine colour by increasing the formation of pyranoanthocyanins and anthocyanin-ethyl-flavan-3-ol adducts and increased the scores for the plum/currant and spicy attributes, and for the tobacco and nutty notes wines. The typical oak chip aromas (vanilla and woody) were reduced by micro-oxygenation. In the already cited work of Arfelli *et al*. (2011) on Sangiovese wines, micro-oxygenation combined with the use of oak chips and yeast lees reduced astringency, increased balance, and enhanced vanilla perception. Devatine *et al*. (2007) investigated the incidence of dissolved carbon dioxide in wine on oxygen transfer. This parameter must be considered when the micro-oxygenation is applied during or after alcoholic fermentation. Authors established that the presence of dissolved carbon dioxide affected the efficiency of the oxygen transfer to the liquid decreasing it of one order of magnitude when carbon dioxide concentration changed from 0 to 1.4 g/L.

3.2.4 Wine aging on lees

It consists in leaving yeast lees into the wine after alcoholic fermentation (Fornairon-Bonnefond *et al*., 2002). During autolysis, yeast cells release polysaccharides, nitrogen compounds, fatty and nucleic acids that enrich the wine. Furthermore, mannoproteins may stabilize wine against protein haze and tartrate crystallization and can decrease bitterness and astringency because they link tannins (Dupin *et al*., 2000). Aging in presence of yeast lees changes the wine aroma profile (Escot *et al*., 2001) since lees are able to adsorb some volatile thiols (methanethiol, ethanethiol) on their cellular walls (Lavigne and Dubourdieu, 1996) and to release aromatic compounds such as β-ionone and ethyl octanoate (Lubbers *et al*., 1996).

Doco *et al*. (2003) investigated the evolution of polysaccharides originating from the cell walls of grape berry (arabinans, arabinogalactan-proteins, RG-II, and RG-I) or released during fermentation or after autolysis of yeasts (mannoproteins) during aging on lees of a red wine from Madiran. Authors found a certain degradation of grape pectic arabinans and polysaccharides, the latter consisting of dearabinosylation of arabinogalactan-proteins (AGPs). The arabinose to galactose molar ratio of AGPs decreased from 0.8 (control wine) to 0.3 (wine aged on lees). When a stirring was performed during aging on lees, a slight increase of mannoprotein content occurred and it was sufficient to alter the organoleptic quality of wines. Mazauric and Salmon (2005) studied the interactions between wine

polyphenols and yeast lees because these interactions have a large effect on the reactivity toward oxygen of both polyphenolics and lees. In particular, they studied the chemical composition of polyphenolic compounds remaining in solution or adsorbed on yeast lees after various contact times. According to their results, wine polyphenols adsorption on yeast lees followed biphasic kinetics. An initial and rapid fixation was followed by a slow, constant, and saturating fixation that reaches its maximum after about 1 week. Furthermore, only few monomeric phenolic compounds remained adsorbed on yeast lees. The condensed tannins remained in wine contained fewer epigallocatechin units than the initial tannins, thus indicating that polar condensed tannins were preferentially adsorbed on yeast lees. On the contrary, the adsorption efficiency of anthocyanin on yeast lees was unrelated to its polarity.

Rodríguez *et al*. (2005) studied the effects of the presence of the lees during oak aging on colour and phenolic composition of two red wines, one with a low aging potential (low anthocyanin and proanthocyanidin concentrations) and the other one with a high aging potential (high anthocyanin and proanthocyanidin concentrations). Authors concluded that the aging in presence of lees had the disadvantage of producing wines with a slightly less intense colour and with a lower proanthocyanidin concentration but with the advantages that colour slowly evolved towards yellowish nuances, astringency decreased, and mouthfeel perception increased. In a study of 2009, Palomero *et al*. were aimed to investigate the release of polysaccharides during the autolysis of different genera of non-*Saccharomyces* wine yeasts, for their possible use in the acceleration of aging over-lees. Their effect on the stability of the anthocyanin monomer content in red wine made from *Vitis vinifera* L. cv. Garnacha grapes was also studied. Authors found that *Schizosaccharomyces pombe* 936, *Saccharomycodes ludwigii* 980, *Pichia anomala* 930, and *Pichia membranifaciens* 956 could be used to reduce aging times since the concentration of yeast cell wall polysaccharides and mannoproteins would increase rapidly in wine. The obtained wines had more complex molecular size profiles with beneficial effects on tartaric and protein stability, and sensory properties without any loss of colour.

According to Smit and du Toit (2013), the presence of lees during aging of Pinotage and Cabernet Sauvignon wines generally led to higher final concentrations of biogenic amines in wines.

Synergic effects between lees and micro-oxygenation or between lees and oak chips are known. The combination of lees and micro-oxygenation could decrease the reducing note

and stabilize wine colour, because a higher amount of oxygen is necessary to balance the consumption of oxygen by lees (Fornairon-Bonnefond and Salmon, 2003), whereas the presence of lees reduced the impact of compounds from oak wood on wine aroma (Jiménez Moreno and Ancín Azpilicueta, 2007). In the already cited work of Arfelli *et al*. (2011) on Sangiovese wines, the combined use of micro-oxygenation and yeast lees led to a lower amount of guaiacol and vanillin than in control.

3.2.5 Wine aging in amphorae

This technology, used since Roman times, consists in the use of earthenware amphorae for the storage of wine. This technique was very popular in Georgia, where large earthenware amphorae were employed to allow the first fermentation and then the aging of wines, both red and white. The use of earthenware pots (*Kvevri* in the local language) affords a completely natural treatment and enhances the varietal characteristics. Amphorae, usually made of clay, are produced in different sizes and subjected to different types of treatment (cooking at high temperature, coatings, etc.). They can be buried in the ground, half buried or not buried at all, depending on the temperature control system installed in the cellars. The aim of in-amphora aging is to replicate the beneficial air exchange of wood containers, without the transferring of vanillin, tannins, and toast flavours (typical of oak barrels) to the wine (http://vinieterroir.wordpress.com/2011/10/18/ritorno-alle-origini-il-vino-nelle-anfore/). Therefore, the resulting wines are different, with a cleaner taste and more pronounced minerality and freshness characteristics. Several French (in Corsica, southern Rhone Valley, and Beaujolais), Portuguese (in Alentejo), Croatian (in Istria), U.S. (in the Napa Valley), Slovenian (in 'Goriška Brda region), and Austrian (in the east-central Thermenregion) wine producers have experimented with fermentation and/or aging in amphorae. In Italy this type of aging is used in regions such as Friuli, Campania, Puglia, and Sicily. From an economic point of view, the production of in-amphora wines is becoming increasingly attractive to producers, especially to those belonging to the 'natural wine' movement. The in-amphora wines have received a lot of attention from wine magazines and wine lovers but only few studies about the effects of this type of aging and comparison with conventional processes on wine quality are present in scientific literature.

A study conducted by Lanati *et al*. (2001) concerning six Georgian white wines produced according to the ancient technology *Kakhetiana* showed that the traditional wine-making in earthenware amphorae represents the highest expression of natural winemaking, without

any use of adjuvants commonly used in modern winemaking. All wines are stable and retain their characteristics even after aging, without the need for further action. From a sensory point of view, these white wines are characterized by a strong amber colour, while the taste is closer to red wines.

A study of Baiano *et al*. (2013), performed on Fiano Passito wine produced by cryomaceration in reductive environment and aged on lees in inert containers and different type of earthenware amphorae, showed that the most important differences attributable to the aging container concerned soluble solids, volatile acidity, and concentration of the different phenolic classes. The increase of organic acids, due to concentration by evaporation, didn't show significant differences between samples aged in the various types of container. Wine aged in glazed amphorae resulted better appreciated than the other wines. Furthermore, Baiano *et al*. (2014) recently studied the aging of Falanghina white wines in different types of amphora for twelve months in the presence of lees, in comparison to a control aged in conventional stainless steel tanks. They demonstrated that all the physical-chemical and sensory characteristics of the wines analysed were significantly influenced by the type of container. The only variables that were not affected by the type of container were the concentrations of organic acids and acid hydroxy-cinnamyl-tartaric and the antioxidant activity measured with the DPPH assay. Furthermore, authors have also concluded that the raw amphorae cannot be considered as inert containers, because of the cation exchange and the diffusion of oxygen. The authors suggested that the engobe-type amphora allowed a better preservation of phenolic compounds, in particular of flavans reactive to vanillin.

3.2.6 Wine aging in bottles

Apart from aging in barrels and other vessels, bottling is another important stage for wine aging. Sometimes, wines are directly aged in bottles (Ancín-Azpilicueta *et al*., 2009; Segade *et al*., 2009). During storage in bottles, temperature, illumination, position, and oxygen content influence the composition of final products.

Storage temperature should be considered carefully during bottling. Generally, wines were stored in cellar in which the temperature is between 13 and 15 °C, but the storage in a range from 5 to 18 °C is also considered acceptable provided that temperature is kept constant. Winemakers can store wines at low and controlled temperature whereas when wines are put in sale places or purchased by consumers they commonly remain at room

temperature. Therefore, it is necessary to clarify how wine evolves at different temperatures during bottling.

Hernanz *et al*., 2009 observed that Zalema and Colombard white wines stored at a low temperature (4 °C in the refrigerator) were clearly distinguishible from those stored at room temperature and those stored at a constant temperature from 15 to 20 °C on the base of the linear discriminate analysis of colour parameters, phenolic and volatile compounds. Furthermore, Garde-Cerdán *et al*. (2008) pointed out that low temperature (5°C) could enhance the contents of some important aromatic compounds such as ethyl asters of fatty acids and isoamyl acetate in white wines without the preserving action of SO_2. Blake *et al*. (2010) also confirmed that a relative low temperature (12 °C storage) benefited the quality of final wines compared to a 22 °C-storage since the wines stored at the lower temperature showed higher retention of acetate esters, phenolic compounds (in red wines), free and bound SO_2, and lower browning. In some cases, high temperature is beneficial for accelerating the aging. For example, Loscos *et al*. (2010) found most of aromatic compounds from grape flavour precursors increased significantly in the first week when the wines were heated to 50°C to mimic wine aging in bottle. Nevertheless, high temperatures should be avoided during wine aging process in most cases, since they can sharply reduce the contents of aromatic compounds in wine (Zoecklein *et al*., 1999; D'Auria *et al*., 2009) and accelerate the process of browning of white wine (Berg and Akiyoshi, 1956).

Phenolic and aroma profiles of wine can be modified by radiation with ultraviolet rays and the blue portion of the visible spectrum (in the range from 350 to 500 nm) during storage. The radiation effect on wine is related to the storage temperature. During storage at 25 °C, "fino" sherry wines lost several polyphenolic compounds when they were exposed to ultraviolet-visible (UV-Vis) radiation, whereas the volatile compounds, including esters and several acids showed an ascending tendency during irradiation (Benítez *et al*., 2003). However, when storage temperature was set at 45 °C, the contents of most esters and acids decreased in these wines (Benítez *et al*., 2006). As can been seen, a high temperature appears to counteract the increases in esters and acids promoted by the application of UV-Vis radiation. Another factor that can affect the radiation effect on wine is the nature of the glass bottle. In the study of Benítez *et al*. (2003), the losses in penolic compounds were greater for the sherry wines bottled in transparent glasses than topaz glasses. The former ones cut off the wavelength at 300 nm, while the latter ones cut off the wavelength near

600 nm, but transmit 20% at 350 nm (D'Auria *et al*., 2009). With respect to red wines, coloured bottles can be used to partially protect wines against photodegradation. Therefore, in order to preserve wine quality and prevent photodegradation in both retail and cellar environments, it is essential for winemakers to choose a proper bottle hue. During irradiation of Italian red wines at 20 °C, different rates of photodegradation of volatile compounds were observed depending on grape varieties (D'Auria *et al*., 2009).

During storage, bottles can be placed either in horizontal position or in vertical position without any moving. Wine researchers seem not to pay much attention to bottle position during storage and few papers reported its influences on wine quality. Hernanz *et al*. (2009) found that the position of bottle slightly affected the wine quality, while Mas *et al*. (2002) concluded that wines stored in bottles placed vertically were more oxidized than those stored horizontally. Sometimes vibration of bottles could be applied to accelerate the aging process. However, this treatment also induces some negative effects on wine, such as reduction of aromatic components and formation of undesirable flavour and taste (Chung *et al*., 2008). Therefore, to produce high-quality wines, vibration of bottles should be avoided.

During winemaking process, oxygen is introduced at various stages. Gentle oxygenation can contribute to the enhancement of wine quality while excessive oxygenation is detrimental to wine quality. Wines are usually packaged in glass bottles with air-tight cap to avoid any direct contact with air. During bottling, oxygen exposure is low and bottling has been called "reductive aging" (Jackson, 2008). The oxygen content in bottled wines is dependent on the type of closure and the materials of bottle. There are several types of closure, for example, cork stopper, synthetic closures, and screw caps. The cork system is widely used to seal bottles and limit the permeation of atmospheric oxygen into bottles. Different cork system are used to modify oxygen permeability (Cook *et al*., 1985). Among them, colmated-cork stoppers and polyurethane-powdered-cork stopper, as well as natural-cork stoppers, have already been proved as suitable for wine bottling (Mas *et al*., 2002). Synthetic closures and screw caps are only valuable for short-term bottling since wines sealed by them could be oxidized quickly (Mas *et al*., 2002). Another factor related to oxygen content in bottled wines is the raw material of bottles. Besides the traditional glass bottles, the plastic bottles made of polyethyleneterephtalate (PET) with an oxygen scavenger can also isolate wines from air (Giovanelli and Brenna, 2007). The oxygen permeability can be regulated artificially by using closures of constant oxygen transfer rate

and controlling oxygen level diffused in the storage space (Caillé *et al*., 2010). In this way, either excessive exposure or excessive protection is avoided and the quality of the final products can be satisfied. However, the topic bottle will be discussed below.

Chapter 4

Packaging systems

4.1 Wine packaging: a brief history

The first archaeological evidence of the use of containers for wine are made from ceramic vessels dating from about 7000 years ago. The amphorae were used for centuries by Romans and Greeks for wine commercial transport in the old world, sometimes closed with a clay seal showing the indication of wine region, vintage and producer: an "ancestor" of the modern label. The conquest of the Britain by the Romans favoured the spread of oak barrels that prevailed on ceramic containers. Although all of these containers would ensure the transport function, none of them was suitable to preserve wine from air and spoilage bacteria. During the Middle Ages and the Renaissance, wines were transported by ship in wooden barrels and then decanted into bottles used for serving wine. Only in 1600 AC, for the first time, the wine was bottled and closed with corks. This solution allowed the wine to age without suffering the negative effects of oxidation and microbial contamination. This discovery changed forever and ever the oenological practices. Wineries started producing wines aged for years in order to acquire complex flavour, just like how the consumers demand of our day (Torri, 2011).

4.2 The main problems of wine shelf-life

Wine shelf-life is directly related to the oxygen content to which it is exposed and to its resistance to oxidation (Escudero *et al*., 2002). When wine is not well protected through a sufficient sulfitation, the presence of high oxygen contents could promote an acetic bacteria attack, and the subsequent development of acetic acid and ethyl acetate. However, also chemical oxidation can produce significant organoleptic modifications in wine flavour and colour, mainly consisting of a loss of aromatic freshness and the appearance of brown precipitates of condensed phenolic material (Benítez *et al*., 2006; Cheynier *et al.*, 1989; Gómez *et al*., 1995; Simpson, 1982; Singleton, 1987; Zurbano *et al.*, 1995). The capacity of a wine to take up oxygen and to withstand oxidation is roughly measured by the total content of phenols, being phenols the major substrates for oxygen in wine. Obviously, phenol content decreases as a consequence of oxidation. Furthermore, wine oxidation can also involve the presence of new odorants especially aldehydes, such as (E)-2-hexenal (Culleré *et al*., 2007), methional and phenylacetaldehyde (Ferreira *et al.*, 2003).

Another one important factor that may considerably alter preserved wine's sensory properties is natural or artificial light. The term "goût de lumiere" (also known as "goût de soleil" or the English appellations of "sunlight flavour" and "light sickness"), was coined by French researchers at the beginning of the 1980s to define the off-flavour of the champagne appeared after its exposure to light and due to the formation of compounds (hydrogen sulfide, dimethyl sulfoxide and methyl-mercapatans) arising from the degradation of sulfur aminoacids. Later, the development of unpleasant odours due to light, it has been also found in other sparkling white wines. Wine has been traditionally preserved in bottles with different transparency and colour (different shades of brown or green), moreover dictated by marketing strategies. It has been proved that dark bottles' protection against light is better than protection of clear bottles. In general bright and clear bottles are liked best for white wines, whose shelf life is shorter (Baroni *et al*., 2013).

Other sensory defects can be associated with the packaging type. This is the case of reduced character smell, due to the formation of sulphur compounds that, at low concentrations, may contribute to a higher flavour complexity, but that at high concentrations are unpleasant and remind fustiness. Such compounds are more easily developed in some wines, especially when they are bottled with low OTR[3] closures. When for the same wines high OTR caps are used, sulphur compounds are partially oxidised and thus they are minus perceivable.

Undoubtedly the most recurrent and well-known off-flavour is the so-called *cork taint.* Such a defect, that gives to wine a musty smell, is commonly associated with the presence of halogenated compounds produced by fungi *Armillaria mellea* (Buser *et al*., 1982) including the main responsible for the off-flavour i.e. trichloroanisole (TCA), featuring a very low perception threshold (5 ng/L) and thus easily perceivable when it is present in very small amounts (Baroni *et al*., 2013).

[3] OTR: oxygen transmission rate.

4.3 Glass bottle and glass bottle closures

Glass bottles are the most popular way to package wine today. They are usually preferred for bottling all types of wine being the only material with a superior barrier to gases and vapours, stability in time, transparency and ease of recycling. However, its use as long-lasting package for short-lived products, and its high cost, are not entirely justified.

Glass is made from silicon dioxide, a relatively inexpensive quartz sand. Silicon dioxide and other materials must be mined before being made into glass. After the silica dioxide and the other materials have been extracted, the materials are placed into gas burning kilns. Once the bottles are formed, the interior of the bottles is chemically treated to make the bottles nonporous (Athens, 2009; Colman and Paster, 2007).

Glass wine bottles have been closed with traditional bark cork (obtained from the oak tree *Quercus suber*) for about 300 years. Cork has performed reasonably well although it can present problems such as cork dust, leakage and off-flavours including *cork taint*. The occurrence of cork taint in wines closed with natural corks, variously estimated as 1–5% by Sefton and Simpson (2005), has been a major motivation for the development of agglomerated corks and other alternative closures.

The various so-called "technical corks" have been developed from reformed, frequently treated comminuted cork into more uniform products such as Twin Top® and more recently Diam® closures. The agglomerate cork, originally developed as a closure for sparkling wine, consists of small pieces or granules of clean, natural cork bound together with resin or a chemical binder into a single stopper, frequently with one or more thin discs of intact natural cork stuck on the end intended to be in contact with the wine. Reduced levels of cork taint have resulted from a disinfecting or deodorizing process, which extracts volatile components from the cork material. This operations eliminates the possibility of contaminants being retained inside the lenticels or pores through which gases are exchanged between the atmosphere and plant tissues (Robertson, 2006).

The past 40 years has seen a proliferation of other non-cork closures, including Stelvin®, Zork®, Nomacorc®, and the "Vino-Lok" glass closure, specifically designed for use with table wine. These types of closure can offer reduced cost, easiness of application and removal, uniformity of performance, and lower O_2 ingress. The opportunity to develop a wine closure technically superior to cork was recognized by a French closure

manufacturing company, Le Bouchage Mecanique (LBM), which began research in the late 1950s on a metal closure to replace cork closures. Their Stelcap closure had already gained widespread acceptance in use over aperitifs, spirits, and liqueurs. LBM aimed to modify the Stelcap and develop a quality table wine closure that would completely replace cork. Since the late 1960s, LBM had developed Stelvin; it was made of aluminum, was corrosion resistant, and had a treated and chemically inert wad facing that was completely compatible with wine. The wad consists of three components: an expanded 2-mm LDPE foam substrate to provide controlled and uniform compressibility, a 20-µm layer of tin to provide a gas barrier, and a 19-µm PVdC copolymer facing that isolated the tin film from the product and provided an additional O_2 barrier (Robertson, 2006; Stelzer, 2005).

Natural cork exercised a variable protection from oxidation on wine. Despite the belief of some researchers that corks do not breathe (Limmer, 2006a, 2006b), there are a lot of data confirming the ingress of O_2 through closure systems. Correlated observations were among the early methods for the assessment of the entrance of O_2. In particular, wine properties such as SO_2 status (Casey, 1994) and browning measured by the absorbance at 420 nm have been used (Skouroumounis *et al*., 2005). The most common methods for the measurement of the oxygen transmission rate (OTR) are: colourimetric measurements through the MoCon OX-TRAN, changes in optical properties of O_2-sensitive materials such as dyes (OxyDot developed by Oxysense) and bis-9,10-anthracene-(4-trimethylphenylammonium) dichloride (BPAA) (Skouroumounis and Waters, 2007), and indigo carmine (Lopes *et al*., 2005). Another method to measure OTR consists of the measurement of oxygen pressure decline over a period of 18-72 hours (Aracil, 2004). Most methods have been criticized. Changes in SO_2 and A_{420} are only very loosely correlated with O_2 ingress and changes in sensory performance. Their changes can be influenced by wine composition and the presence of other antioxidants such as ascorbic acid. Skouroumounis and Waters (2007) noted that the MoCon method does not reflect normal cork usage where wine is typically stored horizontally and the cork is in contact with wine, and not with the headspace gas. Moreover, research has shown that cork changes with the bottle storage (horizontal or vertical) position (Gibson, 2005; Lopes *et al*., 2006).

Some of the dye oxidation methods have been criticized because it is considered that other wine components may interfere with the reaction, and so give a false result. Skouroumounis and Waters (2007) considered the absence of O_2 consumption by the

OxyDot reagent could be a disadvantage. They also note that the indigo carmine method is a very careful technique.

There are different approaches for the expression of OTR in the literature. For example, Casey (1994) reported OTR values equivalent to 0.002 mL day^{-1} for natural cork calculated on the base of SO_2 losses in white wine stored horizontally for 21 months. It was assumed that the loss of SO_2 was entirely due to the permeation of O_2 into the wine. Peck (2007) noted that, in presence of screw caps, liner type, layer thicknesses and cap application variables such as top pressure, reform depth, thread roller pressure, and glass finish can affect OTR. Lopes *et al*. (2005) reported an OTR range of 0.24–0.50 mg L^{-1} $month^{-1}$ for natural corks. Instead, Godden (2004) found a range of 0.0001–0.1227 mL day^{-1} and Gibson (2005) quotes Southcorp results of <0.001 to >1.00 mL day^{-1}. It has been noted that, in some cork studies where "selected" corks were used, the OTR values found were both lower and more consistent than where the corks were said to have been taken randomly.

Robertson (2006) provides detailed treatment of the mechanisms by which gases pass though polymeric materials. Two effects, a pore effect and a solution-diffusion effect, are discussed. The pore effect is gas transfer through microscopic pores, pinholes, and cracks in the material, whereas the solution-diffusion effect involves dissolution of gas in a material, diffusion through it under the influence of a concentration gradient and evaporation at the other surface. This latter process is the "true permeability" and varies inversely with thickness.

Although porosity decreases very sharply with increasing material thickness, in the case of natural corks the porosity may still represent a relevant aspect for its oxygen permeability as cork has pores and cracks by virtue of the natural growth process. The extent of these in a given line of corks may be influenced by the visual grading processes employed during manufacture. It is therefore quite conceivable that both pore and solution-diffusion processes will occur in natural corks, whereas only the solution-diffusion process will be found in technical and synthetic cork analogues, most of which should be pore-free by virtue of the manufacturing process.

A third permeation mechanism that may also be present is represented by the transfer of gas through the interface between the glass and the closure material itself (where the two surfaces may have microscopically parted). Nevertheless, its magnitude is determined by individual closure characteristics such as closure diameter, elasticity, dimensional stability

with time, and ability to bond with the glass surface. Variations of the internal diameter of glass bottle necks is also an important variable, but no published data has been found on this. O'Brien (2005) and Lopes *et al*. (2007) portrayed the three mechanisms.

The pattern of change in DO (dissolved oxygen) after bottling depends on initial DO levels, remaining headspace O_2, O_2 in the closure, and subsequent O_2 ingress, with the last two being dependent on the closure system. Oxygen input varies considerably from one closure system to another and also according to how the bottles are stored.

For natural cork, in particular, but also for synthetic analogues, mathematical modelling of the processes that occur after insertion is difficult. Considering that the closure is essentially cellular and that the cells are intact, the increase in internal gas pressure for a typical 45 mm long and 24 mm-diameter natural cork, compressed into a bottle neck of diameter 18.5 ± 0.5 mm, would be 70%. Driven by this pressure increase, cellular gas will gradually permeate out at each end of the cork. At the outside end, this will continue until the internal closure pressure is equal to that of the atmosphere. The inside end process is more complex, being affected by factors such as closure internal pressure, gas flushing if used, the extent of pre-insertion vacuum application, wine composition. In addition, at the inside end, solution-diffusion process will occur, controlled by compositional differences between the closure gas and the headspace gas composition.

Dissolved oxygen in wine must be consumed more quickly than it is supplied by the various processes due to the reactivity of phenolics (Vidal and Moutounet, 2007). Depending on temperature and wine composition, the initial DO, headspace O_2, and O_2 released from compression of the cork are essentially consumed within a few days. The pressure migration process is probably be continuing although it occurs at a decreasing rate for some weeks. The rate of the solution-diffusion process also decrease as the overall concentration of O_2 in the closure decreases. Depletion occurs at the surface nearby to the wine; in the cork body the oxygen concentration is that of the original air. However, as the O_2 surface layer (those in contact with wine) depletes, the O_2 from the adjacent inner layers of the cork body move toward the wine end. Gradually, the depletion zone progress through the cork until it reaches the outer surface (those in contact with the atmosphere). Any depletion is then made up from the atmosphere. As the diffusion through cork is slow, the establishment of this gradient will take time. Some but not all research shows that the overall transfer process is influenced by the storage position of the bottle (Gibson, 2005; Lopes *et al*., 2006).

Once the closure internal pressure is equal to the headspace pressure, pressure-driven transfer cease. However, a steady state situation with regard to O_2 ingress is not reached due to the O_2 removal from the headspace by reaction with wine components. The O_2 concentration at the inside end of the closure is lower than at the outside end, which is at atmospheric concentration. Driven by a concentration difference, O_2 continues to diffuse out of the closure into the wine. The zone of internal O_2 depletion in the cork gradually progresses toward the outside end, and the rate of transfer into the wine decreases as the magnitude of the concentration gradient in the closure decreases.

Depending on closure characteristics, storage position, and temperature, and presuming that wine has the capacity to react with all the transferred O_2, there could need a substantial time before the steady state is reached for the solution-diffusion process.

Skouroumounis and Waters (2007) followed the cumulative O_2 ingress through a synthetic closure using BPAA and identified three stages. An initial rapid loss in BPAA occurred for about 10 days followed by a period of about 25 days after which there was a generally linear increase of total loss. The latter would represent the steady state. A natural cork could display the same behaviour apart from any contribution from pore diffusion. The rates for the three phases are likely to be different due to the fundamental structural differences. Both closure types may allow the O_2 inlet as a result of closure/glass interface diffusion.

For bottles stored horizontally, Lopes *et al*. (2007) found that O_2 in an inserted natural cork continued to be released into the bottle slowly over a period of 12 months, and only a small amount diffused through the cork. The synthetic plastic corks tested (Nomacorc) were permeable to atmospheric O_2 after the first month. They did not discuss possible pore diffusion (a process that vary from cork to cork) and transfer at the closure–glass interface.

After insertion, a cork exerts a sidewall pressure of 1.5-3 kg cm^{-2}; over time pressure lessens as the cell walls begin to collapse and the unrestrained volume of the cork decreases (Casey, 1988). Depending on magnitude, fluctuations in temperature can encourage the egress of wine and ingress of air. This phenomena will be minimized where such fluctuations are seasonal rather than diurnal.

After examining the structure of paraffin wax coatings used on corks, Keenan *et al*. (1999) calculated that O_2 permeation didn't allow ingress of sufficient O_2 to cause oxidation of wine during storage.

Waters *et al*. (2001) measured the ingress of O_2 (as A_{420} values) at the closure-glass interface in wines with natural and synthetic closures partly and totally covered with epoxy resin and compared them with uncovered controls and bottles closed with Stelvin closures. Their results indicated that there was some O_2 ingress along the glass–cork interface for natural corks but little through the cork body. For synthetic closures, it was observed a reverse trend, with a significant O_2 ingress through the closure body. The Stelvin absorbance values were the same as for the 100% covered synthetic closure and the 70% and 100% covered natural cork. However, the authors warned that data interpretation must be made with caution due to the small sample size, which ranged from just 4 to 60 bottles for the different closures.

Lopes *et al*. (2007) investigated ingress routes by different covered zones of the outside cork surface (no coverage, interface cork-glass covered, and fully covered) and assessing O_2 ingress using indigo-carmine dye. They concluded that technical corks were essentially impermeable for the first 24 months, while natural corks showed low permeability for the first 12 months, with O_2 penetrating in very tiny amounts through the glass–cork interface thereafter. They found that Nomacorc synthetic closures were permeable to atmospheric O_2, especially after the first month of storage.

Differences in O_2 ingress caused by bottle storage position may be explained by wine penetration into the cork and the closure-glass interface. Skouroumounis *et al*. (2005) found significant differences after 6 months in the extent of this latter penetration for natural cork purchased from two different suppliers. For one line of corks, the penetration remained constant, after 6 months, at about 10% of the length of the cork for bottles stored in either inverted or upright positions. For the second line of corks, the penetration increased to 70% for bottles stored upright for 5 years and to 90% for the inverted bottles. This difference in wine penetration may influence O_2 ingress via the same path.

Horizontally stored bottles closed with natural corks frequently show a very small percentage with wine leakage through continuous longitudinal channels. Such channels can facilitate the egress of wine and ingress of O_2, even under relatively stable storage temperatures. This is largely influenced by pressure due to wine volume changes with temperature. For a 750-mL bottle of 12% v/v alcohol wine, the pressure increases of about 11% as a consequence of the thermal expansion when the temperature increases from 20 °C to 25 °C.

Such channels could be present to a greater or lesser extent depending on cork grading. Poorer, lower-density corks may be affected to a greater extent. Together with a variable glass-cork interface performance, this could contribute to the higher OTR values observed in trials where corks have been randomly selected, such as the Godden (2004) and Gibson (2005) trials, which both reported a 1000-fold OTR range.

After completion of an OTR trial, Gibson (2005) investigated the natural corks for leakage. Among the 64 corks tested, 45% showed bubbling, ranging from small (16%) to gross (3%). Although the pressures used were not encountered in normal everyday storage, the test supported the wide range of OTR values reported by Hart and Kleinig (2005). Of the gas leakers, 33% leaked through the body and 66% leaked at the glass–cork interface. About 70% of the leakers (32% overall) had OTR values > 0.1 mL day^{-1} compared with just 30% of the non-leakers (17% overall) having OTR values > 0.1 mL day^{-1}. The level of 48% of the corks with OTR values > 0.1 mL day^{-1} is less than the 80% in the 35 random cork trial reported by Hart and Kleinig (2005). To a winemaker, variability in performance is every bit as important as averages in terms of determining shelf life and product acceptability. However, Casey (2008) expressed doubts concerning the real contribution of leakers to high OTR values and hence wine shelf life reduction.

O_2 ingress has been assessed for bottles stored in upright, horizontal, and inverted positions. Lopes *et al*. (2006) concluded that storage position had little effect on O_2 ingress in natural corks and its analogues over 20 months. However, Gibson (2005) found some noticeable differences with upright storage resulting in more rapid wine development with some closures. Gibson discussed the possible effect of the O_2-wine interface actually being located in the interior of the cork as the result of wine penetration over time into the cork body.

4.4 Other packaging materials

In the past decade, wine bottles have been gradually gaining weight because people typically associate heavier glass bottles with higher quality. The total weight gain for a glass wine bottle is about a pound. Despite glass being recyclable, it is actually more economical for wineries to use virgin glass because of the cost required to transport used or recycled glass over long distances.

While glass bottles have traditionally been used to store wine, they also have another important function. Glass bottles protect wine quality by reducing oxygen permeation through the container.

Wine that has the potential to be aged longer than a year should be bottled in glass because of its superior ability to prevent deterioration due to oxygen. An increasing proportion of the world's wine production is packed in other containers, including bag-in-box (BIB), poly-(ethylene-terephthalate) (PET) bottles, paperboard laminates, and aluminum cans. As with bottles, the major influences on shelf life of these alternative packaging formats are temperature, initial DO (dissolved oxygen) before packing, pickup during filling, package headspace, and subsequent O_2 ingress.

4.4.1 PET bottles

Wine packaged in PET bottles is becoming increasingly accepted (Carter, 2007). The manufacturing process of PET bottles is "cleaner" and more energy efficient than glass and the resultant bottles are indistinguishable from glass in appearance, but are extremely lightweight, more compact, almost unbreakable and fully recyclable (Newhouse, 2008).

With an O_2 permeability of around 3×10^{-10} mL cm cm^{-2} s^{-1} $(cm\ Hg)^{-1}$ at 25 °C, monolayer PET does not have an acceptable barrier for highly O_2-sensitive products such as wine. In order to obtain a 2-4 times greater shelf life, its performances can be improved by the use of a barrier layer (MXD6 nylon) between two PET layers and the addition of O_2 scavengers. A comparison can be made with the O_2 ingress values for various closures reported by Godden (2004) and Gibson (2005). A potential shelf life of 1 year is being claimed for 187-mL barrier-coated PET bottles fitted with aluminum caps (Linkplas, 2008). According to Birkby (2006), a scavenger layer can result in less than 10 mg of O_2 permeating into a 2-L PET bottle in a year. New coatings such as oxides of silicon are also claimed to significantly reduce O_2 permeation (Mans, 2008). Atmospheric ingress through the closure should also be controlled so that the O_2 contained into the headspace is scavenged preferentially.

PET bottles offer several advantages over traditional glass wine bottles. They are unbreakable, offer a greater flexibility in design, and are lighter", greener", and 100% recyclable than traditional glass bottles.

In the first few months after bottling in a PET container, the wine will taste as wine bottled in a glass bottle. After eight months to a year, taste change drastically. In comparison to wine packaged in glass bottles, PET bottles allow the inlet of a significantly greater amount of oxygen causing wine oxidation and altering the aroma profile of the packaged product. Although most wine today is consumed less than a year after bottling, some wines are stored for longer periods of time.

4.4.2 Bag-in-Box (BIB)

Bag-in-Box packaging first made their way onto the wine scene in the 1960s where they were traditionally used to package generic bulk wines. Today's bags consist of a five-layer co-extruded bag that includes LDPE and nylon with ethylenevinyl alcohol (EVOH) as O_2 barrier layer (Lingle, 2004). Metalized PET (mPET) can also be used to improve O_2 barrier properties but it has lower flex resistance. Multilayer metalized laminates can be produced with O_2 permeabilities lower than 0.02 mL m^{-2} day^{-1}.

The air-tight bladder and the spigot are both able to protect the wine during short term storage. Bag-in-Box design can offer several advantages with respect to glass bottles. Those advantages include improved distribution, cost reduction, an easiness of opening and of using. BIB come in several sizes (1.5, 3, and 5 L which are equivalent to two, four, and ten 750 mL bottles of wine). A 4-6 L box weighs 40% less than a traditional glass bottle. This does not mean, however, that the carbon footprint over the entire lifecycle of the package is 40% less. Companies who have switched from traditional glass bottles to the BIB design estimate that there is a minimum of 50% reduction in carbon emissions. Although BIB design provides manufacturers and consumers with a lighter weight product, the internal bladder that holds the wine can cause the wine to age even when unopened. This is because the plastic packaging is not hermitically sealed. Fu *et al.* (2009) looked at the oenological properties of white wine packaged in a Bag-in-Box container. The overall quality of the wine was significantly affected by the oxygen transmission rate (OTR) of the package. Colour, free and total SO_2, total aldehyde, and total phenol content were all correlated with an increase of OTR. Time and temperature have a significant effect on the colour development and SO_2 depletion during storage.

4.4.3 Laminated paperboard containers

Semi-rigid paperboard containers laminated with alufoil and LDPE and aseptically filled have been used for many years for wine. Despite offering weight and space savings and easy disposal once empty, they have become extremely popular in certain parts of the world, including Latin America and Scandinavia.

Foil in laminates provides a good O_2 barrier (about 0.02 mL O_2 m^{-2} day^{-1}), providing about 12 months' shelf life even with packs as small as 200 mL (Casey, 1989). As with all other forms of wine packaging, DO and headspace O_2 are significant contributors to loss of initial free SO_2 and hence influence shelf life. Wines can be packed into brick-type packs with the absence of any headspace. This type of packaging allows a good wine handling and filling technology, and allows to obtain initial DO levels similar to those generally present in bottled wine. Buiatti *et al.* (1997) found that wine in laminated paperboard cartons can be stored for up to 24 months, as measured by several quality-related properties, and that this was longer than for PET and BIB packaging. Recent improvements to the barrier and scavenging properties of PET have enhanced the performance of this type of package.

Concerning the Life Cycle Inventory (LCI), Anon (2006) compared laminated paperboard containers, glass bottles, and PET bottles. According to its study, the paperboard containers had the lowest weight per delivered volume of wine and the lowest total energy requirements; the glass bottles had the highest. The production of container materials accounts for the largest share of the total energy for all container systems. Moreover, the glass bottles had significantly higher transportation requirements than the paperboard containers or PET bottles.

4.4.5 Metal cans

Although widely accepted for many beverages, cans have not been especially well received as packaging for wine. Alongside aluminum cans from the cylindrical shape of 250 mL are also emerging containers shaped bottle that can give a touch of elegance to the product. The health and hygienic safety of the packaged product is guaranteed by the aluminum lacquers lining the inner surface of the containers, which are essential to prevent corrosion due to sulfites and anions present in wine. Recently, a new product has been launched (Barokes, 2008) with an epoxy internal coating that can withstand 35 mg/L free SO_2 and

up to 250 mg/L total SO_2. For the packaging of non-sparkly wine in cans, it is necessary to increase the pressure inside of the cans through the injection of N_2 or addition of carbon dioxide with an internal pressure of about 170 kPa, in order to prevent the collapse of the body of the can. This happens because the cans are constructed so as to be in need of significant internal pressure to increase the relatively low resistance of the body of the can. The oxygen content should be close to zero to minimize unwanted oxidation reactions; this can be achieved by a closure with gas flushing of nitrogen. Wine in metal cans may from time to time show problems with SLOs (sulphur-like odours) (Baroni *et al*., 2013).

Chapter 5

Aims of the research

The research described in these pages focused on evaluating the effects of viticultural, technological, aging and packaging variables on the quality of native red wines from the South of Italy.

The research activity of the 1st year of the PhD course included an initial analytical phase consisting in the development of the LC-MS methods and optimization of the HPLC-ESI-MS conditions that should be employed during the 2nd and 3rd PhD years for the evaluation of the effects of the above cited variables on the phenolic composition (in particular on anthocyanins and their derived pigments) of the wines studied.

During the 2nd PhD year, the research activities were focused on the study of the effects of leaf removal and of alternative aging practices such as micro-oxygenation and aging with oak chips.

The 3rd PhD year was dedicated to the evaluation of the single and interactive effects of winemaking technologies, type of closure, and treatment with oak chips on colour, evolution of the phenolic composition (especially anthocyanins), antioxidant activity, and sensory profile of the wines. The effects of timing (before or after malolactic fermentation) of the treatment with oak chips were also studied.

The aims of the PhD research programme are analytically listed below:

- ✓ study of the effects of viticultural practices (in particular leaf removal) on composition of Uva di Troia (*Vitis vinifera* L.) grape and wine;
- ✓ evaluation of aging technologies such as micro-oxygenation and treatment with oak chips on colour stabilization and phenolic evolution of wines derived from native red grape cultivars;
- ✓ influence of winemaking technologies such as traditional red-winemaking, cryomaceration, vinification with pectolytic enzyme treatment, and vinification with extended maceration, on chemical and sensorial parameters in native wines;
- ✓ study of the effect of the treatments with French oak chips performed before or after the malo-lactic fermentation on Nero di Troia wine quality;
- ✓ study of single and interactive effects of leaf removal and aging with French wood chips on physical, chemical, and sensory characteristics of Nero di Troia wines;

- ✓ evaluation of single and interactive effects of winemaking technologies and treatment with French oak chips on colour, phenolic composition and sensory profile of Nero di Troia wines;
- ✓ evaluation of single and interactive effects of treatment with French wood chips and different types of closure on Nero di Troia wine quality.

Chapter 6

Materials and Methods

6.1 Red grapes

The vinifications were performed starting from red grapes native of the South of Italy. Their description is reported in the following paragraphs.

6.1.1 Aglianico

Aglianico grape has probable Greek origins, and in Italy it is mostly diffused in the provinces of Avellino and Benevento in the Campania region. Aglianico grape prefers volcanic soils in the territories in which it offers the best results. From Aglianico grape are produced great wines such as Taurasi D.O.C.G. Wines produced with Aglianico grapes are suitable to wood aging, both in large barrels and in barrique. The aging in wood tends to soften the tannins in young wines and soften the product, making it delicate and harmonious.

The spread of Aglianico grape provided him with a strong intra-zonal and varietal variability, which led to its registration in the National Registry of wine grapes in two distinct varieties: *Aglianico* (mainly grown in Taurasi in Campania) and *Aglianico del Vulture* (common in Basilicata). Recently, in-depth molecular, biochemical, and ampelographic investigations, carried out by the Agricultural Institute of San Michele all'Adige, made possible to establish that Aglianico and Aglianico del Vulture may be biotypes of the same variety having the same genetic identity, and that the differences would then be mostly attributed to the strong genotypic and phenotypic variability resulting from the ancient use of the by-seed reproduction.

The growing of Aglianico grapes is mainly diffused in Campania, Apulia, Molise, Basilicata, and Calabria regions, in the South of Italy. The production is abundant and constant. The following varietal characteristics must be remembered: the leaves are of medium size, elongate, orbicular or three-lobed; the cluster are medium-small, cylindrical, often winged, compact; the berries are of medium-small size and ellipsoidal shapes, with a waxy, thin, strong, black skin.

Vine trellis is the preferred training system but also other types of training are suitable.

The wine obtained from Aglianico grape is ruby red, fruity, fresh and tannic, full-bodied, suitable for aging.

Denominations in which Aglianico grapes are used for the production of the following quality wines:

- ✓ **D.O.C. Basilicata**: Aglianico del Vulture D.O.C.;
- ✓ **D.O.C. Molise**: Biferno D.O.C.; Molise o del Molise D.O.C.;
- ✓ **D.O.C. Campania**: Campi Flegrei D.O.C.; Cilento D.O.C.; Costa d'Amalfi D.O.C.; Falerno del Massico D.O.C.; Galluccio D.O.C.; Irpinia D.O.C.; Penisola Sorrentina D.O.C.; Sannio D.O.C.; Vesuvio D.O.C.;
- ✓ **D.O.C. Apulia**: Castel del Monte D.O.C.; Gravina D.O.C.;
- ✓ **D.O.C. Calabria**: Savuto D.O.C.; Scavigna D.O.C.; Terre di Cosenza D.O.C.;
- ✓ **D.O.C.G Campania**: Aglianico del Taburno o Taburno D.O.C.G; Taurasi D.O.C.G;
- ✓ **D.O.C.G Basilicata**: Aglianico del Vulture Superiore D.O.C.G;
- ✓ **I.G.T. Calabria**: Arghillà I.G.T.; Calabria I.G.T.; Costa Viola I.G.T.; Lipuda I.G.T.; Locride I.G.T.; Palizzi I.G.T.; Pellaro I.G.T.; Scilla I.G.T.; Val di Neto I.G.T.; Valdamato I.G.T.;
- ✓ **I.G.T. Campania**: Benevento o Beneventano I.G.T.; Campania I.G.T.; Colli di Salerno I.G.T.; Dugenta I.G.T.; Epomeo I.G.T.; Paestum I.G.T.; Pompeiano I.G.T.; Roccamorfina I.G.T.; Terre del Volturno I.G.T.;
- ✓ **I.G.T. Abruzzo**: Colli Aprutini I.G.T.; Colli del Sangro I.G.T.; Colline Frentane I.G.T.; Colline Pescaresi I.G.T.; Colline Teatine I.G.T.; del Vastese o Histonium I.G.T.; Terre Aquilane o dell'Aquila I.G.T.; Terre di Chieti I.G.T.;
- ✓ **I.G.T. Pulgia**: Daunia I.G.T.; Murgia I.G.T.; Apulia I.G.T.; Salento I.G.T.; Tarantino I.G.T.; Valle d'Itria I.G.T. (http://www.quattrocalici.it/vitigni/aglianico).

6.1.2 Montepulciano

Montepulciano grape is of quite uncertain origins and it is often confused with the *Sangiovese* vine, probably because of the Tuscan name "Vino Nobile di Montepulciano D.O.C.G". It is grown mostly in Marche, Abruzzo, Emilia-Romagna, Tuscany, Molise, Umbria, Lazio, Apulia, and Basilicata. Its origin is almost certainly Abruzzo, in particular the province of Pescara, although some of its greatest expressions is the Conero D.O.C.G.

The following varietal characteristics must be highlighted: the leaves are of medium size, pentagonal and five lobes; the clusters are medium, conical or cylindrical-conical, with one or two wings, and quite compact; the berries are of medium size, with a sub-oval shape; the skins are very waxy, thick and firm, and of a black-purple colour. Montepulciano vines prefer deep soils and good exposure, and warm and dry weather. The most commonly training systems give a medium expansion and medium-short pruning. The vigour is medium and the ripening is delayed.

When vinified in purity, Montepulciano grapes give a wine with a deep ruby red colour. Its bouquet is vinous and fruity. In the mouth, it is dry and hot, with balanced tannins. It is suitable for aging. It also lends itself to the vinification and production of rosé wines through partial maceration of the grapes.

Denominations in which the vine is present are the following:

- ✓ **D.O.C. Abruzzo**: Abruzzo D.O.C.; Cerasuolo d'Abruzzo D.O.C.; Controguerra D.O.C.; Montepulciano d'Abruzzo D.O.C.; Ortona D.O.C.; Terre Tollesi o Tullum D.O.C.; Villamagna D.O.C.;
- ✓ **D.O.C. Puglia**: Alezio D.O.C.; Brindisi D.O.C.; Castel del Monte D.O.C.; Copertino D.O.C.; Gravina D.O.C.; Leverano D.O.C.; Lizzano D.O.C.; Nardò D.O.C.; Orta Nova D.O.C.; Rosso di Cerignola D.O.C.; San Severo D.O.C.;
- ✓ **D.O.C. Molise**: Biferno D.O.C.; Molise o del Molise D.O.C.; Pentro d'Isernia o Pentro D.O.C.;
- ✓ **D.O.C. Lazio**: Castelli Romani D.O.C.; Cerveteri D.O.C.; Colli della Sabina D.O.C.; Colli Etruschi Viterbesi o Tuscia D.O.C.; Colli Lanuvini D.O.C.; Cori D.O.C.; Roma D.O.C.; Tarquinia D.O.C.; Velletri D.O.C.;
- ✓ **D.O.C. Emilia-Romagna**: Colli di Rimini D.O.C.; Colli Romagna Centrale D.O.C.; D.O.C. Marche: Colli Maceratesi D.O.C.; Esino D.O.C.; I Terreni di San Severino D.O.C.; Rosso Conero D.O.C.; Rosso Piceno D.O.C.; D.O.C. Basilicata: Grottino di Roccanova D.O.C.; D.O.C. Umbria: Rosso Orvietano o Orvietano Rosso D.O.C.;
- ✓ **D.O.C.G Marche**: Conero D.O.C.G; Offida D.O.C.G;
- ✓ **D.O.C. Abruzzo**: Montepulciano d'Abruzzo Colline Teramane D.O.C.G;

- ✓ **I.G.T. Campania**: Benevento o Beneventano I.G.T.; Campania I.G.T.; Colli di Salerno I.G.T.; Dugenta I.G.T.; Epomeo I.G.T.; Paestum I.G.T.; Pompeiano I.G.T.; Roccamorfina I.G.T.; Terre del Volturno;
- ✓ **I.G.T. Lazio**: Colli Cimini I.G.T.; Costa Etrusco Romana I.G.T.; Lazio I.G.T. (http://www.quattrocalici.it/vitigni/montepulciano).

6.1.3 Uva di Troia

The origins of *Uva di Troia* grape is probably in Asia Minor, where the ancient city of Troy was placed. Troy (small village in the province of Foggia) was probably founded by Greek colonists from Asia Minor. Other hypotheses invoke its Albanian origin from the city of Cruja. The spread of Uva di Troia vine is abundantly grown along the Apulia coastal area, in the provinces of Bari and Barletta, and also in the province of Foggia. There are at least two different biotypes: one with bigger and the other with smaller clusters and berries. The grape with smaller clusters is locally known as "*Carmosina*" and has the most promising oenological features.

Uva di Troia grape variety has good vigour, average ripening period and productivity. It easily adapts to any training form and pruning and has no particular soil requirements.

Among the varietal characteristics, the following must be remembered: the leaves are of medium-size, and of pentagonal, five-lobed shape; the clusters are large, pyramidal, or simple winged, moderately compact; the berries are of medium size and spheroidal shape; the skin is covered with bloom, thick and firm, almost leathery, pale violet.

Uva di Troia grapes give a ruby red wine with violet hues, vinous and pleasant tastes, and flowery and licorice olfactory notes. The in-mouth taste is dry, tannic, fresh, alcoholic and with a good body structure.

The Denominations in which Uva di Troia grapes are used alone or in blend are the following:

- ✓ **D.O.C. Apulia**: Barletta D.O.C.; Cacc'e Mmitte di Lucera D.O.C.; Castel del Monte D.O.C.; Gravina D.O.C.; Orta Nova D.O.C.; Rosso di Cerignola D.O.C.; San Severo D.O.C.; Tavoliere delle Puglie o Tavoliere D.O.C.;
- ✓ **D.O.C.G Apulia**: Castel del Monte Nero di Troia Riserva D.O.C.G; Castel del Monte Rosso Riserva D.O.C.G;

- ✓ **I.G.T. Campania**: Benevento o Beneventano I.G.T.; Dugenta I.G.T.; Epomeo I.G.T.; Paestum I.G.T.; Pompeiano I.G.T.; Roccamorfina I.G.T.; Terre del Volturno I.G.T.;
- ✓ **I.G.T. Apulia**: Daunia I.G.T.; Murgia I.G.T.; Apulia I.G.T.; Salento I.G.T.; Tarantino I.G.T.; Valle d'Itria I.G.T.. (http://www.quattrocalici.it/vitigni/uva-di-troia).

6.2 Leaf removal treatment

6.2.1 Vineyard site

The field trial was carried out, in the summer of 2012, at a private farm located in San Ferdinando di Puglia (province of Foggia, 41°19' N, 15°05', altitude 68 m a.s.l.).

The climate of this area is Mediterranean semi-arid according to the De Martonne (1926) scale (aridity index = 18 within the 15-20 range defined as semi-arid). The annual mean temperature is 15.5 °C (maximum temperature 31.8 °C in July and August, minimum temperature 3.0 °C in February); the mean annual rainfall is 470 mm, 34% of which in the warmer period (May-September). (CliNO, 1971-2000). The area totalizes 2170 GDD (growing-degree days) (IV region of the Winkler scale). The soil is deep, calcareous, medium textured, fertile, and retains moisture in the deep layers.

The vineyard was established in 2007. Uva di Troia vines was grafted onto 140 Ru (*V. berlandieri* x *V. rupestris*) rootstock at 1.25 x 2.50 m apart, in N-S oriented rows. Vines were VSP trained and spur-cordon pruned. The cordon was positioned 0.60 m above the ground while the highest trellis wire was at 1.80 cm from the soil and the total canopy height reached about 2.20 m; the average main shoot length was 1.60 m.

Fertilization was provided by means of soil applications, foliar nutrition and fertigation, with a total amount of about 45 kg N, 25 kg P_2O_5, 53 kg K_2O, 32 kg CaO, 20 kg MgO, 25 kg SO_3 per hectare; moreover, foliar application provided also about 50 kg alginic acid and 125 kg organic matter (both strong water soluble) per hectare.

Irrigation supplied about 1700 m^2/ha of water, from July to early September, by a drip system.

6.2.2 Leaf removal treatments and leaf area evaluation

At complete veraison (mid August), the following four leaf removal treatments were manually applied:

- ✓ **N**: no leaf removal;
- ✓ **E**: 75% of fruit-zone leaves removed from the East canopy side;
- ✓ **E/W**: 75% removal of the fruit-zone leaves on the East and also on the West side of the canopy;
- ✓ **F**: Farm defoliation (2 steps), that is, almost 100% removal of fruit-zone leaves on the West side of the canopy at full veraison (1st step), plus almost 100% removal of fruit-zone leaves on the East side of the canopy about 15 days before grape harvest.

Defoliation percentage was visually estimated.

Treatments were replicated in three 4-row blocks; each replicate was assigned to one row and involved 16 vines.

In order to evaluate the amount of leaf area removed and retained on vine after the treatments were imposed, the leaves removed from each replicate were immediately enclosed in plastic bags and transported to the lab where, after weighing, the weight-to-area ratio was applied using 100 leaf dishes (28 mm diameter) per replicate.

Moreover, aiming to express the data in terms of percentage of the total vine leaf area, half canopy of 5 representative vines was entirely defoliated and was subjected to the same procedure already described.

6.2.3 Field measurements

Measurements were taken in cloudless days of late summer (August 30th and 31st).

Air temperature and relative humidity at 2.00 m above the soil were measured (thermo-hygrometer HD 8501 H, Delta Ohm, Padova, Italy) under midday conditions.

During the morning, when the East side of the canopy was fully lighted, the rate of photosynthetic active radiation (PAR) was measured as maximum photosynthetic photon flux (PPF) interceptable by orienting a solar bar (AccuPAR PAR/LAI LP-80, Decagon Dev. Inc. Pullman, WA, USA), and as PPF interceptable at the leaf surface of the East and

of the West side of the vine canopy by positioning the solar bar along the canopy at 0.90 m above the cordon; 30 readings per type of measurements were recorded.

Immediately after, in order to assess the influence of the leaf removal treatments on the fruit-zone microclimate, PAR availability at East and at the West side of the vine canopy was measured by positioning the solar bar along the bunches and, moreover, the surface temperature of exposed bunches was measured using a non-contact infrared thermometer with laser pointer (TRI-88 Lafayette Electronic Supply Inc., Indiana, USA); 10 readings per each replicate and each type of measurement were recorded. The same set of measurements was taken in the afternoon, when the West side of the canopy was fully lighted.

Furthermore, in order to evaluate if leaf removal influenced the vine water status, stem water potential (Ψstem) was measured under midday conditions according to Turner (1981); 10 measurements per replicate were taken.

At farm harvest (October 4th), yield components were assessed on 10 vines per replicate, that is, vine total grape yield, number of bunches per vine, average bunch weight. The grape was immediately sent to the vinification.

6.2.4 Grapes and winemaking

Grapes were picked early in the morning on 4th October 2012 and immediately delivered to a pilot plant (Foggia, Italy) made of a crusher-destemmer, 20 stainless steel vats (100 L-capacity), a temperature management system, and 2 winepresses.

A traditional red wine-making was carried out with crushing-destemming, addition of potassium metabisulphite (10 g/hL of must), fermentation-maceration performed at 25 °C for 7 days by *Saccaromyces cerevisiae* (AEB, Brescia, Italy), and two punching-down per day. Each vinification was repeated two times, and every time, 2 samples were withdrawn.

6.3 Micro-oxygenation and treatment with oak chips

The red wines made from *Vitis vinifera* L. grape cv. Aglianico, Montepulciano, and Uva di Troia grapes were supplied by two local wineries (Aglianico by Casa Maschito, Maschito, PZ, Italy; Montepulciano and Nero di Troia by Valentina Passalacqua, Apricena, FG, Italy).

The wines were homogeneously distributed within stainless steel tanks of 100 L-capacity. For each variety, two tanks were submitted to a treatment with oak chips (3 g/L of French heavy-toasted oak chips - *Quercus petrae* Liebl.; 16 x 5 x 1 mm size; POLICELL 311/2L, CRC Biotek, Orvieto, TR, Italy) for 40 days at 17 °C (referred to as CHIPS) and two tanks contained untreated, control wine (TEST). The addition of oak chips was performed 4 months after racking for Aglianico wine, and 6 months after racking for Montepulciano and Nero di Troia wines.

In the case of Nero di Troia wine, other two tanks were submitted to a micro-oxygenation treatment (referred to as MOX) 7 months after racking, when the malolactic fermentation was already occurred. The micro-oxygenation treatment consisted in an oxygen dose of 3 mg/L/month blown for 20 days at 22 °C by means of a micro-diffusion system EcO_2 *plus* equipped with a technical ceramic protected by a stainless steel case diffuser (Vivelys, Villeneuve lès Maguelone, Languedoc-Roussillon, France).

After the oak chips and/or micro-oxygenation treatments the wines were bottled, closed with silicone closures (22 x 38 mm), kept at 16 ± 2 °C, and periodically analysed during 12-months of storage calculated from racking. Sampling were made in duplicate at each considered time.

6.4 Comparison among winemaking procedures

For the experimental trials described in this paragraph, Uva di Troia grape purchased from a local winery (Valentina Passalacqua, Apricena, FG, Italy) was used.

Grapes were picked early in the morning in the first week of October 2013 and immediately delivered to the same pilot plant described above (see 3.2.4 paragraph). At harvesting, Uva di Troia grapes showed the following characteristics: sugar content 21.2 ± 0.4 °Brix; titratable acidity 2.01 ± 0.08 g tartaric acid/L; pH 4.00 ± 0.03.

The sampling of the starting grapes was made according to the method described by Boulton *et al*. (1995) and Bisson (2001) in order to obtain a sample representative of the vineyard population. Half of the sample was always taken from one side of a row, to have an average between sun and shade exposure. Berries were randomly withdrawn at the top, middle, and bottom part of the bunch. Three 100-berry samples were selected from at least seven 10-cluster selections at similar position of 30 whole vine selections.

For the comparative study described in this paragraph, the following 4 winemaking procedures were applied:

- ✓ a traditional red vinification (referred to as T) including: the addition of potassium metabisulfite (10 g/100 kg) at the beginning of crushing-destemming; the fermentation-maceration performed by *S. cerevisiae* r.f. *cereviasiae* (Fermol 4, 20 g/100 kg, AEB) at 25 °C for 15 days; the addition of yeast activator (20 g/100 kg, preparation based on ammonium sulphate, diammonium phosphate, chemically inert filter and as dispersing agent, Vitamin B1; Enovit, AEB, Brescia, Italy); and two punching-down per day;
- ✓ a vinification with cryomaceration (referred as to C) including: the addition of potassium metabisulfite (10 g/100 kg) at the beginning of crushing-destemming; a 24-h skin cryomaceration at 4 °C (the temperature of the must, which was around 25 °C was lowered to 4 °C by addition of solid carbon dioxide); the fermentation-maceration performed by *S. cerevisiae* r.f. *cereviasiae* (Fermol 4, 20 g/100 kg, AEB) at 25 °C for 15 days; the addition of yeast activator (20 g/100 kg, preparation based on ammonium sulphate, diammonium phosphate, chemically inert filter and as dispersing agent, Vitamin B1; Enovit, AEB, Brescia, Italy); and two punching-down per day;
- ✓ a vinification with enzymes (referred to as E) including: the addition of potassium metabisulfite (10 g/100 kg) at the beginning of crushing-destemming; the addition of pectolytic enzyme preparation (mixture of pectine lyase, polygalacturanase, and pectine methylesterase; Endozym Rouge, 2 g/100 kg, AEB) left to act for 1 h; the fermentation-maceration performed by *S. cerevisiae* r.f. *cereviasiae* (Fermol 4, 20 g/100 kg, AEB) at 25 °C for 15 days; the addition of yeast activator (20 g/100 kg, preparation based on ammonium sulphate, diammonium phosphate, chemically inert filter and as dispersing agent, Vitamin B1; Enovit, AEB, Brescia, Italy); and two punching-down per day;

- ✓ a vinification with prolonged maceration (referred to as PM) including: the addition of potassium metabisulfite (10 g/100 kg) at the beginning of crushing-destemming; the fermentation-maceration performed by *S. cerevisiae* r.f. *cereviasiae* (Fermol 4, 20 g/100 kg, AEB) at 25 °C for 30 days; the addition of yeast activator (20 g/100 kg, preparation based on ammonium sulphate, diammonium phosphate, chemically inert filter and as dispersing agent, Vitamin B1; Enovit, AEB, Brescia, Italy); and two punching-down per day.

Each vinification procedure was repeated two times, and every time, two samples were withdrawn. After fermentation, the wines were submitted to three rackings and, after twelve weeks of decantation at 10 °C, they were bottled into 750 mL green glass bottles, closed with silicone stoppers, kept at 16 ± 2 °C, and periodically analysed during 12-months of storage.

6.5 Comparison among several types of closure having different permeability

For the evaluation of the influence of different types of closure on red wine quality and evolution during bottle aging, Uva di Troia grapes purchased from Valentina Passalacqua winery (Apricena, FG, Italy) were employed (see 3.4 paragraph for the starting grapes characteristics).

A traditional red vinification (as described in the previous paragraph) was applied.

After fermentation, the wines were decanted at 10 °C and submitted to three rackings during the successive twelve weeks, then they were bottled in 750 mL green glass bottles, closed with coextruded synthetic stoppers (Nomacorc® *Select Series*™, 23 x 38 mm; Nomacorc, Zebulon, NC, U.S.A.), and stored vertically at a constant temperature (16 ± 2 °C).

The OTR (oxygen transmission rate) mean values related to 12 months and physical characteristics of the employed stoppers, are given in Table 3.

Each bottling trial was repeated two times and, every time, two samples were withdrawn. Wines were periodically analysed during the 12-months of storage.

Code	Nomacorc stopper	Foam density (g/cm^3)	Total density (g/cm^3)	OTR (mg O_2/bottle)
S100	*Select Series*™ 100	0.261	0.328	1.2
S300	*Select Series*™ 300	0.261	0.328	2.4
S700	*Select Series*™ 700	0.306	0.357	3.4

Table 3 - Physical characteristics of Nomacorc® low density polyethylene (LDPE) stoppers as reported on the technical sheet provided by Nomacorc (Zebulon, NC, U.S.A.).

6.6 Timing of the treatment with oak chips

In order to study the influence of the time of addition of wood fragments on red wine quality and to understand the best time of application of oak chips treatment, Nero di Troia wine was placed into contact with oak chips at 2 different stages of the winemaking process, that is, before malolactic fermentation (BMLF) and after malolactic fermentation (AMLF).

Red grapes of cv. Uva di Troia (*Vitis vinifera* L.) were obtained from the local winery Valentina Passalacqua (Apricena, FG, Apulia, Italy). They harvested at their optimal ripening stage (21.2 ± 0.4 °Brix) and in good sanitary conditions. Five batches of grapes (100 kg each) were elaborated in stainless steel tanks of 100 L-capacity: *a)* according to the already described traditional red winemaking process; b) with the addition of oak chips, whose amount was selected in order to avoid an excessive impact of the wood character and a negative effect on the taster (Perez-Coello *et al*., 2000; Guchu *et al*., 2006; Gómez García-Carpintero *et al*., 2011; 2014). In particular, 3 g/L oak chips of 15 x 6 x 1 mm size were added before or after MLF for a contact time of 30 days at 16 °C. The employed wood fragments were French oak medium-toasted chips (Greensistem S.a.s, Foggia, Italy).

The wines were racked, bottled in green glass bottles, closed with silicone stoppers (22 x 38 mm), and stored in a conditioned room kept at 16 ± 2 °C. Wines were periodically analysed during a 12-month storage and, every time, two samples were withdrawn.

6.7 Treatment with oak wood chips combined with other processing

For the evaluation of the interactive effects of the treatment with oak wood chips with other variables, further experimental trials were performed.

The oak chips treatment was combined with the already described leaf removal. One week after racking (before MLF), the wines were added with 3 g/L oak chips of 16 x 5 x 1 mm size (referred to as "**+ CHIPS**") for a contact time of 70 days at 16 °C. The employed wood fragments were French oak heavy-toasted chips (*Quercus petrae* Liebl., POLICELL 311/2L, CRC Biotek, Orvieto, TR, Italy). Each vinification was performed in duplicate. After the treatment, the wines were racked, bottled in green glass bottles, closed with silicone stoppers (22 x 38 mm), and stored in a conditioned room kept at 16 ± 2 °C. Wines were periodically analysed during 12 months of storage and, every time, two samples were withdrawn.

The oak chips treatment was also combined with the four winemaking technologies described in the 3.4 paragraph and with the different types of closure employed for the bottle aging of Nero di Troia wines (3.5 paragraph). In both the experimental tests, the treatment with oak chips was performed two months after the first racking (before MLF). French medium-toasted oak chips (15 x 6 x 1 mm size; Greensistem s.a.s, Foggia, Italy) were employed for a contact time of 30 days at 16 °C (**+ CHIPS**). After the treatment, the wines were racked, bottled in green glass bottles, closed (see 3.4 paragraph for winemaking technologies and 3.5 paragraph for closure trials), and stored in a conditioned room kept at 16 ± 2 °C. Wines were periodically analysed during 12 months of storage and, every time, two samples were withdrawn.

6.8 Analytical determinations

6.8.1 Physical-chemical analysis

Alcoholic strength at 20 °C (expressed as % vol.), titratable acidity (expressed as g of tartaric acid/L), volatile acidity (g acetic acid/L), density (g/mL), dry extract (g/L), and free and total sulphur dioxide (mg/L) were determined according to the EEC Regulation 2676/90 (1990). The pH was also measured. The concentration of organic acids (g/L) and acetaldehyde (mg/L) were measured through a Hyperlab automatic multi-parametric analyzer (Steroglass, San Martino in Campo, PG, Italy) by means of enzymatic kits.

Dissolved oxygen (mg/L) was measured by using an LDO-HQ10 portable oxygen meter (Hach, Düsseldorf, Germany). The evaluation of the redox potential (E_H) was performed with a CyberScan pH 510 (Eutec Instruments, Nijkerk, Netherlands) equipped with an encapsulated Ag/AgCl electrode (Crison, Lainate, MI, Italy). The E_H were expressed in mV.

6.8.2 Colour measurements and structure indices

Wine colour was evaluated by the measurement of the Glories parameters (1984), which include colour intensity (CI), tonality (T), percentage of yellow, red and blue components (% yellow, % red and % blue, respectively), dA%, dAl%, dAT%, and dTAT% using quartz cells of 0.1 cm path length. Ethanol index, HCl index, gelatin index, and PVPP index were determined according to Glories (1978), while polymeric pigments were quantified as described in Habertson *et al*. (2003). Both colour and structure indices determinations were taken with an UV–visible spectrophotometer (Cary 50 SCAN; Varian, Palo Alto, CA).

6.8.3 Determination of phenolic compounds and antioxidant activity

The extraction of the phenolic fractions from pulps and skins was done according to Di Stefano and Cravero (1991). In order to separate the various parts of berries, the peeling and the separation of seeds from pulps were manually performed with the assistance of stainless steel tweezers and all the operations were performed under a laminar flow cabinet near a Bunsen-type flame thus limiting the contact with oxygen. After peeling, pulps were immediately separated from seeds and crushed, and the recovered juices were centrifuged at 6500 rpm for 15 min. The supernatants were filtered through common filter paper, weighed, and combined with concentrated H_2SO_4 10 N in order to avoid tartaric precipitation (juices/H_2SO_4 10 N, 9:1 v/v). The acidified juices were filtered and immediately analysed. Skins were weighed and combined with 25 mL of a solution of ethanol/water/hydrochloric acid 37% (70:30:1 v/v/v). After 24 h under dark conditions, the mixtures were filtered and immediately analysed.

The total phenolic contents of pulps, skins, and wines were measured at 765 nm through the above cited UV–visible spectrophotometer according to the Folin-Ciocalteu method as reported by Singleton and Rossi (1965). Results were expressed as gallic acid equivalents (mg/L of pulp juice and wine or mg/kg of dry pulps and skins). A calibration line was built on the basis of solutions of known and increasing concentrations of gallic acid. The choice

of gallic acid as standard is based on its availability as a stable and pure substance. Furthermore, the response to gallic acid is equivalent to that of most other phenolics in wine.

The various phenolic classes were analysed according to the methods of Di Stefano *et al*. (1989) and Di Stefano and Cravero (1991). When necessary, extracts were opportunely diluted with aliquots of the extraction solution. Anthocyanins sensistive to SO_2 were measured according to Ribéreau-Gayon and Stonestreet (1965). The results were expressed as mg/L of pulp juice and wine or mg/kg of dry pulps skins, and seeds.

The evaluation of the antioxidant activity was made through the ABTS assays (Re *et al*., 1999), which give a measure of the scavenging radical activity of the phenol extract or wine samples compared with that of a standard antioxidant (Trolox). ABTS is generally used to evaluate the radical scavenging abilities of flavonoids and phenolics. In the present PhD thesis, ABTS was dissolved into water to a 7 mM concentration and the ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The $ABTS^{+\cdot}$ solution was diluted with ethanol to an absorbance of 0.70 (± 0.02) at 734 nm and equilibrated at 30 °C. After addition of 1.0 mL of diluted $ABTS^{+\cdot}$ solution to 30 µL of wine opportunely diluted with ethanol, the absorbance reading was taken at 30 °C, exactly 1 min after the initial mixing and up to 6 min. Appropriate solvent blanks were run in each assay. The Trolox calibration curve of the antioxidant power assay was built by analysing six ethanolic solutions in the range 25-700 µM. Results were expressed as mmol of Trolox equivalents/L of wine, while for pulps, skins and seeds the results were expressed as mmol of Trolox equivalents/kg of dry matter.

6.8.4 HPLC-DAD-ESI-MS/MS analysis of phenolics

The chromatographic analyses were performed according to the method described in Crupi *et al*. (2012) with some modifications. A Capillary HPLC 1100 Series system, equipped with a degasser model G1379A, a binary pump model G1376A solvent delivery, an autosampler model G1377A, a thermostated column compartment model G1316A, a DAD model G1315C, and an XCT-trap Plus mass detector model G2447A (Agilent, Santa Clara, CA) coupled with an ESI interface was used. The reversed stationary phase employed was a Poroshell 120 SB-C18 2.7 µm (150 × 2.1 mm i.d., Agilent Technologies) thermostated at 40 °C. The solvent A was water containing 1% formic acid while the solvent B was

acetonitrile. The following gradient system was applied: 0 min, 0% B; 2 min, 5% B; 10 min, 13% B; 25 min, 15% B; 30 min, 22% B; 50 min, 22% B; 55 min, 95% B; 65 min, 95% B; 66 min, 5% B; stop time to 66 min followed by washing and re-equilibration of the column. The flow was maintained at 200 µL/min. The sample injection was 8 µL. Diode array detection was between 250 and 650 nm, and absorbance were recorded at 280, 313, 350 and 520 nm. Both positive and negative electrospray mode were used for the molecule ionization with a capillary voltage of 3500 V and a skimmer voltage at 40 V. The nebulizer pressure was 40 psi and the nitrogen flow rate was 8 L/min. The temperature of the drying gas was 350 °C. The monitored mass range was from *m/z* 50 to 1200. The wine samples were filtered through a 0.45 µm cellulose acetate filter prior to HPLC injection. Compounds identification was achieved by combining different information: elution pattern, UV-Vis and MS spectra, MS/MS fragmentation patterns and with the help of structural models already hypothesized in the literature. Quantitative determinations were made by using the external standard method with commercial standards. The calibration curves were obtained by injection of standard solutions under the same conditions of the samples analysed, over the range of concentrations observed. The compounds for which no standards were available were quantified on the curves of quercetin-3-rutinoside (flavonols and dihydroflavonols), *trans*-resveratrol (stilbenes), gallic acid (hydroxybenzoic acids), caffeic acid (hydroxycinnamic acids), (+)-catechin (flavan-3-ols) and malvidin-3-*O*-glucoside (anthocyanins). Therefore, flavonols, flavan-3-ols, hydroxybenzoic acids, hydroxycinnamic acids, stilbenes, and anthocyanins were respectively expressed in quercetin-3-rutinoside (QE, mg/L; R^2 = 0.9986), (+)-catechin (CE, mg/L; R^2 = 0.9945), gallic acid (GAE, mg/L; R^2 = 0.997), caffeic acid (CAE, mg/L; R^2 = 0.9954), *trans*-resveratrol (RE, mg/L; R^2 = 0.9894) and malvidin-3-*O*-glucoside (ME, mg/L; R^2 = 0.9941) equivalents.

6.8.5 HS-SPME-GC-MS analysis of free volatile compounds

6.8.5.1 Sample preparation

The free volatile compounds were extracted through the HS-SPME (Headspace solid-phase microextraction) technique according to the method of Canuti *et al*. (2009) opportunely modified. A 5 mL aliquot of wine was transferred into a 20 mL glass headspace sample vial containing 1 g of NaCl. Two point five microliters of a octan-3-ol internal standard solution (83 mg/L in ethanol) were added to each vial. The mixture was carefully shaken to dissolve NaCl and then left to equilibrate 1 h in the dark at room temperature before the analysis.

6.8.5.2 HS-SPME procedures

The SPME fibre coating used in this study was the polydimethylsiloxane (PDMS), 100 μm thickness and 24 gauge. The fibre were purchased from Supelco and thermally conditioned in accordance with the manufacturer's recommendations before the first use. The samples of grapes and wines were warmed up to 40 °C for 10 min before exposing the SPME fibre to the headspace of the sample. Headspace sampling/extraction times of 30 min were evaluated under continuous stirring conditions (250 rpm).

6.8.5.3 GC–MS analysis

A Gerstel MPS autosampler (Gerstel, Baltimore, MD, USA) mounted onto an Agilent 6890N gas chromatograph (Little Falls, DE, USA) paired with an Agilent 5975 mass selective detector constituted the analytical system. The software used was the MSD ChemStation (Agilent). SPME injections were in splitless mode using a SPME injection sleeve (0.75 mm i.d.) at 250 °C for 350 sec during which the thermal desorption of th analytes from the fibre occurred. A DB-Wax column (60 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA) was used for all analyses. Helium carrier gas was used at a flow of 1.0 mL/min. Concerning the oven parameters, the initial temperature of 40 °C was held for 1.0 min, followed by an increase to 200 °C at a rate of 4 °C/min. The oven was then held at 200 °C for 20 min before returning to the initial temperature (40 °C). The total cycle time was 61 min. The MS detector was operated in a

scan mode (mass range 30-500) and the transfer line to the MS system was maintained at 250 °C. The identity of the peaks was assigned using the NIST 05 library.

6.8.5.4 Quantitation

The internal standard (octan-3-ol) quantification method was used. The relative areas of analytes were normalized with the internal standard area. The samples were analysed in duplicate and blank runs were made with empty glass vial before and after each analysis.

6.9 Sensory descriptive analysis

The flavour profiles of wines were generated by a panel of 7 trained judges between 24 and 50 years of age, experienced in food and beverage sensory evaluation using quantitative descriptive analysis. The panellists were initially screened based on: (i) sensitivity to recognise the basic tastes and to perceive astringency in standard solutions containing slightly above-threshold concentrations of pure compounds (sucrose, citric acid, caffeine, sodium chloride and tartaric acid) (Meilgaard *et al.*, 1999); (ii) discrimination ability to determine differences in the flavour of red wine samples made according to different technologies (Meilgaard *et al.*, 1999); and (iii) ability to identify odours usually perceived in red wines.

Samples of red wine (20 mL) were presented in standard wine-tasting glasses according to the standard ISO 3591 (1997). Glasses were covered with a watch-glass to minimize the escape of volatile components. The temperature of the wines was maintained at 18 ± 1 °C, and the evaluations were made in individual booths under white light in a standard sensory-analysis chamber (ISO 8589, 1998). Wines were sniffed and tasted. The trained judges performed a quantitative-descriptive analysis (QDA) and each descriptor was evaluated on a 0 to 4 scale. The data were collected using the descriptive ballot consensually generated by the panel. Overall, each judge evaluated each wine with two repetitions, and for each repetition, a different bottle of wine was tested.

6.10 Statistical analysis

Each analysis was replicated at least three times. The averages and the standard deviations were calculated using the Excel software V. 11.5.1 (Microsoft, Redmond, WA). The data statistical treatment was performed using the package Statistica for Windows ver. 10 (Statsoft Inc., Tulsa, OK). The *t*-Student test ($p < 0.05$), and the analysis of variance (ANOVA) followed by *post-hoc* least significant difference test (LSD) ($p < 0.05$) were applied to determine single (One-way ANOVA) and combined (Factorial ANOVA) effects of the applied treatments and the aging time on the chemical composition of wine. The Principal Component Analysis (PCA) was applied to the data sets in order to check the possibility to discriminate wine samples subjected to different agronomic, technological, and packaging treatments.

Chapter 7

Results and Discussion

7.1 Study of the effects of defoliation on composition of Uva di Troia (*Vitis vinifera* L.) grape and wine

Vines gave a very high grape yield, as expected, that ranged from almost 9 kg per vine with F and E/W treatments and almost 10.6-10.7 kg with N and E treatments (+18%) (Table 2). Since the number of bunches per vine was very constant, the amount of grape was closely dependant on the bunch weight. Differences were not statistically significant; nonetheless, it was noticed that the two treatments that achieved the higher grape yield were those that, by the time of harvest, had less leaf area removed. It is well-known that leaf removal may reduce grape yield (Hunter and Visser, 1990); generally speaking, it can be due to the vine source-sink balance or to the cluster microclimate. With late-time defoliation, i.e. imposed at veraison, microclimate is likely more effective than the leaf-to-fruit ratio.

Muñoz *et al.* (2002) affirmed that the quality of light reaching grape bunches influences fruit quality, and hence its sugar content. In the present study, the highest sugar content was detected in the grapes from vines submitted to the removal of the highest leaf area (F) (Table 3), which also showed the highest sums of the photosynthetic photon flux densities during a typical late summer day (996.13 PPF, μmol m^{-2} s^{-1}). This behaviour was in agreement with TaeJoung *et al*. (2000), who found that photosynthesis increased with photosynthetic photon flux but decreased at high temperatures. The lowest titratable acidity was found in grapes from E and F vines. The grapes deriving from E vines also showed the lowest malic acid concentration whereas the grapes from F wines also had the lowest tartaric acid concentration (Table 3). This behaviour could be explained saying that, among the various grapes, E and F samples received the highest photosynthetic photon flux densities (an average of 873.17 and 996,13 μmol m^{-2} s^{-1}, respectively). According to Valdivia (2001), the exposure of racemes to higher radiation (as is the case with defoliated plants) increases respiration in fruit cells, which induces a higher consumption of organic acids. The F grapes also had the skins with the highest % of dry matter among the samples. In fact, Keller *et al*. (1998) demonstrated that low-light conditions decreased the weight of grape skins and the skin to berry ratio.

The concentrations of total anthocyanins, flavans reactive with vanillin, and proanthocyanidins were not affected by the various defoliation treatments, whereas the total flavonoids, the flavonoids different from anthocyanins, and the total phenolic content

were higher in the skins of the not defoliated thesis (Table 4). These results were in agreement with the findings of Beleski *et al*. (2012), who studied the influence of partial defoliation on skin polyphenol content of Syrah grapevine variety. According to their results, defoliation applied 30 days after blooming allowed achieving highest content of total anthocyanins, total phenols and total flavan-3-ols in the berry skin, while defoliation made 15 days after blooming leaded to lower content of polyphenols. According to Vanden Heuvel *et al*. (2005), the timing of partial defoliation affects carbohydrate concentration of vegetative tissues and concentration of phenolics in berries. In their study, potted De Chaunac grapevines were partially defoliated at three phenological stages, without affect cluster light environment. Partial defoliation at berry set significantly reduced glucose and fructose concentrations in the leaf/shoot tissue, and also reduced total flavonols and total phenolics in the fruit. Vines partially defoliated pre-harvest had increased sucrose concentration in the leaves and roots, increased glucose in the roots, and produced fruit with improved total anthocyanins compared to the control. According to Table 5, the phenolic profile of skins was modified by defoliation. In particular, the flavonols myricetin-3-glucoside, quercetin-3-glucoside, quercetin-3-glucuronide, and laricitrin-3-rhamnose-7-tri-hydroxycinnamic acid decreased while laricitrin-3-glucoside increased as a consequence of all the defoliation treatments. The flavn-3-ols were not affected by leaf removal. Although the concentration of total anthocyanins remained unchanged in the defoliated grapes, the phenolic profile of skins was modified by the different leaf removal treatments. In fact, it has been demonstrated that the exposure of berries to changes of temperature and solar radiation alters the partitioning of anthocyanins between the various forms (Tarara *et al*., 2008). In particular, defoliation determined the increase of concentration of the highest number of anthocyanin species in grapes exposed to a photosynthetic photon flux equal to that of non defoliated grapes and to temperatures only slightly higher than those of the non defoliated grapes. In fact, the F grapes showed the increase of the 3-glucoside forms of delphinidin, cyanidin, petunidin+peonidin, and of peonidin-3-*p*-coumaroylglucoside concentrations. Grapes submitted to the highest photon flux and temperatures exhibited the increases of concentration of only 2 compounds (delphinidin-3-glucosides and malvidin-3-*p*-coumaroylglucoside in E grapes; petunidin-+peonidin-3-glucoside and peonidin-3-*p*-coumaroylglucoside in E/W grapes). Malvidin-3-glucoside decreased with all the defoliation treatments, while malvidin-3-acetylglucoside, malvidin-3-caffeoylglucoside, and petunidin-3-*p*-coumaroylglucoside remained unchanged. The E samples showed the lowest concentrations of all the phenolic classes in

the seeds and the highest total phenolic content in the pulps (Table 6). According to the data shown in Table 7, defoliation significantly affected the composition of flavan-3-ols contained in grape seeds by increasing their concentrations especially as consequence of the E and F treatments.

Table 8 shows the influence of the defoliation treatments on the oenological parameters of the wines. The leaf removal led to wines higher in alcohol content than those produced from not defoliated vines. Furthermore, the alcohol content increased with the increase of the surface of the removed leaves (from E to F). These data were in agreement with data concerning the total soluble solids of the grapes and with the findings of Diago *et al.* (2010), who observed higher sugar and alcohol content in wines from the varieties Tempranillo, Graciano, and Mazuelo when plants were subjected to early defoliation.

The highest values of dry matter were observed in wines from the vines submitted to the more severe defoliation. Since these data related well with the dry matter of the skins, it could be due to the skin thickening (Pastore *et al*., 2013).

The wines made from shaded (not defoliated) fruits had the lowest volatile acidity, according to the findings of Ristic *et al*. (2007) in Shiraz wines, while the highest values were observed in wines from grape subjected to leaf removal in the area of the clusters along the East side at complete veraison (E). The E wines also showed the lowest titratable acidity. The different behaviour showed by the east-side defoliation compared to other defoliations, in terms of acidity, can be related to the different levels of irradiation and temperatures as already described in Table 2. In fact, a higher temperature inside the canopy leads to a lower preservation of the acidity level, as shown in previous studies (Morrison and Noble, 1990). Similar results were obtained by Ferrer *et al*. (2007), who explained that the high temperatures induced organic acids degradation.

The wine pH in the present study showed no significant differences between the various defoliation treatments ($p < 0.05$). Otero *et al*. (2010) reported that early defoliation had no influence on pH value in Albariño wines and Diago *et al.* (2010) affirmed that the pH in wines from Tempranillo, Graciano, and Mazuelo vines was not modified when plants underwent partial defoliation. Similar results were reported by Calderon (2004) and Muñoz *et al*. (2002) in experiments concerning Cabernet Sauvignon vines. The amounts of the residual soluble solids were higher in the wines from the defoliated plants, in particular in E and F samples. It should be supposed that defoliation, by inducing a strong flow of

photo-assimilates toward sink organs, also promoted a mobilization of non-reducing sugars, which remained in the wines after fermentation. The titratable acidity in musts and wine is obviously mainly related to the accumulation of organic acids, especially tartaric and malic ones. Table 9 shows the absence of significant differences among defoliation treatments for L-lactic, citric, pyruvic, and D-gluconic acids, while the lowest concentrations of tartaric and L-malic acids were found, respectively, in the F and E wines, in agreement with the results observed in the starting grapes.

Data concerning the phenolic composition of the wines are reported in Tables from 10 to 12. In particular, Table 10 concerns the concentrations of specific phenolic classes while Table 11 described the distribution of anthocyanins among monomeric and polymeric forms. According to the results of Tables 10 and 11, the E wines showed the highest concentrations of total anthocyanins, antocyanins sensitive to SO_2, monomeric and small polymeric anthocyanins, while the highest concentrations of total flavonoids, flavonoids different from anthocyanins, and proanthocyanidins were detected in the F wines, and the highest total phenolic content was measured in the E/W samples. These results greatly differed from those detected on the grapes and already discussed. Nevertheless, it can be stated that partial defoliation has no marked effect on berry composition and volume but it generally improves wine quality (Hunter *et al*., 1991). Probably the different composition of the anthocyanin pigments in the shaded and not shaded grapes could have affected their extractability and stability during winemaking (Ristic *et al*., 2007). Furthermore, wine colour is the result of a complex series of reactions and is influenced by the amount and type of flavonoids in the fruit, the extent of extraction of these compounds during winemaking, and the stability of the pigments during fermentation and subsequent aging of the wine. While grape anthocyanins (especially monomeric) are initially the prominent contributor to wine colour, the levels and composition of other flavonoids such as tannins and flavonols in the fruit are also important as they influence anthocyanin stability both by acting as co-pigments and through the formation of stable adducts, such as the pigmented polymers. Many studies have shown that the level of polymerisation between anthocyanins and tannins and the stability of these pigments depends on their concentration and composition (Cheynier *et al*., 2000; Romero and Bakker, 2000; Eglinton *et al*., 2004).

Table 11 also concerns some indexes that indicate different tannin attributes. The gelatin index measures the capacity of tannins to react with proteins, forming stable combinations and, since the maximum reactivity occurs with procyanidins that have a molecular weight

around 2500 (eight flavanol units), it may be give an indication of astringency. The highest value of this index was shown by the F wines, in agreement with their highest proanthocyanidin contents and with the statement that tannin polymerization increase with aging. The ethanol index measures the condensed anthocyanin polysaccharides while the hydrochloric acid index measure the degree of polymerization of procyanidins. Both increase with aging. In the present study, there were no significant differences among wines for both the indices, and their intermediate values (the HCl index normally ranges from 5 to 40) are index of enough balanced wines. The PVPP index measure the amounts of anthocyanins bounded to tannins. According to the results of Table 11, they increased with defoliation, showing the highest concentrations in the E wines, which were also the wines with the highest concentration of total anthocyanins. Also the specific phenolic profiles of wines strongly differed from those detected on the grapes (Table 12) and the effects of defoliation treatments was mitigated by wine-making. Among phenolic acids, differences among samples were exhibited only by the caftaric and caffeic acids, whose highest concentrations were shown by the E wines. Defoliation determined significant increases of quercetin-3-glucoside among flavonols, of (-)-epicatechin among flavan-3-ols, and of malvidin-3-*p*-coumaroylglucoside among anthocyanins.

Concerning the colour parameters, the only significant differences were found for the colour intensity, dAl% and dAT% (Table 13). The first parameter exhibited the highest values in the E wines, in agreement with their higher anthocyanin (especially monomeric and small polymeric) contents. The F wines showed the lowest absorbance due to monomeric anthocyanins and the highest values absorbance due to polymeric pigments decolorized with SO_2.

The standardising effect of wine-making can be also inferred by the application of PCA to the all the data set of grapes (Figures 7*a* and *b*) and wines (Figures 7*c* and *d*)., respectively Concerning grapes, the first two components explained about 65% of the total variability in the data and the samples were homogeneously grouped according to the defoliation practices. Concerning wines, the variance explained by the first two components was about 69% but the samples appeared not clearly distinguishable from each other due to the same values of the first components exhibited by E/W and F wines and the same values of the second components showed by E, E/W, and N wines.

Experimental treatments	Stem water potential (Ψ_{stem}, MPa)	Yield components		
		Grape per vine (kg)	Bunch weight (g)	Bunches per vine (n)
N	-1.08 ± 0.05 **a**	10.55 ± 0.48 **a**	324.00 ± 11.31 **a**	32.52 ± 0.36 **a**
E	-1.05 ± 0.04 **ab**	10.72 ± 0.98 **a**	331.60 ± 22.59 **a**	32.15 ± 0.84 **a**
E/W	-0.95 ± 0.04 **b**	8.96 ± 0.74 **a**	280.80 ± 25.49 **a**	32.00 ± 0.45 **a**
F	-1.04 ± 0.04 **ab**	9.03 ± 0.20 **a**	307.50 ± 20.54 **a**	31.84 ± 1.82 **a**

Table 2 – Influence of fruit-zone leaf removal on the midday vine water status (in late summer) and on the vine productivity (at harvest).
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Experimental Treatments	Conventional analysis				% Dry matter			Organic acids (g/L juice)			
	°Brix	pH	Titrat. Acidity	Gluc./Fruct.	Skins	Pulps	Seeds	Tartaric acid	L-Malic acid	Citric acid	D-Gluconic acid
N	20.4 ± 0.4 **a**	4.12 ± 0.01 **b**	6.61 ± 0.05 **b**	0.70 ± 0.01 **a**	34.4 ± 1.1 **a**	18.2 ± 0.7 **a**	58.1 ± 9.9 **a**	2.25 ± 0.01 **b**	2.48 ± 0.01 **d**	0.27 ± 0.04 **a**	0.04 **a**
E	21.0 ± 0.3 **a**	4.03 ± 0.01 **a**	6.01 **a**	0.70 **a**	38.4 ± 1.6 **ab**	17.7 ± 3.0 **a**	64.8 ± 2.0 **a**	2.85 ± 0.00 **c**	1.16 ± 0.01 **a**	0.26 **a**	0.09 ± 0.01 **b**
E/W	20.4 ± 0.8 **a**	4.06 ± 0.06 **ab**	6.61 ± 0.05 **b**	0.72 ± 0.01 **a**	37.1 ± 4.5 **ab**	16.4 ± 2.4 **a**	67.1 ± 2.4 **a**	2.30 ± 0.04 **b**	1.90 ± 0.01 **c**	0.35 **b**	0.23 ± 0.03 **c**
F	22.1 ± 0.7 **b**	4.07 **ab**	6.00 ± 0.01 **a**	0.70 ± 0.01 **a**	43.7 ± 1.6 **b**	15.6 ± 0.7 **a**	64.3 ± 4.7 **a**	2.07 ± 0.04 **a**	1.41 ± 0.02 **b**	0.27 ± 0.02 **a**	0.06 ± 0.01 **a**

Table 3 - Effect of leaf removal on the sugar content, pH, titratable acidity and % of dry matter of skins, pulps and seeds of Uva di Troia grapes.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Experimental Treatments	TA (mg malvidin-3-glucoside/kg dry skins)	TF (mg (+)-catechin/kg dry skins)	FNA (mg (+)-catechin/kg dry skins)	FRV (mg (+)-catechin/kg dry skins)	PA (mg cyanidin chloride/kg dry skins)	TPC (mg gallic acid/kg dry skins)
N	31296 ± 1629 **a**	90574 ± 5930 **b**	45009 ± 7069 **b**	31531 ± 2788 **a**	48242 ± 8557 **a**	77478 ± 3229 **b**
E	26192 ± 5499 **a**	70939 ± 7592 **a**	32805 ± 797 **a**	30488 ± 12147 **a**	51304 ± 14363 **a**	65395 ± 16294 **a**
E/W	27837 ± 4390 **a**	74880 ± 14891 **a**	34351 ± 9407 **a**	27422 ± 5705 **a**	52180 ± 7895 **a**	69215 ± 11474 **ab**
F	29713 ± 3168 **a**	83666 ± 7337 **ab**	40406 ± 3148 **ab**	28804 ± 3843 **a**	44568 ± 9755 **a**	74781 ± 6212 **ab**

Table 4 – Effect of leaf removal on the phenolic composition of skins of Uva di Troia grapes.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
TA: total anthocyanins; TF: total flavonoids; FNA: flavonoids different from anthocyanins; FRV: flavans reactive with vanillin; PA: proanthocyanidins; TPC: total phenolic compounds.
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Phenolic compounds	N	E	E/W	F
Flavonols (mg QE/kg dry skins)				
Myricetin-3-glc	166.16 ± 1.22 **b**	106.62 ± 16.31 **a**	104.07 ± 16.24 **a**	122.60 ± 5.86 **a**
Quercetin-3-glc	52.49 ± 2.77 **c**	35.12 ± 5.94 **b**	23.72 ± 4.45 **a**	26.56 ± 1.84 **ab**
Quercetin-3-glcr	164.23 ± 12.38 **b**	144.00 ± 27.97 **b**	74.62 ± 6.44 **a**	123.82 ± 7.63 **b**
Quercetin-3-galac	174.15 ± 31.99 **a**	163.30 ± 32.72 **a**	154.43 ± 27.32 **a**	153.30 ± 19.00 **a**
Laricitrin-3-glc	38.14 ± 2.57 **a**	62.15 ± 0.33 **c**	47.93 ± 3.13 **b**	53.41 ± 2.77 **b**
Syringetin-3-galac	35.73 ± 0.84 **a**	31.65 ± 1.49 **a**	39.82 ± 7.89 **a**	31.12 ± 0.30 **a**
Laricitrin-3-rhamnose-7-tri-hydroxy-cinnamic acid	1124.22 ± 70.99 **b**	919.50 ± 110.89 **b**	558.02 ± 85.67 **a**	639.24 ± 92.27 **a**
Σ Flavonols	**1755.13 ± 49.11 c**	**1462.34 ± 26.13 b**	**1002.60 ± 83.63 a**	**1150.06 ± 99.01 a**

Flavan-3-ols (mg CE/kg dry skins)				
Procyanidin B3	77.08 ± 6.90 **a**	57.26 ± 10.64 **a**	64.89 ± 4.03 **a**	58.01 ± 7.48 **a**
(+)-Catechin	61.01 ± 6.74 **a**	69.88 ± 13.39 **a**	62.80 ± 4.67 **a**	60.61 ± 4.49 **a**
Σ Flavan-3-ols	**138.09 ± 0.16 a**	**127.14 ± 24.03 a**	**127.68 ± 8.70 a**	**118.62 ± 2.99 a**
Anthocyanins (mg ME/kg dry skins)				
Dp-3-glc	112.48 ± 20.81 **a**	173.09 ± 4.50 **b**	118.77 ± 0.63 **a**	186.18 ± 31.72 **b**
Cy-3-glc	285.76 ± 2.38 **c**	126.30 ± 0.26 **a**	211.80 ± 21.91 **b**	327.51 ± 15.70 **d**
Pt-3-glc + Pn-3-glc	1512.85 ± 115.96 **b**	1070.52 ± 185.70 **a**	2698.75 ± 140.48 **c**	2390.83 ± 25.36 **c**
Mv-3-glc	5887.29 ± 317.52 **b**	4641.30 ± 552.06 **a**	5298.63 ± 92.80 **ab**	5279.52 ± 439.17 **ab**
Mv-3-acetylglc	3659.60 ± 651.52 **a**	3003.86 ± 339.13 **a**	2635.92 ± 204.64 **a**	3035.35 ± 455.89 **a**
Mv-3-caffeoylglc	322.16 ± 37.06 **a**	320.53 ± 43.63 **a**	270.06 ± 20.79 **a**	263.42 ± 12.83 **a**
Pt-3-*p*-coumglc	286.24 ± 22.80 **a**	342.23 ± 18.87 **a**	306.66 ± 60.71 **a**	364.64 ± 69.68 **a**
Pn-3-*p*-coumglc	118.45 ± 1.57 **a**	153.70 ± 2.17 **a**	454.30 ± 44.96 **b**	493.41 ± 0.62 **b**
Mv-3-*p*-coumglc	6647.31 ± 324.88 **bc**	7540.75 ± 622.15 **c**	4782.58 ± 550.49 **a**	5616.91 ± 622.64 **ab**
Σ Anthocyanins	**18832.14 ± 1188.46 a**	**17372.29 ± 23.43 a**	**16777.48 ± 907.98 a**	**17957.76 ± 730.58 a**

Table 5 – Effect of leaf removal on the phenolic profile of skins of Uva di Troia grapes.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
glc: glucoside, glcr: glucuronide, galac: galactoside, Dp: delphinidin, Cy: cyanidin, Pt: petunidin, Pn: peonidin, Mv: malvidin, acetylglc: acetylglucoside, *p*-coumglc: *p*-coumaroylglucoside, caffeoylglc: caffeoylglucoside.
In row, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Experimental Treatments	Seeds				Pulps
	TF (mg (+)-catechin/kg dry matter)	**FRV (mg (+)-catechin/kg dry matter)**	**PA (mg cyanidin chloride/kg dry matter)**	**TPC (mg gallic acid/kg dry matter)**	**TPC (mg gallic acid/kg dry matter)**
N	132416 ± 34576 **b**	78750 ± 21412 **c**	165108 ± 37811 **a**	164974 ± 34821 **b**	2147 ± 73 **b**
E	92938 ± 9630 **a**	56509 ± 10159 **a**	131117 ± 13586 **a**	126447 ± 3218 **a**	2474 ± 6 **c**
E/W	104934 ± 11981 **ab**	62645 ± 3294 **ab**	132941 ± 1533 **a**	141368 ± 7023 **a**	1943 ± 108 **a**
F	126495 ± 16322 **b**	72672 ± 5475 **bc**	223416 ± 41269 **b**	167942 ± 11672 **b**	2477 ± 172 **c**

Table 6 – Effect of leaf removal on the phenolic composition of seeds and pulps of Uva di Troia grapes.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
TF: total flavonoids; FRV: flavans reactive with vanillin; PA: proanthocyanidins; TPC: total phenolic compounds.
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Phenolic compounds	N	E	E/W	F
Flavan-3-ols (mg CE/kg dry seeds)				
Procyanidin B3	851.98 ± 1.04 **a**	4867.19 ± 993.34 **c**	421.83 ± 36.18 **a**	2720.23 ± 335.71 **b**
(+)-Catechin	1525.93 ± 26.85 **b**	6375.43 ± 522.94 **d**	586.66 ± 71.20 **a**	3705.21 ± 218.27 **c**
Procyanidin B1	319.69 ± 14.99 **a**	2248.72 ± 70.48 **c**	631.98 ± 14.63 **b**	2651.20 ± 106.13 **d**
Procyanidin B4	585.61 ± 59.47 **a**	3951.59 ± 688.09 **c**	1254.64 ± 31.61 **ab**	1570.89 ± 143.77 **b**
(-)-Epicatechin	1290.23 ± 52.77 **a**	6519.57 ± 820.57 **c**	744.77 ± 144.78 **a**	2891.24 ± 320.88 **b**
Procyanidin B2	311.96 ± 22.56 **a**	1926.20 ± 298.10 **c**	596.29 ± 13.91 **ab**	864.78 ± 22.66 **b**
(-)-Epicathechin-3-*O*-gallate	421.01 ± 59.30 **a**	1765.37 ± 196.70 **c**	699.25 ± 79.30 **a**	1089.44 ± 138.60 **b**
Σ Flavan-3-ols	**5306.41 ± 72.91 a**	**27654.07 ± 1808.13 c**	**4935.41 ± 363.80 a**	**15492.97 ± 178.06 b**

Table 7 – Effect of leaf removal on the phenolic profile of seeds of Uva di Troia grapes.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
In row, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Experimental Treatments	Alcohol (% vol)	Dry Matter (g/L)	Volatile Acidity (g acetic acid/L)	Titratable Acidity (g tartaric acid/L)	pH	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Total Soluble Solids (°Brix)	Acetaldehyde (mg/L)
N	11.44 ± 0.11 **a**	33.7 ± 2.2 **ab**	0.12 ± 0.01 **a**	6.46 ± 0.10 **b**	3.70 ± 0.05 **a**	48.4 ± 2.0 **b**	53.6 ± 2.8 **bc**	7.0 ± 0.3 **a**	52 ± 3 **a**
E	11.85 ± 0.11 **b**	32.8 ± 0.2 **ab**	0.25 ± 0.03 **c**	6.14 ± 0.02 **a**	3.77 ± 0.01 **a**	46.0 ± 1.5 **b**	51.2 ± 2.3 **b**	7.6 ± 0.2 **b**	61 ± 4 **a**
E/W	11.73 ± 0.07 **b**	30.3 ± 3.7 **a**	0.16 ± 0.01 **b**	6.47 ± 0.08 **b**	3.71 ± 0.02 **a**	40.0 ± 2.9 **a**	45.6 ± 3.8 **a**	7.1 ± 0.3 **ab**	57 ± 8 **a**
F	12.25 ± 0.26 **c**	35.3 ± 1.5 **b**	0.17 ± 0.01 **b**	6.59 ± 0.19 **b**	3.72 ± 0.08 **a**	52.8 ± 2.9 **c**	57.6 ± 2.9 **c**	7.5 ± 0.3 **b**	57 ± 9 **a**

Table 8 – Effect of leaf removal on the quali-quantitative characteristics of Nero di Troia wines at racking.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Experimental Treatments	Tartaric acid	L-Malic acid	L-Lactic acid	Acetic acid	Citric acid	Pyruvic acid	D-Gluconic acid
N	2.40 ± 0.03 **b**	2.66 ± 0.27 **ab**	0.05 **a**	0.11 **b**	0.58 ± 0.05 **a**	0.06 ± 0.01 **a**	0.70 ± 0.16 **a**
E	2.43 ± 0.10 **b**	2.48 ± 0.03 **a**	0.05 ± 0.01 **a**	0.12 ± 0.01 **b**	0.58 ± 0.06 **a**	0.06 **a**	0.65 ± 0.07 **a**
E/W	2.40 ± 0.04 **b**	2.66 ± 0.04 **ab**	0.07 ± 0.01 **a**	0.13 ± 0.01 **c**	0.57 ± 0.01 **a**	0.06 ± 0.01 **a**	0.58 ± 0.08 **a**
F	2.27 ± 0.05 **a**	2.86 ± 0.12 **b**	0.06 ± 0.01 **a**	0.09 ± 0.01 **a**	0.66 ± 0.06 **a**	0.06 **a**	1.03 ± 0.28 **a**

Table 9 – Effect of leaf removal on the organic acids content of Nero di Troia wines at racking (data are expressed as g per L of wine).
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Experimental Treatments	TA	SA	MA	TF	FNA	FRV	PA	TPC	ABTS assay
N	411 ± 56 **a**	393 ± 24 **a**	255 ± 11 **b**	1899 ± 265 **a**	1300 ± 184 **a**	1537 ± 92 **b**	2604 ± 148 **a**	2379 ± 72 **a**	16.09 ± 1.73 **b**
E	491 ± 50 **b**	486 ± 42 **b**	286 ± 18 **c**	1931 ± 379 **a**	1096 ± 320 **a**	1256 ± 144 **a**	2513 ± 179 **a**	2420 ± 105 **ab**	16.71 ± 1.60 **b**
E/W	472 ± 9 **ab**	393 ± 19 **a**	243 ± 13 **ab**	2301 ± 280 **ab**	1615 ± 285 **a**	1466 ± 176 **b**	2422 ± 338 **a**	2520 ± 251 **b**	15.64 ± 1.75 **ab**
F	428 ± 24 **a**	384 ± 26 **a**	236 ± 16 **a**	2776 ± 319 **b**	2153 ± 353 **b**	1189 ± 185 **a**	5601 ± 791 **b**	2438 ± 202 **ab**	14.62 ± 1.52 **a**

Table 10 – Effect of leaf removal on phenolic composition of Nero di Troia wines at racking.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
TA: total anthocyanins (as mg malvidin-3-glucoside/L); SA: anthocyanins sensitive to SO_2 (as mg malvidin-3-glucoside/L); MA: monomeric anthocyanins (as malvidin-3-glucoside mg/L); TF: total flavonoids (as mg (+)-catechin/L); FNA: flavonoids different from anthocyanins (as mg (+)-catechin/L); FRV: flavans reactive with vanillin (as mg (+)-catechin/L); PA: proanthocyanidins (as mg cyanidin chloride/L); TPC: total phenolic compounds (as mg gallic acid/L); ABTS assay: antioxidant activity by ABTS assay (as mmol Trolox eq/L).
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Experimental Treatments	MP	SPP	LPP	$I_{gelatin}$	I_{EtOH}	I_{HCl}	I_{PVPP}
N	0.54 ± 0.06 **a**	0.16 ± 0.02 **a**	0.12 ± 0.02 **a**	62.8 ± 4.1 **a**	20.7 ± 3.7 **a**	28.3 ± 4.5 **a**	35.0 ± 2.0 **a**
E	0.72 ± 0.12 **b**	0.24 ± 0.03 **c**	0.13 ± 0.01 **a**	58.9 ± 6.6 **a**	24.2 ± 3.2 **a**	25.4 ± 4.7 **a**	40.9 ± 3.0 **c**
E/W	0.63 ± 0.01 **a**	0.19 ± 0.00 **ab**	0.13 ± 0.02 **a**	60.3 ± 5.0 **a**	20.6 ± 0.6 **a**	29.5 ± 4.6 **a**	38.2 ± 0.6 **b**
F	0.64 ± 0.03 **a**	0.21 ± 0.01 **b**	0.13 ± 0.03 **a**	87.4 ± 1.7 **b**	21.0 ± 2.3 **a**	31.1 ± 5.3 **a**	38.6 ± 2.6 **b**

Table 11 – Effect of leaf removal on monomeric and polymeric pigments and structure indices of Nero di Troia wines at racking.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
MP: absorbance at 520 nm due to monomeric pigments; SPP: absorbance 520 nm due to small polymeric pigments; LPP: absorbance 520 nm due to large polymeric pigments; $I_{gelatin}$: gelatin index; I_{EtOH}: ethanol index; I_{HCl}: hydrochloric acid index; I_{PVPP}: PVPP index.
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

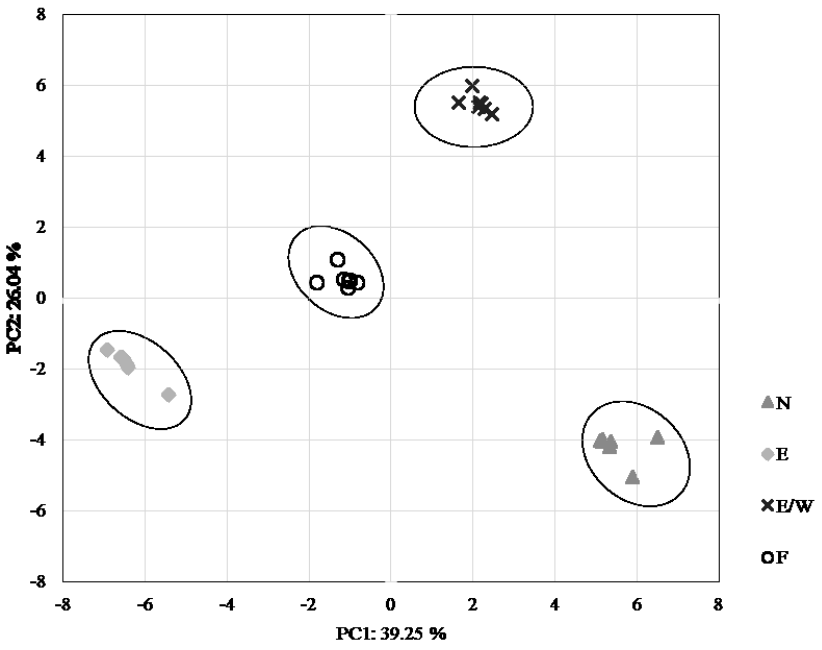
Phenolic compounds	t_r (min)	MS (*m/z*)	MS–MS fragments (*m/z*)	**N**	**E**	**E/W**	**F**
Phenolic acids (mg GAE/L; mg CAE/L)		$[M-H]^-$					
Gallic acid	5.6	169	125	18.8 ± 1.5 **a**	18.6 ± 1.1 **a**	19.0 ± 1.8 **a**	19.4 ± 0.9 **a**
Caftaric acid	12.5	311	179, 149	9.7 ± 0.5 **a**	17.8 ± 3.2 **b**	11.1 ± 0.4 **a**	11.2 ± 1.7 **a**
Caffeic acid	12.8	179	135	13.5 ± 0.7 **a**	17.7 **c**	14.9 ± 0.2 **b**	15.6 ± 0.6 **b**
p-Coumaric acid	15.3	163	119	6.7 ± 0.4 **a**	7.3 ± 1.9 **a**	7.3 ± 0.1 **a**	7.0 ± 0.1 **a**
Ferulic acid	17.2	193	178, 149, 134	6.8 ± 0.3 **a**	7.7 ± 1.3 **a**	7.6 ± 0.3 **a**	7.0 ± 0.3 **a**
Σ Phenolic acids				**55.5 ± 0.3 a**	**69.1 ± 1.1 b**	**59.8 ± 1.4 a**	**60.1 ± 2.8 a**
Stilbens (mg RE/L)		$[M-H]^-$					
cis-Piceid	24.7	389	227	0.6 ± 0.1 **a**	0.8 ± 0.1 **a**	0.7 **a**	0.8 ± 0.1 **a**
trans-Piceid	35.3	389	227	0.2 **a**	0.4 **b**	0.3 **b**	0.3 **ab**
Σ Stilbens				**0.8 ± 0.1 a**	**1.2 ± 0.2 b**	**1.1 ± 0.1 ab**	**1.1 ± 0.2 ab**
Flavonols (mg QE/L)		$[M-H]^-$					
Myricetin-3-glc	22.3	479	316/317	6.6 ± 1.7 **a**	9.7 ± 2.4 **a**	10.1 ± 1.8 **a**	10.8 ± 1.2 **a**
Myricetin-3-rha	26.2	463	317	0.5 ± 0.1 **a**	0.6 ± 0.1 **a**	0.4 **a**	0.4 **a**
Quercetin-3-glc	27.4	463	301	0.6 ± 0.1 **a**	1.1 **b**	1.2 ± 0.2 **bc**	1.5 **c**
Quercetin-3-glcr	28.6	477	301	3.9 ± 0.1 **a**	3.6 ± 0.5 **a**	3.4 ± 0.6 **a**	4.3 ± 0.2 **a**
Quercetin-3-galac	28.9	463	301	3.3 **a**	3.6 ± 0.6 **a**	4.5 ± 0.8 **a**	7.4 ± 0.6 **b**
Laricitrin-3-glc	30.7	493	331	2.7 ± 0.2 **a**	3.3 ± 0.4 **a**	3.9 ± 0.5 **a**	3.4 ± 0.6 **a**
Quercetin-3-rha	35.1	447	301	0.8 ± 0.2 **a**	1.1 ± 0.2 **a**	0.9 **a**	1.5 **b**
Syringetin-3-galac	36.5	507	344/345	2.8 ± 0.1 **a**	3.4 ± 0.6 **a**	3.6 ± 0.4 **a**	3.0 ± 0.4 **a**
Laricitrin-3-rhamnose-7-tri-hydroxycinnamic acid	37.5	655	509, 501, 475, 347, 329, 314, 303	1.6 **a**	2.0 ± 0.4 **a**	1.9 ± 0.2 **a**	1.9 ± 0.1 **a**
Σ Flavonols				**22.7 ± 2.2 a**	**28.4 ± 5.2 ab**	**30.1 ± 4.5 ab**	**34.3 ± 1.6 b**
Flavan-3-ols (mg CE/L)		$[M-H]^-$					
Procyanidin B3	13.5	577	451, 425, 407, 289	108.4 ± 0.9 **a**	106.1 ± 8.2 **a**	117.7 ± 7.2 **a**	103.9 ± 10.9 **a**
(+)-Catechin	14.5	289	245, 205, 179	13.0 ± 3.4 **a**	12.2 ± 1.4 **a**	15.0 ± 4.8 **a**	13.6 ± 0.3 **a**

Procyanidin B1	15.7	577	451, 425, 407, 289	5.0 ± 1.2 **a**	8.3 ± 1.1 **b**	4.9 ± 0.6 **a**	10.2 ± 0.1 **b**
Procyanidin B4	16.4	577	451, 425, 407, 289	11.3 ± 0.8 **a**	11.0 ± 0.4 **a**	11.3 ± 0.3 **a**	15.0 ± 0.2 **b**
(-)-Epicatechin	17.5	289	245, 205, 179	12.4 ± 3.0 **a**	14.2 ± 3.0 **ab**	18.4 ± 1.2 **ab**	21.9 ± 3.8 **b**
Procyanidin B2	20.0	577	451, 425, 407, 289	8.8 ± 1.3 **a**	8.3 ± 0.7 **a**	8.3 ± 0.1 **a**	7.6 ± 1.2 **a**
Σ Flavan-3-ols				**158.8 ± 3.7 a**	**160.2 ± 14.7 a**	**175.7 ± 12.8 a**	**172.2 ± 5.9 a**
Anthocyanins (mg ME/L)		$[M-2H]^-$					
Dp-3-glc	15.3	463	301	9.7 ± 1.6 **a**	13.8 ± 4.4 **a**	8.6 ± 0.1 **a**	10.6 ± 1.7 **a**
Cy-3-glc	17.2	447	285	0.4 ± 0.1 **ab**	0.7 ± 0.2 **b**	0.3 **a**	0.6 ± 0.1 **ab**
Pt-3-glc	18.0	477	315	17.1 ± 3.2 **a**	24.4 ± 7.7 **a**	15.5 ± 1.5 **a**	18.1 ± 4.7 **a**
Pn-3-glc	20.4	461	299	6.8 ± 1.2 **a**	11.3 ± 2.4 **a**	7.5 ± 0.4 **a**	9.1 ± 2.1 **a**
Mv-3-glc	21.5	491	329	141.2 ± 6.5 **a**	171.8 ± 32.6 **a**	135.9 ± 11.8 **a**	138.8 ± 9.2 **a**
Dp-3-acetylglc	25.1	505	463, 301	1.8 ± 0.4 **a**	2.4 ± 0.5 **a**	2.0 **a**	2.1 ± 0.1 **a**
Pyrano-Mv-3-glc (Vitisin B)	27.6	515	353	0.3 ± 0.1 **c**	0.3 **c**	0.2 **b**	nd **a**
Pt-3-acetylglc	32.8	519	477, 315	4.6 ± 0.4 **a**	6.7 ± 2.2 **a**	4.0 ± 0.5 **a**	4.6 ± 0.4 **a**
Pn-3-acetylglc	35.8	503	299	5.8 **a**	6.5 ± 0.4 **a**	7.1 ± 0.9 **a**	6.0 ± 1.5 **a**
Mv-3-acetylglc	36.1	533	329	65.6 ± 2.1 **a**	70.2 ± 12.3 **a**	62.3 ± 1.4 **a**	58.7 ± 5.9 **a**
Pyrano-Mv-3-*p*-coumglc (*p*-coum-Vitisin B)	37.1	661	353	0.6 **a**	0.8 ± 0.2 **a**	0.7 ± 0.1 **a**	0.7 ± 0.1 **a**
Mv-3-caffeoylglc	38.1	653	491, 329	1.7 ± 0.1 **a**	2.1 ± 0.3 **a**	1.8 ± 0.1 **a**	2.0 ± 0.1 **a**
Pt-3-*p*-coumglc	38.8	623	477, 315	2.3 **a**	3.0 ± 0.6 **a**	2.6 **a**	2.5 **a**
Mv-3-*p*-coumglc-8-ethyl-(epi)cat	41.9	953	801, 663, 645, 355	4.5 **a**	5.4 ± 1.4 **a**	4.8 ± 0.9 **a**	5.2 ± 0.5 **a**
Pn-3-*p*-coumglc	42.4	607	299	18.4 ± 0.9 **a**	22.6 ± 2.7 **b**	19.8 ± 0.4 **ab**	20.5 ± 0.3 **ab**
Mv-3-*p*-coumglc	42.4	637	491, 329				
Σ Anthocyanins				**280.8 ± 10.4 a**	**342.2 ± 67.8 a**	**273.1 ± 13.6 a**	**279.4 ± 11.2 a**

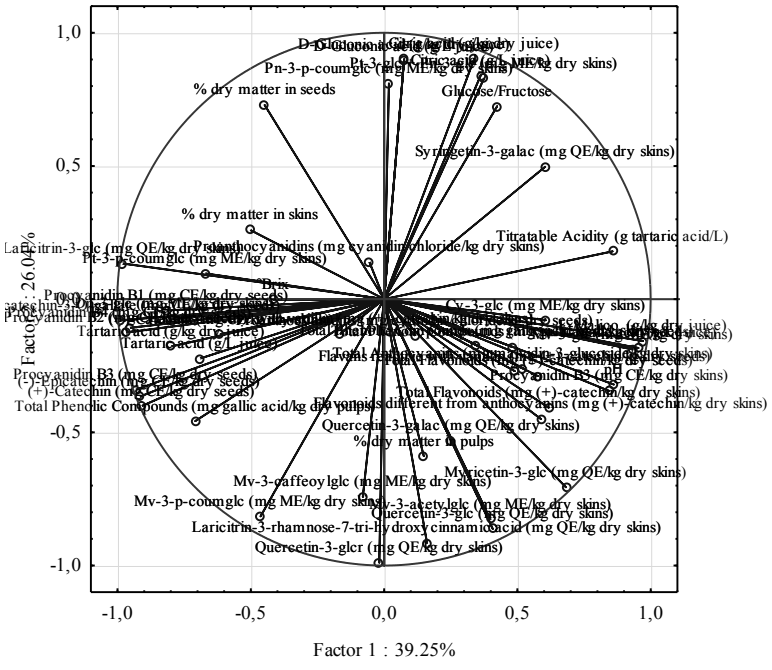
Table 12 – Effect of leaf removal on the phenolic profile of Nero di Troia wines at racking.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
nd: not detected.
glc: glucoside, glcr: glucuronide, gall: rha: rhamnoside, galac: galactoside, Dp: delphinidin, Cy: cyanidin, Pt: petunidin, Pn: peonidin, Mv: malvidin, acetylglc: acetylglucoside, *p*-coumglc: *p*-coumaroylglucoside, caffeoylglc: caffeoylglucoside, cat: catechin.
In row, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Experimental Treatments	CI	T	dA(%)	% yellow	% red	% blue	dAl%	dAT%	dTAT%
N	5.670 ± 0.585 **a**	0.565 ± 0.002 **a**	63.1 ± 0.7 **a**	32.5 ± 0.1 **a**	57.5 ± 0.4 **a**	10.0 ± 0.6 **a**	10.6 ± 0.2 **b**	89.4 ± 0.2 **a**	0.0 **a**
E	7.340 ± 1.024 **b**	0.566 ± 0.013 **a**	62.6 ± 0.7 **a**	32.4 ± 0.5 **a**	57.2 ± 0.4 **a**	10.4 ± 0.2 **a**	10.5 ± 0.3 **b**	89.5 ± 0.2 **a**	0.0 **a**
E/W	6.431 ± 0.042 **ab**	0.565 ± 0.004 **a**	62.6 ± 0.3 **a**	32.3 ± 0.2 **a**	57.2 ± 0.2 **a**	10.5 ± 0.2 **a**	10.9 ± 0.0 **b**	89.2 ± 0.1 **a**	0.0 **a**
F	6.298 ± 0.092 **a**	0.593 ± 0.044 **a**	61.1 ± 3.1 **a**	33.3 ± 1.3 **a**	56.3 ± 2.0 **a**	10.3 ± 0.7 **a**	7.8 ± 1.6 **a**	92.2 ± 1.6 **b**	0.0 **a**

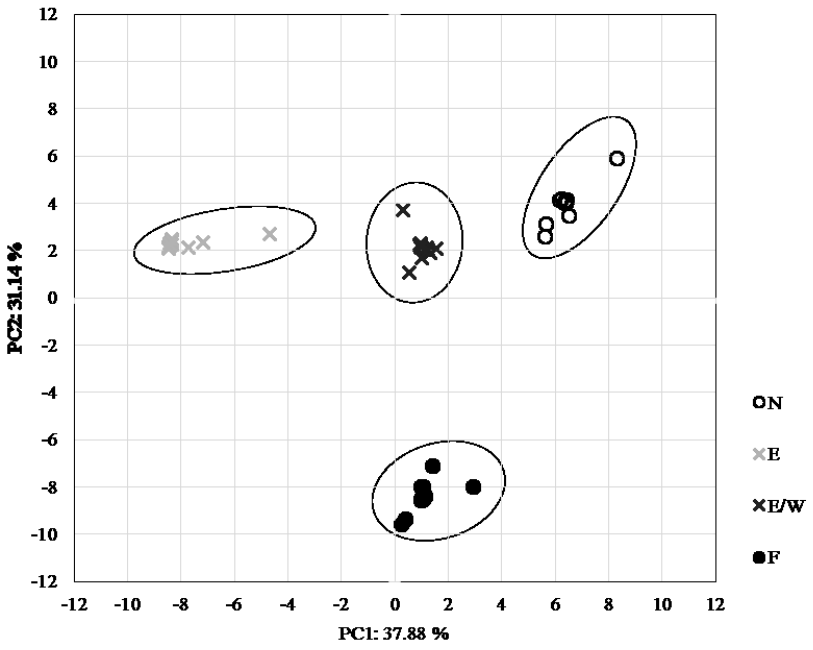
Table 13 – Effect of leaf removal on colour parameters and polymeric pigments of Nero di Troia wines at racking.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
CI: colour intensity; T: tonality; dAl: absorbance at 520 nm due to monomeric anthocyanins; dAT: absorbance at 520 nm due to polymeric pigments decolorized with SO_2; dTAT: absorbance at 520 nm due to polymeric pigments not decolorized; MP: absorbance at 520 nm due to monomeric pigments; SPP: absorbance 520 nm due to small polymeric pigments; LPP: absorbance 520 nm due to large polymeric pigments; LPP+SPP: absorbance 520 nm due to total polymeric pigments; LPP/SPP: ratio between large and small polymeric pigments.
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.


PC2: 26.04 %
PC1: 39.25 %
N
E
E/W
F

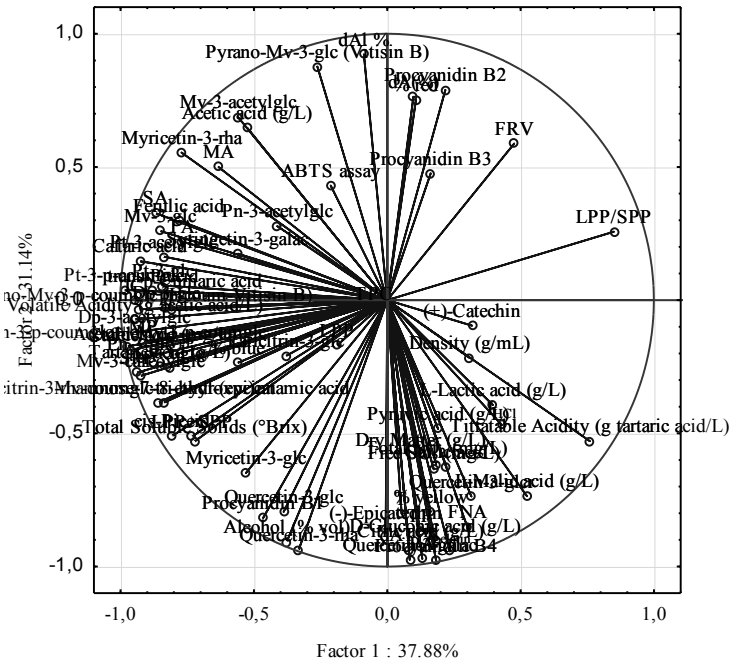
a)


% dry matter in seeds
Glucose/Fructose
Syringetin-3-galac (mg QE/kg dry skins)
% dry matter in skins
Titratable Acidity (g tartaric acid/L)
Cy-3-glc (mg ME/kg dry skins)
Procyanidin B3 (mg CE/kg dry skins)
Total Flavonoids (trs (+)-catechin/kg dry skins)
Quercetin-3-galac (mg QE/kg dry skins)
%dry matter in pulps
Myricetin-3-glc (mg QE/kg dry skins)
Mv-3-caffeoylglc (mg ME/kg dry skins)
Mv-3-p-coumglc (mg ME/kg dry skins)
Quercetin-3-glc (mg QE/kg dry skins)
Laricitrin-3-rhamnose-7-tri-hydroxy cinnamic acid (mg QE/kg dry skins)
Quercetin-3-glcr (mg QE/kg dry skins)
Factor 2 : 26.04%
Factor 1 : 39.25%

b)



c)



d)

Figure 7 - PCA scatter plot for projection on the factor plane of: **a)** Uva di Troia grapes and **b)** the corresponding analytical results; **c)** Uva di Troia wines and **d)** the corresponding analytical results.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.

7.2 Evaluation of treatment with oak chips and micro-oxygenation on colour stabilization and phenolic evolution of wines derived from native red grape cultivars

7.2.1 Aglianico wines

Table 14 shows the main physicochemical parameters of wines at racking, immediately after the treatment with oak chips, and at 12 months of aging. The wines at racking showed the typical characteristics of Aglianico, being in high alcohol content and dry matter. The soluble solids were index of the correct progress of fermentation and the volatile acidity was acceptable. The concentrations of free and total SO_2 were very low. At the end of the treatment with wood chips, the control wine (TEST) exhibited lower ethanol content and higher volatile acidity, free and total SO_2 than the treated wines, while any significant difference was found for the other parameters of oenological interest. The results concerning alcohol content and volatile acitidy, although significant, are probably casual. In fact, they cannot be explained on the basis of oxidation reactions of ethanol by film yeasts (Fugelsang, 1997) or by a coupled autoxidation of phenolics (Wildenradt and Singleton, 1971) since the highest acetaldehyde content was detected in treated wines. In fact, ellagitannins extracted from the wooden chips are involved in oxidation reactions (Moutounet *et al*., 1989), in which they act as oxygen scavenger by producing large amounts of peroxides. The wood ellagitannins, found in several species of oak (*Quercus* sp.) belong to the group of hydrolysable tannins (Haslam, 1981). Vescalagin and castalagin are the main ellagitannins present in the oak wood. Peroxides may react with the ethanol to form acetaldehyde (Vivas and Glories, 1996). After 12 months, the differences among treated and untreated wines were mitigated by the aging process. The increase of volatile acidity with respect to the previous time indicates an increase of oxidation reaction. The acetaldehyde content strongly decreased in both the wines probably as a consequence of acetaldehyde-mediated condensation reactions involving anthocyanins and flavan-3-ols. The only registered difference among the wines concerned the dissolved oxygen that showed the lowest value in CHIPS samples as a consequence of ellagitannins ability to quickly absorb the dissolved oxygen and favouring hyperoxidation of the wine constituents (Vivas and Glories, 1996).

Table 15 includes the concentrations of several organic acids. At racking, they were in a

decreasing order: tartaric > malic > lactic > acetic > D-gluconic > citric and pyruvic. The presence of acetic and pyruvic acids are justified by the statement that they are further by-products of the yeast fermentative metabolism. Small amounts of D-gluconic acid could be explained by the presence of moulds that metabolized part of the glucose present in grape juice. The content of lactic acid can be due to lactic acid bacteria present on the surface of grapes and survived to fermentation. At the time corresponding to end of the treatment with oak chips, significant differences were found only for tartaric acid (whose concentration was lower in the control wine). Compared to the data collected at racking, the concentrations of malic and lactic acids indicate that malolactic fermentation occurred and those concerning acetic acid are index of oxidation reactions. After 12 months of aging, no significant differences were found among treated and untreated wines, while for both the wines, precipitation of tartaric acid occurred.

The phenolic composition of wines is shown in Table 16. At racking, the total free anthocyanins is consistent with the typical concentrations of in full-bodied young red, which is around 500 mg/L although, in some cases, they can be higher than 2000 mg/L (He *et al.*, 2012). The wine at racking also had high amounts of all the considered phenolic classes, and high values of antioxidant activity. At the time corresponding to the end of the treatment with oak chips, strong decrease of concentrations was detected for all the phenolic classes, with the exception of that of flavonoids different from anthocyanins. The wines treated with oak chips showed a lower contents of monomeric anthocyanins and flavans reactive with vanillin (about -14% and -17%, respectively), probably due to polymerization reactions occurred, and a higher content of total phenolics (+4.5%) with respect to the control wines, which was probably due to the extraction of phenolic compounds from wood. Any significant difference was highlighted for the other classes of phenolics and for the antioxidant activity value. Significant changes of the concentrations of the phenolic classes occurred during storage in all the types of wines, especially in those ones treated with wood. In particular after 12 months from the racking, monomeric anthocyanins decreased by about 82.5% in TEST wines and by 94% in CHIPS ones with respect to the initial concentration (400±18 mg/L at racking), while flavans reactive with vanillin decreased by about 29% in untreated samples and by 63.5% in wood-treated ones (2863±158 mg/L at racking). Furthermore, after 12 months of aging (Table 16), the untreated wines exhibited higher concentrations of all the phenolic classes (except for flavonoids different from anthocyanins) than the wines treated with chips, probably as a

consequence of condensation reactions between anthocyanins and tannins. Thus, results indicate that the typical reactions of aging that decrease levels of anthocyanins take place more quickly in wine treated with oak chips. These data are confirmed by the results concerning the phenolic profiles of wine (Table 17). Immediately after the end of the treatment, oak-treated samples exhibited higher concentrations of caffeic and *p*-coumaric acids, *trans*-piceid, myricetin-3-rhamnoside, quercetin-3-glucuronide, and (-)-(epi)gallocatechin, and lower content of *cis*-piceid, quercetin-3-rhamnoside, laricitrin-3-rhamnose-7-tri-hydroxy-cinnamic acid, and (+)-catechin compared to the control ones. The decrease of free anthocyanins and the increase of pyranoanthocyanins were observed. Contemporary, a higher anthocyanin polymerization was observed in the oak-treated samples. This behaviour was in agreement with the studies of Piracci *et al.* (2001), which showed the loss of free anthocyanins during vinification in the presence of woody material and the increase of the more stable forms. After 12 months, the wines treated with oak chips exhibited the lowest concentration of almost all phenolic compounds, especially of monomeric and dimeric flavan-3-ols, and of all the anthocyanins (both free and polymerized forms). The loss of anthocyanins was in agreement with the study of Del Alamo Sanza and Nevares Domínguez (2006).

Table 18 includes the distribution of pigments among monomeric and polymeric forms in terms of contribution to the absorbance at 520 nm by monomeric and polymeric pigments. The monomeric anthocyanins predominated at racking, and then their decreae during aging was accompanied by the increases of small and large polymeric pigments. At the same time, the ratio between large and small polymeric forms changed from values < 1 to values > 1, thus indicating the increase of pigment polymerization. After 12 months, the concentrations of monomeric and small polymeric pigments in untreated wines were twofold those of the wines treated with chips. Table 18 also concerns gelatin, hydrochloric, and PVPP indexes. The highest value of the gelatin index was calculated at racking, corresponding to the higher concentration of proanthocyanidins, molecules responsible for astringency. The increase of HCl index and PVPP index, their higher values in wines treated with oak chips, as well as the results observed in the phenolic composition discussed above, indicated a higher degree of tannins and anthocyanins polymerization mediated by acetaldehyde in wine treated with oak fragments. The gelatin index value decrease when wines were put into contact with oak chips and than increase, although it didn't reach the value measured at racking. This behaviour could be related to the toasting

degree of the oak chips employed for the treatment. Also Tao *et al*. (2014) stated that heavy-toasted wood gave wines higher wood-related properties, but also made wines more astringent and bitter.

As observed for monomeric and polymeric pigments, the treatment with oak chips did not immediately affect colour parameters (Table 19). The differences were highlighted, instead, after 12 months. Colour intensity and tonality increased at the time corresponding to the end of the treatment with oak chips but without significant differences about control and oak-treated wines. After 12 months, the colour intensity remained unchanged in the control wines while decreased in the wines treated with chips as a consequence of the decrease of monomeric anthocyanins and the increase of condensation reactions between anthocyanins with highly polymerized tannins that led to the formation of unstable pigments (Zironi *et al*., 2006). The higher intensity of colour in control wine was mainly due to the yellow and red components. Tonality increased during all the considered aging time but, after 12 months, was higher in treated than in untreated wines thanks to the contribution of the blue components. The percentage of absorbance at 520 nm due to monomeric anthocyanins and polymeric pigments decolorized with SO_2 decreased during aging while that due to polymeric pigments not decolorized increased. In particular, after 12 months, the percentage of absorbance at 520 nm due to monomeric anthocyanins and polymeric pigments decolorized with SO_2 was higher in the control wines, while that due to polymeric pigments not decolorized was higher in the treated ones.

Changes in the volatile components of Aglianico wine aged with or without oak chips were investigated at racking and after 12 months. The 61 volatile compounds found by means of HS-SPME-GC-MS were grouped into the following 12 different classes (Table 21): acids (7 compounds), alcohols (17), acetic esters (3), ethyl esters (18), other esters (5), carbonyl compounds (2), terpenes (2), ethers (1), aromatics (2), hydrocarbons (2), sulphur compounds (1), and lactones (1). The more representative compounds of each class were: acetic acid (acids); 1-butanol,3-methyl alcohol and phenylethyl alcohol (alcohols); isoamyl acetate (acetic esters); ethyl octanoate (ethyl esters); isoamyl caprylate at racking and in final wine with traditional aging, isopropyl myristate in final wine with chips (other esters); nonanal (carbonyl compounds); cyclooctane (hydrocarbons) and D-nerolidol (terpenes); octyl ether (ethers); oxime-, methoxy-phenyl- at ranking and in final wine traditionally aged while 4-ethylphenol in the final wine with chips (aromatics); methionol (sulphur compounds) and whiskey lactone (lactones).

In the aged wines dramatic decreases in esters and alcohols were measured while the acids increased. This behaviour was more evident in wine treated with chips; in fact, Vivas *et al*. (1995) documented an increase in volatile acidity in wine with chips due to two reasons: mainly, acidity may originate from oak as a result of the toasting process, and secondly it can be due to the metabolism of acetic acid bacteria in wine. Moreover, the release of tannins from chips changed the redox balance in the wine and thus significant quantitative differences in the n-decanoic acid and dodecanoic acid were detected during storage in wine with chips as well as the formation of complex terpenes (D-nerolidol). Finally, the wine aged with chips was characterized by a greater presence of whiskey lactone directly released from chips. The Principal Component Analysis applied to the data concerning the volatile fraction (Figure 8) shows the distance between wines at racking and control and treated wines after 12 months of aging. They resulted clearly separated from each other based on the first components, which explained about 60% of the total variance.

As already mentioned, the extraction of tannins from oak wood (especially those heavy-toasted) may involve an increase of astringency if the treatment is performed in the wrong aging phase of a wine (Zironi *et al*., 2006). This phenomen occurred in the experimental wines that, immediately after the treatment with oak chips, obtained a negative sensory judgement due to their excessive "tannic character" (Table 21). This finding was in disagreement with those observed by Pèrez-Coello *et al*. (2000), who instead found a greater acceptability of wines treated with the chips. The result was otherwise in agreement with that observed by Quinn and Singleton (1985), who reported that high amounts of ellagitannins increased wine astringency and bitterness. Treatment with the chips also immediately determined an increase of spicy, woody, and vanilla flavours, and reduced the perception of floral and fruity notes (Figure 9*a*) in agreement with the findings of Guchu *et al*. (2006) and Gómez García-Carpintero *et al*. (2011) for Chardonnay and Bobal wines, respectively. The aging time reduced the flavour complexity and also reduced the citation frequency of the various flavours in both control and treated wines (Figure 9*b*). Moreover, the judges perceived a reduction of astringency in oak-treated samples in a greater extension that in control wines (Table 21) although the woody and vanilla aromas were perceived yet (Figure 9*b*).

The Principal Component analysis (PCA) was applied to the all standardize data (with the only exception of the volatile compounds which were separately evaluated) and the obtained results are reported in Figure 10. The first two principal components accounted

for about 75% of the total variance, and showed the evolution of control and treated wines during the aging time. At the time corrensponding to end of the treatments with oak chips, control and treated samples resulted indistinguishable, as stated by their representative symbols, which were overlapped. After12 months of aging, untreated and treated wines strongly differed from each others.

Sample	Alcohol (% vol)	Density (g/mL)	Dry Matter (g/L)	Volatile Acidity (g acetic acid/L)	Titratable Acidity (g tartaric acid/L)	pH	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Total Soluble Solids (°Brix)	Acetaldehyde (mg/L)	Dissolved O_2 (mg/L)
					At racking						
	14.99 ± 0.08	0.993 ± 0.002	32.1± 4.5	0.47 ± 0.04	4.92 ± 0.13	3.72 ± 0.01	27.2 ± 2.3	31.6 ± 2.8	8.6 ± 0.2	82.08 ± 14.74	2.99 ± 0.01
					After the treatment with oak chips						
TEST	14.28 ± 0.07 **a**	0.994 ± 0.001 **a**	32.0 ± 2.1 **a**	0.49 **b**	3.89 ± 0.06 **a**	3.64 ± 0.04 **a**	38.4 ± 0.0 **b**	46.4 **b**	8.9 **a**	11.50 ± 0.71 **a**	2.75 ± 0.20 **a**
CHIPS	14.61 ± 0.06 **b**	0.994 **a**	31.1 ± 0.1 **a**	0.43 ± 0.02 **a**	3.77 ± 0.15 **a**	3.67 ± 0.01 **a**	30.4 ± 2.3 **a**	38.4 ± 2.3 **a**	8.9 ± 0.1 **a**	51.50 ± 0.71 **b**	2.84 ± 0.01 **a**
					After 12 months						
TEST	15.11 ± 0.69 **a**	0.993 **a**	30.0 ± 1.1 **a**	0.63 ± 0.06 **a**	4.72 ± 0.05 **a**	3.64 ± 0.01 **a**	31.2 ± 1.1 **a**	36.8 **a**	8.6 **a**	2.00 **a**	2.78 ± 0.01 **b**
CHIPS	14.63 ± 0.06 **a**	0.992 **a**	29.2 ± 0.1 **a**	0.75 ± 0.05 **a**	4.71 ± 0.04 **a**	3.64 ± 0.01 **a**	24.0 ± 4.5 **a**	29.6 ± 5.7 **a**	8.5 ± 0.1 **a**	3.00 **a**	1.89 ± 0.01 **a**

Table 14 – Physicochemical parameters, acetaldehyde content, and dissolved oxygen of Aglianico wine at racking, immediately after the treatment with oak chips and at 12 months of aging.
In column, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by t Student test.

Sample	Tartaric acid	L-Malic acid	L-Lactic acid	Acetic acid	Citric acid	Pyruvic acid	D-Gluconic acid
				At racking			
	2.85 ± 0.01	0.90	0.38± 0.01	0.29 ± 0.01	0.08	0.08	0.12
				After the treatment with oak chips			
TEST	2.46 ± 0.03 **a**	0.09 ± 0.01 **a**	0.69 **a**	0.66 **a**	0.09 **a**	0.06 **a**	0.13 **a**
CHIPS	2.96 ± 0.04 **b**	0.03 **a**	0.70 **a**	0.69 ± 0.01 **a**	0.06 ± 0.01 **a**	0.08 ± 0.01 **a**	0.12 ± 0.01 **a**
				After 12 months			
TEST	2.35 ± 0.09 **a**	0.09 ± 0.01 **a**	0.63 ± 0.01 **a**	0.53 **a**	nd **a**	0.04 **a**	0.10 **a**
CHIPS	2.44 ± 0.10 **a**	0.10 ± 0.01 **a**	0.64 ± 0.01 **a**	0.58 ± 0.01 **a**	nd **a**	0.04 **a**	0.12 **a**

Table 15 – Organic acids content of Aglianico wine at racking, immediately after the treatment with oak chips and at 12 months of aging.
nd: not detected.
In column, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by t Student test.

Sample	TA	SA	MA	TF	FNA	FRV	PA	TPC	ABTS assay
					At racking				
	571 ± 26	817 ± 37	400 ± 18	3863 ± 97	3031 ± 68	2863 ± 158	4904 ± 18	4162 ± 122	26.43 ± 3.66
					After the treatment with oak chips				
TEST	294 ± 10 **a**	350 ± 5 **a**	148 ± 1 **b**	3390 ± 74 **a**	2962 ± 85 **a**	2008 ± 160 **b**	4147 ± 52 **a**	3868 ± 150 **a**	23.26 ± 2.04 **a**
CHIPS	284 ± 10 **a**	348 ± 10 **a**	127 ± 7 **a**	3348 **a**	2935 ± 15 **a**	1676 ± 113 **a**	4269 ± 157 **a**	4042 ± 96 **b**	26.22 ± 2.40 **a**
					After 12 months				
TEST	247 ± 6 **b**	228 ± 9 **b**	70 ± 7 **b**	2690 ± 33 **b**	2330 ± 25 **a**	2032 ± 81 **b**	4396 ± 61 **b**	4158 ± 76 **b**	21.64 ± 3.41 **a**
CHIPS	140 ± 10 **a**	97 ± 8 **a**	23 ± 4 **a**	2387 ± 150 **a**	2184 ± 156 **a**	1043 ± 71 **a**	3113 ± 167 **a**	3079 ± 32 **a**	19.17 ± 2.72 **a**

Table 16 – Phenolic composition and antioxidant activity of Aglianico wine at racking, immediately after the treatment with oak chips and at 12 months of aging.
TA: total anthocyanins (as mg malvidin-3-glucoside/L); SA: anthocyanins sensitive to SO_2 (as mg malvidin-3-glucoside/L); MA: monomeric anthocyanins (as malvidin-3-glucoside mg/L); TF: total flavonoids (as mg (+)-catechin/L); FNA: flavonoids different from anthocyanins (as mg (+)-catechin/L); FRV: flavans reactive with vanillin (as mg (+)-catechin/L); PA: proanthocyanidins (as mg cyanidin chloride/L); TPC: total phenolic compounds (as mg gallic acid/L); ABTS assay: antioxidant activity by ABTS assay (as mmol Trolox eq/L).
In column, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by t Student test.

Phenolic compounds	MS (m/z)	MS–MS fragments (m/z)	*At racking*	*After the treatment with oak chips*		*After 12 months*	
				TEST	CHIPS	TEST	CHIPS
Phenolic acids (mg GAE/L; mg CAE/L)	$[M-H]^-$						
Gallic acid	169	125	82.54 ± 1.00	75.88 ± 0.76 **a**	77.44 ± 1.90 **a**	78.83 ± 0.96 **b**	72.89 ± 0.71 **a**
Caftaric acid	311	179, 149	23.23 ± 0.60	22.23 ± 0.10 **a**	21.67 ± 0.75 **a**	12.03 ± 0.71 **b**	2.96 ± 0.01 **a**
Caffeic acid	179	135	35.69 ± 1.10	31.08 ± 0.23 **a**	33.31 ± 0.29 **b**	18.94 ± 0.83 **b**	3.56 ± 0.09 **a**
p-Coumaric acid	163	119	13.38 ± 0.35	11.42 ± 0.17 **a**	12.06 ± 0.53 **b**	8.28 ± 0.52 **b**	2.39 ± 0.06 **a**
Ferulic acid	193	178, 149, 134	9.65 ± 0.28	10.23 ± 0.21 **a**	10.57 ± 0.29 **a**	3.27 ± 0.23 **a**	6.34 ± 0.03 **b**
Σ Phenolic acids			**164.48 ± 2.12**	**150.84 ± 0.85 a**	**155.05 ± 3.76 a**	**121.36 ± 1.37 b**	**88.14 ± 0.84 a**
Stilbens (mg RE/L)	$[M-H]^-$						
cis-Piceid	389	227	1.15	1.53 ± 0.01 **b**	1.39 ± 0.01 **a**	1.37 ± 0.02 **b**	0.74 ± 0.01 **a**
trans-Piceid	389	227	1.28 ± 0.04	2.02 ± 0.01 **a**	2.10 ± 0.02 **b**	1.57 ± 0.42 **a**	1.02 ± 0.04 **a**
Σ Stilbens			**2.43 ± 0.04**	**3.55 ± 0.01 b**	**3.49 ± 0.01 a**	**2.93 ± 0.44 a**	**1.77 ± 0.05 a**
Flavonols (mg QE/L)	$[M-H]^-$						
Myricetin-3-glc	479	316/317	1.54 ± 0.04	nd **a**	nd **a**	nd **a**	nd **a**
Myricetin-3-rha	463	317	3.55 ± 0.02	1.77 ± 0.04 **a**	2.40 ± 0.04 **b**	2.80 ± 0.36 **a**	2.08 ± 0.09 **a**
Quercetin-3-glc	463	301	1.27 ± 0.01	0.25 ± 0.02 **a**	0.19 ± 0.01 **a**	nd **a**	nd **a**
Quercetin-3-glcr	477	301	12.16 ± 1.30	7.38 ± 0.06 **a**	8.35 ± 0.18 **b**	4.72 ± 0.49 **b**	1.77 ± 0.01 **a**
Quercetin-3-galac	463	301	1.35 ± 0.13	nd **a**	nd **a**	nd **a**	nd **a**
Laricitrin-3-glc	493	331	1.68 ± 0.01	1.40 ± 0.06 **a**	1.35 ± 0.06 **a**	0.73 ± 0.17 **a**	0.42 ± 0.10 **a**
Quercetin-3-rha	447	301	4.35 ± 0.60	4.05 **b**	3.64 ± 0.10 **a**	4.41 ± 0.86 **b**	1.02 ± 0.01 **a**
Syringetin-3-galac	507	344/345	4.82 ± 0.09	4.64 ± 0.04 **a**	4.49 ± 0.07 **a**	4.40 ± 0.49 **b**	1.77 ± 0.13 **a**
Laricitrin-3-rhamnose-7-tri-hydroxy-cinnamic acid	655	509, 501, 475, 347, 329, 314, 303	3.22 ± 0.16	1.17 ± 0.02 **b**	0.88 ± 0.01 **a**	0.62 ± 0.11 **b**	0.39 ± 0.08 **a**
Σ Flavonols			**33.95 ± 0.79**	**21.66 ± 0.04 a**	**21.31 ± 0.37 a**	**17.68 ± 1.16 b**	**7.45 ± 0.26 a**
Flavan-3-ols (mg CE/L)	$[M-H]^-$						
(-)-(Epi)Gallocatechin	305	179, 125	14.67 ± 0.36	20.29 ± 0.49 **a**	28.33 ± 1.17 **b**	13.10 ± 3.47 **a**	13.96 ± 0.10 **a**
Procyanidin B3	577	451, 425, 407, 289	119.00 ± 1.82	113.31 ± 1.06 **a**	99.14 ± 5.25 **a**	65.62 ± 0.08 **b**	23.38 ± 0.13 **a**
(+)-Catechin	289	245, 205, 179	144.53 ± 3.35	104.88 ± 1.80 **b**	96.85 ± 1.89 **a**	67.40 ± 8.57 **b**	31.90 ± 1.77 **a**
Procyanidin B1	577	451, 425, 407, 289	144.93 ± 0.14	147.40 ± 6.01 **a**	129.90 ± 2.61 **a**	145.79 ± 6.53 **b**	6.78 ± 0.81 **a**
Procyanidin B4	577	451, 425, 407, 289	37.15 ± 2.29	38.65 ± 3.32 **a**	32.91 ± 0.13 **a**	32.30 ± 0.19 **b**	11.57 ± 1.24 **a**

(-)-Epicatechin	289	245, 205, 179	95.84 ± 0.70	59.79 ± 0.07 **a**	56.41 ± 1.26 **a**	42.18 ± 0.60 **b**	17.53 ± 0.54 **a**
Procyanidin B2	577	451, 425, 407, 289	31.51 ± 0.32	38.65 ± 1.43 **a**	36.58 ± 0.32 **a**	9.93 ± 2.41 **b**	3.11 **a**
Σ Flavan-3-ols			**587.63 ± 5.05**	**522.98 ± 14.16 a**	**480.12 ± 12.63 a**	**376.33 ± 14.37 b**	**108.24 ± 1.65 a**
Anthocyanins (mg ME/L)	$[M-2H]^-$						
Dp-3-glc	463	301	12.09 ± 0.12	8.17 ± 0.35 **b**	6.73 ± 0.08 **a**	3.80 ± 0.06 **b**	0.34 **a**
Cy-3-glc	447	285	nd	nd **a**	nd **a**	nd **a**	nd **a**
Pt-3-glc	477	315	18.48 ± 0.11	9.92 ± 0.80 **a**	7.68 ± 0.09 **a**	2.95 ± 0.37 **b**	0.30 ± 0.01 **a**
Pn-3-glc	461	299	5.36 ± 0.35	3.31 ± 0.09 **a**	2.35 ± 0.37 **a**	1.18 ± 0.08 **b**	0.16 ± 0.02 **a**
Mv-3-glc	491	329	186.85 ± 3.63	98.02 ± 0.24 **b**	82.04 ± 1.09 **a**	34.51 ± 0.17 **b**	3.26 ± 0.06 **a**
Dp-3-acetylglc	505	463, 301	4.35 ± 0.09	4.32 ± 0.03 **a**	4.56 ± 0.10 **a**	3.58 ± 0.01 **b**	1.84 ± 0.01 **a**
Pyrano-Mv-3-glc (Vitisin B)	515	353	0.99 ± 0.03	0.82 **a**	1.51 ± 0.01 **b**	0.39 ± 0.02 **b**	0.25 ± 0.02 **a**
Cy-3-acetylglc	489	447,285	nd	nd **a**	nd **a**	nd **a**	nd **a**
Pt-3-acetylglc	519	477, 315	1.54 ± 0.02	1.01 ± 0.03 **a**	1.30 ± 0.02 **b**	nd **a**	nd **a**
Pyrano-Mv-3-acetylglc (acetyl-Vitisin B)	557	353	0.20 ± 0.01	0.29 **a**	0.34 ± 0.01 **b**	nd **a**	nd **a**
Mv-3-*p*-coumglc-(epi)cat	925	617, 491	nd	nd **a**	nd **a**	0.46 ± 0.06 **a**	0.43 ± 0.04 **a**
Pn-3-acetylglc	503	299	4.44 ± 0.20	2.38 **a**	2.60 ± 0.12 **a**	0.69 ± 0.03 **b**	0.30 ± 0.02 **a**
Mv-3-acetylglc	533	329	14.19 ± 0.10	7.78 ± 0.17 **b**	6.30 ± 0.04 **a**	2.70 ± 0.13 **b**	0.60 ± 0.01 **a**
Pyrano-Mv-3-*p*-coumglc (*p*-coum-Vitisin B)	661	353	0.81 ± 0.14	0.93 ± 0.01 **a**	0.99 ± 0.01 **b**	1.06 ± 0.06 **b**	0.49 ± 0.03 **a**
Mv-3-caffeoylglc	653	491, 329	0.68 ± 0.04	nd **a**	nd **a**	nd **a**	nd **a**
Pt-3-*p*-coumglc	623	477, 315	1.97 ± 0.06	0.76 ± 0.03 **a**	0.66 ± 0.01 **a**	0.56 ± 0.03 **b**	0.28 ± 0.05 **a**
Mv-3-*p*-coumglc-8-ethyl-(epi)cat	953	801, 663, 645, 355	nd	1.38 ± 0.07 **a**	1.39 ± 0.03 **a**	1.00 ± 0.01 **b**	0.34 **a**
Pn-3-*p*-coumglc	607	299	1.28 ± 0.20	11.37 ± 0.21 **b**	8.93 ± 0.02 **a**	2.75 ± 0.07 **b**	nd **a**
Mv-3-*p*-coumglc	637	491, 329	29.76 ± 0.66				
Mv-3-glc-4-vinyl(epi)cat	803	641	nd	0.95 ± 0.04 **a**	1.07 ± 0.02 **a**	1.07 ± 0.01 **b**	0.52 ± 0.01 **a**
Mv-3-*p*-coumglc-4-vinyl(epi)cat	949	641	nd	0.30 ± 0.04 **a**	0.35 ± 0.04 **a**	0.56 ± 0.01 **b**	0.18 ± 0.01 **a**
Mv-3-glc-4-vinylphenol/Pn-3-glc-vinylguaiacol	607	445	nd	0.60 ± 0.01 **a**	0.61 ± 0.03 **a**	0.96 **b**	0.28 ± 0.02 **a**
Σ Anthocyanins			**282.98 ± 5.25**	**152.29 ± 1.00 b**	**129.40 ± 1.79 a**	**58.21 ± 0.21 b**	**9.56 ± 0.20 a**

Table 17 – Phenolic profile of Aglianico wine at racking, immediately after the treatment with oak chips and at 12 months of aging.
nd: not detected; *: significant difference; ns: no significant difference; glc: glucoside, glcr: glucuronide, rha: rhamnoside, galac: galactoside, Dp: delphinidin, Cy: cyanidin, Pt: petunidin, Pn: peonidin, Mv: malvidin, acetylglc: acetylglucoside, *p*-coumglc: *p*-coumaroylglucoside, caffeoylglc: caffeoylglucoside, cat: catechin.
In row, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by t Student test.

Sample	MP	SPP	LPP	LPP/SPP	$I_{gelatin}$	I_{HCl}	I_{PVPP}
				At racking			
	1.10 ± 0.03	0.35 ± 0.01	0.32 ± 0.04	0.90 ± 0.13	68.8 ± 2.2	17.4 ± 1.4	36.3 ± 4.9
				After the treatment with oak chips			
TEST	0.91 ± 0.07 **a**	0.43 ± 0.01 **a**	0.51 ± 0.07 **a**	1.20 ± 0.14 **a**	25.5 ± 4.3 **a**	26.7 ± 3.5 **a**	57.6 ± 0.5 **a**
CHIPS	1.00 ± 0.03 **a**	0.44 ± 0.01 **a**	0.47 ± 0.02 **a**	1.07 ± 0.08 **a**	37.5 ± 4.2 **b**	40.9 ± 2.0 **b**	63.4 ± 3.0 **b**
				After 12 months			
TEST	0.64 ± 0.07 **b**	0.65 ± 0.07 **b**	0.68 ± 0.12 **a**	1.19 ± 0.20 **a**	40.4 ± 1.1 **a**	21.0 ± 2.0 **a**	69.3 ± 2.3 **a**
CHIPS	0.28 ± 0.01 **a**	0.36 ± 0.04 **a**	0.71 ± 0.06 **a**	1.76 ± 0.09 **a**	57.9 ± 1.5 **b**	40.2 ± 4.8 **b**	75.7 ± 2.1 **b**

Table 18 – Monomeric and polymeric pigments and structure indices of Aglianico wine at racking, immediately after the treatment with oak chips and at 12 months of aging.
MP: absorbance at 520 nm due to monomeric pigments; SPP: absorbance 520 nm due to small polymeric pigments; LPP: absorbance 520 nm due to large polymeric pigments; LPP+SPP: absorbance 520 nm due to total polymeric pigments; LPP/SPP: ratio between large and small polymeric pigments; $I_{gelatin}$: gelatin index; I_{EtOH}: ethanol index; I_{HCl}: hydrochloric acid index; I_{PVPP}: PVPP index.
In column, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by t Student test.

Sample	CI	T	dA(%)	% yellow	% red	% blue	dAl%	dAT%	dTAT%
				At racking					
	11.433 ± 0.221	0.606 ± 0.004	57.2 ± 0.3	32.6 ± 0.1	53.9 ± 0.2	13.5 ± 0.1	5.5	87.6 ± 4.1	9.3 ± 0.7
				After the treatment with oak chips					
TEST	14.938 ± 0.128 **a**	0.643 ± 0.003 **a**	54.5 ± 0.2 **a**	33.7 ± 0.1 **a**	52.4 ± 0.1 **a**	13.9 **a**	2.6 **a**	54.5 ± 4.2 **a**	43.1 ± 4.2 **a**
CHIPS	15.939 ± 0.539 **a**	0.638 ± 0.003 **a**	54.4 ± 0.8 **a**	33.3 ± 0.4 **a**	52.3 ± 0.4 **a**	14.4 ± 0.8 **a**	2.4 **a**	60.1 ± 0.3 **a**	37.5 ± 0.3 **a**
				After 12 months					
TEST	15.506 ± 0.003 **b**	0.712 ± 0.001 **a**	50.6 ± 0.1 **b**	35.8 **b**	50.3 **b**	13.9 **a**	1.2 ± 0.2 **a**	23.7 ± 3.2 **b**	75.2 ± 3.0 **a**
CHIPS	10.716 **a**	0.742 **b**	45.9 **a**	35.7 **a**	48.0 **a**	16.3 **b**	0.7 ± 0.1 **a**	7.7 ± 1.2 **a**	91.6 ± 1.2 **b**

Table 19 – Colour parameters of Aglianico wine at racking, immediately after the treatment with oak chips and at 12 months of aging.
CI: colour intensity; T: tonality; dA(%): percentage of red color due to flavilium cation of free and combined anthocyanins; % yellow: percentage of yellow component; % red: percentage of red component; % blue: percentage of blue component; dAl%: percentage of absorbance at 520 nm due to monomeric anthocyanins; dAT%: percentage of absorbance at 520 nm due to polymeric pigments decolorized with SO_2; dTAT%: percentage of absorbance at 520 nm due to polymeric pigments not decolorized.
In column, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by t Student test.

Compounds	t_r (min)	Mean concentration (AU x 10^5) ± SD (n=2)			Odour description
		T_0	T_5	C_5	
Acids					
Acetic acid	23.8	100 ± 31	103.8 ± 1.9	202 ± 33	Vinegar
Hexanoic acid	35.2	16.7 ± 2.8	14.12 ± 1.2	14.98 ± 0.34	Cheese, fatty, sour
Hexanoic acid, 2-ethyl	37.8	0	10.15 ± 0.41	0	n.d.
Octanoic Acid	40.6	96.4 ± 1.1	88.7 ±8.5	99.4 ± 1.8	Fatty acid, dry, dairy
Nonanoic acid	43.2	7.7 ± 2.2	7.6 ± 2.0	9.0 ± 1.9	Cheese, waxy
n-Decanoic acid	46.5	20 ± 19	35 ± 10	97 ± 19	Fatty acid, dry, woody
Dodecanoic acid	56.0	10.6 ± 6.7	6.1 ± 2.4	24.9 ± 6.2	Fatty
Total		252	266	448	
Percentage (%)		2.74	3.26	8.42	
Alcohols					
1-Propanol	9.8	6.7 ± 3.8	6.23 ± 0.15	8.7 ± 1.5	Alcohol, ripe fruit
1-Propanol, 2-methyl-	11.5	124 ± 23	120.1 ± 7.1	95.1 ± 6.4	Alcohol, solvent
1-Butanol	13.1	4.31 ± 0.34	3.950 ± 0.040	6.13 ± 0.84	Medicinal, phenolic
1-Butanol, 3-methyl-	15.2	2573 ± 66	2641 ± 130	2107 ± 51	Fusel, alcohol, sweet, fruity
1 Hexanol	20.0	71.2±2.2	56.8 ± 2.7	49.5 ± 1.3	Herbaceous
2-Heptanol	23.4	6.650 ± 0.050	8.110 ± 0.080	5.69 ± 0.16	Oily; earthy; banana; butter; herbaceous; meaty
1-Hexanol, 2-ethyl	24.5	7.08 ± 0.12	46.56 ± 0.90	10.120 ± 0.010	Oily; rose; sweet
2,3-Butanediol	26.3	28 ± 21	23 ± 13	53.09 ± 0.69	Fruity
1-Octanol	26.6	13.86 ± 0.46	14.49 ± 0.43	12.20 ± 0.34	Orange, floral
2,3-Butanediol	27.4	10.1 ± 5.6	10.4 ± 4.9	15.92 ± 0.10	Fruity
1-Nonanol	29.6	14.1 ± 2.6	6.8 ± 7.5	10.00 ± 0.47	citrus; rose
2-Furanmethanol	30.1	1.630 ± 0.090	1.38 ± 0.29	1.100 ± 0.040	n.d.
2-Octen-1-ol, 3,7-dimethyl-	32.7	21.1 ± 2.9	10.47 ± 0.40	9.9 ± 1.3	n.d
Benzyl Alcohol	36.1	1.91 ± 2.9	2.49 ± 0.60	2.990 ± 0.040	Flowery, sweet
Phenylethyl Alcohol	37.0	803 ± 63	718 ± 17	689 ± 43	Flowery, rose, honey
Glycerin	48.5	0	332 ± 398	0	
Farnesol	49.3	8.4 ± 4.2	0	11.3 ± 3.9	Anise; apricot; balsam; clove; grapefruit; oily
Total		3695	3823	3088	
Percentage (%)		40.12	46.86	58.04	
Acetic Esters					
Isoamyl acetate	12.3	461.2 ± 7.5	351.1 ± 7.5	214 ± 59	Banana
Acetic acid, hexyl ester	17.3	14.0.0 ± 0.060	8.390 ± 0.070	3.7 ± 1.4	
2-Phenethyl acetate	34.4	52.7 ± 43	34.77 ± 0.66	27.74 ± 0.77	Floral
Total		528	394	246	
Percentage (%)		5.73	4.83	4.62	
Ethyl Esters					
Ethyl butyrate	9.8	29.4 ± 1.6	32.9 ± 2.7	22.2 ± 5.7	Banana, pineapple, sweet, ethereal
Ethyl 2-methylbutyrate	10.2	2.710 ± 0.040	20.0 ± 2.5	8.06 ± 2.7	fruity; green
Ethyl 3-methylbutyrate	10.7	5.8 ± 1.3	22.0 ± 1.1	13.1 ± 3.8	apple
Ethyl hexanoate	16.0	493.1 ± 9.0	361 ± 16	206 ± 63	apple; banana; wine-like
Ethyl heptanoate	19.4	12.45 ± 0.23	21 ± 15	4.9 ± 1.6	Fruity
Ethyl lactate	19.9	14.88 ± 0.84	47.55 ± 0.67	28 ± 32	Lactic
Ethyl octanoate	22.8	2301 ± 57	1430 ± 110	338 ± 89	Banana, floral, pear, pineapple, wine-like

Ethyl decanoate	29.0	1226 ± 94	389 ± 27	30.3 ± 2.2	Grap, oily, wine-like
Diethyl succinate	30.3	228 ± 28	708 ± 23	549 ± 36	Spicy
Ethyl 9-decenoate	30.6	5.47 ± 0.16	8.05 ± 0.49	2.47 ± 0.71	
Diethyl glutarate	33.2	0	1.75 ± 0.31	1.09 ± 0.12	Apple, apricot, chocolate, cranberry
Ethyl dodecanoate	34.8	43 ± 19	27 ± 17	1.950 ± 0.050	green; fruity; floral
Ethyl myristate	40.0	46.9 ± 16.9	119.5 ± 1.6	15.2 ± 1.0	Waxy, soapy
Ethyl pentadecanoate	42.5	4.2 ± 0.6	8.3 ± 4.8	3.0 ± 1.7	
Ethyl 4-ethoxybenzoate	43.4	10.42 ± 0.20	0	0	
Ethyl hexadecanoate	45.4	87.0 ± 3.9	121.9 ± 1.3	14.8 ± 2.0	waxy
Ethyl hydrogen succinate	51.0	93 ± 91	92 ± 66	61.4 ± 2.4	Chocolate
Ethyl stearate	53.8	8.19 ± 0.56	14.7 ± 3.3	3.98 ± 0.34	Waxy
Total		4613	3428	1330	
Percentage (%)		50.08	42.01	24.99	
Other Esters					
Isoamyl hexanoate	27.7	9.43 ± 0.47	2.400 ±0.060	0	Apple; green; pineapple; sweet
Methyl caprylate	29.6	4.02 ± 0.27	1.520 ± 0.050	0	Green; citrus; fruity
Isoamyl caprylate	35.3	31.9 ± 6.5	17.0 ± 7.2	7.3 ± 1.3	Fruity
Isoamyl n-decanoate	39.6	7.2 ± 8.1	5.5 ± 2.0	0.960 ± 0.070	
Isopropyl myristate	23.5	8.8 ± 1.0	11.3 ± 2.6	12.01 ± 0.36	Cheese; cherry; cinnamon; wine-like; waxy
Total		61	38	20	
Percentage (%)		0.67	0.46	0.38	
Carbonyl Compounds					
Nonanal	21.5	4.98 ± 0.21	2.340 ± 0.040	2.71 ± 0.51	Apple, coconut, grapefruit, lemon
Isoamyl acetamide	33.5	1.59 ± 0.11	2.4 ± 1.5	1.90 ± 0.50	
Total		7	5	5	
Percentage (%)		0.07	0.06	0.09	
Hydrocarbons					
2,6-Dimethyl 2,6-octadiene	29.7	1.75 ± 0.19	0	0	
Cyclooctane	32.6	10.54 ± 0.69	9.42 ± 0.06	9.23 ± 0.42	
Total		12	9	9	
Percentage (%)		0.13	0.12	0.17	
Terpenes					
Geraniol	35.0	3.74 ± 0.42	1.35 ± 0.37	1.23 ± 0.27	Fruity, rose, sweet
D-Nerolidol	39.8	11.11 ± 0.45	5.4 ± 1.0	20.78 ± 0.95	Apple, green, woody, citrus, rose
Total		15	7	22	
Percentage (%)		0.16	0.08	0.41	
Ethers					
Octyl ether	32.1	3.045 ± 0.37	4.1 ± 1.9	3.84 ± 0.12	
Total		3	4	4	
Percentage (%)		0.04	0.05	0.07	
Aromatics					
Benzene, 1,3-bis[1,1-dimethylethyl]-	22.5	n.d.	20.9 ± 1.5	13.7 ± 9.4	
xime-, methoxy-phenyl-	32.4	5.800 ± 0.080	7.61 ±0.36	12.6 ± 2.4	
4-Ethylphenol	43.8	0	64.550 ± 0.090	1.53 ± 0.23	Alcohol; medicinal
2,4-di-t-Butylphenol	47.8	10.82 ± 0.61	78.7 ± 8.7	109 ± 15	Phenolic

Total		17	172	137	
Percentage (%)		0.18	2.11	2.57	
Sulphur Compounds					
Methionol	31.6	4.12 ± 0.59	4.45 ± 0.52	4.08 ± 0.16	Earthy; alliaceous (onion, garlic); vegetable; meaty
Total		4	5	4	
Percentage (%)		0.04	0.05	0.08	
Lactones					
Whiskey lactone	38.2	3.01 ± 0.76	5.2 ± 1.5	8.12 ± 0.16	Woody
Total		3	5	8	
Percentage (%)		0.03	0.06	0.15	

Table 20 - Volatile compounds (AU x10^5) isolated from Aglianico wine at racking (T_0) and after 12 months of aging wine in untreated wines (T_5) and wines treated with oak chips (C_5) (mean ± standard deviation). Odour descriptors have been obtained from Capone *et al*. (2013) and SAFC "Flavors and Fragrances. European Ed. Catalogue 2007–2008". Percentages are referred to the volatile compounds respect to each chemical group in different packaging.

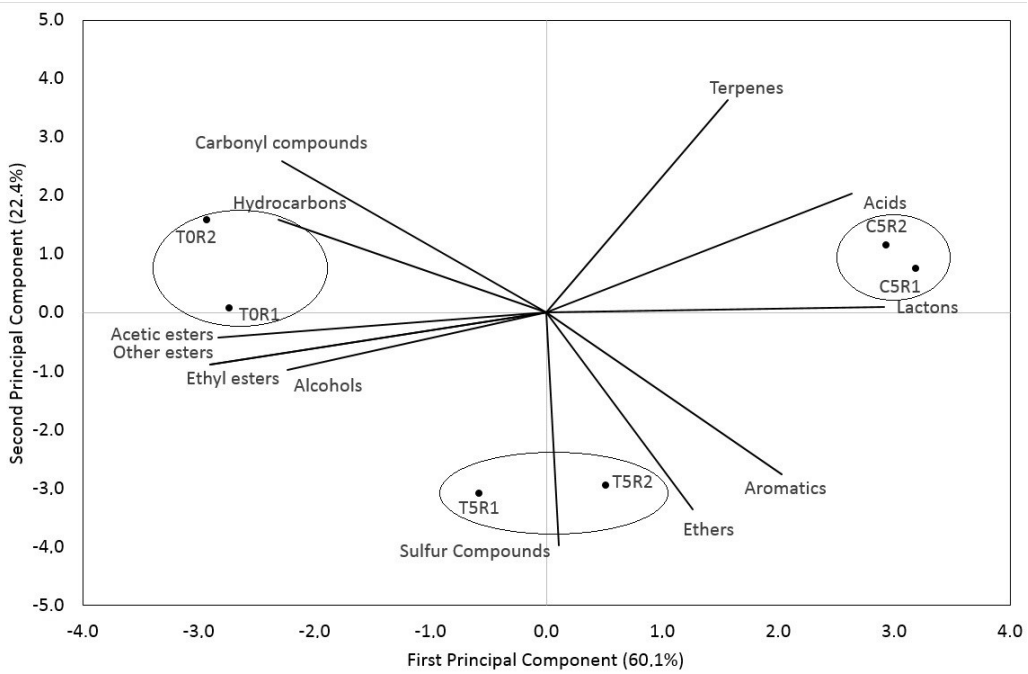
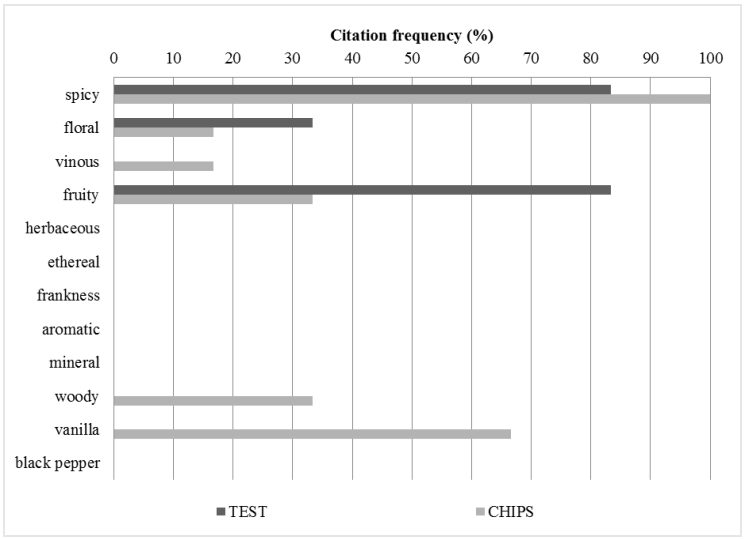


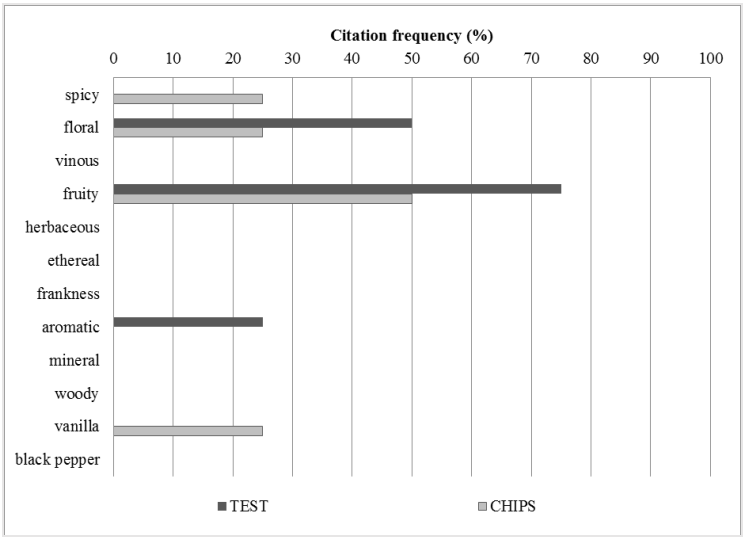
Figure 8 - Principal component analysis of volatile compounds among the wine at racking (T_0) and after 12 months of aging wine in untreated wines (T_5) and wines treated with oak chips (C_5).

Sensory descriptors	*After the treatment with oak chips*		*After 12 months*	
	TEST	CHIPS	TEST	CHIPS
Visual descriptors				
Clearness	2.0 **a**	2.0 **a**	2.0 **a**	2.0 **a**
Texture	2.7 ± 0.4 **a**	2.8 ± 0.4 **a**	2.5 ± 0.4 **a**	2.6 ± 0.5 **a**
Olfactory descriptors				
Olfactory intensity	2.3 ± 0.6 **a**	2.8 ± 0.3 **a**	2.0 **a**	1.9 ± 0.3 **a**
Olfactory complexity	2.1 ± 0.4 **a**	2.3 ± 0.5 **a**	2.1 ± 0.3 **a**	2.0 **a**
Olfactory quality	2.4 ± 0.4 **a**	2.4 ± 0.5 **a**	2.0 **a**	2.0 ± 0.4 **a**
Gustatory-olfactory descriptors				
Sugars	0.0 **a**	0.0 **a**	0.0 **a**	0.0 **a**
Alcohols	2.9 ± 0.2 **a**	2.7 ± 0.4 **a**	2.5 ± 0.4 **a**	2.6 ± 0.5 **a**
Polyols	2.3 ± 0.3 **a**	2.0 **a**	1.8 ± 0.5 **a**	2.1 ± 0.3 **a**
Acids	2.0 ± 0.5 **a**	1.9 ± 0.6 **a**	2.1 ± 0.9 **a**	2.1 ± 0.3 **a**
Tannins	3.3 ± 0.4 **a**	3.8 ± 0.4 **b**	2.9 ± 0.6 **a**	2.5 ± 0.6 **a**
Minerals	2.1 ± 0.4 **a**	1.9 ± 0.2 **a**	1.6 ± 0.5 **a**	1.9 ± 0.3 **a**
Structure	2.7 ± 0.3 **a**	2.8 ± 0.3 **a**	2.8 ± 0.5 **a**	2.8 ± 0.5 **a**
Balance	1.9 ± 0.2 **a**	1.8 ± 0.3 **a**	1.5 ± 1.1 **a**	2.3 ± 0.5 **a**
Gustatory-olfactory intensity	2.3 ± 0.4 **a**	2.7 ± 0.3 **a**	2.6 ± 0.5 **a**	2.3 ± 0.3 **a**
Gustatory-olfactory persistence	2.6 ± 0.4 **a**	2.8 ± 0.3 **a**	2.8 ± 0.5 **a**	2.8 ± 0.3 **a**
Gustatory-olfactory quality	2.6 ± 0.5 **a**	2.3 ± 0.5 **a**	1.8 ± 1.2 **a**	2.5 ± 0.4 **a**
Final considerations				
Evolutionary state	2.0 ± 0.5 **a**	2.7 ± 0.8 **a**	3.5 ± 0.6 **a**	3.0 **a**
Harmony	2.2 ± 0.3 **b**	1.4 ± 0.5 **a**	1.5 ± 1.2 **a**	2.1 ± 0.3 **a**

Table 21 – Sensory characteristics of Aglianico wine at the time corresponding to the end of the treatment with oak chips and after 12 months of aging. In row, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by t Student test.

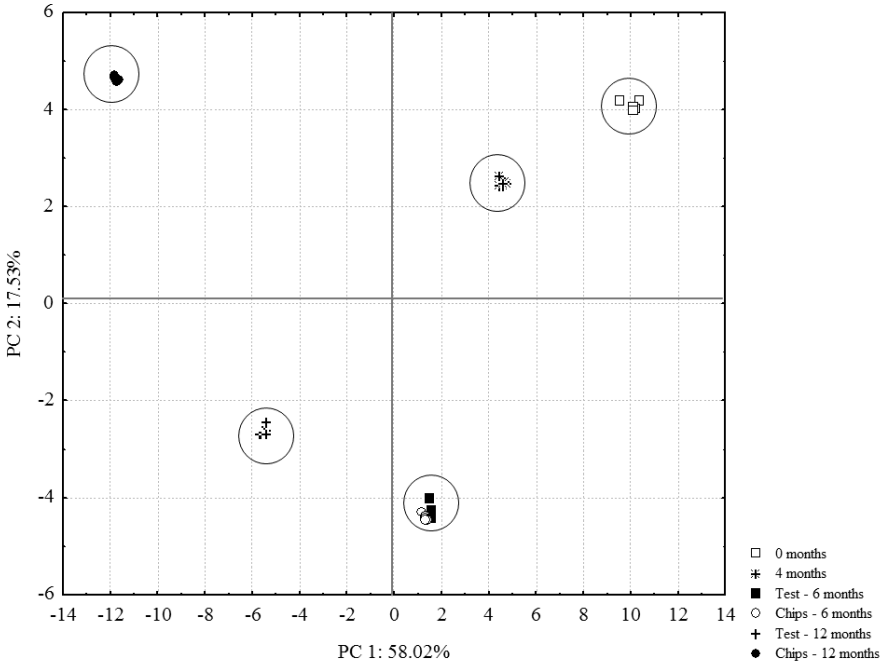


a)

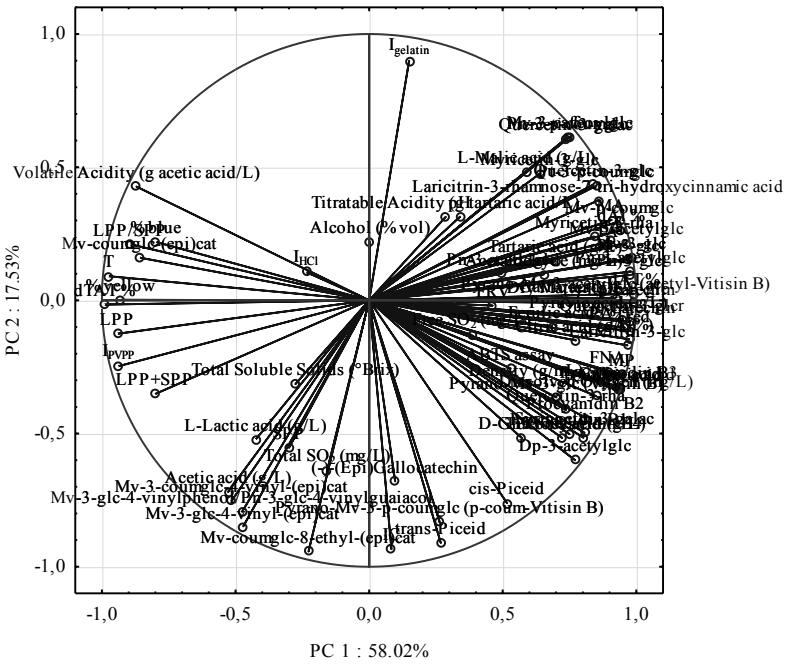


b)

Figure 9 – Citation frequency (%) of sensory attributes detected in control and treated wines at the time corresponding to end of the treatment with oak chips (*a*) and after 12 months of aging (*b*).



a)



b)

Figure 10 - PCA scatter plot for projection on the factor plane of: **a)** Aglianico wine samples at different stage of aging process and **b)** analytical results.

7.2.2 Montepulciano wines

The physicochemical characteristics of Montepulciano wines are reported in Table 22. At racking, it had: high alcohol content and dry matter, although they were lower than those measured in Aglianico wines; low values of volatile acidity, acetaldehyde, free and total SO_2; good titratable acidity; total soluble solids that indicates the correct progress of fermentation; dissolved oxygen contents that were double that those of the Aglianico wines. At the end of the treatment with wood chips, the control wine (TEST) exhibited lower volatile acidity, and higher free and total SO_2, acethaldehyde, and dissolved oxygen than the treated wines, while any significant difference was found for the other parameters of oenological interest. This behaviour could be due to a first oxygen scavenging effect exercised by ellagitannins arising from chips. Already 1 month after treatment with oak chips (data not shown), acetaldehyde was higher in treated wines than in control ones. This trend could be depend by a subsequent production of acetaldehyde by the action of hydrogen peroxide generated from the oxidation of ellagitannins, in agreement with the study of Ribéreau-Gayon *et al*. (1983). After 12 months of aging, control wines exhibited lower volatile and titratable acidity, and higher pH, acetaldehyde, and dissolved oxygen than the treated wines. During aging, the volatile acidity increase up to the time corresponding to the treatment with chips and then stabilized, while the acetaldehyde content always decreased, and the dissolved oxygen gradually increased.

The concentrations of various organic acids are shown in Table 23. At racking, they were in a decreasing order: tartaric > malic > citric, lactic, and acetic > D-gluconic and pyruvic. At the time corresponding to end of the treatment with oak chips, significant differences were found only for tartaric acid (whose concentration was higher in the control wine), and for lactic and acetic acids (whose concentrations were lower in the control wine). Compared to the data collected at racking, the concentrations of malic and lactic acids indicate that malolactic fermentation occurred and those concerning acetic acid are index of oxidation reactions. After 12 months of aging, the wines treated with woody fragments still had higher concentrations of acetic and L-lactic acids, and a higher concentration of tartaric acid with respect to the control (for both the wines, precipitation of tartaric acid occurred). The citric acid content decreased during the aging time as a result of its metabolism by lactic acid bacteria (Ribéreau-Gayon *et al*., 2000), as well as the acetic acid content.

The phenolic composition of Montepulciano wine is shown in Table 24. At racking, the concentration of all the phenolic classes was lower in Montepulciano than in Aglianico wines. At the time corresponding to the end of the treatment with oak chips, decreases of total anthocyanins, anthocyanins sensitive to SO_2, monomeric anthocyanins, and total flavonoids were observed with respect to racking, and the treated wines showed the highest concentrations of total anthocyanins (+15.5% respect to the control wines), anthocyanins sensitive to SO_2 (+44%), monomeric anthocyanins (+49.6%), flavonoids (+13%), flavonoids different from anthocyanins (+11.8%), and flavans reactive with vanillin (+12.5%). No significant differences were found for proanthocyanidins, total phenolic contents, and antioxidant activity. The addition of the chips and, therefore, the extraction and diffusion of the related phenolic compounds (ellagitannins) into the wine determined an initial protection of the wine phenolic compounds against oxygen. This behaviour was in agreement with the findings of Vivas and Glories (1996), Del Alamo Sanza and Nevares Domínguez (2006), and Del Alamo Sanza *et al.* (2004), who stated the antioxidant capacity of phenolic compounds extracted from oak. The protection effect was more evident in Montepulciano than in Aglianico wines, in agreement with Versari *et al*. (2013), who observed that the antioxidant activity of ellagic tannins extracted from wood is greater in wines with lower phenolic content. The woody tannins have a greater affinity for oxygen than the other typical grape phenolic compounds and their oxidation rate to quinones is even higher than that of iron from ferrous (Fe^{2+}) to ferric (Fe^{3+}). Therefore, they can constitute the true protection from oxidation for anthocyanins and other phenolic compounds (Vivas and Glories, 1996). After this protection effect against oxidation, a subsequent oxidizing effect of the hydrogen peroxide formed by the ellagitannins was observed. In fact, wood tannins have numerous hydroxyl functions in the *ortho* position on the peripheries of their molecular structures. For this reason, in wine, they are involved in oxidation reactions, acting as a consumer of oxygen, and finally cause the production of a massive amount of peroxides and acetaldehyde (Vivas and Glories, 1996). Therefore, the ellagitannins may participate in colour changes during wine aging, by helping to improve wine colour stability and protecting it against oxidation (Vivas and Glories, 1996; Del Alamo Sanza and Nevares Domínguez, 2006; Del Alamo Sanza *et al*., 2004). After 12 months of aging, the untreated wines exhibited higher concentrations of all the phenolic classes (except for flavonoids different from anthocyanins) than the wines treated with chips, as a consequence of condensation reactions between anthocyanins and tannins, the typical reactions of aging that take place more quickly in wine treated with oak chips. The

already described double effect (first protection against oxidation, then oxidizing effect) also emerged from the data concerning the phenolic profiles of wines, especially for flavan-3-ols and anthocyanins (Table 26). In fact, at the time corresponding to the end of the treatment with oak chips, the control wines had lower flavan-3-ol and anthocyanin concentrations than the treated wines, while no significant dfferences were highlighted for flavonols, stilbens, and phenolic acids. After 12 months of aging, apart from a decrease of concentrations of all the phenolic classes, the control wines exhibited higher concentrations of phenolic acids, flavonols, and anthocyanins. At racking, the most representative compounds, for each phenolic classes were: gallic and caffeic acids (phenolic acids), myricetin-3-rhamnoside and syringetin-3-galac (flavonols), procyanidin B3, catechin, and procyanidin B1 (flavan-3-ols), malvidin-3-glucoside (anthocyanins). Table 26 shows the distribution of anthocyanins between monomeric and polymeric forms. With the time, the first decreased while the second increased and, after 12 months, the polymeric forms were higher in the wines treated with chips. In Montepulciano wine, immediately after the treatment with oak chips, there was an increase of the gelatin index, as already observed in Aglianico wine, and a decrease of the HCl index (Table 26). This decrease could be due to the precipitation of condensed tannins. In fact, Collins (2003) reported that as the number of units within the polymers increases, their solubility decreases, and the polymers can precipitate. No significant differences were observed in PVPP index among control and treated wines, thus indicating an initial lower anthocyanins polymerization. After 12 months of aging, the PVPP index increased in both wines, although in a greater extent in treated wines.

The colour parameters are listed in Table 27. At the end of the treatment with oak chips, lower colour intensity and % of red component, and higher value of tonality, % of yellow component, and percentage of absorbance due to monomeric anthocyanins were found. Subsequently, the production of acetaldehyde by means of ethanol oxidation the acetaldehyde-mediated polymerization occurred, with the production of compounds such as pyranoanthocyanins (B-type vitisins) and flavanyl-pyranoanthocyanins, which determined a deepening of the crimson colour. After 12 months of aging, the treated wines showed higher colour intensity and percentage of absorbance due to polymeric pigments not decolorized and lower values of absorbance by anthocyanin monomers and polymeric pigments decolorized with SO_2.

Changes in the volatile components of Montepulciano wine aged with or without oak chips were investigated during one year of aging. The 73 volatile compounds identified by means of HS-SPME-GC-MS were grouped into the following 10 different classes (Table 28 and Figure 11)): acids (6 compounds), alcohols (17), acetic esters (4), ethyl esters (22), other esters (8), carbonyl compounds (3), terpenes (4), aromatics (3), hydrocarbons (4), and lactones (1). The more representative compounds of each class were: octanoic acid (acids); 1-butanol,3-methyl alcohol and phenylethyl alcohol (alcohols); isoamyl acetate (acetic esters); ethyl octanoate (ethyl esters); isoamyl n-decanoate (other esters); ß-damascenone (carbonyl compounds); cyclooctane (hydrocarbons) and D-nerolidol (terpenes); 2,4-t-butylphenol and whiskey lactone (lactones).

In the aged wines, dramatic decreases in esters were measured while acids increased. This behaviour was more evident in wine treated with chips; in fact, Vivas *et al*. (1995) documented an increase in volatile acidity in wine with chips due to two reasons: acidity firstly originate from oak as a result of the toasting process, and, in addition, it can be produced by the metabolism of acetic acid bacteria in wine. The release of wood tannins from chips changes the redox potential in the wine. For this reason, significant quantitative differences in the n-decanoic acid were found between control and treated wines. Finally, the wine treated with oak chips was characterized by a greater presence of whiskey lactone directly released from chips. According to the Principal Component Analysis (Figure 11), the first two components explained about 90% of the total variance. The wine at racking, and after 12 months of aging (control and treated with oak chips) were clearly distinguishable on the first component. At racking, the wines exhibited higher esters content, while treated wines after 12 months of aging were characterized by the amounts of carbonyl compounds and control wines by the concentrations of acids, lactone, aromatics, and ethers.

Wines were sensory analyzed after the treatment with oak chips and after 12 months of aging. At the time corresponding to the end of the treatments, the treated wines showed higher olfactory and gustatory quality than the control, and were judged as more acid, and tannic (due to the extraction of ellagitannins (Quinn and Singleton, 1985)) but also they were more appreciated by judges for their higher balance and harmony than the control (Table 29). As already noted for Aglianico wine, at the time corresponding to the end of the treatment with oak chips, the treated Montepulciano wines were judged as more spicy and woody, with vanilla and black pepper notes (Figure 12*a*) in agreement with the

literature (Guchu *et al*., 2006; Gómez García-Carpintero *et al*., 2011). These results were in contrast with those found in Aglianico wine and in agreement with those of Pérez-Coello *et al.* (2000). After 12 months of storage, the main significant differences concerned the higher gustatory-olfactory quality, spicy flavour, and vanilla note of the treated wines (Table 29, Figure 12*b*). During aging, the spicy note reduced in treated wines and disappeared in control wines while the floral, fruity and vinous notes increased in the control wines.

The results of the application of the Principal Component Analysis (PCA) to all the standardized data (with the only exception of volatile compounds, which were evaluated separately) are shown in Figure 13. The first two PCs explained almost 70% of the total variability of Montepulciano wines and describe the trend of the control and treated wines. Wines analysed after 6 months of aging and wine (control and treated) at the time corresponding to the end of the treatment are place close to each other in the factor plane, as well as the control and treted wines after 12 months of aging.

Sample	Alcohol (% vol)	Density (g/mL)	Dry Matter (g/L)	Volatile Acidity (g acetic acid/L)	Titratable Acidity (g tartaric acid/L)	pH	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Total Soluble Solids (°Brix)	Acetaldehyde (mg/L)	Dissolved O_2 (mg/L)
					At racking						
	13.61 ± 0.17	0.995	31.5 ± 0.5	0.30 ± 0.04	5.76	3.48 ± 0.01	11.2	17.6	7.8 ± 0.1	59.69 ± 8.47	4.61 ± 0.35
					After the treatment with oak chips						
TEST	13.36 ± 0.01 **a**	0.994 **a**	27.7 ± 0.1 **a**	0.48 ± 0.02 **a**	5.04 ± 0.04 **a**	3.51 ± 0.01 **a**	42.9 ± 0.9 **b**	51.2 **b**	7.9 ± 0.3 **a**	34.00 **b**	5.33 ± 0.02 **b**
CHIPS	13.21 ± 0.21 **a**	0.994 **a**	28.8 ± 0.5 **a**	0.87 ± 0.03 **b**	5.06 ± 0.02 **a**	3.59 ± 0.02 **a**	27.2 ± 2.3 **a**	38.4 ± 2.3 **a**	7.9 ± 0.3 **a**	8.00 **a**	5.27 ± 0.01 **a**
					After 12 months						
TEST	13.28 ± 0.01 **a**	0.993 ± 0.001 **a**	27.7 ± 1.6 **a**	0.44 ± 0.07 **a**	4.29 **a**	3.64 **b**	41.6 ± 6.8 **a**	47.2 ± 5.7 **a**	7.8 **a**	4.50 ± 0.71 **b**	6.95 ± 0.02 **b**
CHIPS	13.11 ± 0.11 **a**	0.994 **a**	28.2 ± 0.1 **a**	0.85 ± 0.11 **b**	5.69 ± 0.02 **b**	3.61 ± 0.01 **a**	25.6 **a**	31.6 ± 1.7 **a**	7.8 ± 0.1 **a**	2.00 **a**	6.33 ± 0.01 **a**

Table 22 – Physicochemical parameters of Montepulciano wine at racking, immediately after the treatment with oak chips and at 12 months of aging.
In column, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by t Student test.

Sample	Tartaric acid	L-Malic acid	L-Lactic acid	Acetic acid	Citric acid	Pyruvic acid	D-Gluconic acid
				At racking			
	3.81	1.65 ± 0.01	0.21	0.19 ± 0.02	0.27 ± 0.01	0.03	0.07
				After the treatment with oak chips			
TEST	2.93 ± 0.01 **b**	0.03 **a**	0.31 **a**	0.65 ± 0.02 **a**	0.03 **a**	0.04 **a**	0.03 **b**
CHIPS	2.59 ± 0.01 **a**	0.05 ± 0.01 **b**	0.47 ± 0.01 **b**	0.84 ± 0.04 **b**	0.03 ± 0.01 **a**	0.05 ± 0.01 **a**	0.02 **a**
				After 12 months			
TEST	1.89 ± 0.01 **a**	0.01 **b**	0.39 ± 0.01 **a**	0.43 ± 0.01 **a**	nd **a**	0.01 **b**	0.01 **b**
CHIPS	2.04 ± 0.01 **b**	nd **a**	0.47 **b**	0.74 ± 0.02 **b**	nd **a**	0.01 **a**	nd **a**

Table 23 – Organic acids content of Montepulciano wine at racking, immediately after the treatment with oak chips and at 12 months of aging.
nd: not detected.
In column, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by t Student test.

Sample	TA	SA	MA	TF	FNA	FRV	PA	TPC	ABTS assay
					At racking				
	551 ± 11	733 ± 15	397 ± 8	2318 ± 64	1515 ± 47	1154 ± 70	2543 ± 363	2606 ± 78	14.27 ± 1.12
					After the treatment with oak chips				
TEST	238 ± 19 **a**	288 ± 7 **a**	133 ± 2 **a**	1803 ± 0 **a**	1456 ± 28 **a**	921 ± 87 **a**	2664 ± 105 **a**	2603 ± 103 **a**	15.87 ± 1.16 **a**
CHIPS	275 ± 7 **b**	415 ± 16 **b**	199 ± 2 **b**	2039 ± 37 **b**	1628 ± 27 **b**	1036 ± 36 **b**	2482 ± 210 **a**	2711 ± 123 **a**	15.88 ± 0.88 **a**
					After 12 months				
TEST	329 ± 19 **b**	358 ± 23 **b**	133 ± 4 **b**	1781 ± 33 **a**	1302 ± 6 **a**	1268 ± 100 **b**	2646 **b**	2673 ± 20 **b**	15.75 ± 2.48 **a**
CHIPS	272 ± 7 **a**	239 ± 19 **a**	70 ± 4 **a**	1743 ± 87 **a**	1346 ± 82 **a**	1055 ± 38 **a**	2138 ± 122 **a**	2630 ± 27 **a**	14.15 ± 1.44 **a**

Table 24 – Phenolic composition and antioxidant activity of Montepulciano wine at racking, immediately after the treatment with oak chips and at 12 months of aging.
TA: total anthocyanins (as mg malvidin-3-glucoside/L); SA: anthocyanins sensitive to SO_2 (as mg malvidin-3-glucoside/L); MA: monomeric anthocyanins (as malvidin-3-glucoside mg/L); TF: total flavonoids (as mg (+)-catechin/L); FNA: flavonoids different from anthocyanins (as mg (+)-catechin/L); FRV: flavans reactive with vanillin (as mg (+)-catechin/L); PA: proanthocyanidins (as mg cyanidin chloride/L); TPC: total phenolic compounds (as mg gallic acid/L); ABTS assay: antioxidant activity by ABTS assay (as mmol Trolox eq/L).
In column, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by t Student test.

Phenolic compounds	MS (m/z)	MS–MS fragments (m/z)	*At racking*	*After the treatment with oak chips*		*After 12 months*	
				TEST	CHIPS	TEST	CHIPS
Phenolic acids (mg GAE/L; mg CAE/L)	$[M-H]^-$						
Gallic acid	169	125	46.67 ± 1.12	48.85 ± 0.19 **a**	50.29 ± 1.59 **a**	49.69 ± 0.15 **b**	22.93 ± 0.84 **a**
Caftaric acid	311	179, 149	22.49 ± 0.57	26.22 ± 2.71 **a**	24.77 ± 0.77 **a**	19.53 ± 0.71 **b**	16.70 ± 0.03 **a**
Caffeic acid	179	135	39.59 ± 1.76	37.27 ± 2.34 **a**	39.72 ± 1.02 **a**	28.93 ± 0.11 **b**	25.18 ± 0.63 **a**
p-Coumaric acid	163	119	14.02 ± 0.21	12.82 ± 0.14 **a**	12.71 ± 0.21 **a**	11.75 ± 0.01 **b**	9.72 ± 0.23 **a**
Ferulic acid	193	178, 149, 134	11.88 ± 0.19	10.30 ± 0.01 **a**	11.19 ± 0.59 **a**	6.74 ± 0.10 **b**	6.37 ± 0.05 **a**
Σ Phenolic acids			**134.65 ± 2.70**	**135.47 ± 0.69 a**	**138.67 ± 3.01 a**	**116.65 ± 0.85 b**	**80.90 ± 1.72 a**
Stilbens (mg RE/L)	$[M-H]^-$						
cis-Piceid	389	227	0.86 ± 0.12	0.59 **b**	0.50 ± 0.01 **a**	0.48 **a**	0.40 ± 0.04 **a**
trans-Piceid	389	227	0.85 ± 0.16	0.82 ± 0.05 **a**	0.65 ± 0.07 **a**	0.81 ± 0.02 **b**	0.70 ± 0.01 **a**
Σ Stilbens			**1.70 ± 0.05**	**1.41 ± 0.05 a**	**1.15 ± 0.08 a**	**1.29 ± 0.03 b**	**1.11 ± 0.03 a**
Flavonols (mg QE/L)	$[M-H]^-$						
Myricetin-3-glc	479	316/317	0.82 ± 0.10	nd **a**	nd **a**	nd **a**	nd **a**
Myricetin-3-rha	463	317	4.33 ± 0.19	3.83 ± 0.04 **a**	3.79 ± 0.20 **a**	1.92 ± 0.03 **a**	1.80 ± 0.15 **a**
Quercetin-3-glc	463	301	1.52 ± 0.02	0.19 ± 0.02 **a**	0.34 ± 0.05 **a**	nd **a**	nd **a**
Quercetin-3-glcr	477	301	3.04 ± 0.03	0.95 ± 0.14 **a**	1.17 ± 0.06 **a**	0.73 ± 0.05 **a**	0.51 ± 0.09 **a**
Quercetin-3-galac	463	301	nd	nd **a**	nd **a**	nd **a**	nd **a**
Laricitrin-3-glc	493	331	1.26 ± 0.05	0.29 ± 0.01 **b**	nd **a**	nd **a**	nd **a**
Quercetin-3-rha	447	301	2.49 ± 0.47	2.58 ± 0.14 **b**	1.86 ± 0.03 **a**	3.02 ± 0.49 **a**	2.27 ± 0.55 **a**
Syringetin-3-galac	507	344/345	3.92 ± 0.10	4.18 ± 0.06 **a**	4.07 ± 0.04 **a**	4.21 ± 0.20 **b**	3.58 ± 0.03 **a**
Laricitrin-3-rhamnose-7-tri-hydroxy-cinnamic acid	655	509, 501, 475, 347, 329, 314, 303	2.51 ± 0.02	0.47 ± 0.03 **a**	1.45 ± 0.04 **b**	0.44 ± 0.01 **a**	0.44 ± 0.02 **a**
Σ Flavonols			**19.90 ± 0.74**	**12.48 ± 0.37 a**	**12.68 ± 0.32 a**	**10.32 ± 0.20 a**	**8.61 ± 0.83 a**
Flavan-3-ols (mg CE/L)	$[M-H]^-$						
(-)-(Epi)Gallocatechin	305	179, 125	16.93 ± 0.17	16.11 ± 0.32 **a**	21.26 ± 0.29 **b**	12.54 ± 0.69 **a**	12.25 ± 0.26 **a**
Procyanidin B3	577	451, 425, 407, 289	47.12 ± 1.81	30.85 ± 0.09 **a**	40.44 ± 2.68 **b**	22.15 ± 0.40 **b**	17.57 ± 1.13 **a**
(+)-Catechin	289	245, 205, 179	50.54 ± 0.12	47.78 ± 0.41 **a**	54.13 ± 1.53 **b**	16.82 ± 0.13 **a**	15.40 ± 0.96 **a**
Procyanidin B1	577	451, 425, 407, 289	40.32 ± 0.68	30.89 ± 1.84 **a**	35.86 ± 1.24 **a**	28.03 ± 5.58 **a**	39.88 ± 6.91 **a**
Procyanidin B4	577	451, 425, 407, 289	19.23 ± 0.41	17.70 ± 0.12 **a**	19.57 ± 1.01 **a**	16.19 ± 2.55 **a**	14.82 ± 0.32 **a**

(-)-Epicatechin	289	245, 205, 179	30.15 ± 7.26	13.48 ± 0.79 **a**	17.55 ± 0.42 **b**	14.87 ± 2.68 **a**	18.46 ± 0.93 **a**
Procyanidin B2	577	451, 425, 407, 289	24.62 ± 0.39	19.57 ± 0.36 **a**	20.72 ± 0.88 **a**	20.15 ± 0.18 **a**	24.52 ± 4.51 **a**
Σ Flavan-3-ols			**228.92 ± 8.90**	**176.37 ± 3.04 a**	**209.54 ± 2.66 b**	**130.75 ± 10.82 a**	**142.89 ± 8.46 a**
Anthocyanins (mg ME/L)	$[M-2H]^-$						
Dp-3-glc	463	301	8.09 ± 0.01	3.70 ± 0.07 **a**	7.24 ± 0.19 **b**	5.31 ± 0.54 **b**	2.31 ± 0.03 **a**
Cy-3-glc	447	285	0.23 ± 0.02	nd **a**	nd **a**	nd **a**	nd **a**
Pt-3-glc	477	315	15.72 ± 1.68	5.13 ± 0.04 **a**	10.68 ± 0.35 **b**	6.71 ± 0.01 **b**	2.49 ± 0.03 **a**
Pn-3-glc	461	299	4.75 ± 0.02	1.38 ± 0.10 **a**	3.34 ± 0.05 **b**	2.24 ± 0.06 **b**	0.73 ± 0.11 **a**
Mv-3-glc	491	329	136.80 ± 1.85	39.24 ± 0.04 **a**	87.29 ± 1.52 **b**	56.44 ± 0.24 **b**	22.73 ± 0.02 **a**
Dp-3-acetylglc	505	463, 301	1.72 ± 0.07	2.18 ± 0.02 **b**	1.42 ± 0.01 **a**	0.90 ± 0.01 **a**	1.02 ± 0.04 **a**
Pyrano-Mv-3-glc (Vitisin B)	515	353	1.12 ± 0.04	3.84 ± 0.03 **b**	0.71 ± 0.03 **a**	0.24 ± 0.04 **a**	0.57 ± 0.02 **b**
Cy-3-acetylglc	489	447,285	0.48 ± 0.02	0.22 ± 0.02 **a**	0.27 **a**	nd **a**	nd **a**
Pt-3-acetylglc	519	477, 315	3.21 ± 0.02	1.38 ± 0.16 **a**	2.44 ± 0.06 **b**	1.59 ± 0.05 **b**	0.49 **a**
Pyrano-Mv-3-acetylglc (acetyl-Vitisin B)	557	353	0.31 ± 0.01	2.18 ± 0.02 **b**	0.31 ± 0.02 **a**	nd **a**	0.22 ± 0.01 **b**
Mv-3-*p*-coumglc-(epi)cat	925	617, 491	nd	nd **a**	nd **a**	0.35 ± 0.03 **a**	0.85 ± 0.03 **b**
Pn-3-acetylglc	503	299	5.37 ± 0.58	2.50 ± 0.16 **a**	4.46 ± 0.44 **b**	1.95 ± 0.19 **a**	1.49 ± 0.07 **a**
Mv-3-acetylglc	533	329	46.23 ± 2.08	11.40 ± 0.14 **a**	26.12 ± 0.13 **b**	18.07 ± 0.18 **b**	6.52 ± 0.02 **a**
Pyrano-Mv-3-*p*-coumglc (*p*-coum-Vitisin B)	661	353	0.42 ± 0.08	0.60 ± 0.01 **b**	0.34 ± 0.04 **a**	0.60 ± 0.01 **a**	0.66 ± 0.10 **a**
Mv-3-caffeoylglc	653	491, 329	0.74 ± 0.07	0.16 ± 0.02 **a**	0.49 ± 0.02 **b**	nd **a**	nd **a**
Pt-3-*p*-coumglc	623	477, 315	2.69 ± 0.14	0.80 ± 0.02 **a**	1.50 ± 0.04 **b**	0.91 **b**	0.56 **a**
Mv-3-*p*-coumglc-8-ethyl-(epi)cat	953	801, 663, 645, 355	nd	0.53 ± 0.01 **b**	0.44 ± 0.02 **a**	0.14 **a**	0.31 ± 0.06 **a**
Pn-3-*p*-coumglc	607	299	4.58 ± 0.22	0.50 ± 0.01 **a**	1.79 ± 0.12 **b**	0.90 ± 0.02 **a**	0.67 ± 0.09 **a**
Mv-3-*p*-coumglc	637	491, 329	22.57 ± 0.55	5.30 **a**	12.32 ± 0.21 **b**	8.70 ± 0.05 **b**	2.75 ± 0.17 **a**
Mv-3-glc-4-vinyl(epi)cat	803	641	nd	0.22 ± 0.02 **b**	nd **a**	0.22 ± 0.02 **a**	0.17 ± 0.02 **a**
Mv-3-*p*-coumglc-4-vinyl(epi)cat	949	641	nd	0.14 ± 0.01 **b**	nd **a**	nd **a**	nd **a**
Mv-3-glc-4-vinyl-phenol/Pn-3-glc-vinyl-guaiacol	607	445	nd	0.84 ± 0.01 **b**	0.50 ± 0.07 **a**	0.61 **b**	0.48 ± 0.01 **a**
Σ Anthocyanins			**255.03 ± 2.76**	**82.23 ± 0.11 a**	**161.65 ± 3.03 b**	**105.87 ± 0.33 b**	**45.02 ± 0.16 a**

Table 25 – Phenolic profile of Montepulciano wines at racking, immediately after the treatment with oak chips and at 12 months of aging.
nd: not detected.
glc: glucoside, glcr: glucuronide, rha: rhamnoside, galac: galactoside, Dp: delphinidin, Cy: cyanidin, Pt: petunidin, Pn: peonidin, Mv: malvidin, acetylglc: acetylglucoside, *p*-coumglc: *p*-coumaroylglucoside, caffeoylglc: caffeoylglucoside, cat: catechin.
In row, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by t Student test.

Sample	MP	SPP	LPP	LPP+SPP	LPP/SPP	$I_{gelatin}$	I_{HCl}	I_{PVPP}
				At racking				
	1.10 ± 0.01	0.31 ± 0.02	0.21 ± 0.01	0.52 ± 0.01	0.69 ± 0.07	69.6 ± 7.6	27.9 ± 4.3	36.6 ± 0.4
				After the treatment with oak chips				
TEST	1.09 ± 0.05 **b**	0.42 **b**	0.51 ± 0.09 **a**	0.93 ± 0.09 **b**	1.22 ± 0.20 **a**	16.5 ± 3.2 **a**	54.7 ± 0.4 **b**	53.6 ± 0.5 **a**
CHIPS	0.94 ± 0.01 **a**	0.34 **a**	0.37 ± 0.02 **a**	0.72 ± 0.02 **a**	1.10 ± 0.05 **a**	40.6 ± 6.3 **b**	27.1 ± 2.4 **a**	52.0 ± 1.5 **a**
				After 12 months				
TEST	0.55 ± 0.03 **a**	0.41 ± 0.04 **a**	0.40 ± 0.06 **a**	0.81 ± 0.03 **a**	0.84 ± 0.05 **a**	29.5 ± 2.6 **a**	16.9 ± 2.9 **a**	62.7 ± 2.5 **a**
CHIPS	0.70 ± 0.02 **b**	0.58 ± 0.08 **b**	0.54 ± 0.10 **b**	1.11 ± 0.01 **b**	1.13 ± 0.10 **a**	28.1 ± 3.9 **a**	26.6 ± 3.1 **a**	70.7 ± 1.8 **b**

Table 26 – Monomeric and polymeric pigments and structure indices of Montepulciano wine at racking, immediately after the treatment with oak chips and at 12 months of aging.
MP: absorbance at 520 nm due to monomeric pigments; SPP: absorbance 520 nm due to small polymeric pigments; LPP: absorbance 520 nm due to large polymeric pigments; LPP+SPP: absorbance 520 nm due to total polymeric pigments; LPP/SPP: ratio between large and small polymeric pigments; $I_{gelatin}$: gelatin index; I_{HCl}: hydrochloric acid index; I_{PVPP}: PVPP index.
In column, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by t Student test.

Sample	CI	T	dA(%)	% yellow	% red	% blue	dAl%	dAT%	dTAT%
				At racking					
	13.688 ± 0.057	0.466 ± 0.003	67.9 ± 0.5	28.4	60.9 ± 0.4	10.7 ± 0.4	13.1	57.0 ± 6.1	30.0 ± 6.1
				After the treatment with oak chips					
TEST	15.113 ± 0.144 **b**	0.585 ± 0.007 **a**	58.9 ± 0.3 **b**	32.1 ± 0.3 **a**	54.9 ± 0.2 **b**	13.0 ± 0.1 **a**	1.8 **a**	58.7 ± 2.9 **a**	39.5 ± 2.9 **a**
CHIPS	13.183 ± 0.027 **a**	0.637 ± 0.007 **b**	56.2 ± 0.3 **a**	34.0 ± 0.3 **b**	53.3 ± 0.2 **a**	12.7 ± 0.2 **a**	6.4 **b**	61.8 ± 0.9 **a**	31.8 ± 0.9 **a**
				After 12 months					
TEST	12.797 ± 0.004 **a**	0.695 ± 0.001 **b**	52.4 **b**	35.6 **b**	51.2 **b**	13.2 **a**	2.4 ± 0.1 **b**	40.2 ± 4.6 **b**	57.5 ± 4.6 **a**
CHIPS	13.712 ± 0.001 **b**	0.686 **a**	52.1 **a**	35.0 **a**	51.1 **a**	13.9 **b**	1.6 **a**	3.8 ± 0.7 **a**	96.7 ± 2.0 **b**

Table 27 – Colour parameters of Montepulciano wine at racking, immediately after the treatment with oak chips and at 12 months of aging.
CI: colour intensity; T: tonality; dA(%): percentage of red color due to flavilium cation of free and combined anthocyanins; % yellow: percentage of yellow component; % red: percentage of red component; % blue: percentage of blue component; dAl%: percentage of absorbance at 520 nm due to monomeric anthocyanins; dAT%: percentage of absorbance at 520 nm due to polymeric pigments decolorized with SO_2; dTAT%: percentage of absorbance at 520 nm due to polymeric pigments not decolorized.
In column, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by t Student test.

Compounds	t_r (min)	Mean concentration (AU x 10^5) ± SD (n=2)			Odour description
		T_0	T_6	C_6	
Acids					
Acetic acid	23.8	118 ± 45	136.7 ± 6.8	416 ± 13	Vinegar
Hexanoic acid	35.2	41.8 ± 6.3	43.7 ± 3.1	51.0 ± 5.5	Cheese, fatty, sour
Octanoic Acid	40.6	320 ± 14	488 ± 14	397 ± 42	Fatty acid, dry, dairy
Nonanoic acid	43.2	31 ± 14	10.11 ± 0.67	16.9 ± 1.0	Cheese, waxy
n-Decanoic acid	46.5	157 ± 21	328.7 ± 8.0	284 ± 20	Fatty acid, dry, woody
Dodecanoic acid	56.0	16.185 ± 0.027	17.0 ± 1.2	11.8 ± 3.8	Fatty
Total		684	1025	1177	
Percentage (%)		2.98	11.30	14.21	
Alcohols					
1-Propanol	9.8	17.3 ± 8.7	14.69 ± 1.69	12.93 ± 0.93	Alcohol, ripe fruit
1-Propanol, 2-methyl-	11.5	132.2 ± 1.6	110.82 ± 0.22	120.3 ± 3.3	Alcohol, solvent
1-Butanol	13.1	5.589 ± 0.047	5.660 ± 0.025	6.10 ± 0.43	Medicinal, phenolic
1-Butanol, 3-methyl-	15.2	2562 ± 228	2355 ± 39	2463 ± 19	Fusel, alcohol, sweet, fruity
1 Hexanol	20.0	32.5 ± 2.1	31.89 ± 0.76	34.7 ± 1.8	Herbaceous
Heptanol	23.4	8.85 ± 0.91	9.77 ± 0.57	13.4 ± 1.7	Oily; earthy/ banana; butter; herbaceous; meaty
1-Hexanol, 2-ethyl	24.5	n.d.	13.275 ± 0.029	9.66 ± 0.13	Oily; rose; sweet
2,3-Butanediol	26.3	44.4 ± 6.5	31 ± 14	39.5 ± 5.4	Fruity
1-Octanol	26.6	28.27 ± 0.47	27.1 ± 1.6	36.1 ± 9.8	Orange, floral
2,3-Butanediol	27.4	16.8 ± 1.3	15.0 ± 5.1	37.1 ± 8.8	Fruity
1-Nonanol	29.6	n.d.	7.8 ± 1.0	n.d	citrus; rose
2-Octen-1-ol, 3,7-dimethyl-	32.7	11.22 ± 0.26	12.3 ± 2.0	12.3 ± 2.0	n.d.
Benzyl Alcohol	36.1	2.18 ± 0.37	1.46 ± 0.20	n.d.	Flowery, sweet
Phenylethyl Alcohol	37.0	549 ± 123	469 ± 13	475 ± 35	Flowery, rose, honey
(E)-dihydrofarnesol	46.0	3.44 ± 0.54	n.d.	n.d.	Floral, green
Glycerin	48.5	15.00 ± 0.23	14 ± 10	n.d.	n.d.
Farnesol	49.3	10.7 ± 1.9	3.2 ± 1.3	n.d	Anise; apricot; balsam; clove; grapefruit; oily
Total		3439	3123	3261	
Percentage (%)		15.01	34.45	39.35	
Acetic Esters					
1-Butanol, 3-methyl-, acetate	12.3	805 ± 220	478 ± 23	450 ± 23	Banana
Hexyl acetate	17.3	11.6 ± 3.4	5.22 ± 0.27	4.4 ± 1.0	Apple; cherry; floral; pear; sweet
Isobornyl acetate	27.7	4.85 ± 0.37	n.d	n.d.	n.d.
2-Phenethyl acetate	34.4	64 ± 11	30.1 ± 2.4	33.7 ± 2.1	Floral
Total		886	514	488	
Percentage (%)		3.87	5.66	5.89	
Ethyl Esters					
Ethyl butyrate	9.8	68 ± 14	65.4 ± 5.9	67.6 ± 5.7	Banana, pineapple, sweet, eterea
Ethyl 2-methylbutyrate	10.2	n.d	6.59 ± 0.65	5.6 ± 1.3	fruity; green
Ethyl 3-methylbutyrate	10.7	3.1 ± 1.9	12.3 ± 1.4	15.00 ± 0.53	Apple
Ethyl hexanoate	16.0	1400 ± 350	793 ± 42	493 ± 18	apple; banana; wine-like
Ethyl heptanoate	19.4	12.5 ± 5.1	2.53 ± 0.54	3.50 ± 0.74	Fruity
Ethyl lactate	19.9	2.98 ± 0.83	60.50 ± 0.77	68.6 ± 7.6	Lactic
Ethyl octanoate	22.8	8707 ± 2000	1233 ± 160	979 ± 37	Banana, floral, pear,

					pineapple, wine-like
Ethyl nonanoate	26.0	33.7 ± 9.4	18.8 ± 1.2	n.d.	Oily; fruity; nutty
Ethyl decanoate	29.0	5655 ± 910	353 ± 65	323 ± 30	Grap, oily, wine-like
Diethyl succinate	30.3	31.9 ± 8.0	968 ± 43	964 ± 58	Spicy
Ethyl 9-decenoate	30.6	80 ± 11	n.d	n.d.	n.d.
Ethyl undecanoate	32.0	7.44 ± 0.80	n.d.	n.d.	Coconut
Ethyl dodecanoate	34.8	1075 ± 92	156.7 ± 3.6	75.9 ± 6.6	green; fruity; floral
Ethyl 3-phenylpropionate	36.2	4.8 ± 1.5	4.29 ± 0.68	5.1 ± 1.2	Ethereal; fruity; wine-like
Ethyl 3-hydroxyhexanoate	41.5	6.494 ± 0.070	4.43 ± 0.23	4.8 ± 1.3	Waxy, soapy
Ethyl 4-ethoxybenzoate	43.4	14.8 ± 2.3	n.d.	n.d.	n.d.
Ethyl hexadecanoate	45.4	138.7 ± 9.2	145 ± 34	26.9 ± 1.8	Waxy
Ethyl E-11-hexadecenoate	46.4	17.6 ±1.3	9.31 ± 0.41	n.d.	n.d.
Ethyl hydrogen succinate	51.0	10.4 ± 4.4	71.8 ± 2.6	39.3 ± 1.0	Chocolate
Ethyl stearate	53.8	13.9 ± 1.3	18.7 ± 3.1	n.d.	Waxy
Ethyl oleate	55.0	6.37 ± 0.22	10.4 ± 3.1	n.d.	n.d.
Ethyl linoleate	58.0	43.77 ± 0.71	62 ± 16	7.7 ± 1.1	
Total		17399	4038	3097	
Percentage (%)		75.92	44.54	37.37	
Other Esters					
Methyl caprylate	21.3	24.2 ± 1.6	4.30 ± 0.52	n.d.	
Methylbutyl caproate	23.5	56 ± 20	3.044 ± 0.044	n.d.	
Isobutyl caprylate	26.4	19.9 ± 6.2	n.d.	n.d	
Methyl caprate	27.7	12.7 ± 3.2	1.34 ± 0.27	n.d.	Apple; green; pineapple; sweet
Isoamyl caprylate	29.6	213 ± 30	34 ± 15	n.d	Green; citrus; fruity
Isobutyl caprate	32.3	5.9 ± 1.3	0.41 ± 0.57	n.d.	Fruity
Isoamyl n-decanoate	35.2	63.6 ± 5.5	20.5 ±v1.2	n.d.	
Isoamyl laurate	40.4	3.56 ± 0.78	2.77 ± 0.72	n.d.	Cheese; cherry; cinnamon; wine-like; waxy
Total		399	66	0	
Percentage (%)		1.74	0.73	0	
Carbonyl Compounds					
Nonanal	21.5	6.32 ± 0.11	5.11 ± 0.43	2.77 ± 0.10	Apple, coconut, grapefruit, lemon
Alpha-ionone	26.0	n.d	11.16 ± 1.08	n.d	
β-Damascenone	34.5	15.8 ± 2.5	339 ± 2.4	13.70 ± 0.52	
Total		22	50	16	
Percentage (%)		0.10	0.55	0.20	
Hydrocarbons					
Tridecane	18.0	5.44 ± 0.57	8.18 ± 0.56	19.0 ± 7.3	
2,6-Dimethyl 2,6-octadiene	29.7	4.66 ± 0.13	n.d.	n.d.	
Cyclooctane	32.6	26.8 ± 1.9	22.75 ± 0.37	8.8 ± 1.6	
Cyclodecane	38.0	11.13 ± 0.72	5.14 ± 0,11	n.d.	
Total		48	36	28	
Percentage (%)		0.21	040	0.34	
Terpenes					
Terpinen-4-ol	28.2	3.37 ± 0.19	4.75 ± 0,13	3.76 ± 0.24	
Terpinyl acetate	30.9	n.d.	4.8 ± 1.3	n.d.	
Geraniol	35.0	4.34 ± 0.80	2.92 ± 0.15	n.d.	Fruity, rose, sweet
D-Nerolidol	39.8	8.37 ± 0.35	10.65 ± 0.76	n.d.	Apple, green, woody, citrus, rose
Total		16	23	4	

Percentage (%)		0.07	0.25	0.05	
Ethers					
Diphenyl ether	39.6	n.d.	n.d.	n.d.	
Percentage (%)		0	0	0	
Aromatics					
Benzene, 1,3-bis[1,1-dimethylethyl]-	22.5	n.d.	38.89 ± 0.58	68 ± 22	
Oxime-, methoxy-phenyl-	32.4	7.0 ± 2.3	13.7 ± 3.2	22.8 ± 1.1	
2,4-di-t-Butylphenol	47.8	12.19 ± 0.64	133 ±	117.1 ± 6.2	
Total		19	185	207	
Percentage (%)		0.08	2.05	2.50	
Lactones					
Whiskey lactone	38.2	5.30 ± 0.42	5.59 ± 0.78	8.47 ± 0.59	Woody
Total		5	6	9	
Percentage (%)		0.02	0.06	0.10	

Table 28 - Volatile compounds (AU x10^5) isolated from Montepulciano wine at racking (T_0) and after 12 months of aging wine in untreated wines (T_6) and wines treated with oak chips (C_6) (mean ± standard deviation).
n.d.: not detected.
Odour descriptors were obtained from Capone *et al*. (2013) and SAFC "Flavors and Fragrances. European Ed. Catalogue 2007–2008". Percentages are referred to the volatile compounds respect to each chemical group in different packaging.

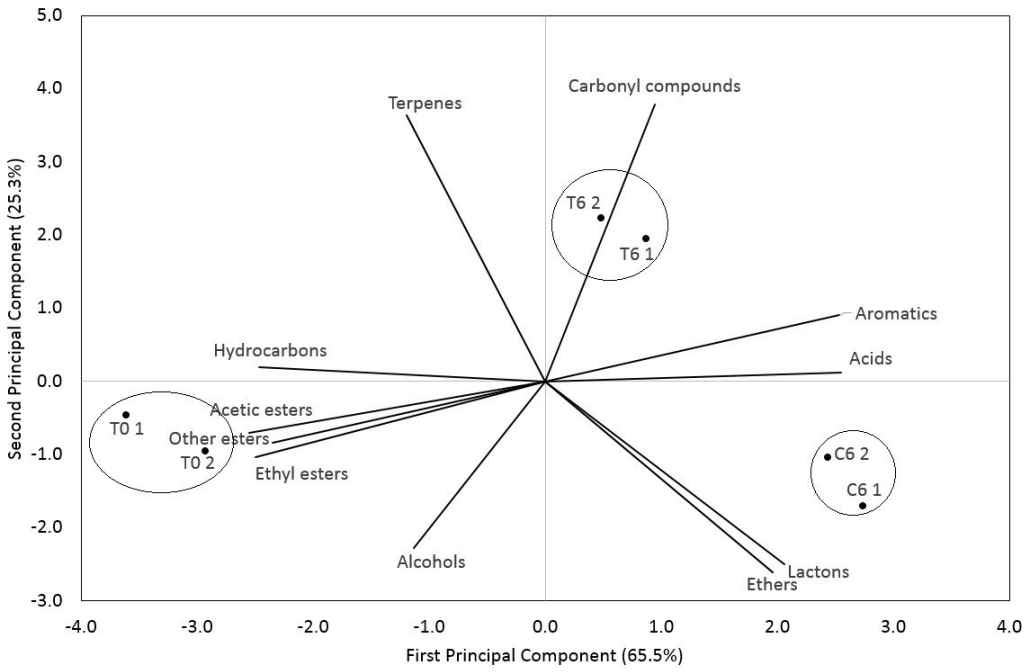
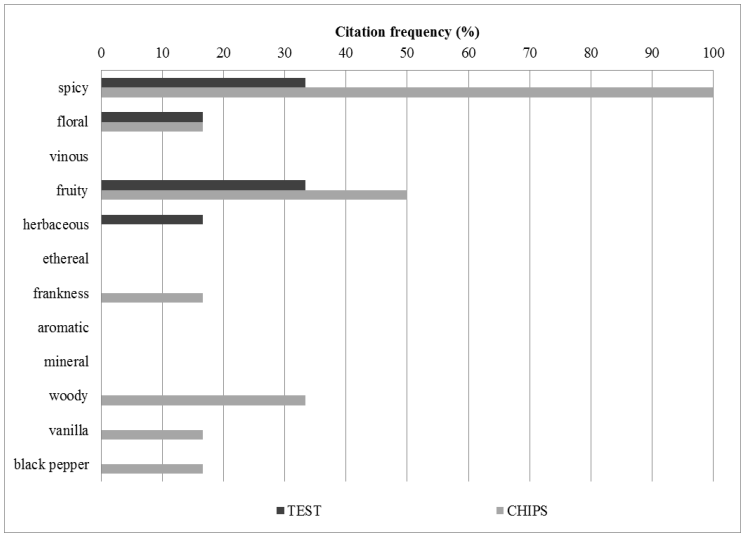


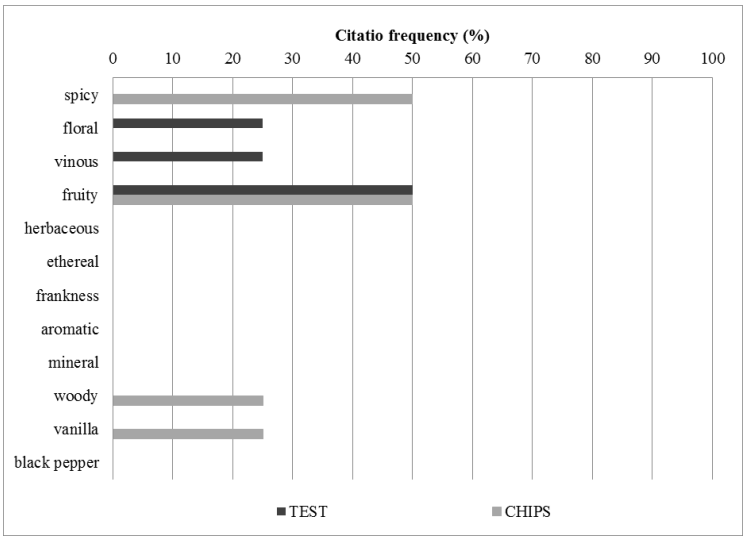
Figure 11 - Principal component analysis of volatile compounds among the wine at racking (T_0) and after 12 months of aging wine in untreated wines (T_6) and wines treated with oak chips (C_6).

Sensory descriptors	***After the treatment with oak chips***		***After 12 months***	
	TEST	CHIPS	TEST	CHIPS
Visual descriptors				
Clearness	2.0 **a**	1.9 ± 0.5 **a**	2.0 **a**	2.0 **a**
Texture	2.4 ± 0.7 **a**	2.4 ± 0.6 **a**	2.4 ± 0.5 **a**	2.6 ± 0.5 **a**
Olfactory descriptors				
Olfactory intensity	2.3 ± 0.4 **a**	2.6 ± 0.8 **a**	2.6 ± 0.5 **a**	2.8 ± 0.6 **a**
Olfactory complexity	2.0 **a**	2.7 ± 0.8 **a**	2.4 ± 0.5 **a**	2.8 ± 0.6 **a**
Olfactory quality	1.4 ± 0.5 **a**	2.8 ± 0.8 **b**	2.3 ± 0.6 **a**	2.8 ± 0.6 **a**
Gustatory-olfactory descriptors				
Sugars	0.0 **a**	0.1 ± 0.2 **a**	0.0 **a**	0.0 **a**
Alcohols	2.5 ± 0.5 **a**	2.6 ± 0.5 **a**	2.5 ± 0.6 **a**	2.5 ± 0.4 **a**
Polyols	1.8 ± 0.4 **a**	2.1 ± 0.2 **a**	2.0 **a**	1.8 ± 0.5 **a**
Acids	3.9 ± 0.2 **b**	2.6 ± 0.6 **a**	1.8 ± 0.3 **a**	1.9 ± 0.6 **a**
Tannins	1.7 ± 0.4 **a**	2.4 ± 0.5 **b**	1.5 ± 0.6 **a**	2.3 ± 0.5 **a**
Minerals	1.8 ± 0.3 **a**	1.8 ± 0.5 **a**	1.9 ± 0.6 **a**	1.8 ± 0.6 **a**
Structure	2.3 ± 0.6 **a**	2.3 ± 0.7 **a**	2.5 ± 0.6 **a**	2.4 ± 0.5 **a**
Balance	0.8 ± 0.3 **a**	2.3 ± 0.5 **b**	1.9 ± 0.6 **a**	2.8 ± 0.5 **a**
Gustatory-olfactory intensity	2.8 ± 0.8 **a**	2.6 ± 0.5 **a**	2.4 ± 0.8 **a**	2.8 ± 0.5 **a**
Gustatory-olfactory persistence	2.8 ± 0.8 **a**	2.5 ± 0.6 **a**	2.4 ± 0.8 **a**	2.6 ± 0.5 **a**
Gustatory-olfactory quality	0.8 ± 0.5 **a**	2.3 ± 0.6 **b**	1.8 ± 0.5 **a**	2.6 ± 0.5 **b**
Final considerations				
Evolutionary state	3.0 ± 1.1 **a**	2.3 ± 0.5 **a**	3.0 **a**	2.9 ± 0.6 **a**
Harmony	0.5 ± 0.5 **a**	2.3 ± 0.9 **b**	1.8 ± 0.5 **a**	2.9 ± 0.9 **a**

Table 29 – Sensory characteristics of Montepulciano wine immediately after the treatment with oak chips and at 12 months of aging.
In row, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by t Student test.

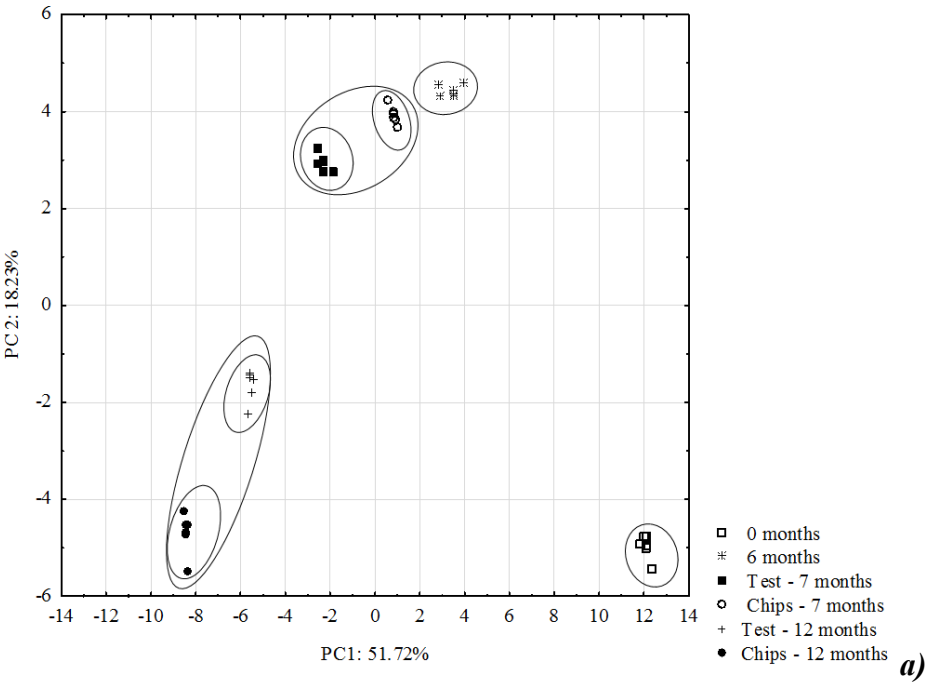


a)

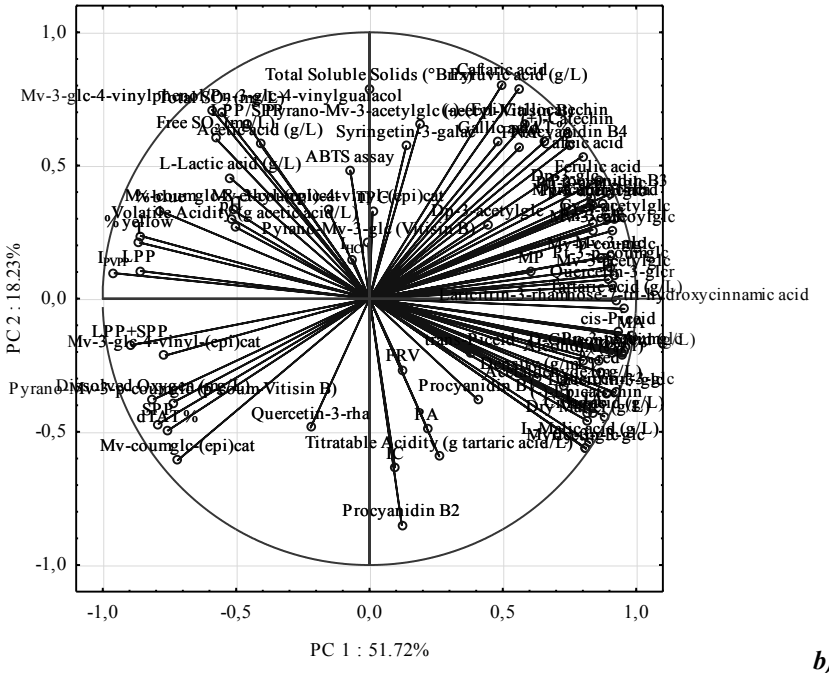


b)

Figure 12 – Citation frequency (%) of Montepulciano wine immediately after the treatment with oak chips (*a*) and after 12 months of aging (*b*).



a)



b)

Figure 13 - PCA scatter plot for projection on the factor plane of: **a)** Montepulciano wine samples at different stage of aging process and **b)** analytical results.

7.2.3 Nero di Troia wines

The physicochemical characteristics of Nero di Troia wines are shown in Table 30. At racking, it had: high alcohol content and dry matter, typical of strong red wines; low values of volatile acidity, free and total SO_2; high titratable acidity and acetaldehyde concentration (the latter being a by-product of the yeast fermentation); concentrations of total soluble solids that indicate the correct progress of fermentation; low dissolved oxygen content. At the time corresponding to the end of the treatment with oak chips, no significant differences were highlighted between control and treated wines with the exception of the free and total SO_2 contents and of the dissolved oxygen content, which were higher in the treated samples. According to the results, the concentration of the free form is higher than that of the bound form for all the wines, data confirmed by the low concentration of acetaldehyde. These data, together with the high concentration of dissolved oxygen in the treated samples, indicate that there was not an immediate action of ellagitannins on oxidative reactions that lead to the formation of acetaldehyde and the consumption of oxygen. At the time corresponding to the end of the micro-oxygenation treatment, no significant differences were highlighted between control and treated wines with the exception of the free and total SO_2 contents (which were higher in the treated samples) and of the dissolved oxygen content (which was higher in the control wine). These data and the presence of acetaldehyde below the detection limit could be explained by the lower oxygen solubility at 22 °C, temperature at which the micro-oxigenation treatment was performed. In fact, the oxygen solubility increases as the temperature decreases (approximately 10% for each lowering of 5 °C) and several authors (Nel, 2001; Berta *et al*., 1999) have shown that the optimal temperatures for the micro-oxygenation treatment are between 15 and 18 °C, and should never be below 10 °C and above 22-25 °C. Therefore, the controlled oxidation of phenolic compounds and their aldehyde-mediated polymerization reactions did not occur. After 12 months of aging, the only significant differences among samples concerned titratable acidity (higher in the samples treated with chips, lower in the control), volatile acidity (higher in wines treated with chips), free and total SO_2 (higher in micro-oxygenated wines), dissolved oxygen content (higher in the control, lower in the micro-oxygenated wines).

Table 31 shows the wine organic acid content. At racking, they were in a decreasing order: tartaric > malic > lactic > D-gluconic, citric, acetic > pyruvic. At the time corresponding to

the end of the treatment with oak chips, the reduction of the tartaric acid content and the simultaneous rise in the concentrations of L-lactic and pyruvic acids observed in the treated wines could be related to the metabolism of tartaric acid by some *Lactobacillus* species (Kandler, 1983) and/or by the partial oxidation of tartaric acid to glyoxylic acid (Fulcrand *et al*., 1997/a). As ethanal, this aldehyde acts as a bridge between two flavanol units, which have then dehydrated and oxidized with the production of yellow xanthylium pigments (Ribéreau-Gayon *et al*., 2004). The hypothesis could be confirmed by the higher value of % yellow measured in the treated wines immediately after 40 days of contact with oak chips. The application of micro-oxygenation did not extert effects on the organic acid content. After 12 months of aging, the differences among samples only concerned acetic acid (whose concentrations was higher in the wines treated with chips), and D-gluconic acid (whose concentration was lower in the wines treated with chips), probably due to the metabolism of acetic acid bacteria (Ribéreau-Gayon *et al*., 2000).

The phenolic classes and the analytical phenolic composition of Nero di Troia wines are shown in Tables 32 and 33. At racking, the concentrations of all the phenolic classes of Nero di Troia wines were lower than those of Aglianico wines. Furthermore, the wine from Uva di Troia had concentrations of anthocyanins lower than those of Montepulciano, but higher concentrations of all the other phenolic classes and higher antioxidant activity. Immediately after treatment with oak chips, the treated wines had higher concentrations of anthocyanins sensitive to SO_2 and of total phenolics than the control wines, probably as a consequence of the antioxidant effect of ellagitannins extracted from oak wood. Similar to what already highlighted for Montepulciano, the extraction of the ellagic tannins from oak determined an initial protection of wine phenolic compounds against oxidative phenomena and a subsequent hyperoxidant effect with the consequent production of acetaldehyde. The ethanal, so formed, determined the onset of via-acetaldehyde condensation reactions, which led to the formation of more stable high molecular weight pigments. In fact, about one month after the treatment (data not shown), the treated wine had lower concentrations of anthocyanins sensitive to SO_2, monomeric anthocyanins and flavans reactive with vanillin, thus indicating a greater degree of polymerization of phenolic compounds respect to the control wine. Furthermore, 12 months after racking, the treated wines exibhited the lowest concentration of all the anthocyanin forms (total, SO_2 sensitive, and monomeric) and of flavans reactive with vanillin and total phenols, while the concentrations of the other phenolic classes were similar to those detected in the control wines. Concerning the

effect of time, the decrease of concentration was evident between racking and the treatment with oak chips.

Immediately after the micro-oxygenation treatment, no differences were highlighted between control and treated wines. As a consequence, the micro-oxygenation treatment did not influence the antioxidant capacity of wines, in disagreement with the finding of de Beer *et al*. (2008) in Pinotage wines, where the application of micro-oxygenation resulted in a reduction of the total antioxidant capacity, and in agreement with Rivero-Pérez *et al*. (2008) who did not observe any change in the total antioxidant capacity in Tinta de Toro, Mencia, and Tempranillo Tinta del Pais wines. However, these discrepancies may be explained by the different winemaking technologies, grape varieties and quantification methods employed (Kallithraka *et al*., 2009). After 12 months of aging, the micro-oxygenated wines showed lower concentrations of anthocyanins sensitive to SO_2 and higher concentration of monomeric anthocyanins than the control.

Concerning the analytical phenolic profiles (Table 33), at racking, the most representative compounds, for each phenolic classes were: gallic and caffeic acids (phenolic acids), quercetin-3-glucuronide (flavonols), procyanidin B3, procyanidin B2, and catechin (flavan-3-ols), malvidin-3-glucoside, malvidin-3-acetylglucoside, and malvidin-*3*-*p*-coumaroylglucoside (anthocyanins). At the time corresponding to the end of the treatment with oak chips, it was observed a higher concentration of free anthocyanins in the treated wine and a lower concentration of stable pigments (B-type vitisins, flavanyl-pyranoanthocyanins, and anthocyanin-flavan-3-ol complexes) with respect to the control. Moreover, the treated wines wine initially presented higher content in (-)-(epi)gallocatechin, (+)-catechin and (-)-epicatechin, laricitrin-3-rhamnose-7-tri-hydroxy-cinnamic acid, and a lower content in caftaric, caffeic, *p*-coumaric acids, myricetin-3-rhamnoside, quercetin-3-glucuronide, quercetin-3-rhamnoside and syringetin-3-galctoside than the control. After 12 months of aging, the stable forms in control wine decreased, while higher concentrations of pyranoanthocyanins (especially visin B) and anthocyanin-tannin adducts (malvidin-3-glucoside-8-ethyl-gallocatechin) were found in the treated wine, thus indicating a greater degree of polymerization of the pigments. Furthermore, the treated wines exhibited lower concentration of almost all phenolic acids, stilbens, flavonols, procyanidins B3 and B2, and (+)-catechin than the control wines.

Immediately after the micro-oxygenation treatment, the phenolic profiles of control and treated wines showed significant differences. In particular, they concerned the total

concentrations of stilbens (higher in the control wines), the concentrations of myrycetin-3-rhamnoside and syryngetin-3-galactoside (respectively higher in the treated wines and higher in the control wines), the concentration of procyanidin B1 (lower in the treated wines); the concentrations of the glucosidic forms of peonidin and malvidin, of the acetylglucosidic forms of cyanidin, petunidin, peonidin and malvidin, of the *p*-coumaoylglucosidic form of petunidin, and of vitisin B (higher in the treated wines) and the concentrations of delphinidin-3-acetylglucoside, malvidin-3-*p*-coumaroylglucoside-4-vinyl-(epi)catechin and malvidin-3-glucoside-4-vinyl-phenol/peonidin-3-glucoside-4-vinylguaiacol (lower in the treated wines). The anthocyanin profile data confirmed the absence of any colour stabilization effect. That result was in agreement with the finding of Gómez-Plaza and Cano-López (2011), who stated that micro-oxygenation seems to be much more effective in improving wine structure if applied before malolactic fermentation, when tannins and anthocyanins are still mostly in their monomeric forms. After 12 months of aging, the micro-oxygenated wines exhibited higher free anthocyanin content and lower amount of stable forms. Furthermore, the micro-oxygenated wines exhibited higher concentration of flavan-3-ols than the control.

Table 34 shows the distribution of anthocyanins between monomeric and polymeric forms. At the time corresponding to the end of the treatment with oak chips, both control and treated wines had lower values of monomeric anthocyanins, and of the ratio between large and small polymeric anthocyanins respect to the racking. Furthermore, treated wines had lower values of absorbance due to monomeric, small polymeric, and large polymeric pigments than the control. After 12 months of aging, the treated wines showed lower values of monomeric, and higher values of small, large polymeric pigments, and the ratio between large and small, thus indicating an increase in the degree of pigments polymerization, in agreement with the HPLC-MS data, the spectrophotometric analysis (decrease of anthocyanins sensitive to sulfur dioxide and free anthocyanins). Regarding the structure indices, at the time corresponding to the end of the period of contact with oak chips, it was observed a lower value of the HCl index (similar effect observed in Montepulciano wine) in the treated wines. That result was a only apparent reduction of the degree of tannins polymerization, probably due to the precipitation of larger polymers (Collins, 2003). In fact, generally the values of I_{HCl} in wine are between 5 and 40; values above 35 and 40 indicate the precipitation and are followed by reductions of the index (Ribéreau-Gayon *et al*., 2004). Also for Nero di Troia, the treatment with oak chips did not

affect the PVPP index indicating, therefore, a low degree of polymerization of anthocyanins. No statistically significant differences were observed for the gelatin index between control and treated wines, contrarily to what observed, instead, in Aglianico and Montepulciano wines. After 12 months of aging, the treated wines showed higher HCl index than control, while no significant differences in gelatin and PVPP indexes were highlighted.

Concerning the effects of micro-oxygenation, immediately after the treatment, both control and treated wines had lower values of monomeric anthocyanins, large polymeric anthocyanins, and of the ratio between large and small polymeric anthocyanins respect to the racking. The control also showed higher values of the absorbance due to the monomeric pigments, in agreement with the decrease of the free anthocyanin content observed in literature for micro-oxygenated wines (Atanasova *et al*., 2002; Tao *et al*., 2007; Cano-López *et al*., 2007; Cano-López *et al*., 2010; Cejudo-Bastante *et al*., 2011a; Cejudo-Bastante *et al*., 2011b). The same behaviour was observed after 12 months of aging. With reference to the effects of micro-oxygenation on the structure indices, no significant differences were observed immediately after the end of the treatment. After 12 months of aging, however, all the structure indices were lower in the micro-oxygenated wines than in the control.

Concerning the colour parameters (Table 35), at the time corresponding to the treatment with oak chips, increases of tonality, % yellow, percentage of absorbance at 520 nm due to polymeric pigments not decolorized, and decreases of % red, percentage of absorbance at 520 nm due to monomeric anthocyanins, percentage of absorbance at 520 nm due to polymeric pigments decolorized with SO_2 were observed with respect to racking. Furthermore, the treated wines showed lower colour intensity, and higher tonality, % yellow, and percentage of absorbance at 520 nm due to monomeric anthocyanins than the control. Even in this case, the colour parameters confirmed the absence of a colour stabilization effect but, subsequently, the production of acetaldehyde, as by-product of peroxidation reactions, favoured the formation of stable wine pigments (such as vitisins) (Vivas and Glories, 1996) which led to an increase of colour intensity and % of red and blue components by darkening the crimson hues (Zironi *et al*., 2006). After 12 months of aging, the treated wines had higher colour intensity, percentage of absorbance at 520 nm due to polymeric pigments not decolorized, % red, % blue, and lower tonality, % yellow, percentage of absorbance at 520 nm due to monomeric anthocyanins and to polymeric

pigments decolorized with SO_2 than the control.

The micro-oxygenation treatment, instead, resulted in an immediate decrease of the colour intensity, in agreement with the finding of Llaudy *et al*. (2006) and in agreement with the lowest content in free anthocyanins measured in treated wines. Nevertheless, after 12 months of bottle aging, higher colour intensity, % of blue component, percentage of absorbance at 520 nm due to monomeric anthocyanins, and lower values of tonality and % of the yellow component were observed.

Changes in the volatile components of Nero di Troia wines treated with oak chips, with micro-oxygenation, and untreated were investigated during 12 months of aging. The 67 volatile compounds found by means of HS-SPME-GC-MS were grouped into the following 10 different classes (see Table 36): acids (6 compounds), alcohols (15), acetic esters (3), ethyl esters (22), other esters (7), carbonyl compounds (3), terpenes (4), aromatics (3), hydrocarbons (3), and lactones (1). The more representative compounds for each class were: acetic acid at racking and octanoic acid after 12 months (acids); 1-butanol, 3-methyl alcohol and phenylethyl alcohol (alcohols); isoamyl acetate (acetic esters); ethyl decanoate at racking and diethyl succinate after 12 months for all the treatments (ethyl esters); isoamyl caprate among the other esters at racking (after 12 months these compounds were not detectable); nonanal at racking and benzaldehyde, 2,-dimethyl- in all the wines at 12 months (carbonyl compounds); 2,6-dimethyl-2,6-octadiene at racking and tetradecane after 12 months (hydrocarbons); D-nerolidol and farnesol (terpenes); oxime-, methoxy-phenyl- at racking and 4-ethylphenol after 12 months (aromatics); butyrolactone (lactones) except for micro-oxygenated wines. In the aged wines, dramatic decreases in esters and alcohols were measured while the acids increased. This behaviour was more evident in micro-oxygenated wine. Furthermore, carbonyl and aromatics compounds increased with time (the latter greatly increase in the micro-oxygenated wines) while, as already written, all the "other esters" fell under their detection limit. Finally, the wine treated with oak chips was characterized by a greater presence of butyrolactone directly released from oak wood. The evolution of volatile compounds is also evident in Figure 14, which shows the results of the Principal Component Analysis applied to the data set of the volatile compounds. The first two components explained about 82% of the total variance. The wine at racking, and after 12 months of aging (control and treated with oak chips) were clearly distinguishable on the first component.

At the time corresponding to the end of the treatment with oak chips, no significant

differences were highlithed between control and treated wines for visual, olfactory, and gustatory properties (Table 37), while the treatment affected the nature of the flavours perceived by the trained judges (Figure 15*a*). The oak chip addition increased the citation frequency for spicy, aromatic, woody, vanilla and black pepper descriptors, while decreased the citation frequency for vinous and fruity notes, in agreement with literature (Guchu *et al*., 2006). At the time corresponding to the end of the micro-oxygenation treatment, the treated wines were less astringent than the TEST ones (Table 37), in agreement with Atanasova *et al*. (2002) and Llaudy *et al*. (2006). As reported by Gómez-Plaza and Cano-López (2011), micro-oxygenation also determined an increase of the fruity notes (Figure 15*b*). Furthermore, the micro-oxygenated wines were better appreciated by the judges for their olfactory intensity, complexity, and quality and these results were in agreement with the finding of Parpinello *et al.* (2012). After 12 months of bottle aging, control wines, wines treated with oak chips, and micro-oxygenated wines didn't show significant differences for any of the sensory descriptors resulting from QDA. Nevertheless, the wines treated with oak chips were show spicy, vanilla, and woody notes in a greater extent than the control wines and floral, herbaceous, and fruity flavours in a lower extent than the control wines. The micro-oxygenated wines were judged as more spicy and fruity and less herbaceous than the control ones (Figure 15*c*).

As reported for Montepulciano wine, the PCA was to the all the standardize data (with the exception of the data concerning the volatile compounds) during the aging time. The results are reported in Figure 16, which shown that the first two principal components explained only about 63% of the total variance. Concerning the evolution of Nero di Troia wines during the aging time, wines at racking was clearly grouped in the factor plane described by both neagtive values of PC1 and PC2. Wines after 6 months were also well separated from the others in the factor plane described by negative values of PC1 and positive values of PC2. Immediately after treatment with oak chips treated and control wines were undistinguishable on the first component and only slightly distinguishable on the second component. Immediately after micro-oxygenation, control and treated wines were overlapped. After 12 months of aging, control and micro-oxygenated wines were still overlapped, while control and wines treated with oak chips showed greater differences based on both the first two principal components. Control, micro-oxygenated, and oak-treated wines after 12 months of aging were located in the factor plane described by positive values of PC1 and negative values of PC2.

Sample	Alcohol (% vol)	Density (g/mL)	Dry Matter (g/L)	Volatile Acidity (g acetic acid/L)	Titratable Acidity (g tartaric acid/L)	pH	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Total Soluble Solids (°Brix)	Acetaldehyde (mg/L)	Dissolved O_2 mg/L)
					At racking						
	14.86 ± 0.01	0.995	37.5 ± 0.6	0.35 ± 0.01	6.06 ± 0.10	3.73 ± 0.03	16.0 ± 2.3	23.2 ± 3.4	9.0 ± 0.1	136.97 ± 3.18	2.03 ± 0.26
					After the treatment with oak chips						
TEST	14.52 ± 0.05 **a**	0.995 ± 0.002 **a**	34.8 ± 4.0 **a**	0.52 ± 0.05 **a**	4.88 ± 0.02 **a**	3.84 ± 0.01 **a**	33.6 **a**	41.4 ± 0.2 **a**	9.3 ± 0.1 **a**	26.50 ± 2.12 **a**	1.88 ± 0.01 **a**
CHIPS	14.48 ± 0.10 **a**	0.994 **a**	35.7 ± 2.7 **a**	0.62 ± 0.01 **a**	4.88 ± 0.02 **a**	3.82 ± 0.02 **a**	47.2 ± 3.4 **b**	56.0 ± 2.3 **b**	9.3 **a**	27.00 ± 1.41 **a**	5.27 ± 0.01 **b**
					After the micro-oxygenation						
TEST	14.53 ± 0.04 **a**	0.994 **a**	34.6 ± 4.3 **a**	0.54 ± 0.02 **a**	4.92 **a**	3.81 ± 0.01 **a**	34.4 ± 1.1 **a**	40.8 ± 1.1 **a**	9.3 ± 0.1 **a**	nd **a**	2.21 ± 0.01 **b**
MOX	14.49 ± 0.01 **a**	0.994 **a**	31.8 ± 0.1 **a**	0.58 ± 0.04 **a**	5.03 ± 0.15 **a**	3.82 ± 0.01 **a**	57.6 **b**	64.8 ± 1.1 **b**	9.3 ± 0.1 **a**	nd **a**	1.81 ± 0.02 **a**
					After 12 months						
TEST	14.34 ± 0.28 **a**	0.995 **a**	33.8 ± 0.4 **a**	0.83 ± 0.12 **a**	4.58 ± 0.11 **a**	3.82 **a**	32.8 ± 3.4 **a**	40.0 ± 2.3 **a**	9.1 ± 0.1 **a**	4.25 ± 0.35 **a**	7.04 ± 0.01 **c**
MOX	14.45 ± 0.04 **a**	0.995 **a**	34.2 ± 1.1 **a**	0.81 ± 0.11 **a**	4.71 ± 0.08 **ab**	3.82 ± 0.02 **a**	47.2 ± 3.4 **b**	54.4 ± 2.3 **b**	9.0 ± 0.1 **a**	4.75 ± 0.35 **a**	1.87 ± 0.01 **a**
CHIPS	14.25 ± 0.29 **a**	0.995 **a**	34.4 ± 0.5 **a**	0.93 ± 0.11 **b**	4.86 ± 0.04 **b**	3.85 ± 0.01 **a**	36.0 ± 3.4 **a**	40.8 ± 3.4 **a**	9.0 ± 0.1 **a**	5.50 ± 0.71 **a**	5.17 ± 0.01 **b**

Table 30 – Physicochemical parameters of Nero di Troia wine at racking, immediately after the treatment with oak chips (7 months) and the micro-oxygenation (8 months), and after 12 months of aging.
nd: not detected.
In column, at the same time of aging, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Sample	Tartaric acid	L-Malic acid	L-Lactic acid	Acetic acid	Citric acid	Pyruvic acid	D-Gluconic acid
				At racking			
	3.37 ± 0.01	2.12 ± 0.01	0.66 ± 0.02	0.31	0.38 ± 0.01	0.06	0.39 ± 0.01
				After the treatment with oak chips			
TEST	2.77 ± 0.04 **b**	0.02 **a**	0.70 ± 0.01 **a**	0.69 ± 0.05 **a**	0.07 ± 0.01 **a**	0.04 ± 0.01 **a**	0.16 **a**
CHIPS	2.57 ± 0.01 **a**	0.03 ± 0.01 **a**	0.97 ± 0.01 **b**	0.81 ± 0.01 **a**	0.06 **a**	0.08 **a**	0.18 ± 0.01 **a**
				After the micro-oxygenation			
TEST	2.78 ± 0.04 **a**	0.03 **a**	0.95 ± 0.01 **a**	0.59 **a**	0.03 **a**	0.05 ± 0.01 **a**	0.04 **a**
MOX	2.52 ± 0.10 **a**	0.05 ± 0.01 **a**	0.93 **a**	0.58 ± 0.01 **a**	0.03 **a**	0.06 **a**	0.04 **a**
				After 12 months			
TEST	2.05 ± 0.09 **a**	0.01 **a**	0.83 ± 0.05 **a**	0.65 ± 0.06 **a**	nd **a**	0.05 **a**	0.15 **b**
MOX	2.04 ± 0.01 **a**	nd **a**	0.78 ± 0.08 **a**	0.70 **a**	nd **a**	0.05 **a**	0.14 **b**
CHIPS	1.98 **a**	0.02 **a**	0.85 ± 0.07 **a**	0.90 ± 0.01 **b**	nd **a**	0.05 **a**	0.05 **a**

Table 31 – Organic acids content of Nero di Troia wine at racking, immediately after the treatment with oak chips (7 months) and the micro-oxygenation (8 months), and after 12 months of aging.
nd: not detected.
In column, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Sample	TA	SA	MA	TF	FNA	FRV	PA	TPC	ABTS assay
					At racking				
	485 ± 16	606 ± 20	286 ± 9	3219 ± 148	2512 ± 129	1991 ± 150	4844 ± 277	4194 ± 180	21.74 ± 2.05
					After the treatment with oak chips				
TEST	225 ± 19 **a**	266 ± 11 **a**	95 ± 16 **a**	2704 ± 223 **a**	2376 ± 203 **a**	1698 ± 187 **a**	3088 ± 182 **a**	3461 ± 98 **a**	22.05 ± 1.10 **b**
CHIPS	253 ± 16 **a**	310 ± 10 **b**	121 ± 15 **a**	2865 ± 46 **a**	2484 ± 64 **a**	1638 ± 54 **a**	3391 ± 105 **a**	3739 ± 108 **b**	18.67 ± 1.34 **a**
					After the micro-oxygenation				
TEST	230 ± 25 **a**	291 ± 3 **a**	80 ± 15 **a**	2768 ± 91 **a**	2434 ± 54 **a**	1748 ± 157 **a**	3512 ± 210 **a**	3674 ± 66 **a**	23.48 ± 1.44 **a**
MOX	217 ± 13 **a**	320 ± 12 **a**	76 ± 13 **a**	2704 **a**	2387 ± 18 **a**	1658 ± 285 **a**	3814 ± 182 **a**	3752 ± 46 **a**	22.32 ± 1.13 **a**
					After 12 months				
TEST	254 ± 38 **b**	306 ± 9 **c**	60 ± 8 **b**	2197 ± 174 **a**	1827 ± 133 **a**	2019 ± 62 **b**	3608 ± 145 **a**	3699 ± 45 **b**	20.24 ± 3.91 **a**
MOX	259 ± 10 **b**	254 ± 18 **b**	88 ± 6 **c**	2387 ± 98 **a**	2010 ± 103 **a**	2061 ± 71 **b**	3661 ± 46 **a**	3807 ± 153 **b**	21.54 ± 3.47 **a**
CHIPS	202 ± 6 **a**	127 ± 7 **a**	23 ± 3 **a**	2197 ± 174 **a**	1903 ± 166 **a**	1767 ± 63 **a**	3554 ± 447 **a**	3544 ± 110 **a**	19.66 ± 3.33 **a**

Table 32 – Phenolic composition and antioxidant activity of Nero di Troia wine at racking, immediately after the treatment with oak chips (7 months) and the micro-oxygenation (8 months), and after 12 months of aging.
TA: total anthocyanins (as mg malvidin-3-glucoside/L); SA: anthocyanins sensitive to SO_2 (as mg malvidin-3-glucoside/L); MA: monomeric anthocyanins (as malvidin-3-glucoside mg/L); TF: total flavonoids (as mg (+)-catechin/L); FNA: flavonoids different from anthocyanins (as mg (+)-catechin/L); FRV: flavans reactive with vanillin (as mg (+)-catechin/L); PA: proanthocyanidins (as mg cyanidin chloride/L); TPC: total phenolic compounds (as mg gallic acid/L); ABTS assay: antioxidant activity by ABTS assay (as mmol Trolox eq/L).
In column, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Phenolic compounds	*At racking*	*After the treatment with oak chips*		*After the micro-oxygenation*		*After 12 months*		
		TEST	CHIPS	TEST	MOX	TEST	MOX	CHIPS
Phenolic acids (mg GAE/L; mg CAE/L)								
Gallic acid	70.32 ± 0.74	64.05 ± 0.68 **a**	64.62 ± 0.72 **a**	68.98 ± 1.60 **a**	68.30 ± 2.18 **a**	62.34 ± 1.46 **ab**	64.80 ± 0.71 **b**	59.68 ± 0.79 **a**
Caftaric acid	25.99 ± 0.01	19.20 ± 0.45 **b**	16.40 ± 0.73 **a**	18.41 ± 1.68 **a**	17.72 ± 0.57 **a**	13.70 ± 0.21 **c**	12.01 ± 0.31 **b**	3.19 ± 0.23 **a**
Caffeic acid	40.39 ± 0.49	20.59 ± 0.08 **b**	18.61 ± 0.04 **a**	18.84 ± 0.40 **a**	18.37 ± 0.62 **a**	17.66 ± 0.40 **c**	15.99 ± 0.23 **b**	3.28 ± 0.22 **a**
p-Coumaric acid	13.44 ± 0.33	7.99 ± 0.03 **b**	7.02 ± 0.18 **a**	8.07 ± 0.03 **a**	8.10 ± 0.10 **a**	7.57 ± 0.09 **b**	7.44 ± 0.10 **b**	2.58 ± 0.08 **a**
Ferulic acid	11.32 ± 0.02	10.38 ± 0.23 **a**	9.90 ± 0.11 **a**	10.19 ± 0.16 **a**	9.94 ± 0.22 **a**	7.27 ± 0.08 **a**	7.06 ± 0.18 **a**	7.51 ± 0.35 **a**
Σ Phenoli acids	**161.46 ± 0.90**	**122.21 ± 1.25 b**	**116.54 ± 1.12 a**	**124.49 ± 0.19 a**	**122.43 ± 2.55 a**	**108.53 ± 1.83 b**	**107.30 ± 1.52 b**	**76.24 ± 1.68 a**
Stilbens (mg RE/L)								
cis-Piceid	3.47 ± 0.03	2.36 ± 0.02 **a**	2.21 ± 0.08 **a**	2.58 ± 0.06 **a**	2.61 ± 0.03 **a**	2.24 **b**	2.19 ± 0.15 **b**	1.69 **a**
trans-Piceid	2.46 ± 0.07	2.17 ± 0.01 **a**	2.17 ± 0.01 **a**	2.23 ± 0.07 **a**	2.07 ± 0.06 **a**	2.07 ± 0.02 **b**	1.70 ± 0.06 **a**	1.82 ± 0.03 **a**
Σ Stilbens	**5.93 ± 0.04**	**4.53 a**	**4.38 ± 0.09 a**	**4.81 ± 0.01 b**	**4.68 ± 0.03 a**	**4.31 ± 0.02 b**	**3.89 ± 0.21 a**	**3.52 ± 0.03 a**
Flavonols (mg QE/L)								
Myricetin-3-glc	nd	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**
Myricetin-3-rha	3.04	2.20 ± 0.03 **b**	1.84 ± 0.07 **a**	2.03 ± 0.31 **a**	4.21 ± 0.12 **b**	2.48 ± 0.03 **a**	2.60 ± 0.05 **a**	2.13 ± 0.28 **a**
Quercetin-3-glc	1.64	0.30 ± 0.03 **a**	0.35 ± 0.04 **a**	0.94 ± 0.07 **a**	0.83 ± 0.03 **a**	0.78 ± 0.10 **a**	0.93 ± 0.16 **a**	0.83 ± 0.08 **a**
Quercetin-3-glcr	7.60 ± 0.09	6.93 ± 0.03 **b**	6.23 ± 0.09 **a**	6.56 ± 0.11 **a**	6.20 ± 0.12 **a**	5.60 ± 0.09 **c**	1.69 ± 0.12 **a**	3.50 ± 0.05 **b**
Quercetin-3-galac	nd	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**
Laricitrin-3-glc	1.08 ± 0.03	0.81 ± 0.01 **a**	0.69 ± 0.04 **a**	0.71 ± 0.04 **a**	0.63 ± 0.04 **a**	nd **a**	nd **a**	nd **a**
Quercetin-3-rha	2.89 ± 0.02	2.52 ± 0.11 **b**	2.17 ± 0.02 **a**	2.42 ± 0.22 **a**	3.08 ± 0.05 **a**	2.71 ± 0.02 **b**	3.07 ± 0.02 **c**	2.09 **a**
Syringetin-3-galac	5.95	4.91 ± 0.02 **b**	4.32 ± 0.07 **a**	4.83 ± 0.03 **b**	4.56 ± 0.01 **a**	5.99 ± 0.06 **c**	4.29 ± 0.04 **b**	3.68 ± 0.07 **a**
Laricitrin-3-rhamnose-7-tri-hydroxy-cinnamic acid	1.69 ± 0.01	0.93 **a**	1.15 ± 0.02 **b**	1.09 ± 0.18 **a**	1.27 ± 0.03 **a**	0.84 ± 0.05 **b**	0.84 ± 0.02 **b**	0.36 ± 0.01 **a**
Σ Flavonols	**23.88 ± 0.04**	**18.60 ± 0.14 b**	**16.75 ± 0.26 a**	**18.59 ± 0.73 a**	**20.77 ± 0.16 a**	**18.40 ± 0.07 b**	**13.42 ± 0.41 a**	**13.42 ± 0.41 a**
Flavan-3-ols (mg CE/L)								
(-)-(Epi)Gallocatechin	20.99 ± 0.69	13.02 ± 0.22 **a**	19.29 ± 0.19 **b**	13.09 ± 0.93 **a**	16.76 ± 1.08 **a**	13.77 ± 0.41 **a**	18.59 ± 0.30 **b**	13.29 ± 2.08 **a**
Procyanidin B3	78.68 ± 1.27	55.32 ± 0.53 **a**	59.82 ± 1.99 **a**	62.94 ± 0.03 **a**	64.33 ± 0.90 **a**	28.12 ± 0.07 **b**	29.93 ± 0.05 **c**	21.46 ± 0.75 **a**
(+)-Catechin	58.56 ± 1.76	52.64 ± 0.40 **a**	54.15 ± 0.24 **b**	49.01 ± 1.27 **a**	47.74 ± 0.02 **a**	38.40 ± 0.73 **b**	50.09 ± 1.24 **c**	12.25 ± 2.06 **a**
Procyanidin B1	15.86 ± 0.33	19.57 ± 0.30 **b**	17.83 ± 0.06 **a**	19.87 ± 0.95 **b**	15.15 ± 0.28 **a**	11.57 ± 0.07 **a**	15.70 ± 2.62 **a**	11.64 ± 0.42 **a**
Procyanidin B4	21.38 ± 0.32	10.40 ± 0.03 **a**	15.39 ± 3.75 **a**	14.57 ± 0.53 **a**	14.80 ± 0.56 **a**	12.37 ± 0.68 **a**	14.59 ± 0.03 **b**	11.12 ± 0.09 **a**

(-)-Epicatechin	23.57 ± 4.67	17.31 ± 0.06 **a**	19.64 ± 0.59 **b**	18.45 ± 2.79 **a**	18.60 ± 3.21 **a**	13.06 ± 0.60 **a**	16.45 ± 0.82 **b**	10.50 ± 1.18 **a**
Procyanidin B2	46.38 ± 0.16	13.86 ± 0.24 **a**	13.02 ± 0.58 **a**	16.80 ± 0.80 **a**	18.28 ± 0.57 **a**	15.96 ± 0.30 **b**	19.39 ± 0.64 **c**	4.28 ± 0.41 **a**
Σ Flavan-3-ols	**265.42 ± 2.50**	**182.10 ± 1.17 a**	**199.14 ± 1.78 b**	**194.74 ± 1.66 a**	**195.66 ± 2.62 a**	**133.26 ± 1.35 b**	**164.74 ± 2.78 c**	**84.54 ± 4.67 a**
Anthocyanins (mg ME/L)								
Dp-3-glc	8.36 ± 0.13	4.68 ± 0.01 **a**	5.43 ± 0.11 **b**	6.52 ± 0.12 **a**	6.98 ± 0.02 **a**	3.66 ± 0.04 **b**	4.55 ± 0.01 **c**	0.89 ± 0.01 **a**
Cy-3-glc	2.85 ± 0.11	1.25 ± 0.02 **a**	1.45 ± 0.02 **b**	1.55 ± 0.02 **a**	1.35 ± 0.08 **a**	0.97 ± 0.01 **b**	1.21 **c**	0.35 ± 0.02 **a**
Pt-3-glc	11.61 ± 0.63	5.95 ± 0.02 **a**	6.62 ± 0.05 **b**	7.73 ± 0.22 **a**	8.20 ± 0.09 **a**	3.71 ± 0.04 **b**	4.60 ± 0.01 **c**	0.80 ± 0.03 **a**
Pn-3-glc	10.52 ± 0.09	4.86 ± 0.05 **a**	6.30 ± 0.17 **b**	7.32 ± 0.10 **a**	7.84 ± 0.05 **b**	3.37 ± 0.04 **b**	4.36 ± 0.04 **c**	0.48 ± 0.02 **a**
Mv-3-glc	82.65 ± 0.37	44.04 ± 0.09 **a**	50.15 ± 0.63 **b**	56.82 ± 0.97 **a**	62.09 ± 0.13 **b**	29.02 ± 0.26 **b**	35.62 ± 0.45 **c**	6.41 ± 0.09 **a**
Dp-3-acetylglc	1.76 ± 0.02	1.67 ± 0.01 **b**	1.04 **a**	1.21 ± 0.02 **b**	1.15 ± 0.02 **a**	0.94 ± 0.01 **b**	0.89 ± 0.06 **ab**	0.77 ± 0.02 **a**
Pyrano-Mv-3-glc (Vitisin B)	1.00 ± 0.02	1.63 **b**	0.71 ± 0.02 **a**	nd **a**	0.15 ± 0.01 **b**	0.19 ± 0.01 **a**	0.18 ± 0.03 **a**	0.27 ± 0.01 **b**
Cy-3-acetylglc	0.87 ± 0.01	0.36 ± 0.05 **a**	0.46 ± 0.03 **a**	0.65 ± 0.01 **a**	0.73 ± 0.03 **b**	nd **a**	nd **a**	nd **a**
Pt-3-acetylglc	3.17 ± 0.25	0.71 ± 0.03 **a**	1.59 **b**	1.86 ± 0.07 **a**	2.07 ± 0.01 **b**	0.38 **b**	0.39 **b**	0.33 **a**
Pyrano-Mv-3-acetylglc (acetyl-Vitisin B)	0.65	0.86 ± 0.02 **b**	0.40 ± 0.01 **a**	nd **a**	nd **a**	1.55 ± 0.09 **a**	1.71 ± 0.11 **a**	1.90 ± 0.21 **a**
Mv-3-glc-8-ethyl-gallocat	nd	nd **a**	nd **a**	nd **a**	nd **a**	0.35 ± 0.02 **ab**	0.30 ± 0.03 **a**	0.49 ± 0.10 **b**
Mv-coumglc-(epi)cat	nd	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**
Pn-3-acetylglc	6.51 ± 0.76	3.37 ± 0.07 **a**	3.83 ± 0.12 **b**	4.16 ± 0.04 **a**	4.54 ± 0.13 **b**	2.20 ± 0.06 **b**	2.90 ± 0.11 **c**	0.91 ± 0.11 **a**
Mv-3-acetylglc	21.75 ± 0.42	15.58 ± 0.24 **a**	18.35 ± 0.03 **b**	22.30 ± 0.41 **a**	23.90 ± 0.19 **b**	11.02 ± 0.24 **b**	13.36 ± 0.03 **c**	2.31 ± 0.01 **a**
Pyrano-Mv-3-*p*-coumglc (*p*-coum-Vitisin B)	nd	0.61 ± 0.01 **b**	0.30 **a**	0.44 ± 0.02 **b**	0.39 **a**	0.39 ± 0.01 **b**	0.37 ± 0.04 **ab**	0.29 ± 0.01 **a**
Mv-3-caffeoylglc	2.73 ± 0.17	0.45 ± 0.02 **a**	0.60 ± 0.07 **a**	0.97 ± 0.04 **a**	1.01 **a**	0.37 ± 0.02 **b**	0.44 ± 0.01 **c**	nd **a**
Pt-3-*p*-coumglc	1.09 ± 0.14	0.99 **a**	1.02 **b**	1.33 ± 0.05 **a**	1.44 ± 0.02 **b**	0.66 **b**	0.99 ± 0.05 **c**	nd **a**
Mv-3-*p*-coumglc-8-ethyl-(epi)cat	nd	0.51 **b**	0.43 ± 0.03 **a**	0.57 ± 0.03 **a**	0.60 ± 0.02 **a**	1.76 ± 0.19 **a**	1.86 ± 0.02 **a**	1.63 ± 0.03 **a**
Pn-3-*p*-coumglc	6.72 ± 0.71	1.84 ± 0.10 **a**	2.43 ± 0.12 **b**	2.86 ± 0.26 **a**	2.97 ± 0.11 **a**	1.24 ± 0.01 **b**	1.85 ± 0.07 **c**	0.17 ± 0.02 **a**
Mv-3-*p*-coumglc	21.95 ± 0.84	7.58 ± 0.07 **a**	8.65 ± 0.11 **b**	12.50 ± 0.04 **a**	12.85 ± 0.39 **a**	5.55 ± 0.01 **b**	6.88 ± 0.07 **c**	0.91 ± 0.05 **a**
Mv-3-glc-4-vinyl(epi)cat	nd	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**
Mv-3-*p*-coumglc-4-vinyl(epi)cat	nd	0.13 ± 0.01 **b**	nd **a**	0.13 **b**	nd **a**	nd **a**	nd **a**	nd **a**
Mv-3-glc-4-vinylphenol/Pn-3-glc-vinylguaiacol	nd	0.57 ± 0.01 **b**	0.31 ± 0.01 **a**	0.48 **b**	0.41 **a**	0.41 **b**	0.40 ± 0.04 **b**	0.26 ± 0.02 **a**
Σ Anthocyanins	**184.18 ± 0.38**	**97.61 ± 0.26 a**	**110.05 ± 1.04 b**	**129.39 ± 2.39 a**	**138.65 ± 0.13 b**	**58.93 ± 0.19 b**	**72.39 ± 1.08 c**	**17.80 ± 0.62 a**

Table 33 – Phenolic profile of Nero di Troia wines at racking, immediately after the treatment with oak chips (7 months) and the micro-oxygenation (8 months), and after 12 months of aging.
nd: not detected, glc: glucoside, glcr: glucuronide, rha: rhamnoside, galac: galactoside, Dp: delphinidin, Cy: cyanidin, Pt: petunidin, Pn: peonidin, Mv: malvidin, acetylglc: acetylglucoside, *p*-coumglc: *p*-coumaroylglucoside, caffeoylglc: caffeoylglucoside, cat: catechin.
In row, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Sample	MP	SPP	LPP	LPP+SPP	LPP/SPP	$I_{gelatin}$	I_{HCl}	I_{PVPP}
				At racking				
	1.02 ± 0.03	0.35 ± 0.02	0.36	0.70 ± 0.02	1.03 ± 0.05	69.7 ± 2.4	49.2 ± 3.2	26.0 ± 5.2
				After the treatment with oak chips				
TEST	0.84 ± 0.02 **b**	0.43 **b**	0.36 ± 0.02 **b**	0.79 ± 0.02 **b**	0.84 ± 0.03 **a**	22.6 ± 0.8 **a**	58.2 ± 0.6 **b**	63.9 ± 7.3 **a**
CHIPS	0.77 ± 0.02 **a**	0.37 ± 0.02 **a**	0.31 ± 0.02 **a**	0.68 ± 0.01 **a**	0.85 ± 0.09 **a**	19.7 ± 1.9 **a**	50.0 ± 1.9 **a**	60.8 ± 5.1 **a**
				After the micro-oxygenation				
TEST	0.63 ± 0.02 **b**	0.37 ± 0.01 **a**	0.27 ± 0.04 **a**	0.64 ± 0.03 **a**	0.75 ± 0.14 **a**	23.9 ± 4.7 **a**	26.1 ± 5.2 **a**	72.4 ± 5.3 **a**
MOX	0.57 ± 0.02 **a**	0.34 **a**	0.25 ± 0.01 **a**	0.60 ± 0.01 **a**	0.74 ± 0.05 **a**	17.4 ± 3.0 **a**	20.4 ± 2.8 **a**	76.2 ± 3.4 **a**
				After 12 months				
TEST	0.50 ± 0.04 **b**	0.39 ± 0.01 **a**	0.49 ± 0.04 **a**	0.88 ± 0.04 **a**	1.25 ± 0.12 **a**	27.4 ± 0.9 **b**	47.4 ± 2.5 **b**	80.4 ± 2.6 **b**
MOX	0.43 ± 0.02 **a**	0.38 ± 0.01 **a**	0.45 ± 0.02 **a**	0.84 ± 0.01 **a**	1.19 ± 0.10 **a**	14.0 ± 2.5 **a**	41.2 ± 1.7 **a**	65.2 ± 3.7 **a**
CHIPS	0.37 ± 0.05 **a**	0.50 ± 0.04 **b**	0.82 ± 0.05 **b**	1.31 ± 0.05 **b**	1.65 ± 0.19 **b**	21.6 ± 4.0 **b**	67.7 ± 2.8 **c**	81.6 ± 3.2 **b**

Table 34 – Monomeric and polymeric pigments and structure indices of Nero di Troia wine at racking, immediately after the treatment with oak chips (7 months) and the micro-oxygenation (8 months), and after 12 months of aging.
MP: absorbance at 520 nm due to monomeric pigments; SPP: absorbance 520 nm due to small polymeric pigments; LPP: absorbance 520 nm due to large polymeric pigments; LPP+SPP: absorbance 520 nm due to total polymeric pigments; LPP/SPP: ratio between large and small polymeric pigments; $I_{gelatin}$: gelatin index; I_{HCl}: hydrochloric acid index; I_{PVPP}: PVPP index.
In column, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test

Sample	CI	T	dA(%)	% yellow	% red	% blue	dAl%	dAT%	dTAT%
				At racking					
	11.370 ± 0.123	0.622 ± 0.001	57.5	33.6 ± 0.1	54.1	12.4 ± 0.1	4.1	62.6 ± 6.4	33.4 ± 6.4
				After the treatment with oak chips					
TEST	12.478 ± 0.154 **b**	0.698 ± 0.007 **a**	53.1 ± 0.8 **a**	36.0 **a**	51.6 ± 0.4 **a**	12.4 ± 0.4 **a**	2.0 **a**	58.3± 2.7 **a**	39.7 ± 2.7 **a**
CHIPS	11.341 ± 0.067 **a**	0.728 ± 0.007 **b**	51.0 ± 0.5 **a**	36.8 ± 0.2 **b**	50.5 ± 0.2 **a**	12.7 ± 0.1 **a**	2.3 **b**	57.7 ± 0.4 **a**	40.1 ± 0.4 **a**
				After the micro-oxygenation					
TEST	10.553 ± 0.022 **b**	0.739 ± 0.004 **a**	51.2 ± 0.3 **a**	37.4 ± 0.1 **a**	50.6 ± 0.1 **a**	12.0 ± 0.1 **a**	1.9 **b**	61.5 ± 1.2 **a**	36.5 ± 1.3 **a**
MOX	9.561 ± 0.016 **a**	0.745 ± 0.005 **a**	50.8 ± 0.3 **a**	37.6 ± 0.1 **a**	50.4 ± 0.2 **a**	12.0 **a**	1.2 **a**	57.2 ± 2.2 **a**	41.6 ± 2.2 **a**
				After 12 months					
TEST	11.695 **a**	0.829 **c**	44.2 **a**	39.2 **c**	47.3 **a**	13.6 **a**	1.4 **b**	43.1 ± 2.6 **b**	55.6 ± 2.6 **a**
MOX	11.934 ± 0.001 **b**	0.820 **b**	44.3 **a**	38.8 **b**	47.3 **a**	14.0 **b**	1.6 **c**	39.6 ± 0.9 **b**	58.8 ± 1.0 **a**
CHIPS	12.960 ± 0.003 **c**	0.758 ± 0.001 **a**	46.7 ± 0.1 **b**	36.7 **a**	48.4 **b**	14.9 **c**	0.4 ± 0.1 **a**	19.3 ± 0.7 **a**	80.3 ± 0.8 **b**

Table 35 – Colour parameters of Nero di Troia wine at racking, immediately after the treatment with oak chips (7 months) and the micro-oxygenation (8 months), and after 12 months of aging.
CI: colour intensity; T: tonality; dA(%): percentage of red color due to flavilium cation of free and combined anthocyanins; % yellow: percentage of yellow component; % red: percentage of red component; % blue: percentage of blue component; dAl%: percentage of absorbance at 520 nm due to monomeric anthocyanins; dAT%: percentage of absorbance at 520 nm due to polymeric pigments decolorized with SO_2; dTAT%: percentage of absorbance at 520 nm due to polymeric pigments not decolorized.
In column, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Compounds	t_r (min)	Mean concentration (AU x 10^5) ± SD (n=2)				Odour description
		T_0	T_6	C_6	M_6	
Acids						
Acetic acid	23.8	129 ± 22	144 ± 40	187 ± 77	93.3 ± 4.7	Vinegar
Hexanoic acid	35.2	21.3 ± 2.0	39.92 ± 0.56	42.8 ± 3.6	50.7 ± 3.6	Cheese, fatty, sour
Octanoic Acid	40.6	121 ± 16	212 ± 35	262.6 ± 5.7	334 ± 10	Fatty acid, dry, dairy
Nonanoic acid	43.2	27 ± 18	76 ± 42	50 ± 10	47 ± 14	Cheese, waxy
n-Decanoic acid	46.5	59 ± 20	115.6 ± 2.8	75.4 ± 7.1	212 ± 48	Fatty acid, dry, woody
Dodecanoic acid	56.0	7.96 ± 0.68	14.7 ± 5.1	9.29 ± 0.052	23.0 ± 5.3	Fatty
Total		365	588	584	853	
Percentage (%)		2.34	6.58	5.17	6.22	
Alcohols						
1-Propanol	9.8	9.1 ± 2.7	13.6 ± 7.2	17.0 ± 6.8	12.64 ± 0.44	Alcohol, ripe fruit
1-Propanol, 2-methyl-	11.5	138.0 ± 2.0	204 ± 58	263 ± 75	223.0 ± 2.3	Alcohol, solvent
1-Butanol	13.1	5.61 ± 0.80	6.6 ± 1.3	10.68 ± 0.86	7.95 ± 0.10	Medicinal, phenolic
1-Butanol, 3-methyl-	15.2	2590 ± 280	3460 ± 370	4636 ± 433	4598 ± 200	Fusel, alcohol, sweet, fruity
1-Pentanol, 4-methyl-	18.7	2.67 ± 0.15	4.48 ± 0.50	5.70 ± 0.24	6.876 ± 0.019	
1 Hexanol	20.0	51.0 ± 5.2	56.7 ± 3.1	79 ± 14	79.0 ± 8.7	Herbaceous
Heptanol	23.4	13.27 ± 0.18	13.54 ± 0.65	22.2 ± 1.2	22.94 ± 0.62	Oily; earthy/banana; butter; herbaceous; meaty
1-Hexanol, 2-ethyl	24.5	2.60 ± 0.11	14.87 ± 0.28	25.65 ± 0.13	20.16 ± 0.53	Oily; rose; sweet
2,3-Butanediol	26.3	55 ± 10	n.d.	n.d.	n.d.	Fruity
1-Octanol	26.6	23.4 ± 1.0	21.80 ± 0.43	29.4 ± 3.3	34.17 ± 0.47	Orange, floral
2,3-Butanediol	27.4	18.8 ± 4.1	n.d.	n.d.	n.d.	Fruity
2-Furanmethanol	30.0	1.489 ± 0.032	n.d.	n.d.	n.d.	
2-Octen-1-ol, 3,7-dimethyl-	32.7	18.64 ± 0.67	15.59 ± 0.22	13.16 ± 0.58	26.8 ± 1.4	
Benzyl Alcohol	36.1	3.86 ± 0.52	4.38 ± 0.50	4.06 ± 0.24	4.33 ± 0.43	Flowery, sweet

Phenylethyl Alcohol	37.0	682 ± 70	805 ± 40	1102 ± 47	1134 ± 30	Flowery, rose, honey
Total		3613	4621	6208	6170	
Percentage (%)		23.20	51.73	54.88	44.94	
Acetic Esters						
Isoamyl acetate	12.3	585 ± 34	480.0 ± 7.2	500.3 ± 1.5	754 ± 32	Banana
Acetic acid, hexyl ester	17.3	12.5 ± 1.7	5.904 ± 0.017	7.10 ± 0.37	13.38 ± 0.42	Apple; cherry; floral; pear; sweet
2-Phenethyl acetate	34.4	50.0 ± 3.2	36.3 ± 1.2	62.2 ± 3.0	64.000 ± 0.090	Floral
Total		647	522	569	831	
Percentage (%)		4.16	5.85	5.03	6.06	
Ethyl Esters						
Ethyl butyrate	9.8	10.14 ± 0.18	14.12 ± 0.80	17.35 ± 0.75	21.30 ± 0.90	Banana, pineapple, sweet, ethereal
Ethyl 2-methylbutyrate	10.2	n.d.	9.48 ± 0.60	6.68 ± 0.42	13.10 ± 0.40	fruity; green
Ethyl 3-methylbutyrate	10.7	n.d.	10.866 ± 0.078	10.35 ± 0.90	16.20 ± 0.30	apple
Ethyl pentanoate	12.7	3.40 ± 0.80	2.70 ± 0.30	3.27 ± 0.25	4.16 ± 0.25	apple
Ethyl hexanoate	16.0	680 ± 48	410 ± 10	395 ± 10	719 ± 32	apple; banana; wine-like
Ethyl heptanoate	19.4	33.0 ± 6.3	4.42 ± 0.65	7.66 ± 0.35	10.3 ± 1.2	Fruity
Ethyl lactate	19.9	12.34 ± 0.78	120 ± 10	139.4 ± 5.2	149.2 ± 3.0	Lactic
Ethyl octanoate	22.8	4820 ± 680	505 ± 50	730 ± 23	1421 ± 81	Banana, floral, pear, pineapple, wine-like
Ethyl nonanoate	25.9	113 ± 20	n.d	n.d.	43.2 ± 2.4	oily; fruity; nutty
Ethyl decanoate	29.0	3650 ± 450	190 ± 20	170.5 ± 8.5	660 ± 48	Grap, oily, wine-like
Diethyl succinate	30.3	206 ± 26	1370 ± 55	1858 ± 89	2103.2 ± 8.3	Spicy
Ethyl 9-decenoate	30.6	43.7 ± 7.5	n.d.	n.d.	n.d	
Ethyl undecanoate	31.9	12.4 ± 7.5	n.d.	18.2 ± 3.0	n.d.	coconut
Ethyl dodecanoate	34.8	565 ± 50	6.7 ±2.3	n.d.	27.6 ± 6.4	green; fruity; floral
Diethyl succinate	36.4	66.6 ± 1.3	125.4 ± 1.2	158 ± 30	215 ± 12	Spicy
Ethyl myristate	40.0	98 ± 13	27.214 ± 0.084	n.d.	42 ± 12	Waxy, soapy
Ethyl 4-ethoxybenzoate	43.4	12.210 ± 0.016	n.d.	n.d.	n.d.	
Ethyl hexadecanoate	45.4	200 ± 31	30.9 ± 1.0	n.d.	19.7 ± 4.3	Waxy

Ethyl hexadecenoate	46.4	35.35 ± 0.37	n.d.	n.d.	n.d.	Waxy
Ethyl hydrogen succinate	51.0	26.0 ± 4.8	142 ± 50	105.5 ± 5.4	119 ± 17	Chocolate
Ethyl oleate	55.0	11.0 ± 2.4	n.d.	n.d.	n.d.	
Ethyl linoleate	58.0	52.2 ± 0.9	n.d.	n.d.	n.d	
Total		10660	2969	3620	5587	
Percentage (%)		68.43	33.23	32.00	40.70	
Other Esters						
Methyl caprylate	21.3	12.8 ± 2.8	n.d.	n.d.	n.d.	Green; citrus; fruity
Isoamyl caproate	23.5	26.0 ± 5.8	n.d.	n.d.	n.d.	Apple; green; pineapple; sweet
Propyl caprylate	25.4	3.64 ± 0.50	n.d.	n.d.	n.d.	
Isobutyl caprylate	26.4	17.8 ± 1.1	n.d.	n.d.	n.d.	
Methyl caprynate	27.7	11.5 ± 2.1	n.d.	n.d.	n.d.	
Isoamyl caprylate	29.6	103 ± 12	n.d.	n.d.	n.d.	Fruity
Isoamyl caprate	35.2	40.6 ± 5.3	n.d.	n.d.	n.d.	
Total		216	0	0	0	
Percentage (%)		1.39	0	0	0	
Carbonyl Compounds						
5-Hepten-2-one, 6-methyl-	19.6	2.70 ± 0.17	n.d.	n.d.	n.d.	Oily; herbaceous; green
Nonanal	21.5	3.66 ± 0.16	n.d.	n.d.	n.d.	Apple, coconut, grapefruit, lemon
Benzaldehyde, 2,4-dimethyl-	34.4	n.d.	8.90 ± 0.47	40.16 ± 0.99	34.5 ± 1.7	Almond; cherry; spicy; sweet
Total		6	9	40	34	
Percentage (%)		0.04	0.10	0.36	0.25	
Hydrocarbons						
Tridecane	18.0	5.02 ± 0.40	n.d.	39.6 ± 2.2	2.93 ± 0.23	
Tetradecane	21.5	n.d.	3.90 ± 0.32	111 ± 14	25.8 ± 5.9	
2,6-Dimethyl 2,6-octadiene	29.7	7.9 ± 1.8	n.d.	n.d.	n.d.	
Total		13	4	151	29	

Percentage (%)		0.08	0.04	1.34	0.21	
Terpenes						
α-Terpineol	28.2	1.88 ± 0.29	3.23 ± 0.85	n.d.	n.d.	
Geraniol	35.0	7.05 ± 0.37	11.0 ± 5.5	n.d.	n.d.	Fruity, rose, sweet
D-Nerolidol	39.8	8.6 ± 2.4	8.84 ± 0.56	n.d.	17.4 ± 2.2	Apple, green, woody, citrus, rose
Farnesol	49.3	12.7 ± 6.5	6.188 ± 0.067	11.02 ± 0.13	9.07 ± 0.16	Anise; apricot; balsam
Total		30.20	29.24	11.02	26.46	
Percentage (%)		0.19	0.33	0.10	0.19	
Aromatics						
Benzene, 1,3-bis[1,1-dimethylethyl]-	22.5	n.d.	23.4 ± 1.7	21.3 ± 3.8	57.9 ± 1.4	
Oxime-, methoxy-phenyl-	32.4	13.0 ± 2.0	n.d.	n.d.	n.d.	
2,4-di-t-Butylphenol	47.8	9.9 ± 1.4	141 ± 12	27.40 ± 0.85	232 ± 30	
Total		23	165	49	289	
Percentage (%)		0.15	1.85	0.43	2.11	
Lactones						
Butyrolactone	29.4	3.78 ± 0.77	9.53 ± 0.18	35.5 ± 1.0	n.d.	Woody
Percentage (%)		0.02	0.11	0.21	0	

Table 36 - Volatile compounds (AU x 10^5) isolated from Nero di Troia wines at racking (T_0) and after 12 months of aging in untreated wines (T_6), wines treated with oak chips (C_6), and micro-oxygenated wines (M_6) (mean ± standard deviation).
n.d.: not detected.
Odour descriptors were obtained from Capone *et al*. (2013) and SAFC "Flavors and Fragrances. European Ed. Catalogue 2007–2008". Percentages are referred to the volatile compounds respect to each chemical group in different packaging.

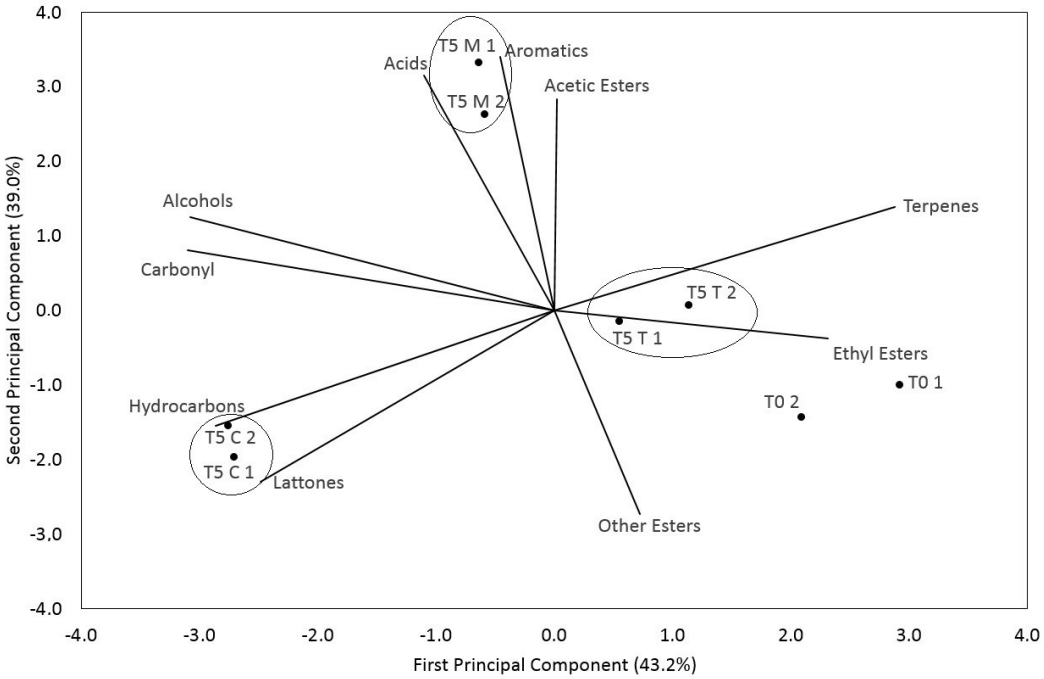


Figure 14 - Principal component analysis of volatile compounds at racking (T_0) and after 12 months of aging wine in untreated wines (T_5T), wines treated with oak chips (T_5C), and micro-oxygenated wines (T_5M).

Sensory descriptors	*After the treatment with oak chips*		*After the micro-oxygenation*		*After 12 months*		
	TEST	CHIPS	TEST	MOX	TEST	MOX	CHIPS
Visual descriptors							
Clearness	2.1 ± 0.2 **a**	1.9 ± 0.5 **a**	2.0 **a**	2.1 ± 0.2 **a**	2.0 **a**	2.0 **a**	2.0 **a**
Texture	2.8 ± 0.4 **a**	2.5 ± 0.8 **a**	2.0 **a**	2.2 ± 0.3 **a**	2.5 ± 0.4 **a**	2.6 ± 0.5 **a**	2.5 ± 0.4 **a**
Olfactory descriptors							
Olfactory intensity	2.7 ± 0.9 **a**	2.9 ± 0.7 **a**	2.0 **a**	2.8 ± 0.3 **b**	2.8 ± 0.3 **a**	2.1 ± 0.5 **a**	2.8 ± 0.6 **a**
Olfactory complexity	2.4 ± 0.5 **a**	2.8 ± 0.4 **a**	1.6 ± 0.2 **a**	3.0 **b**	2.5 ± 0.4 **a**	2.4 ± 0.6 **a**	2.5 ± 0.6 **a**
Olfactory quality	2.8 ± 0.7 **a**	3.1 ± 0.7 **a**	1.8 ± 0.3 **a**	3.0 **b**	2.9 ± 0.3 **a**	2.6 ± 0.5 **a**	2.5 ± 0.6 **a**
Gustatory-olfactory descriptors							
Sugars	0.0 **a**	0.0 **a**	0.0 **a**	0.0 **a**	0.0 **a**	0.0 **a**	0.0 **a**
Alcohols	3.0 ± 0.8 **a**	2.8 ± 0.7 **a**	2.1 ± 0.2 **a**	2.8 ± 0.3 **b**	2.8 ± 0.5 **a**	2.6 ± 0.5 **a**	2.9 ± 0.3 **a**
Polyols	2.2 ± 0.3 **a**	2.1 ± 0.2 **a**	1.8 ± 0.3 **a**	2.7 ± 0.3 **b**	2.1 ± 0.3 **a**	2.1 ± 0.3 **a**	1.9 ± 0.3 **a**
Acids	1.9 ± 0.8 **a**	2.5 ± 0.5 **a**	2.0 **a**	2.0 **a**	1.6 ± 0.5 **a**	2.1 ± 0.3 **a**	1.6 ± 0.5 **a**
Tannins	2.6 ± 0.7 **a**	2.6 ± 0.9 **a**	3.1 ± 0.2 **b**	2.4 ± 0.4 **a**	2.8 ± 0.5 **a**	2.9 ± 0.6 **a**	2.6 ± 0.8 **a**
Minerals	1.9 ± 0.4 **a**	2.2 ± 0.4 **a**	2.0 **a**	1.9 ± 0.2 **a**	1.8 ± 0.5 **a**	2.0 ± 0.7 **a**	1.9 ± 0.5 **a**
Structure	2.8 ± 0.7 **a**	3.0 ± 0.6 **a**	2.2 ± 0.3 **a**	2.2 ± 0.3 **a**	2.6 ± 0.5 **a**	3.0 **a**	2.6 ± 0.5 **a**
Balance	2.4 ± 0.5 **a**	2.5 ± 0.5 **a**	2.0 **a**	2.7 ± 0.3 **b**	2.9 ± 0.3 **a**	2.6 ± 0.5 **a**	3.0 ± 0.4 **a**
Gustatory-olfactory intensity	2.8 ± 0.9 **a**	3.2 ± 0.7 **a**	2.3 ± 0.3 **a**	2.3 ± 0.4 **a**	2.9 ± 0.3 **a**	2.8 ± 0.5 **a**	2.9 ± 0.3 **a**
Gustatory-olfactory persistence	2.8 ± 0.8 **a**	2.9 ± 0.8 **a**	2.6 ± 0.5 **a**	2.9 ± 0.2 **a**	2.9 ± 0.3 **a**	2.5 ± 0.4 **a**	2.8 ± 0.6 **a**
Gustatory-olfactory quality	2.8 ± 0.6 **a**	2.8 ± 0.4 **a**	2.0 **a**	2.7 ± 0.7 **b**	2.9 ± 0.3 **a**	2.6 ± 0.5 **a**	2.8 ± 0.6 **a**
Final considerations							
Evolutionary state	1.8 ± 0.8 **a**	2.0 ± 0.9 **a**	2.0 ± 0.0 **a**	2.2 ± 0.4 **a**	2.6 ± 0.5 **a**	2.5 ± 0.6 **a**	2.9 ± 0.6 **a**
Harmony	2.8 ± 0.8 **a**	3.3 ± 0.8 **a**	2.0 ± 0.0 **a**	2.9 ± 0.2 **b**	3.1 ± 0.9 **a**	2.8 ± 0.6 **a**	3.0 ± 0.9 **a**

Table 37 – Sensory characteristics of Nero di Troia wine immediately after the treatment with oak chips (7 months) and the micro-oxygenation (8 months), and after 12 months of aging. In row, among the same time of aging, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

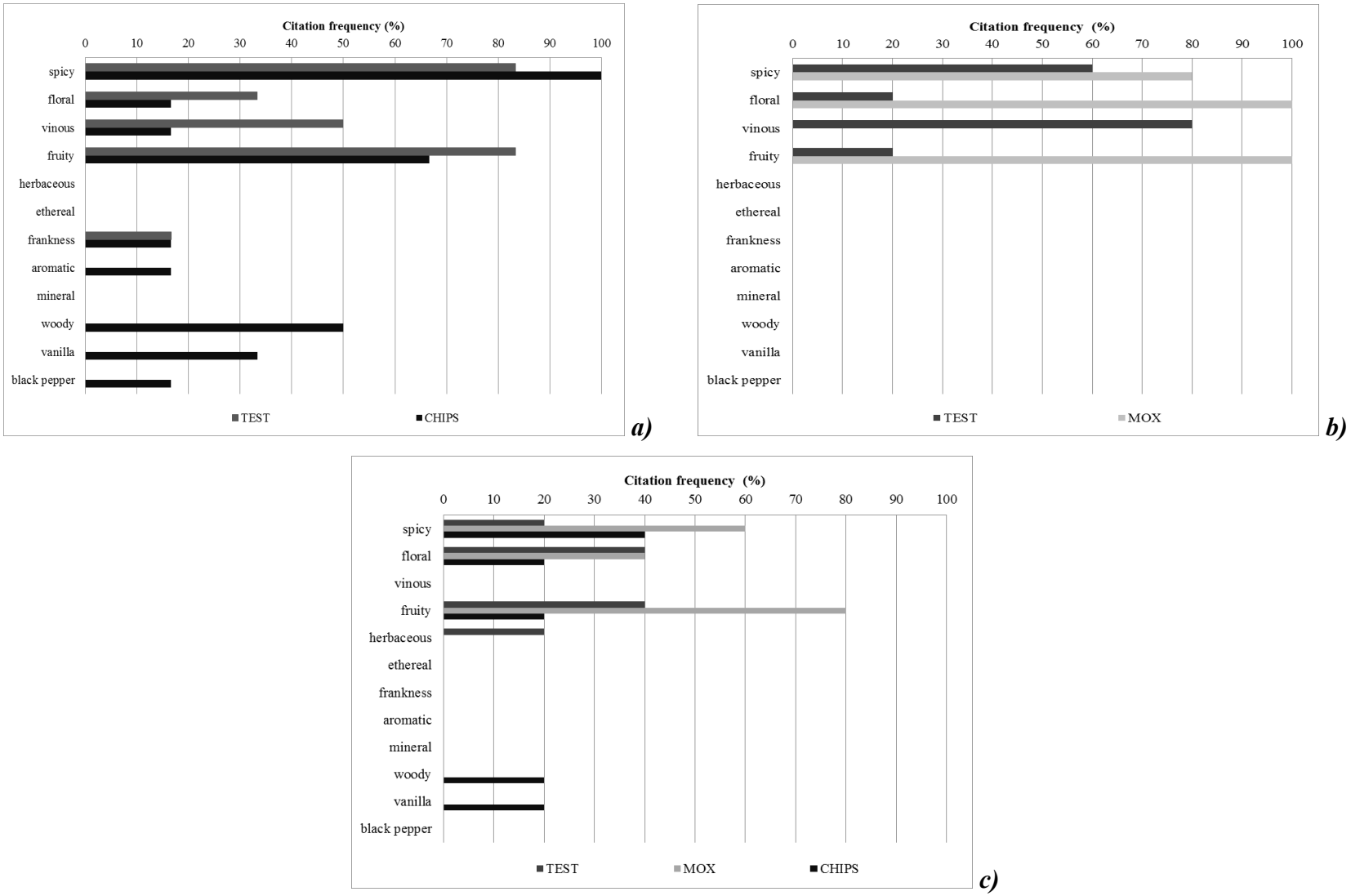


Figure 15 – Citation frequency (%) of flavour perceived in Nero di Troia wines immediately after the treatment with oak chips (7months) (*a*) and the micro-oxygenation (8 months) (*b*), and at 12 months of aging (*c*).

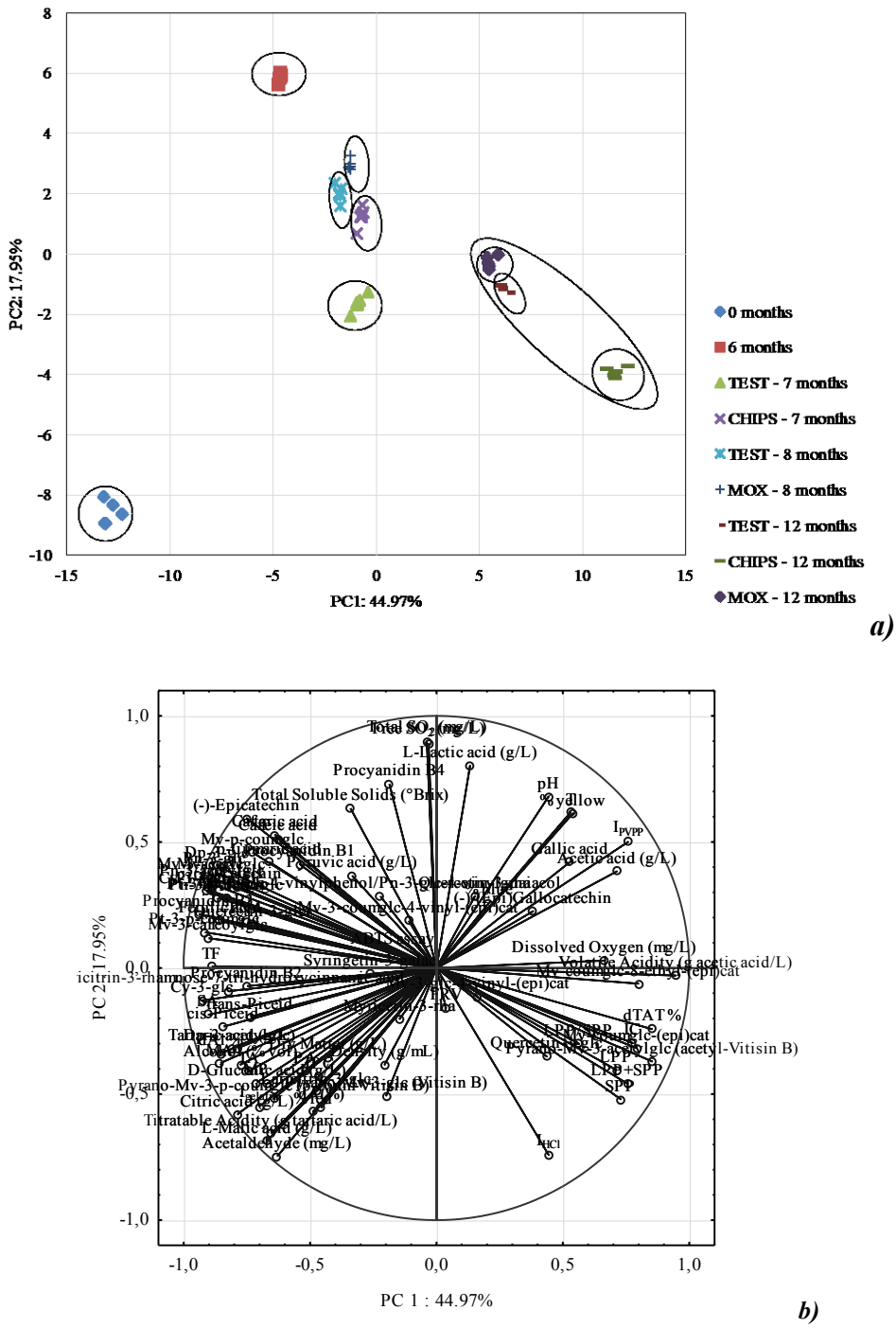


Figure 16 - PCA scatter plot for projection on the factor plane of: **a)** Nero di Troia wine samples at different stages of aging process and **b)** analytical results.

7.3 Influence of winemaking technologies on chemical and sensorial parameters in native wines

Some important physico-chemical parameters of oenological interest concerning wines produced from Uva di Troia grapes through different vinification technologies are reported in Table 38 with their statistical evaluation. Wines produced through cryomaceration (C) showed the highest alcohol strength while extended macerated wines (PM) exhibited the lowest alcohol content. According to Puertas *et al.* (2013), the effect of cryomaceration on this parameter may be related with the low temperatures occurred during dry ice maceration, which may inhibit autochthonous micro-flora, favouring the whole implantation of commercial inoculated yeast. Analogous results were obtained by Gordillo *et al*. (2010) and Álvarez *et al*. (2006) for Tempranillo and Monastrell wines. Concerning the lowest alcohol content of wine produced by extended maceration, similar results were highlighted by Gambacorta *et al*. (2011) and cannot be explained on the basis of oxidation reactions of ethanol that general occurr by film yeasts (Fugelsang, 1997) or by a coupled autoxidation of phenolics present in wine (Wildenradt and Singleton, 1971) since it was not accompanied by the increase of acetaldehyde content. The lowest alcohol content could be due to a slow fermentation activity. The volatile acidity was very low, due to the antiseptic effect of sulphur dioxide, and without significant differences among wines produced according to the different vinification procedures. As reported by Coletta *et al*. (2013) for Negramaro wine and by Baiano *et al.* (2013) for Falanghina and Bombino bianco wines, titratable acidity was significantly lower in the wines produced through cryomaceration, which also showed the highest values of pH. In fact, solubility of tartaric acid, which strongly contributes to wine acidity, decreased at low temperature, although, as discussed hereafter, the concentration of tartaric acid did not exhibit significant differences among T and C wines (Table 40). This result was in agreement with the finding of Puertas *et al*. (2013), who observed that cryomaceration affected titatrable acidity and pH in Tempranillo musts but did not influence their tartaric acid concentration. A low titratable acidity was also detected in wines obtained through extended maceration, probably as a result of oxidation-reduction reactions taking place (Stegăruş *et al.,* 2014) or as a consequence of the prolonged contact of must with the pomace that induced tartaric precipitation (Gambacorta *et al*., 2011). Free and total sulphur dioxide contents, density, and total soluble solids were not affected by the vinification technologies.

Acetaldehyde, whose levels in red wines generally range from 4 to 212 mg/L (Liu and Pilone, 2000) with an average value of 30 mg/L (McCloskey and Mahaney, 1981), is one of the major metabolic intermediates in alcoholic fermentation by yeasts (Margalith, 1981). For example, *Saccharomyces cerevisiae* produces 0.5–286 mg/L (Liu and Pilone, 2000). At low levels, acetaldehyde gives a pleasant fruity aroma, whereas at high concentrations it produces a green, grassy or apple-like off-flavour. In the present study (Table 39), all the samples showed acetaldehyde concentrations lower than the flavour threshold, which amounts to 100–125 mg/L (Zoecklein *et al*., 1995). More specifically, the wines produced through extended maceration exhibited the lowest concentration of acetaldehyde. In fact, during fermentation, there is a rapid accumulation of acetaldehyde when the rate of carbon dissimilation is at its maximum; after that time, it falls to low levels. The wines from cryomacerated grapes showed the highest acetaldehyde content. The wines produced through extended maceration also exhibited the highest concentration of dissolved oxygen (due to the slight micro-oxygenation produced by the punching-down performed until the racking) and a high value of the redox potential.

Table 40 illustrates the organic acid composition of Nero di Troia wines. Wines produced through extended maceration showed the lowest concentration of tartaric acid for the reason already explained. The concentration of L-malic acid was not influenced by the vinification procedures while Baiano *et al*. (2013) and Carillo *et al*. (2011) found a higher malic acid concentrations in white wines produced through cryomaceration. The highest concentrations of citric acid were found in wines from cryomacerated grapes due to a higher solubilisation effect. The D-gluconic acid was mostly detected in extended maceration and it corresponded to the lowest alcohol content. This finding could be explained by the presence of moulds that metabolized part of the glucose present in grape juice, thus producing this organic acid.

Data concerning the phenolic composition of the wines are reported in Tables from 41 to 43. In particular, Table 41 concerns the concentrations of specific phenolic classes while Table 42 described the distribution of anthocyanins among monomeric and polymeric forms. The data reported in Table 41 highlight that the wines produced through extended maceration had the lowest concentration of all the considered phenolic classes, and the lowest antioxidant activity. These results disagree with the finding of previous studies that highlighted a greater extraction of phenolic compounds in wines with longer skin maceration times (Francesca *et al*., 2014; Gómez-Plaza *et al.*, 2001). In the present

experimental work, the generalized decrease of phenolic compound concentration could be related to the longer contact of wine with oxygen with respect to the other trials. The other vinification procedures gave the same results for all the considered parameters with the exception of the concentration of proanthocyanidins, which was higher in wines obtained from cryomacerated grapes (in agreement with Alexaidre *et al*., 2012) and from grapes to whom pectolytic enzymes were added (in agreement with Favre *et al*., 2014). This behaviour could be explained considering that proanthocyanidins are located in skin and seeds. The extraction of skin proanthocyanidins, which are contained in the vacuoles of skin cells, requires the cells walls to be broken (physically by cryo-maceration, or enzymatically, by pectolytic enzymes) to allow their vacuole content to be extracted and to diffuse into the wine. According to the results of Table 42, the wine obtained through prolonged maceration showed the lowest concentrations of monomeric and small polymeric anthocyanins (the latter together with wines from grapes added with pectolytic enzymes), while no significant differences were highlighted for large polymeric anthocyanins. The highest LPP/SPP ratio was found in wines from grapes added with pectolytic enzymes. These data are in agreement with Bucelli *et al*. (2006), who found that, in Sangiovese wine, the treatment with enzymes had no effect on the extraction of anthocyanins during fermentation but allowed the extraction of greater amounts of tannins, thus leading to the formation of more stable polymerized pigments. Table 42 also concerns gelatin, hydrochloric, and PVPP indices that did not show significant differences among samples. Table 43 illustrates the specific phenolic profile of wines. The wines obtained through prolonged maceration were characterized by the lowest concentrations of flavonols, flavan-3-ols, and anthocyanins (about -26, -17, -24%, respectively, in comparison with the control wines).

Concerning the colour parameters (Table 44), the wines produced through extended maceration exhibited a low colour intensity consequent to the low anthocyanin concentration (in particular of monomeric and small polymeric pigments) while the wines from traditional vinification showed the highest colour intensity. According to the results concerning the LPP/SPP ratio, the wines from grapes added with enzymes presented the highest values of absorbance percentage due to polymeric pigments decolorized with SO_2 (dAT%).

In order to gain a better comprehension of the whole variability of the results, PCA was applied to the whole data set. The first two PCs explained almost 73% of the total

variability of Nero di Troia wines (Figure 17*a*). PCA was suitable to visualise the principal groupings, and the samples were clearly grouped according to the vinification procedure. The products obtained through traditional wine-making, cryo-maceration, and treatment with pectolytic enzymes showed similar values of the first principal component, thus indicating that the application of these winemaking technologies conferred similar characteristics to the resulting wines, which were, instead, distinguishable for the values of the second component. The weights of each original variable (eigenvectors) in the PCA were determined, in order to highlight the analytical indices significantly related to the PCs. The corresponding loading plots (Figure 17*b*) showed that all the indices, except those immediately around the centre of the plan (such as volatile acidity, citric acid, dA%, % red, LPP/SPP, caftaric acid+caffeic acid, *p*-coumaric acid) could be employed for discriminating the wine samples.

Sample	Alcohol (% vol)	Density (g/mL)	Dry Matter (g/L)	Volatile Acidity (g acetic acid/L)	Titratable Acidity (g tartaric acid/L)	pH	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Total Soluble Solids (°Brix)
T	13.11 ± 0.04 **b**	0.994 **b**	29.9 ± 0.2 **b**	0.25 ± 0.03 **a**	6.77 ± 0.03 **b**	3.48 ± 0.01 **a**	21.4 ± 1.8 **a**	28.0 ± 2.7 **a**	7.6 ± 0.1 **a**
C	13.26 ± 0.06 **c**	0.994 **a**	29.1 ± 0.2 **a**	0.23 ± 0.03 **a**	6.20 ± 0.11 **a**	3.55 ± 0.01 **b**	20.4 ± 2.8 **a**	26.4 ± 2.3 **a**	7.7 **a**
E	13.05 **b**	0.994 **b**	29.8 ± 0.1 **ab**	0.27 ± 0.03 **a**	6.78 ± 0.08 **b**	3.47 ± 0.01 **a**	21.6 ± 1.1 **a**	26.4 ± 3.4 **a**	7.6 **a**
PM	12.88 ± 0.04 **a**	0.995 **b**	29.4 ± 0.8 **ab**	0.24± 0.02 **a**	6.40 ± 0.18 **a**	3.50 ± 0.02 **a**	22.4 **a**	28.8 **a**	7.7 ± 0.1 **a**

Table 38 – Effect of winemaking technologies on physicochemical characteristics of Nero di Troia wines at first racking.
T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: prolonged maceration.
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Sample	Acetaldehyde mg/L)	E_H (mV)	Dissolved O_2 (mg/L)
T	54 ± 2 **bc**	135.3 ± 11.8 **b**	1.27 ± 0.03 **a**
C	56 **c**	112.5 ± 0.4 **a**	1.25 ± 0.01 **a**
E	51 ± 2 **b**	111.9 ± 0.2 **a**	1.21 ± 0.02 **a**
PM	31 **a**	124.7 ± 0.5 **ab**	2.12 ± 0.14 **b**

Table 39 – Effect of winemaking technologies on acetaldehyde content, redox potential and dissolved oxygen of Nero di Troia wines at first racking.
T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: prolonged maceration.
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Sample	Tartaric acid	L-Malic acid	L-Lactic acid	Acetic acid	Citric acid	Pyruvic acid	D-Gluconic acid
T	2.14 ± 0.07 **b**	1.81 ± 0.09 **a**	0.07 ± 0.01 **ab**	0.18 ± 0.01 **a**	0.22 ± 0.01 **a**	0.03 **b**	0.11 ± 0.02 **a**
C	2.18 ± 0.04 **b**	1.72 ± 0.06 **a**	0.08 ± 0.01 **b**	0.19 ± 0.01 **a**	0.27 ± 0.01 **b**	0.05 **d**	0.12 **a**
E	2.12 ± 0.01 **b**	1.77 ± 0.07 **a**	0.06 **ab**	0.21 ± 0.01 **b**	0.24 **ab**	0.04 **c**	0.13 ± 0.01 **a**
PM	1.87 **a**	1.77 ± 0.08 **a**	0.05 **a**	0.19 ± 0.01 **a**	0.23 ± 0.03 **a**	0.02 **a**	0.16 **b**

Table 40 – Effect of winemaking technologies on organic acids content of Nero di Troia wines at first racking (data are expressed as g per L of wine).
T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: prolonged maceration.
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Sample	TA	SA	MA	TF	FNA	FRV	PA	TPC	ABTS assay
T	326 ± 44 **b**	437 ± 32 **b**	126 ± 20 **b**	2444 ± 144 **b**	1970 ± 108 **ab**	2474 ± 166 **b**	3808 ± 104 **a**	3386 ± 176 **b**	20.09 ± 1.63 **b**
C	364 ± 31 **b**	474 ± 2 **b**	148 ± 20 **b**	2595 ± 33 **b**	2065±16 **b**	2590 ± 203 **b**	4196 ± 258 **b**	3470 ± 43 **b**	19.86 ± 1.43 **b**
E	353 ± 26 **b**	435 ± 26 **b**	127 ± 12 **ab**	2406 ± 87 **b**	1892 ± 82 **b**	2577 ± 107 **b**	4276 ± 122 **b**	3419 ± 36 **b**	19.48 ± 1.92 **b**
PM	226 ± 4 **a**	307 **a**	104 ± 7 **a**	2046 ± 57 **a**	1716 ± 51 **a**	2207 ± 149 **a**	3795 ± 46 **a**	2875 ± 97 **a**	15.80 ± 2.47 **a**

Table 41 – Effect of winemaking technologies on phenolic composition and antioxidant activity of Nero di Troia wines at first racking (data are expressed per L of wine).
TA: total anthocyanins (as mg malvidin-3-glucoside/L); SA: anthocyanins sensitive to SO_2 (as mg malvidin-3-glucoside/L); MA: monomeric anthocyanins (as malvidin-3-glucoside mg/L); TF: total flavonoids (as mg (+)-catechin/L); FNA: flavonoids different from anthocyanins (as mg (+)-catechin/L); FRV: flavans reactive with vanillin (as mg (+)-catechin/L); PA: proanthocyanidins (as mg cyanidin chloride/L); TPC: total phenolic compounds (as mg gallic acid/L); ABTS assay: antioxidant activity by ABTS assay (as mmol Trolox eq/L).
T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: prolonged maceration.
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Sample	MP	SPP	LPP	LPP/SPP	$I_{gelatin}$	I_{HCl}	I_{PVPP}
T	0.70 ± 0.04 **b**	0.27 ± 0.03 **b**	0.22 ± 0.03 **a**	0.73 ± 0.19 **a**	47.3 ± 5.1 **a**	39.2 ± 8.8 **a**	70.9 ± 5.0 **a**
C	0.69 ± 0.03 **b**	0.23 ± 0.02 **ab**	0.15 ± 0.02 **a**	0.75 ± 0.02 **a**	41.5 ± 2.4 **a**	32.8 ± 3.3 **a**	68.8 ± 4.3 **a**
E	0.66 ± 0.04 **ab**	0.20 **a**	0.23 ± 0.02 **a**	1.16 ± 0.12 **b**	39.3 ± 5.8 **a**	33.7 ± 4.0 **a**	70.7 ± 4.2 **a**
PM	0.58 ± 0.02 **a**	0.22 ± 0.02 **a**	0.19 ± 0.02 **a**	0.92 ± 0.16 **ab**	45.0 ± 7.7 **a**	37.0 ± 2.2 **a**	66.2 ± 2.2 **a**

Table 42 – Effect of winemaking technologies on structure indices and polymeric pigments of Nero di Troia wines at racking.
MP: absorbance at 520 nm due to monomeric pigments; SPP: absorbance at 520 nm due to small polymeric pigments; LPP: absorbance at 520 nm due to large polymeric pigments; LPP/SPP: ratio between large and small polymeric pigments; $I_{gelatin}$: gelatin index; I_{HCl}: hydrochloric acid index; I_{PVPP}: PVPP index.
T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: extended maceration.
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

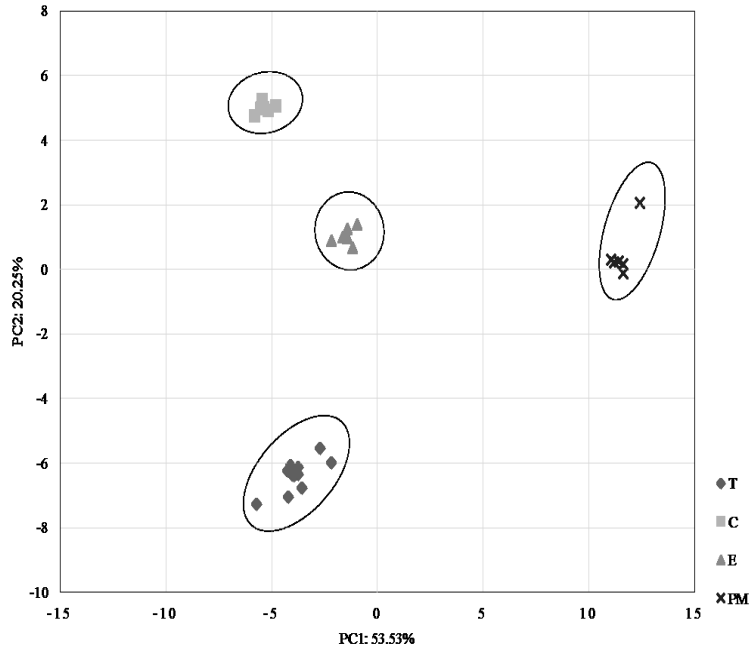
Phenolic compounds	MS (*m/z*)	MS–MS fragments (*m/z*)	T	C	E	PM
Phenolic acids and derivatives (mg GAE/L; mg CAE/L)	$[M-H]^-$					
Gallic acid	169	125	24.92 ± 0.61 **b**	24.40 ± 0.38 **ab**	23.05 ± 0.31 **a**	27.26 ± 0.22 **c**
2-S-Glutathionyl-Caftaric acid	616	484, 440, 272	0.20 ± 0.01 **ab**	0.21 ± 0.01 **b**	0.19 ± 0.01 **a**	0.19 ± 0.01 **ab**
Caftaric acid	311	179, 149	19.09 ± 1.73 **a**	18.93 ± 0.17 **a**	17.41 ± 0.06 **a**	19.80 ± 0.25 **a**
Caffeic acid	179	135				
p-Coumaric acid	163	119	8.01 ± 0.86 **a**	7.86 ± 0.06 **a**	7.20 ± 0.03 **a**	7.75 ± 0.25 **a**
Ferulic acid	193	178, 149, 134	7.27 ± 0.81 **a**	7.24 ± 0.13 **a**	7.11 ± 0.02 **a**	6.13 ± 0.77 **a**
Σ Phenolic acids and derivatives			**59.49 ± 4.01 a**	**58.64 ± 0.75 a**	**54.97 ± 0.41 a**	**61.14 ± 1.05 a**
Stilbens (mg RE/L)	$[M-H]^-$					
cis-Piceid	389	227	2.99 ± 0.30 **a**	3.23 ± 0.06 **a**	2.91 ± 0.01 **a**	2.80 ± 0.02 **a**
trans-Piceid	389	227	3.02 ± 0.17 **a**	3.23 ± 0.66 **a**	2.87 **a**	3.61 ± 0.71 **a**
Σ Stilbens			**6.01 ± 0.47 a**	**6.46 ± 0.60 a**	**5.78 ± 0.02 a**	**6.41 ± 0.69 a**
Flavonols (mg QE/L)	$[M-H]^-$					
Myricetin-3-glc	479	316/317	2.86 ± 0.11 **c**	2.57 ± 0.07 **b**	2.43 ± 0.07 **b**	2.04 ± 0.12 **a**
Myricetin-3-glcr	493	317	5.10 ± 0.35 **b**	5.12 **b**	4.47 ± 0.01 **b**	3.62 ± 0.45 **a**
Myricetin-3-galac	479	316/317	18.35 ± 1.11 **b**	14.57 ± 0.12 **a**	16.71 ± 0.50 **ab**	14.82 ± 1.13 **ab**
Myricetin-3-rha	463	317	nd **a**	nd **a**	nd **a**	nd **a**
Quercetin-3-glc	463	301	2.18 ± 0.17 **b**	1.71 ± 0.04 **a**	1.70 **a**	1.68 ± 0.04 **a**
Quercetin-3-glcr	477	301	12.70 **c**	11.42 ± 0.08 **b**	11.12 ± 0.22 **b**	8.02 ± 2.26 **a**
Quercetin-3-galac	463	301	7.69 ± 1.32 **a**	5.29 ± 0.03 **a**	7.55 ± 0.07 **a**	6.85 ± 0.39 **a**
Laricitrin-3-glc	493	331	4.67 ± 0.18 **c**	3.76 ± 0.02 **a**	4.20 ± 0.05 **b**	3.86 ± 0.17 **ab**
Quercetin-3-rha	447	301	9.28 ± 0.77 **a**	8.49 ± 0.17 **a**	8.72 ± 0.05 **a**	7.38 ± 0.35 **a**
Isorhamnetin-3-glc	477	315	7.28 ± 1.24 **b**	6.25 ± 0.16 **b**	7.11 ± 0.01 **b**	4.99 ± 0.02 **a**
Syringetin-3-galac	507	344/345	10.09 ± 0.19 **b**	10.07 ± 0.14 **b**	9.55 ± 0.19 **b**	7.73 ± 0.70 **a**
Laricitrin-3-rhamnose-7-tri-hydroxycinnamic acid	655	509, 501, 475, 347, 329, 314, 303	7.28 ± 1.24 **a**	6.25 ± 0.16 **a**	7.11 ± 0.01 **a**	4.99 ± 0.02 **a**
Σ Flavonols			**86.69 ± 5.41 c**	**75.17 ± 0.27 b**	**79.59 ± 0.39 bc**	**65.43 ± 2.06 a**
Flavan-3-ols (mg CE/L)	$[M-H]^-$					
(-)-(Epi)Gallocatechin	305	179, 125	31.03 ± 0.09 **c**	27.71 ± 0.16 **b**	26.56 ± 0.41 **a**	27.17 ± 1.62 **a**
Procyanidin B3	577	451, 425, 407, 289	67.29 ± 4.12 **b**	54.91 ± 1.09 **a**	51.92 ± 2.06 **a**	55.22 ± 5.93 **a**

(+)-Catechin	289	245, 205, 179	82.62 ± 3.18 **b**	75.69 ± 1.44 **b**	75.17 ± 3.33 **b**	69.66 ± 8.72 **a**
Procyanidin B1	577	451, 425, 407, 289	59.77 ± 1.81 **d**	54.85 ± 0.42 **c**	50.72 ± 0.67 **b**	45.65 ± 8.04 **a**
Procyanidin B4	577	451, 425, 407, 289	60.48 ± 2.80 **c**	56.39 ± 1.39 **bc**	52.75 ± 0.35 **ab**	56.24 ± 9.86 **a**
(-)-Epicatechin	289	245, 205, 179	104.79 ± 9.24 **b**	104.92 ± 2.49 **b**	103.42 ± 2.10 **b**	81.48 ± 12.52 **a**
Procyanidin B2	577	451, 425, 407, 289	23.35 ± 1.15 **a**	20.43 ± 1.60 **a**	20.15 ± 0.73 **a**	22.01 ± 2.61 **a**
Σ Flavan-3-ols			**429.32 ± 22.22 c**	**394.90 ± 8.60 bc**	**380.69 ± 4.74 ab**	**357.42 ± 44.08 a**
Anthocyanins (mg ME/L)	$[M-2H]^-$					
Dp-3-glc	463	301	4.43 ± 0.36 **b**	4.62 ± 0.06 **b**	4.07 ± 0.04 **b**	2.95 ± 0.03 **a**
Cy-3-glc	447	285	0.90 ± 0.12 **b**	0.91 ± 0.02 **b**	0.85 ± 0.01 **ab**	0.59 **a**
Pt-3-glc	477	315	6.77 ± 0.55 **b**	7.18 ± 0.05 **b**	6.33 ± 0.02 **b**	4.62 ± 0.13 **a**
Pn-3-glc	461	299	4.28 ± 0.38 **b**	4.28 ± 0.04 **b**	3.89 ± 0.03 **b**	2.81 **a**
Mv-3-glc	491	329	63.33 ± 3.23 **b**	65.90 ± 0.48 **b**	60.11 ± 0.36 **b**	47.32 ± 0.52 **a**
Dp-3-acetylglc	505	463, 301	1.98 ± 0.04 **c**	2.14 ± 0.03 **d**	1.85 ± 0.03 **b**	1.34 ± 0.01 **a**
Pyrano-Mv-3-glc (Vitisin B)	515	353	1.07 ± 0.06 **a**	1.01 ± 0.02 **a**	0.98 ± 0.02 **a**	1.53 ± 0.29 **b**
Cy-3-acetylglc	489	447,285	1.17 ± 0.08 **a**	1.15 ± 0.02 **a**	1.22 ± 0.07 **a**	1.09 ± 0.11 **a**
Pt-3-acetylglc	519	477, 315	4.55 ± 0.16 **b**	4.50 **b**	4.33 ± 0.11 **b**	3.73 ± 0.04 **a**
Pyrano-Mv-3-acetylglc (acetyl-Vitisin B)	557	353	3.32 ± 0.07 **b**	3.00 ± 0.03 **a**	2.90 **a**	3.08 ± 0.65 **ab**
Pn-3-acetylglc	503	299	9.43 ± 0.01 **b**	9.74 ± 0.18 **b**	9.05 ± 0.05 **ab**	7.61 ± 0.27 **a**
Mv-3-acetylglc	533	329	35.03 ± 1.58 **b**	36.04 ± 0.56 **b**	33.64 ± 0.02 **b**	27.56 ± 0.12 **a**
Pyrano-Mv-3-*p*-coumglc (*p*-coum-Vitisin B)	661	353	2.70 ± 0.27 **a**	2.71 ± 0.13 **a**	2.56 ± 0.03 **a**	2.32 ± 0.21 **a**
Mv-3-caffeoylglc	653	491, 329	2.31 ± 0.03 **b**	2.23 ± 0.06 **b**	2.26 ± 0.03 **b**	1.96 ± 0.06 **a**
Pt-3-*p*-coumglc	623	477, 315	4.12 ± 0.19 **b**	4.02 ± 0.06 **b**	3.97 ± 0.03 **b**	3.38 ± 0.04 **a**
Pn-3-*p*-coumglc	607	299	4.35 ± 0.15 **b**	4.60 ± 0.03 **b**	4.41 ± 0.11 **b**	2.47 ± 0.05 **a**
Mv-3-*p*-coumglc	637	491, 329	9.68 ± 0.90 **b**	10.14 ± 0.08 **b**	8.96 ± 0.12 **b**	6.39 ± 0.13 **a**
Σ Anthocyanins			**159.41 ± 7.06 b**	**164.17 ± 1.07 b**	**151.37 ± 0.31 b**	**120.75 ± 0.15 a**

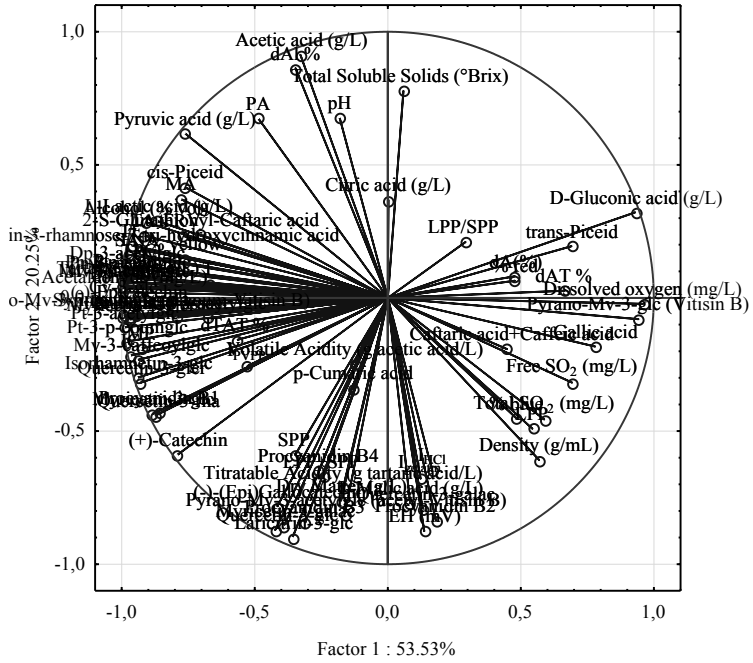
Table 43 – Effect of winemaking technologies on the phenolic profile of Nero di Troia wines at first racking.
nd: not detected.
T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: prolonged maceration.
glc: glucoside, glcr: glucuronide, rha: rhamnoside, galac: galactoside, Dp: delphinidin, Cy: cyanidin, Pt: petunidin, Pn: peonidin, Mv: malvidin, acetylglc: acetylglucoside, *p*-coumglc: *p*-coumaroylglucoside, caffeoylglc: caffeoylglucoside, cat: catechin.
In row, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Sample	CI	T	dA(%)	% yellow	% red	% blue	dAl%	dAT%	dTAT%
T	11.803 ± 0.098 **c**	0.549 ± 0.022 **a**	63.2 ± 1.5 **a**	31.6 ± 0.7 **a**	57.6 ± 1.0 **a**	10.8 ± 0.3 **a**	3.9 ± 0.4 **a**	52.3 ± 3.6 **a**	43.3 ± 3.7 **ab**
C	11.183 ± 0.001 **b**	0.552 **a**	63.1 **a**	31.8 **a**	57.5 **a**	10.7 **a**	5.9 ± 1.5 **b**	53.4 ± 3.4 **a**	40.7 ± 2.0 **ab**
E	11.088 ± 0.002 **b**	0.543 **a**	63.7 **a**	31.5 **a**	58.0 **a**	10.6 **a**	4.6 ± 0.8 **ab**	48.4 ± 4.4 **a**	47.1 ± 3.6 **b**
PM	8.530 ± 0.026 **a**	0.539 ± 0.012 **a**	63.7 ± 0.7 **a**	31.2 ± 0.4 **a**	57.9 ± 0.5 **a**	10.9 ± 0.1 **a**	4.3 ± 0.6 **ab**	58.1 ± 2.6 **a**	37.6 ± 3.1 **a**

Table 44 – Effect of winemaking technologies on colour parameters of Nero di Troia wines at first racking.
CI: colour intensity; T: tonality; dA(%): percentage of red colour due to flavilium cation of free and combined anthocyanins; % yellow: percentage of yellow component; % red: percentage of red component; % blue: percentage of blue component; dAl%: percentage of absorbance at 520 nm due to monomeric anthocyanins; dAT%: percentage of absorbance at 520 nm due to polymeric pigments decolorized with SO_2; dTAT%: percentage of absorbance at 520 nm due to polymeric pigments not decolorized.
T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: prolonged maceration.
In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.



a)



b)

Figure 17 - PCA scatter plot for projection on the factor plane of: **a)** Nero di Troia wine samples and **b)** analytical results.
T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: prolonged maceration.

7.4 Effect of the treatments with French oak chips performed before or after the malo-lactic fermentation on Nero di Troia wine quality

The main oenological parameters of the wines at racking and after 12 months of aging are listed in Table 44. At racking, wines were characterized by: medium alcohol content and dry matter; low values of volatile acidity and acetaldehyde concentration, free and total SO_2; high titratable acidity, concentrations of total soluble solids that indicate the correct progress of fermentation; low dissolved oxygen content, and redox potential values similar to those of wines stored in absence of air, to indicate that they were well protected against oxidation during fermentation. The values of SO_2 stated the high amount of active SO_2 (the free form). After 12 months of aging, few significant differences were observed among control and treated wines and concerned titatrable acidity, dissolved oxygen and redox potential. The last two parameters were in the following order: control > wine treated with oak chips after the malo-lactic fermentation > wine treated with oak chips before the malo-lactic fermentation. This behaviour was probably due to the ellagitannins extracted from oak wood, which are able to a fast absorption of the dissolved oxygen and to protect wine from an excessive oxidation (Vivas and Glories, 1996). Their effect increased as more precocious the addition of chips occurred. During aging, volatile acidity increased due to the AAB (acetic acid bacteria) metabolism, titatrable acidity decreased and pH increased due to the malo-lactic conversion from lactic acid bacteria, totable soluble solids increased probably due to an enrichment in polysaccharides derived from lactic acid bacteria metabolism, free and total sulphur dioxide content decreased as a consequence of oxidation, acetaldehyde decreased as consequence of the partecipation of ethanal in polymerization reactions involving wine phenolic compounds (Liu and Pilone, 2000), dissolved oxygen and red-ox potential decreased as a consequence of the in-bottle aging.

The organic acid profiles of the wines are described in Table 45. At racking, they were in a decreasing order: tartaric > malic > citric > acetic > D-gluconic > lactic and pyruvic. After 12 months of aging, the results highlighted the absence of any effect of the treatments with French oak chips on the organic acids composition of Nero di Troia wines. Concerning the effect of aging time, the concentration of tartaric acid remained almost invariate, while L-malic and citric acids were metabolized by lactic acid bacteria that produced L-lactic acid (Ribéreau-Gayon *et al*., 2000), the acetic acid content increased and the pyruvic and D-gluconic acids concentration decreased. The acetic acid generally derives from the

oxidation of ethanol by acetic acid bacteria but may also derive from lactic acid bacteria metabolism of citric acid during the malo-lactic fermentation (Ribéreau-Gayon *et al*., 2000). Pyruvic and α-ketoglutaric acids are secondary products of the alcoholic fermentation. They may combine SO_2 (Ribéreau-Gayon *et al*., 2000), and the decrease of pyruvic acid content could be due both to this phenomena and/or to the combination with anthocyanins to originate more stable pigments (such as vitisins) (Fulcrand *et al*., 1998).

Data concerning the phenolic composition of the wines are reported in Tables from 46 to 48. At racking, the concentrations of all the phenolic classes of these Nero di Troia wines were lower than those of the Nero di Troia wines use in the experiments concerning the effects of treatment with oak chips and of micro-oxygenation. In the oak-treated wines (independently on the time at which the treatments was applied), higher contents of monomeric and sensitive to SO_2 anthocyanins, of flavans reactive with vanillin, and of total phenolic compounds (only in wines treated after the malo-lactic fermentation) were detected with respect to the control. These results can be explained by the antioxidant activity of ellagitannins extracted from oak wood chips. This type of tannins acts as *oxygen-scavenger*, thus preserving anthocyanins and flavans from oxidation (Vivas and Glories, 1996; Guerra *et al*., 1996). These data indicated that the contact with oak chips didn't increase the anthocyanin polymerization in contrast with the findings of Del Alamo Sanza and Nevares Domínguez (2006) (who instead observed a faster decrease of anthocyanins in wine treated with oak chips with respect to the control) and with the results already discussed in sections 7.2.1, 7.2.2 and 7.2.3 for Aglianico, Montepulciano, and Nero di Troia wines. This behaviour could be due to the different tannins/anthocyanins ratio (T/A) found in the studied wines. Ribéreau-Gayon *et al*. (2004) reported that for a harmonious development of a wine, this ratio should be between 1 and 4, because a T/A ratio lower and/or equal to 1 means that the medium has a low tannin content and anthocyanins can be degradated, while a T/A ratio upper and/or equal to 4 means that the tannin concentration is much higher than the anthocyanin content, and then tannins polymerize between them, without the inclusion of anthocyanin whithin the molecule. In the Nero di Troia wines discussed in the present paragraph, at racking, this ratio was about 9, while those found in in Aglainico, Montepulciano, and the other Nero di Troia were 5, 2, and 4, respectively. During aging, all the phenolic classes suffered of strong decreases of concentration, except for total flavonoids and flavans reactive with vanillin. The antioxidant activity slightly increased with aging. Table 47 shows the distribution of

anthocyanins among monomeric and polymeric forms. After 12 months of aging, no significant differences were found among control and treated wines for monomeric, small polymeric, and large polymeric anthocyanins, thus confirming the absence of any polymerization effect due to the ellagitannins deriving from oak chips. Concerning the effects of aging, from racking to 12 months, the monomeric forms decreased, while the polymeric forms increased.

The treatment with oak chips didn't have effects on the structure indices, confirming the absence of polymerization between tannins and anthocyanins. The HCl index measure the state of polymerization of tannins in the wine. A wine with a high amount of highly polymerized tannins has a value of this index higher than 25 (Ribéreau-Gayon *et al*., 2004). From the data of Table 47, it was evident that already at racking this index was high (43.1 ± 2.5), as well as the PVPP index (70.1 ± 0.5) (which instead measure the degree of anthocyanin polymerization), thus indicating a high degree of polymerization of phenolic compounds. After 12 months of aging, in fact, HCl and PVPP indices changed little. After 12 months of aging, the gelatin index significantly decreased in all the wines. That change indicated the decrease of tannins responsible for astringency. In fact, during aging, tannins combined with each other, increasing their degree of polymerization.

The phenolic profiles of the wines (Table 48) further highlight the absence of a greater degree of polymerization. At racking, the most representative compounds, for each phenolic classes were: gallic acid (phenolic acids), myricetin-3-galactoside (flavonols), epicatechin, catechin, and procyanidin B3 (flavan-3-ols), malvidin-3-glucoside and malvidin-3-acetylglucoside, (anthocyanins). During the aging time, all the phenolic classes decreased, although the concentrations of phenolic acids and flavan-3-ols decreased less than the other. In particular, phenolic acids and derivatives decreased by about 2% in all the wines; stilbens decreased by about 45% in control wine, 54% in wines treated before the malolactic fermentation, and 53% in wines treated after the malolactic fermentation; flavonols decreased by 32.7%, 37.9% and 33.2% in control, wines treated before and wines treated after the malolactic fermentation, respectively; the decrement of flavan-3-ols was about -7%, -5%, and -3% in control, wines treated before, and wines treated after the malolactic fermentation; the total anthocyanin content decreased more than the other phenolics: -72.5% (control), -75% (wines treated before), and -72.8% (wines treated after).

After 12 months of aging, both the treated wines exhibited higher contents of (-)-(epi)gallocatechin, (+)-catechin, and procyanidin B1 than the control wines, although their

total flavan-3-ols content did not significantly differed between the control and the oak-treated samples. The control wines exhibited higher concentrations of acetyl-vitisin A and B-type vitisin of malvidin-3-glucoside, flavanyl-pyranoanthocyanins and anthocyanin/tannin complexes via acetaldehyde than the treated wines. Furthermore, control wines had the highest concentrations of delphinidin-3-acetylglucoside, malvidin-3-caffeoylglucoside, petunidin-3-*p*-coumaroylglucoside. Among the oak-treated samples, those treated with oak chips before the malolactic fermentation showed the highest content of delphinidin-3-acetylglucoside and malvidin-3-glucoside-4-vinyl-(epi)catechin (flavanyl-pyranoanthocyanins), whereas those treated after the malolactic fermentation showed the highest content in glucosidic forms of delphinidin, peonidin and malvidin, malvidin 3-acetylglucoside and 3-*p*-coumaroylglucoside, and acetyl-Vitisin A.

Table 49 reports the main colour parameters of Nero di Troia wines. As can been seen, few significant differences were found between the samples. In particular, the wine treated with oak chips after the malolactic fermentation showed the lowest values of absorbance due to polymeric pigments resistant to SO_2 bleaching and the highest values of absorbance due to polymeric pigments sensitive to SO_2 in agreement with the data concerning the anthocyanin sensitive to SO_2 (Table 46) and the low molecular weight polymeric pigments (Table 47). Observing the colour indexes at racking and after 12 months of storage, it was possible to notice that colour intensity did not suffer changes while tonality increased from approximately 0.5 to 0.7. The percentage of the various chromatic components suffered of changes due to the aging time: % red decreased while % yellow and % blue increased as a consequence of the decrement of free anthocyanins and the increase of vitisins, flavanyl-pyranoanthocyanins, and ethyl-linked derivatives, which shift colour from red to orange or purple tints and enhances colour intensity and resistance to pH changes and sulfite bleaching (Cheynier *et al.*, 2006). As expected, during aging, a reduction of the absorbance due to monomeric and polymeric pigments (both bleachable with sulphite) and an increase of the absorbance due to the unbleachable polymeric pigments (more stable) were observed.

Table 50 shows the results of the QDA applied to visual, olfactory and gustatory parameters of wines after 12 months of aging and highlights the absence of significant differences between control and treated wines. Concerning the frequency of citation of some selected flavours (Figure 18), the control wine showed a flavour profile characterised by floral, vinous, fruity, and ethereal notes. Nero di Troia wines in contact with oak chips

before or after malo-lactic fermentation had lower floral and fruity notes, and higher spicy and woody notes in comparison with the control samples, in agreement with the results observed by Gómez García-Carpintero *et al*. (2011; 2012) in Bobal and Moravia Agria wines treated with oak chips during the malolactic fermentation. The treatment with oak chips (both before and after the malolactic fermentation) determined the attenuation of the vinous attribute. Nevertheless, in disagreement with the finding of Gómez García-Carpintero *et al*. (2011), who stated that wines treated with oak chips during malolactic fermentation had a stronger oak character than wines treated after malolactic fermentation, the Nero di Troia wines treated before malolactic fermentation had softer oak and spicy characters, more fruity notes, and less ethereal character than the wines treated after malolactic fermentation.

In order to gain a better comprehension of the whole variability of the results, the Principal Components Analysis was applied to the whole data set. The first two principal components explained only 66% of the total variability of Nero di Troia wines (Figure 19). The wines were clearly grouped according to the vinification procedures. The control wines were grouped in the factor plane described by positive values of both PCs, while the wines obtained through the application of the treatment with oak chips before the malolactic fermentation were placed on the factor plane determined by positive values of PC1 and negative values of PC2, and the wines treated after the malolactic were grouped in the factor plane described by negative values of PC1 and positive values of PC2. Thus, through the choice of the time at which the treatment with oak chips must be made, it was possible to modify the quality parameters of Nero di Troia wines.

Sample	Alcohol (% vol)	Density (g/mL)	Dry Matter (g/L)	Vol. Ac.	Titr. Ac.	pH	Free SO_2 (mg/L)	Total SO_2 (mg/L)	TSS (°Brix)	Dissolved O_2 (mg/L)	E_H (mV)	Acetaldehyde (mg/L)
						At racking						
	12.96 ± 0.01	0.994	29.1 ± 0.1	0.28 ± 0.03	6.57 ± 0.04	3.50	25.6 ± 2.3	32.0 ± 2.3	6.7 ± 0.1	2.44 ± 0.10	128.0 ± 1.5	24.00 ± 0.02
						After 12 months						
Control	12.86 ± 0.16 **a**	0.993 ± 0.001 **a**	26.0 ± 2.1 **a**	0.65 **a**	5.51 ± 0.06 **ab**	3.75 ± 0.06 **a**	18.4 ± 1.1 **a**	25.2 ± 0.6 **a**	7.4 **a**	2.55 ± 0.13 **c**	51.0 ± 0.4 **b**	2.82 ± 0.07 **a**
BMLF	12.76 ± 0.17 **a**	0.994 ± 0.001 **a**	27.1 ± 1.1 **a**	0.68 ± 0.04 **a**	5.63 ± 0.02 **b**	3.76 ± 0.05 **a**	18.8 ± 0.6 **ab**	25.6 **a**	7.4 ± 0.1 **a**	0.76 ± 0.03 **a**	31.8 ± 3.3 **a**	2.50 ± 0.30 **a**
AMLF	12.81 ± 0.07 **a**	0.994 ± 0.001 **a**	26.9 ± 0.2 **a**	0.64 ± 0.03 **a**	5.38 ± 0.05 **a**	3.75 ± 0.02 **a**	20.8 **b**	27.2 ± 1.1 **a**	7.3 ± 0.1 **a**	1.67 ± 0.14 **b**	37.5 ± 6.7 **ab**	2.72 ± 0.28 **a**

Table 45 – Physicochemical characteristics of Nero di Troia wines at racking and after 12 months of aging.
Control: control wine; BMLF: wine treated with oak chips before the malo-lactic fermentation; AMLF: wine treated with oak chips after the malo-lactic fermentation.
Vol. Ac.: volatile acidity (g acetic acid/L); Titr. Ac.: titratable acidity (g tartaric acid/L); TSS: total soluble solids (°Brix).
In column, different letters indicate significant differences at $p < 0.05$ by LSD test.

Sample	Tartaric acid	L-Malic acid	L-Lactic acid	Acetic acid	Citric acid	Pyruvic acid	D-Gluconic acid
				At racking			
	2.11 ± 0.02	1.74 ± 0.01	0.06 ± 0.01	0.19 ± 0.01	0.25	0.034 ± 0.004	0.14 ± 0.01
				After 12 months			
Control	2.28 ± 0.23 **a**	nd **a**	1.29 ± 0.04 **a**	0.56 ± 0.01 **a**	0.13 ± 0.01 **a**	nd **a**	nd **a**
BMLF	2.15 ± 0.23 **a**	nd **a**	1.35 ± 0.18 **a**	0.54 ± 0.02 **a**	0.12 ± 0.02 **a**	nd **a**	nd **a**
AMLF	2.12 ± 0.09 **a**	nd **a**	1.21 ± 0.13 **a**	0.53 ± 0.01 **a**	0.12 ± 0.02 **a**	nd **a**	nd **a**

Table 46 – Organic acids content of Nero di Troia wines at racking and after 12 months of aging (data are expressed as g per L of wine).
nd: not detected.
Control: control wine; BMLF: wine treated with oak chips before the malo-lactic fermentation; AMLF: wine treated with oak chips after the malo-lactic fermentation.
In column, different letters indicate significant differences at $p < 0.05$ by LSD test.

Sample	TA	SA	MA	TF	FNA	FRV	PA	TPC	ABTS assay
					At racking				
	286 ± 30	384 ± 1	115 ± 2	1989 ± 57	1572 ± 99	2585 ± 133	4276 ± 93	3287 ± 128	17.71 ± 3.23
					After 12 months				
Control	139 ± 2 **a**	80 ± 5 **a**	24 ± 4 **a**	1776 ± 49 **a**	1574 ± 46 **a**	1084 ± 84 **a**	3888 ± 212 **a**	2899 ± 122 **a**	20.40 ± 2.79 **a**
BMLF	138 ± 6 **a**	88 ± 3 **ab**	29 ± 6 **ab**	1818 ± 130 **a**	1617 ± 131 **a**	1232 ± 112 **b**	3908 ± 160 **a**	2831 ± 185 **a**	21.29 ± 3.01 **a**
AMLF	147 ± 7 **a**	102 ± 7 **b**	32 ± 1 **b**	1847 ± 219 **a**	1633 ± 211 **a**	1229 ± 114 **b**	3668 ± 139 **a**	3433 ± 184 **b**	21.03 ± 2.08 **a**

Table 47 – Phenolic composition and antioxidant activity of Nero di Troia wines at racking and after 12 months of aging.
TA: total anthocyanins (as mg malvidin-3-glucoside/L); SA: anthocyanins sensitive to SO_2 (as mg malvidin-3-glucoside/L); MA: monomeric anthocyanins (as malvidin-3-glucoside mg/L); TF: total flavonoids (as mg (+)-catechin/L); FNA: flavonoids different from anthocyanins (as mg (+)-catechin/L); FRV: flavans reactive with vanillin (as mg (+)-catechin/L); PA: proanthocyanidins (as mg cyanidin chloride/L); TPC: total phenolic compounds (as mg gallic acid/L); ABTS assay: antioxidant activity by ABTS assay (as mmol Trolox eq/L).
Control: control wine; BMLF: wine treated with oak chips before the malo-lactic fermentation; AMLF: wine treated with oak chips after the malo-lactic fermentation.
In column, different letters indicate significant differences at $p < 0.05$ by LSD test.

Sample	MP	SPP	LPP	LPP/SPP	$I_{gelatin}$	I_{HCl}	I_{PVPP}
				At racking			
	0.61 ± 0.02	0.26 ± 0.03	0.14 ± 0.01	0.50	52.5 ± 1.0	43.1 ± 2.5	70.1 ± 0.5
				After 12 months			
Control	0.30 ± 0.06 **a**	0.35 ± 0.01 **ab**	0.73 ± 0.08 **a**	2.11 ± 0.25 **a**	16.2 ± 3.1 **a**	48.8 ± 1.4 **b**	70.4 ± 4.8 **a**
BMLF	0.32 ± 0.01 **a**	0.33 ± 0.01 **a**	0.67 ± 0.03 **a**	2.05 ± 0.06 **a**	12.8 ± 1.5 **a**	47.8 ± 1.8 **ab**	67.1 ± 7.3 **a**
AMLF	0.33 ± 0.04 **a**	0.36 ± 0.02 **b**	0.73 ± 0.03 **a**	2.05 ± 0.13 **a**	14.8 ± 3.1 **a**	45.0 ± 1.5 **a**	68.6 ± 1.9 **a**

Table 48 – Monomeric and polymeric pigments and structure indices and polymeric pigments of Nero di Troia wines at racking and after 12 months of aging.
MP: absorbance at 520 nm due to monomeric pigments; SPP: absorbance at 520 nm due to small polymeric pigments; LPP: absorbance at 520 nm due to large polymeric pigments; LPP/SPP: ratio between large and small polymeric pigments; $I_{gelatin}$: gelatin index; I_{HCl}: hydrochloric acid index; I_{PVPP}: PVPP index.
Control: control wine; BMLF: wine treated with oak chips before the malo-lactic fermentation; AMLF: wine treated with oak chips after the malo-lactic fermentation.
In column, different letters indicate significant differences at $p < 0.05$ by LSD test.

Phenolic compounds	*At racking*	*After 12 months*		
		Control	**BMLF**	**AMLF**
Phenolic acids and derivatives (mg GAE/L; mg CAE/L)				
Gallic acid	47.19 ± 0.56	45.21 ± 0.48 **a**	45.97 ± 2.04 **a**	45.75 ± 0.03 **a**
2-S-Glutathionyl-Caftaric acid	0.38 ± 0.01	0.34 ± 0.03 **a**	0.39 ± 0.03 **a**	0.31 ± 0.02 **a**
Caftaric acid + Caffeic acid	21.85 ± 0.04	21.50 ± 0.03 **a**	21.81 ± 0.85 **a**	21.46 ± 0.01 **a**
p-Coumaric acid	8.33 ± 0.03	8.67 **a**	8.83 ± 0.37 **a**	8.69 ± 0.02 **a**
Ferulic acid	7.46 ± 0.01	7.09 ± 0.09 **a**	7.19 ± 0.23 **a**	7.00 ± 0.06 **a**
Σ Phenolic acids and derivatives	**85.21 ± 0.61**	**82.81 ± 0.33 a**	**84.18 ± 3.52 a**	**83.21 ± 0.03 a**
Stilbens (mg RE/L)				
cis-Piceid	3.24 ± 0.03	0.58 ± 0.04 **a**	0.50 ± 0.04 **a**	0.54 ± 0.01 **a**
trans-Piceid	4.07 ± 0.11	3.40 ± 0.13 **b**	2.87 ± 0.08 **a**	2.94 ± 0.01 **a**
Σ Stilbens	**7.31 ± 0.09**	**3.98 ± 0.16 b**	**3.38 ± 0.05 a**	**3.48 ± 0.02 a**
Flavonols (mg QE/L)				
Myricetin-3-glc	2.44 ± 0.32	1.35 ± 0.03 **a**	1.33 ± 0.10 **a**	1.34 **a**
Myricetin-3-glcr	4.54 ± 0.05	2.77 ± 0.02 **a**	2.71 ± 0.05 **a**	2.78 ± 0.09 **a**
Myricetin-3-galac	15.93 ± 0.16	8.55 ± 0.02 **a**	8.26 ± 0.32 **a**	8.39 ± 0.01 **a**
Quercetin-3-glc	1.99 ± 0.03	1.81 ± 0.02 **b**	1.50 ± 0.11 **a**	1.80 ± 0.06 **b**
Quercetin-3-glcr	12.07	9.97 ± 0.01 **a**	9.54 ± 0.43 **a**	9.89 ± 0.04 **a**
Quercetin-3-galac	7.29 ± 0.01	1.26 ± 0.01 **a**	1.17 ± 0.08 **a**	1.17 ± 0.09 **a**
Laricitrin-3-glc	3.81 ± 0.19	3.23 ± 0.05 **a**	3.09 ± 0.04 **a**	3.14 ± 0.13 **a**
Quercetin-3-rha	8.15 ± 1.42	7.87 ± 1.14 **a**	6.94 ± 0.43 **a**	7.96 ± 0.13 **a**
Isorhamnetin-3-glc	4.88 ± 0.17	3.92 ± 0.13 **a**	3.92 ± 0.23 **a**	3.94 ± 0.27 **a**
Syringetin-3-galac	9.35 ± 0.54	7.31 ± 0.15 **a**	7.02 ± 0.23 **a**	7.30 ± 0.05 **a**
Laricitrin-3-rhamnose-7-tri-hydroxycinnamic acid	5.66 ± 0.43	3.18 ± 0.05 **b**	1.79 ± 0.08 **a**	3.14 ± 0.13 **b**
Σ Flavonols	**76.10 ± 1.82**	**51.21 ± 1.04 a**	**47.26 ± 1.94 a**	**50.83 a**
Flavan-3-ols (mg CE/L)				
(-)-(Epi)Gallocatechin	30.34 ± 0.63	28.05 ± 0.34 **a**	30.05 ± 0.68 **b**	30.14 ± 0.57 **b**
Procyanidin B3	62.34 ± 0.30	58.82 ± 0.91 **a**	59.73 ± 3.00 **a**	60.12 ± 0.58 **a**
(+)-Catechin	79.70 ± 0.49	65.99 ± 0.15 **a**	69.53 ± 0.97 **b**	68.81 ± 0.03 **b**
Procyanidin B1	58.98 ± 1.03	38.88 ± 1.15 **a**	44.15 ± 1.41 **b**	44.23 ± 1.75 **b**

Procyanidin B4	56.84 ± 1.65	61.91 ± 1.55 **a**	62.77 ± 3.44 **a**	66.64 ± 2.09 **a**
(-)-Epicatechin	102.65 ± 4.12	107.09 ± 3.97 **a**	103.24 ± 3.01 **a**	106.25 ± 1.65 **a**
Procyanidin B2	18.89 ± 1.06	18.57 ± 1.38 **a**	19.24 ± 0.91 **a**	21.40 ± 2.83 **a**
Σ Flavan-3-ols	**409.74 ± 7.71**	**379.32 ± 9.44 a**	**388.71 ± 13.41 a**	**397.59 ± 9.51 a**
Anthocyanins (mg ME/L)				
Dp-3-glc	3.56 ± 0.03	0.58 **a**	0.56 ± 0.01 **a**	0.72 ± 0.02 **b**
Cy-3-glc	0.70 ± 0.04	nd **a**	nd **a**	nd **a**
Pt-3-glc	5.58 ± 0.07	0.49 ± 0.03 **a**	0.45 ± 0.01 **a**	0.57 ± 0.05 **a**
Pn-3-glc	3.46 ± 0.09	0.35 ± 0.03 **ab**	0.33 ± 0.05 **a**	0.45 ± 0.03 **b**
Mv-3-glc	53.98 ± 0.25	4.88 ± 0.06 **a**	4.67 ± 0.22 **a**	6.09 ± 0.03 **b**
Dp-3-acetylglc	1.90 ± 0.02	0.39 ± 0.04 **b**	0.33 ± 0.02 **ab**	0.25 **a**
Pyrano-Mv-3-glc (Vitisin B)	1.44 ± 0.04	2.49 ± 0.02 **b**	2.20 ± 0.08 **a**	2.26 ± 0.02 **a**
Carboxypyrano-Mv-3-acetylglc (acetyl-Vitisin A)	nd	1.38 ± 0.01 **b**	1.21 ± 0.07 **a**	1.25 ± 0.02 **ab**
Cy-3-acetylglc	1.15 ± 0.02	nd **a**	nd **a**	nd **a**
Pt-3-acetylglc	4.29 ± 0.07	2.27 ± 0.22 **a**	1.82 ± 0.07 **a**	2.10 ± 0.24 **a**
Pyrano-Mv-3-acetylglc (acetyl-Vitisin B)	4.03 ± 0.03	2.22 ± 0.13 **a**	2.38 ± 0.22 **a**	2.44 ± 0.14 **a**
Pn-3-acetylglc	8.97 ± 0.40	3.78 ± 0.18 **a**	3.64 ± 0.17 **a**	3.35 ± 0.50 **a**
Mv-3-acetylglc	31.98 ± 0.43	9.16 ± 0.18 **ab**	8.55 ± 0.43 **a**	9.53 ± 0.08 **b**
Pyrano-Mv-3-*p*-coumglc (*p*-coum-Vitisin B)	3.00 ± 0.30	3.88 ± 0.21 **a**	3.70 ± 0.09 **a**	3.56 ± 0.08 **a**
Mv-3-caffeoylglc	2.36 ± 0.07	2.84 ± 0.07 **b**	2.32 ± 0.14 **a**	2.33 ± 0.11 **a**
Pt-3-*p*-coumglc	4.18 ± 0.07	1.76 ± 0.09 **b**	1.31 ± 0.12 **a**	1.16 ± 0.09 **a**
Mv-3-*p*-coumglc-8-ethyl-(epi)cat	nd	0.73 ± 0.03 **b**	0.55 ± 0.05 **a**	0.61 ± 0.01 **a**
Pn-3-*p*-coumglc	3.77 ± 0.28	0.41 ± 0.03 **a**	0.36 ± 0.01 **a**	0.44 ± 0.06 **a**
Mv-3-*p*-coumglc	7.73 ± 0.21	1.34 ± 0.07 **b**	0.86 ± 0.07 **a**	1.34 ± 0.01 **b**
Mv-3-glc-4-vinyl(epi)cat	nd	0.16 ± 0.01 **b**	0.16 **b**	0.13 ± 0.01 **a**
Σ Anthocyanins	**142.08 ± 0.15**	**39.13 ± 0.15 b**	**35.40 ± 0.90 a**	**38.58 ± 0.65 b**

Table 49 – Phenolic profile of Nero di Troia wines at racking and after 12 months of aging.
nd: not detected; glc: glucoside, glcr: glucuronide, rha: rhamnoside, galac: galactoside, Dp: delphinidin, Cy: cyanidin, Pt: petunidin, Pn: peonidin, Mv: malvidin, acetylglc: acetylglucoside, *p*-coumglc: *p*-coumaroylglucoside, caffeoylglc: caffeoylglucoside, cat: catechin;
Control: control wine; BMLF: wine treated with oak chips before the malo-lactic fermentation; AMLF: wine treated with oak chips after the malo-lactic fermentation.
In row, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Sample	CI	T	dA(%)	% yellow	% red	% blue	dAl%	dAT%	dTAT%
				At racking					
	9.224 ± 0.001	0.578	59.7	32.0	55.3	12.7	4.1	64.6 ± 1.5	31.3 ± 1.4
				After 12 months					
Control	9.244 ± 0.047 **a**	0.705 ± 0.015 **a**	50.0 ± 1.1 **a**	35.2 ± 0.4 **a**	50.0 ± 0.6 **a**	14.8 ± 0.2 **a**	0.9 **a**	26.1 ± 0.2 **a**	73.0 ± 0.2 **b**
BMLF	9.162 ± 0.078 **a**	0.702 ± 0.013 **a**	50.0 ± 0.2 **a**	35.1 ± 0.6 **a**	50.0 ± 0.1 **a**	14.9 ± 0.5 **a**	1.2 ± 0.2 **a**	24.1 ± 0.2 **a**	74.8 ± 0.2 **b**
AMLF	9.347 ± 0.061 **a**	0.713 ± 0.014 **a**	49.4 ± 1.3 **a**	35.4 ± 0.3 **a**	49.7 ± 0.6 **a**	14.9 ± 0.4 **a**	1.1 **a**	32.7 ± 1.5 **b**	66.1 ± 1.5 **a**

Table 50 – Colour parameters of Nero di Troia wines at racking and after 12 months of aging.
CI: colour intensity; T: tonality; dA(%): percentage of red colour due to flavilium cation of free and combined anthocyanins; % yellow: percentage of yellow component; % red: percentage of red component; % blue: percentage of blue component; dAl%: percentage of absorbance at 520 nm due to monomeric anthocyanins; dAT%: percentage of absorbance at 520 nm due to polymeric pigments decolorized with SO_2; dTAT%: percentage of absorbance at 520 nm due to polymeric pigments not decolorized.
Control: control wine; BMLF: wine treated with oak chips before the malo-lactic fermentation; AMLF: wine treated with oak chips after the malo-lactic fermentation.
In column, different letters indicate significant differences at $p < 0.05$ by LSD test.

Sensory descriptors	Control	BMLF	AMLF
	Visual descriptors		
Clearness	2.0 **a**	1.8 ± 0.4 **a**	2.0 **a**
Texture	2.2 ± 0.4 **a**	2.1 ± 0.5 **a**	2.1 ± 0.5 **a**
	Olfactory descriptors		
Olfactory intensity	2.0 **a**	2.1 ± 0.2 **a**	2.3 ± 0.3 **a**
Olfactory complexity	1.8 ± 0.3 **a**	1.9 ± 0.4 **a**	2.1 ± 0.2 **a**
Olfactory quality	1.8 ± 0.6 **a**	2.1 ± 0.4 **a**	2.2 ± 0.4 **a**
	Gustatory-olfactory descriptors		
Sugars	0.0 **a**	0.0 **a**	0.0 **a**
Alcohols	2.0 ± 0.6 **a**	2.1 ± 0.5 **a**	2.3 ± 0.4 **a**
Polyols	1.6 ± 0.4 **a**	1.5 ± 0.5 **a**	1.6 ± 0.4 **a**
Acids	2.3 ± 0.4 **a**	2.2 ± 0.4 **a**	2.3 ± 0.4 **a**
Tannins	2.2 ± 0.9 **a**	2.4 ± 1.1 **a**	2.8 ± 0.4 **a**
Minerals	2.1 ± 0.4 **a**	2.3 ± 0.4 **a**	2.1 ± 0.4 **a**
Structure	2.5 ± 0.5 **a**	2.4 ± 0.5 **a**	2.3 ± 0.4 **a**
Balance	1.5 ± 0.5 **a**	1.3 ± 0.8 **a**	1.5 ± 0.9 **a**
Gustatory-olfactory intensity	2.3 ± 0.6 **a**	2.0 ± 0.8 **a**	2.4 ± 0.4 **a**
Gustatory-olfactory persistence	2.3 ± 0.4 **a**	2.1 ± 0.7 **a**	2.3 ± 0.6 **a**
Gustatory-olfactory quality	1.5 ± 0.5 **a**	1.4 ± 0.7 **a**	1.6 ± 0.4 **a**
	Final considerations		
Evolutionary state	2.0 ± 0.4 **a**	2.4 ± 0.5 **a**	2.1 ± 0.3 **a**
Harmony	1.5 ± 0.9 **a**	1.6 ± 0.9 **a**	1.6 ± 0.9 **a**

Table 51 – Effect of the treatment with oak chips performed before or after the malo-lactic fermentation on sensory characteristics of Nero di Troia wines after 12 months from the racking.
Control: control wine; BMLF: wine treated with oak chips before the malo-lactic fermentation; AMLF: wine treated with oak chips after the malo-lactic fermentation.
In column, different letters indicate significant differences at $p < 0.05$ by LSD test.

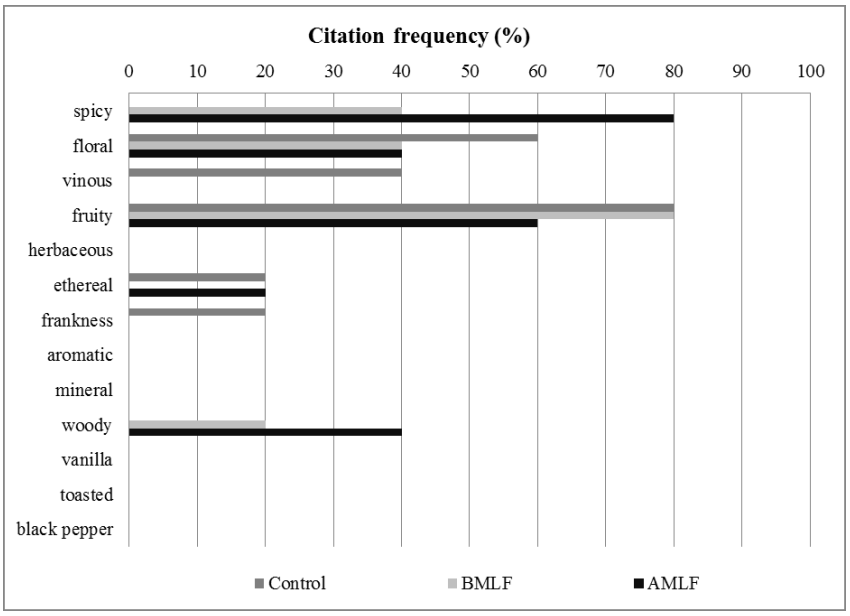
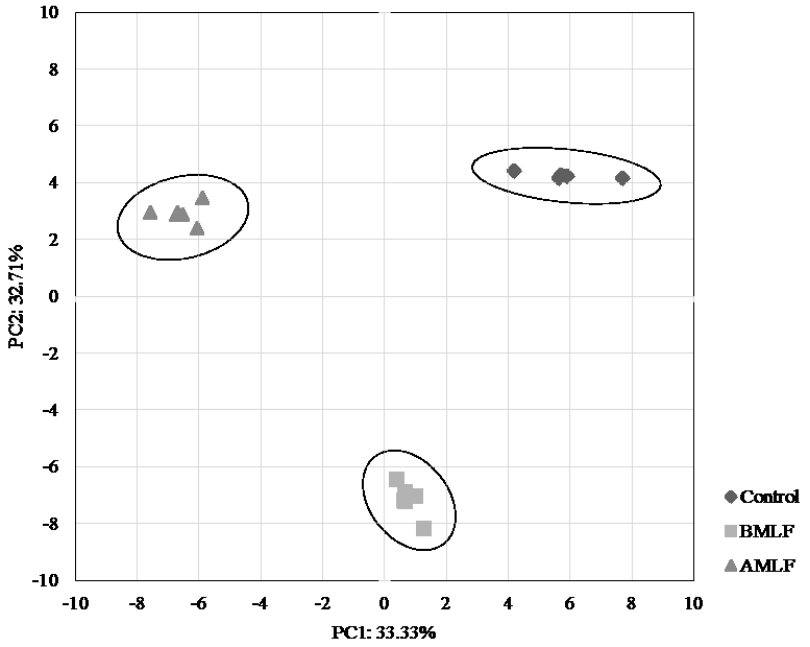
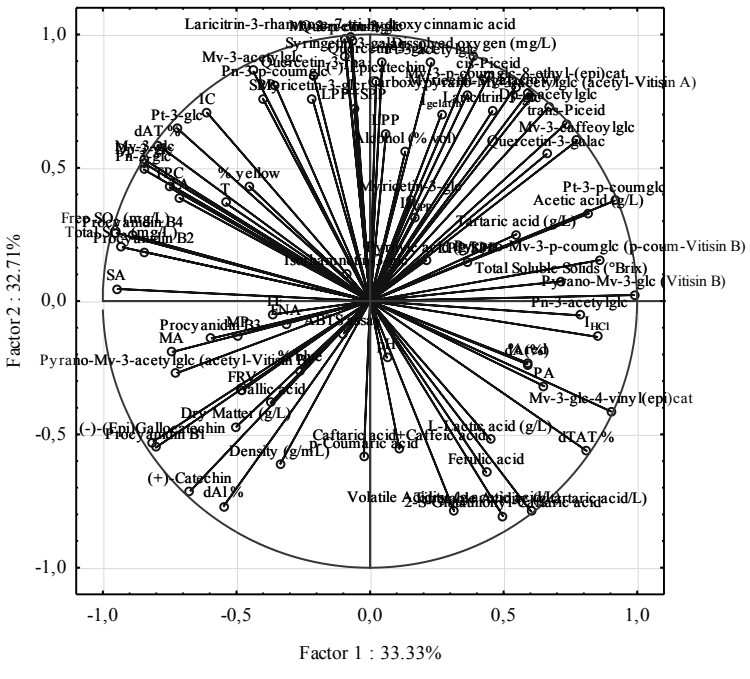


Figure 18 - Citation frequency (%) of Nero di Troia wines after 12 months from the racking.



a)



b)

Figure 19 - PCA scatter plot for projection on the factor plane of: **a)** Nero di Troia wine samples after 12 months from the racking and **b)** analytical results.
Control: control wine; BMLF: wine treated with oak chips before the malo-lactic fermentation; AMLF: wine treated with oak chips after the malo-lactic fermentation.

7.5 Study of single and interactive effects of leaf removal and aging with French oak chips on physical, chemical, and sensory characteristics of Nero di Troia wines

Table 52 reports the main physicochemical parameters of Nero di Troia wines 12 months after racking. It can be inferred that the differences highlighted among the wines were due only to the leaf removal. Even after 12 months, the wines produced from E and F grapes exhibited the highest alcohol % and dry matter. In particular, the F wines, which was obtained from the almost complete defoliated grapes, showed the highest values of dry matter, in agreement with the results at racking in section 7.1. In contrast to what detected at racking, after 12 months the E and F wines had the lowest and highest volatile acidity values, respectively. As already observed at racking, the defoliation treatments did not affect the wine pH and the amounts of the residual soluble solids were higher in the wines from defoliated vines (especially from E and F grapes). The E/W wines showed the highest free/total SO_2 ratio (thus indicating lower content of the bound form), while the F wines exhibited the lowest values of this ratio. However, in all the samples, the concentration of the free form remained over the recommended values for a correct wine preservation during storage (20-30 mg/L; Ribéreau-Gayon *et al*., 2000). Observing the contents of acetaldehyde and dissolved oxygen it was possible to notice that the E wines exhibited the lowest concentrations of acetaldehyde content. The F samples, instead, had the highest acetaldehyde content. The interactive effects of leaf removal and oak chips addition were highlighted for all the oenological parameters, with the exception of density, volatile acidity, and pH. Compared to the racking (Table 8; section 7.1), in all the wines, the aging time determined the rise of volatile acidity, the decrease of titratable acidity, the simultaneous increase of pH, the decrease of free and total sulphur dioxide content and of their ratio due to oxidation reactions (Ribéreau-Gayon *et al*., 2000), and the decreases of acetaldehyde concentration as consequence of its reaction with sulphur dioxide and its participation in polymerization reactions with anthocyanins and tannins (Liu and Pilone, 2000).

Table 53 shows the single and interactive effects of leaf removal and treatment with oak chips on the organic acid profiles. The composition in organic acids strongly changed with respect to the racking time. The leaf removal had significant effects on all the considered organic acids, except for malic and pyruvic acids, while the effects of chips were

significant only for tartaric acid, whose concentration was the lowest in the treated wines. This effect could be due to the metal (Cu^{2+} or Fe^{2+})-induced oxidation of tartaric acid to glyoxylic acid (Fulcrand *et al*., 1997). Glyoxylic acid, similar to acetaldehyde, can bind catechins and other phenolics into increasingly long polymers. The reaction can generate both colourless and yellow polyphenols (Jackson, 2008). Concerning the single effect of leaf removal, E wines showed the highest concentration of tartaric and D-gluconic acids, while the F wines still showed the lowest content of tartaric acid and, furthermore, the highest concentrations of L-lactic, acetic, and citric acids.

Comparing the data at racking (Table 9, section 7.1) with those at 12 months, decreases of the tartaric acid content occurred. This phenomenon was a consequence of the precipitation of crystallized tartrates and/or of its metal ion-catalyzed oxidation. L-malic and citric acids fell under the detection limit. The increase of acetic acid content, in agreement with the increase of the volatile acidity of the wines, and the consumption of D-gluconic acid were essentially due to the acetic acid bacteria activity, while the disappearance of pyruvic acid was probably due to its binding with sulphur dioxide and phenolics (especially anthocyanins).

The phenolic composition, the distribution of pigments between monomeric and polymeric forms of wines, and the structure indices after 12 months of aging are reported in Tables from 54 to 56. Even after 12 months of aging, the wines produced from E vines had the highest content of total anthocyanins and anthocyanins sensitive to SO_2 (Table 54), together with the highest values of absorbance due to monomeric and small polymeric pigments, and, among the wines produced from defoliated grapes, the highest content in large polymeric pigments (Table 55). The E wines also exhibited the lowest content of flavonoids different from anthocyanins, and the highest flavan content and radical scavenging activity values. F and E/W wines, instead, showed the lowest total anthocyanin and flavan reactive with vanillin contents, and the lowest antioxidant activity. Furthermore, the wines produced from defoliated grapes exhibited greater concentrations of anthocyanins sensible to SO_2 than the not defoliated ones. Concerning the single effect of the oak chip treatment, the oak-treated wines exhibited higher contents of monomeric anthocyanins, anthocyanins sensitive to SO_2, total flavonoids, flavonoids different from anthocyanins, and total phenolic compounds, and higher antioxidant capacity than the untreated wines. This behaviour, as already discussed in the preceding sections, can be explained by the presence of ellagitannins extracted from oak wood. Vivas and Glories

(1996), in fact, reported that ellagitannins revealed as notable oxidation regulators, rapidly absorbing the dissolved oxygen and facilitating the hydroperoxidation of wine constituents, while Guerra *et al*. (1996) reported that the oak tannins can influence indirectly the colour stabilization, through the protection of anthocyans and flavanols from oxidative degradation. The protective effect of ellagitannins was highlighted only in wines produced from defoliated grapes, while the wines produced from shaded grapes and treated with oak chips exhibited a loss of anthocyanins, flavans reactive with vanillin, total phenolics and antioxidant capacity if compared with the respective control. Proanthocyanidins was the only phenolic class that did not suffer neither the single not the interactive effects of leaf removal and treatment with oak chips.

The E wines also exhibited the lowest LPP/SPP ratio. These results indicate a low polymerization degree of wine phenolics, as also established by the lowest value of HCl and PVPP indices of the same wines (which give a measure of the polymerization state of tannins and anthocyanins, respectively). Among the wines from defoliated grapes, the E/W and F wines showed the highest values of LPP/SPP ratio, HCl and PVPP indices, thus indicating a higher phenolic polymerization degree. Concerning the single effect of the treatment with oak chips on the structure indices and the distribution of wine pigments among monomeric and polymeric forms, no significant differences were found. Instead, leaf removal and treatment with oak chips exerted significant interactive effects on all the above mentioned indices. The wines from shaded grapes which were submitted to the treatment with oak chips exhibited the highest tannin polymerization degree and the highest concentrations of tannins responsible for astringency (high gelatin index).

The effects of defoliation and treatment with oak chips on the analytical phenolic composition are quantified in Table 56. It is evident that the leaf removal exerted significant single effects on a greater number of phenolic compounds. The wines from shaded grapes and from grapes submitted to the mild defoliation (E) showed the highest contents of phenolic acids and anthocyanins while the wines from grapes submitted to the strong defoliation treatments (E/W and F) exhibited the highest contents of stilbens and flavanols. All the wines from defoliated grapes showed higher flavanol contents than the wines from shaded grapes. The flavan-3-ols content suffered a dramatic decrease in all the wines in comparison with the racking (Table 12; section 7.1) (average about -73%), but both E/W and F wines had a greater content with respect to the other wines. Even the total anthocyans content drastically declined compared to the racking (average about -92%), the

E wines showed the highest content both in free and more stable forms, while E/W and F wines the lowest ones.

The single effects of treatment with oak chips were general not significant with the exception of: the concentrations of *cis*-piceid and total stilbens, which were higher in the treated wines; the concentrations of total flavanols and of procyanidins B1 and B4, which were higher in the untreated wines, and of catechin and procyanidin B2, which were higher in the treated wines.

Concerning the interactive effects of the defoliation and oak chips treatment: the highest phenolic acid contents were exhibited by the wines from shaded grapes; the highest stilben contents by the wines from vines submitted to the stronger defoliation treatments (E/W and F) and treated with oak chips; the highest flavonol contents by the F wines treated with oak chips; the highest flavanol contents by the wines from vines submitted to the more severe defoliation treatments (E/W and F); the highest anthocyanin content by the E wines treated or not with oak chips.

The colour parameters are listed in Table 57. Also for these parameters, the effects of defoliation were always significant while those of the treatment with oak chips were always not significant. The E wines still showed the highest colour intensity and tonality as a consequence of the highest anthocyanins content. Concerning the three chromatic components (yellow, red and blue), in E wines the contribute to the colour due to the yellow component was the highest while the blue component was the lowest. These data could be explained by the lowest ratio between the total anthocyanin and the portisin type contents in E wines compared to the other wines. Indeed, this ratio was around 1.1 % in E wines and around 1.8, 3.3 and 3.5 % in N, E/W and F wines, respectively. Portisins, so called because they were first isolated from Port wines, are particular type of blue flavanyl-vinylpyranoanthocyanins (λmax 575 nm) (Mateus *et al*., 2003). The E wines also showed the highest values of dAl% and dAT% and the lowest values of dTAT% thus highligthing the lowest degree of polymerization of anthocyanins in these wines.

The scores of the visual, olfactory, and gustatory attributes evaluated by a trained panels through the QDA are reported in Table 58, while the citation frequency of the flavours are showed in Figure 20. Neither leaf removal nor treatment with oak chips exerted significant interactive effects on visual and olfactory parameters. Concerning the gustatory parameters: the wines from shaded grapes and from grapes submitted to the mildest

defoliation were judged as more tannic if treated with chips; the wines from grapes almost completely defoliated were the most mineral and the less balanced and harmonic because they exhibited the lowest scores of gustatory-olfactory intensity, persistence and quality. Concerning the wines not treated with oak chips (Figure 20*a*), they exhibited sensory profiles characterised by spicy (F), floral (N), vinous (F), fruity (N), herbaceous (F), ethereal (E/W), aromatic (all), and mineral (F) notes. Concerning the wines treated with oak chips (Figure 20*b*), they exhibited sensory profiles characterised by spicy (N), floral (E), vinous (E and E/W), fruity (N), ethereal (N and E), woody, and vanilla aromas.

The wines in contact with oak chips for 70 days had lower floral notes, and higher spicy, woody and vanilla attributes in comparison with the untreated samples, in agreement with the results observed by Guchu *et al*. (2006) and Gómez García-Carpintero *et al*. (2011; 2012) in Chardonnay, Bobal and Moravia Agria wines treated with oak chips, respectively. These results are in line with those observed and already discussed for Aglianico, Montepulciano and Nero di Troia wines (sections 7.2.1, 7.2.2, and 7.2.3).

In order to gain a better comprehension of the complete variability of the results, the PCA was applied to the whole data set. The first two PCs explained only 52% of the total variability (Figures 21*a* and *b*). Even after 12 months of aging, the PCA was suitable to visualise the principal groupings, according to the specific defoliation and oak chip treatment. The wines produced from shaded grapes (N), from grapes defoliated along the east and west side (E/W) and from full defoliated vines (F) showed similar values for the PC1, thus indicating that after one year of aging these canopy management practices conferred similar characteristics to the resulting wines, which were, instead, distinguishable for the values of the PC2. E and E/W wines, instead, showed similar values of the PC2 and different values of the PC1.

	Alcohol (% vol)	Density (g/mL)	Dry Matter (g/L)	Vol. Ac.	Titr. Ac.	pH	Free SO_2 (mg/L)	Total SO_2 (mg/L)	TSS (°Brix)	Acetaldehyde (mg/L)	Dissolved O_2 (mg/L)
					Leaf removal						
N	11.20 ± 0.16 **a**	0.996 **a**	27.7 ± 1.3**a**	0.65 ± 0.10 **ab**	4.80 ± 0.13 **b**	3.87 ± 0.05 **a**	36.2 ± 3.5 **ab**	46.2 ± 5.8 **ab**	7.0 ± 0.2 **a**	7.88 ± 3.9 **ab**	1.44 ± 0.34 **a**
E	11.79 ± 0.04 **b**	0.996 **a**	29.0 ± 0.2 **ab**	0.51 ± 0.10 **a**	4.38 ± 0.07 **a**	3.94 ± 0.02 **a**	25.6 ± 6.5 **a**	32.8 ± 5.9 **a**	7.2 ± 0.1 **b**	5.13 ± 3.52 **a**	2.56 ± 0.64 **b**
E/W	11.39 ± 0.18 **a**	0.995 **a**	27.4 ± 0.7**a**	0.62 ± 0.12 **ab**	4.37 ± 0.15 **a**	3.94 ± 0.03 **a**	47.2 ± 8.7 **c**	56.8 ± 9.3 **b**	6.9 **a**	11.50 ± 7.05 **b**	1.49 ± 0.51 **a**
F	11.91 ± 0.26 **b**	0.996 **a**	29.8 ± 1.1 **b**	0.83 ± 0.25 **c**	4.97 ± 0.23 **b**	3.95 ± 0.08 **a**	42.6 ± 5.4 **bc**	59.0 ± 12.7 **b**	7.5 ± 0.2 **c**	18.13 ± 7.32 **c**	1.50 ± 0.47 **a**
Significance	*	**ns**	*	*	*	**ns**	*	*	*	*	*
					Treatment with oak chips						
No CHIPS	11.61 ± 0.30 **a**	0.996 **a**	28.5 ± 1.2 **a**	0.64 ± 0.18 **a**	4.62 ± 0.21 **a**	3.92 ± 0.05 **a**	38.1 ± 7.8 **a**	47.5 ± 10.2 **a**	7.2 ± 0.3 **a**	10.00 ± 6.59 **a**	1.69 ± 0.54 **a**
+ CHIPS	11.53 ± 0.39 **a**	0.996 **a**	28.4 ± 1.5 **a**	0.67 ± 0.20 **a**	4.64 ± 0.39 **a**	3.92 ± 0.06 **a**	37.7 ± 12.6 **a**	49.9 ± 16.6 **a**	7.1 ± 0.3 **a**	11.31 ± 8.20 **a**	1.80 ± 0.80 **a**
Significance	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
					Leaf removal * Treatment with oak chips						
N	11.23 ± 0.20 **a**	0.996 **a**	28.2 ± 1.1 **ab**	0.63 ± 0.11 **a**	4.77 ± 0.04 **bc**	3.88 ± 0.07 **a**	36.0 ± 3.4 **abc**	44.8 **abc**	7.1 ± 0.2 **ab**	9.50 ± 1.53 **abc**	1.52 ± 0.03 **a**
N+chips	11.16 ± 0.17 **a**	0.996 **a**	27.3 ± 1.8 **a**	0.66 ± 0.14 **a**	4.83 ± 0.21 **cd**	3.86 ± 0.06 **a**	36.4 ± 5.1 **abc**	47.6 ± 9.6 **abc**	6.9 ± 0.1 **a**	6.25 ± 5.06 **ab**	1.37 ± 0.50 **a**
E	11.76 ± 0.03 **b**	0.996 **a**	28.8 **ab**	0.45 ± 0.11 **a**	4.37 ± 0.10 **a**	3.94 ± 0.02 **a**	29.6 ± 7.9 **ab**	36.0 ± 7.9 **ab**	7.2 ± 0.1 **b**	5.75 ± 4.35 **ab**	2.31 ± 0.73 **bc**
E+chips	11.82 ± 0.02 **b**	0.996 **a**	29.1 ± 0.1 **ab**	0.58 ± 0.03 **a**	4.38 ± 0.08 **a**	3.94 ± 0.01 **a**	21.6 ± 1.1 **a**	29.6 ± 1.1 **a**	7.3 ± 0.1 **bc**	4.50 ± 3.00 **a**	2.82 ± 0.50 **c**
E/W	11.51 ± 0.09 **ab**	0.995 **a**	27.4 ± 0.4 **ab**	0.65 ± 0.05 **a**	4.50 **ab**	3.92 ± 0.03 **a**	43.2 ± 9.1 **bc**	53.6 ± 12.4 **bc**	6.9 **a**	10.00 ± 9.38 **abc**	1.30 ± 0.38 **a**
E/W+chips	11.27 ± 0.16 **a**	0.996 ± 0.001 **a**	27.4 ± 1.1 **a**	0.59 ± 0.19 **a**	4.25 ± 0.06 **a**	3.97 ± 0.01 **a**	51.2 ± 9.1 **c**	60.0 ± 7.9 **c**	7.0 ± 0.1 **a**	13.00 ± 4.69 **bc**	1.68 ± 0.59 **ab**
F	11.93 ± 0.16 **b**	0.996 **a**	29.8 ± 1.5 **ab**	0.83 ± 0.23 **a**	4.83 **cd**	3.96 ± 0.08 **a**	43.6 ± 1.7 **bc**	55.6 ± 5.1 **bc**	7.5 ± 0.3 **c**	14.75 ± 7.27 **cd**	1.64 ± 0.16 **ab**
F+chips	11.89 ± 0.42 **b**	0.996 **a**	29.9 ± 1.3 **b**	0.84 ± 0.37 **a**	5.10 ± 0.30 **d**	3.93 ± 0.10 **a**	41.6 ± 9.1 **bc**	62.4 ± 20.4 **c**	7.5 ± 0.2 **c**	21.50 ± 6.45 **d**	1.35 ± 0.66 **a**
Significance	*	**ns**	*	**ns**	*	**ns**	*	*	*	*	*

Table 52 – Single and interactive effects of leaf removal and treatment with oak chips on the physicochemical characteristics of Nero di Troia wines analysed 12 months after racking.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
Vol. Ac.: volatile acidity (g acetic acid/L); Titr. Ac.: titratable acidity (g tartaric acid/L); TSS: total soluble solids (°Brix).
In column, as part of leaf removal or treatment with oak chips or combination of leaf removal-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference; ns: no significant difference.

	Tartaric acid	L-Malic acid	L-Lactic acid	Acetic acid	Citric acid	Pyruvic acid	D-Gluconic acid
				Leaf removal			
N	1.50 ± 0.14 **bc**	nd **a**	1.56 ± 0.17 **a**	0.74 ± 0.11 **a**	nd **a**	nd **a**	0.02 ± 0.02 **ab**
E	1.58 ± 0.18 **c**	nd **a**	1.50 ± 0.06 **a**	0.62 ± 0.03 **a**	nd **a**	nd **a**	0.04 ± 0.05 **b**
E/W	1.47 ± 0.11 **b**	nd **a**	1.51 ± 0.10 **a**	0.68 ± 0.07 **a**	nd **a**	nd **a**	nd **a**
F	1.37 ± 0.13 **a**	nd **a**	1.73 ± 0.09 **b**	0.91 ± 0.22 **b**	0.02 ± 0.04 **b**	nd **a**	0.03 ± 0.05 **ab**
Significance	*	**ns**	*	*	*	**ns**	*
				Treatment with oak chips			
No CHIPS	1.56 ± 0.14 **b**	nd **a**	1.59 ± 0.12 **a**	0.71 ± 0.12 **a**	nd **a**	nd **a**	0.03 ± 0.04 **a**
+ CHIPS	1.40 ± 0.12 **a**	nd **a**	1.56 ± 0.16 **a**	0.76 ± 0.20 **a**	0.01 ± 0.03 **a**	nd **a**	0.02 ± 0.03 **a**
Significance	*	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
				Leaf removal * Treatment with oak chips			
N	1.61 ± 0.11 **cd**	nd **a**	1.59 ± 0.17 **ab**	0.65 ± 0.08 **ab**	nd **a**	nd **a**	0.04 ± 0.01 **ab**
N+chips	1.39 ± 0.01 **ab**	nd **a**	1.54 ± 0.19 **a**	0.83 ± 0.01 **bcd**	nd **a**	nd **a**	nd **a**
E	1.71 ± 0.12 **d**	nd **a**	1.53 ± 0.05 **a**	0.62 ± 0.04 **a**	nd **a**	nd **a**	0.07 ± 0.06 **b**
E+chips	1.45 ± 0.12 **b**	nd **a**	1.47 ± 0.04 **a**	0.62 ± 0.03 **a**	nd **a**	nd **a**	0.01 ± 0.01 **a**
E/W	1.47 ± 0.09 **bc**	nd **a**	1.53 ± 0.03 **a**	0.70 ± 0.05 **abc**	nd **a**	nd **a**	nd **a**
E/W+chips	1.48 ± 0.13 **bc**	nd **a**	1.49 ± 0.15 **a**	0.66 ± 0.09 **ab**	nd **a**	nd **a**	nd **a**
F	1.47 ± 0.11 **bc**	nd **a**	1.72 ± 0.07 **b**	0.88 ± 0.11 **cd**	nd **a**	nd **a**	nd **a**
F+chips	1.27 ± 0.06 **a**	nd **a**	1.74 ± 0.12 **b**	0.94 ± 0.31 **d**	0.04 ± 0.05 **b**	nd **a**	0.06 ± 0.06 **b**
Significance	*	**ns**	*	*	*	**ns**	*

Table 53 – Single and interactive effects of leaf removal and treatment with oak chips on organic acid content of Nero di Troia wines analysed 12 months after racking (data are expressed as g per L of wine).
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
In column, as part of leaf removal or treatment with oak chips or combination of leaf removal-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
nd: not detected; *: significant difference; ns: no significant difference.

	TA	SA	MA	TF	FNA	FRV	PA	TPC	ABTS assay
					Leaf removal				
N	154 ± 21 **a**	104 ± 22 **a**	33 ± 6 **a**	1165 ± 53 **a**	940 ± 52 **b**	622 ± 81 **ab**	2275 ± 269 **a**	2343 ± 317 **b**	10.14 ± 1.11 **b**
E	206 ± 37 **b**	158 ± 48 **b**	30 ± 6 **a**	1094 ± 108 **a**	795 ± 59 **a**	669 ± 82 **b**	2165 ± 235 **a**	2274 ± 351 **b**	11.31 ± 1.39 **c**
E/W	147 ± 19 **a**	136 ± 59 **ab**	30 ± 6 **a**	1108 ± 91 **a**	894 ± 99 **b**	579 ± 89 **a**	2195 ± 217 **a**	2332 ± 294 **b**	9.40 ± 0.90 **a**
F	144 ± 31 **a**	137 ± 26 **ab**	34 ± 8 **a**	1122 ± 154 **a**	913 ± 133 **b**	549 ± 203 **a**	2014 ± 510 **a**	2046 ± 269 **a**	9.74 ± 1.46 **ab**
Significance	*	*	**ns**	**ns**	*	*	**ns**	*	*
					Treatment with oak chips				
No CHIPS	154 ± 29 **a**	118 ± 35 **a**	30 ± 6 **a**	1076 ± 89 **a**	852 ± 99 **a**	600 ± 102 **a**	2089 ± 259 **a**	2172 ± 277 **a**	9.75 ± 1.38 **a**
+ CHIPS	171 ± 42 **a**	150 ± 46 **b**	34 ± 7 **b**	1169 ± 104 **b**	919 ± 100 **b**	615 ± 146 **a**	2235 ± 378 **a**	2326 ± 355 **b**	10.47 ± 1.39 **b**
Significance	**ns**	*	*	*	*	**ns**	**ns**	*	*
					Leaf removal * Treatment with oak chips				
N	159 ± 26 **ab**	122 ± 18 **ab**	35 ± 4 **bc**	1151 ± 28 **ab**	919 ± 35 **b**	651 ± 69 **b**	2335 ± 155 **a**	2460 ± 302 **cd**	10.34 ± 1.42 **c**
N+chips	150 ± 16 **ab**	90 ± 15 **a**	32 ± 7 **ab**	1179 ± 72 **b**	961 ± 63 **b**	588 ± 85 **ab**	2215 ± 368 **a**	2226 ± 304 **bc**	9.90 ± 0.56 **bc**
E	181 ± 31 **b**	128 ± 53 **ab**	27 ± 3 **a**	1023 ± 104 **a**	760 ± 59 **a**	647 ± 104 **b**	2024 ± 148 **a**	1970 ± 158 **a**	10.72 ± 1.43 **c**
E+chips	230 ± 23 **c**	187 ± 15 **c**	33 ± 6 **b**	1165 ± 57 **ab**	829 ± 38 **ab**	692 ± 49 **b**	2305 ± 233 **a**	2578 ± 169 **d**	11.96 ± 1.06 **d**
E/W	139 ± 19 **a**	83 ± 13 **a**	27 ± 3 **a**	1051 ± 73 **ab**	849 ± 89 **ab**	579 ± 79 **ab**	2125 ± 293 **a**	2137 ± 195 **ab**	8.97 ± 0.93 **ab**
E/W+chips	155 ± 18 **ab**	176 ± 43 **c**	33 ± 6 **b**	1165 ± 73 **ab**	940 ± 96 **b**	579 ± 106 **ab**	2265 ± 106 **a**	2527 ± 246 **d**	9.83 ± 0.65 **abc**
F	137 ± 22 **a**	130 ± 22 **ab**	30 ± 7 **ab**	1080 ± 104 **ab**	880 ± 133 **ab**	478 ± 78 **a**	1874 ± 235 **a**	2119 ± 195 **ab**	8.91 ± 0.54 **a**
F+chips	151 ± 41 **ab**	144 ± 31 **bc**	40 ± 6 **c**	1165 ± 200 **ab**	945 ± 143 **b**	593 ± 247 **ab**	2155 ± 706 **a**	1973 ± 324 **a**	10.57 ± 1.63 **c**
Significance	*	*	*	*	*	*	**ns**	*	*

Table 54 – Single and interactive effects of leaf removal and treatment with oak chips on the phenolic composition and antioxidant activity of Nero di Troia wines analysed 12 months after racking.
TA: total anthocyanins (as mg malvidin-3-glucoside/L); SA: anthocyanins sensitive to SO_2 (as mg malvidin-3-glucoside/L); MA: monomeric anthocyanins (as malvidin-3-glucoside mg/L); TF: total flavonoids (as mg (+)-catechin/L); FNA: flavonoids different from anthocyanins (as mg (+)-catechin/L); FRV: flavans reactive with vanillin (as mg (+)-catechin/L); PA: proanthocyanidins (as mg cyanidin chloride/L); TPC: total phenolic compounds (as mg gallic acid/L); ABTS assay: antioxidant activity by ABTS assay (as mmol Trolox eq/L).
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
In column, as part of leaf removal or treatment with oak chips or combination of leaf removal-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference; ns: no significant difference.

	MP	SPP	LPP	LPP/SPP	$I_{gelatin}$	I_{HCl}	I_{PVPP}
				Leaf removal			
N	0.30 ± 0.07 **a**	0.39 ± 0.07 **a**	0.68 ± 0.08 **c**	1.83 ± 0.23 **c**	58.2 ± 2.9 **a**	33.5 ± 10.0 **b**	67.8 ± 5.1 **b**
E	0.45 ± 0.08 **b**	0.51 ± 0.07 **b**	0.65 ± 0.09 **bc**	1.28 ± 0.25 **a**	52.7 ± 4.7 **a**	24.7 ± 5.2 **a**	62.6 ± 2.8 **a**
E/W	0.28 ± 0.04 **a**	0.38 ± 0.04 **a**	0.56 ± 0.06 **a**	1.47 ± 0.19 **b**	54.6 ± 5.2 **a**	32.9 ± 6.9 **b**	72.4 ± 11.0 **c**
F	0.28 ± 0.05 **a**	0.40 ± 0.07 **a**	0.59 ± 0.08 **ab**	1.53 ± 0.18 **b**	54.8 ± 7.6 **a**	34.0 ± 4.7 **b**	74.6 ± 5.2 **c**
Significance	*	*	*	*	**ns**	*	*
				Treatment with oak chips			
No CHIPS	0.32 ± 0.06 **a**	0.43 ± 0.07 **a**	0.63 ± 0.09 **a**	1.51 ± 0.22 **a**	54.8 ± 6.5 **a**	31.1 ± 7.2 **a**	68.8 ± 7.5 **a**
+ CHIPS	0.33 ± 0.12 **a**	0.41 ± 0.09 **a**	0.60 ± 0.09 **a**	1.54 ± 0.36 **a**	55.7 ± 4.1 **a**	31.9 ± 8.6 **a**	69.9 ± 8.6 **a**
Significance	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
				Leaf removal * Treatment with oak chips			
N	0.34 ± 0.06 **bc**	0.42 ± 0.04 **b**	0.69 ± 0.07 **d**	1.65 ± 0.12 **d**	56.2 ± 2.0 **ab**	24.4 ± 3.1 **ab**	70.4 ± 2.2 **b**
N+chips	0.24 ± 0.03 **a**	0.36 ± 0.08 **a**	0.66 ± 0.10 **cd**	2.04 ± 0.13 **e**	60.2 ± 2.2 **b**	42.7 ± 2.9 **e**	65.1 ± 5.8 **a**
E	0.39 ± 0.04 **c**	0.50 ± 0.03 **c**	0.69 ± 0.12 **bcd**	1.36 ± 0.33 **ab**	51.7 ± 5.0 **a**	27.3 ± 4.0 **b**	62.5 ± 3.7 **a**
E+chips	0.50 ± 0.07 **d**	0.52 ± 0.09 **c**	0.62 ± 0.03 **abcd**	1.20 ± 0.17 **a**	54.5 ± 5.0 **ab**	20.9 ± 4.7 **a**	62.8 ± 1.7 **a**
E/W	0.29 ± 0.03 **ab**	0.38 ± 0.04 **ab**	0.57 ± 0.02 **ab**	1.53 ± 0.18 **cd**	55.2 ± 7.6 **ab**	38.4 ± 5.1 **de**	64.9 ± 8.7 **a**
E/W+chips	0.27 ± 0.04 **a**	0.38 ± 0.05 **ab**	0.54 ± 0.09 **a**	1.41 ± 0.20 **bc**	54.0 ± 2.3 **ab**	27.4 ± 2.6 **b**	79.9 ± 7.5 **d**
F	0.28 ± 0.04 **a**	0.41 ± 0.10 **ab**	0.59 ± 0.10 **abc**	1.47 ± 0.17 **bcd**	56.4 ± 11.9 **ab**	35.2 ± 5.0 **cd**	77.2 ± 2.9 **cd**
F+chips	0.27 ± 0.07 **a**	0.39 ± 0.04 **ab**	0.59 ± 0.06 **abcd**	1.63 ± 0.17 **d**	53.6 ± 4.0 **ab**	33.0 ± 4.6 **c**	71.9 ± 5.8 **bc**
Significance	*	*	*	*	*	*	*

Table 55 – Single and interactive effects of leaf removal and treatment with oak chips on monomeric and polymeric pigments, and structure indices of Nero di Troia wines analysed 12 months after racking.
MP: absorbance at 520 nm due to monomeric pigments; SPP: absorbance at 520 nm due to small polymeric pigments; LPP: absorbance at 520 nm due to large polymeric pigments; LPP/SPP: ratio between large and small polymeric pigments; $I_{gelatin}$: gelatin index; I_{HCl}: hydrochloric acid index; I_{PVPP}: PVPP index.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
In column, as part of leaf removal or treatment with oak chips or combination of leaf removal-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference; ns: no significant difference.

Phenolic compounds	*Leaf removal*					*Treatment with oak chips*		
	N	**E**	**E/W**	**F**	*Significance*	**No CHIPS**	**+ CHIPS**	*Significance*
Phenolic acids and derivatives								
(mg GAE/L; mg CAE/L)								
Gallic acid	29.87 ± 4.97 **ab**	33.09 ± 2.07 **b**	26.12 ± 6.46 **a**	31.73 ± 1.86 **ab**	*	31.73 ± 2.60 **a**	28.67 ± 5.95 **a**	**ns**
Caftaric acid	11.93 ± 1.66 **b**	9.21 ± 1.58 **a**	9.02 ± 1.07 **a**	8.96 ± 1.05 **a**	*	9.74 ± 2.40 **a**	9.82 ± 1.00 **a**	**ns**
Caffeic acid	17.51 ± 3.14 **a**	14.59 ± 0.65 **a**	15.98 ± 0.60 **a**	15.82 ± 0.63 **a**	**ns**	16.19 ± 2.44 **a**	15.76 ± 1.06 **a**	**ns**
p-Coumaric acid	nd **a**	nd **a**	nd **a**	nd **a**	**ns**	nd **a**	nd **a**	**ns**
Ferulic acid	5.36 ± 0.59 **a**	5.25 ± 0.64 **a**	4.46 ± 1.36 **a**	5.39 ± 1.29 **a**	**ns**	5.12 ± 1.16 **a**	5.11 ± 0.90 **a**	**ns**
Σ Phenolic acids and derivatives	**64.67 ± 7.34 b**	**62.14 ± 3.97 b**	**55.59 ± 7.13 a**	**61.90 ± 1.28 ab**	*	**62.79 ± 5.97 a**	**59.36 ± 5.96 a**	**ns**
Stilbens (mg RE/L)								
cis-Piceid	0.12 ± 0.06 **a**	0.11 ± 0.02 **a**	0.35 ± 0.29 **b**	0.38 ± 0.33 **b**	*	0.09 ± 0.02 **a**	0.39 ± 0.27 **b**	*
trans-Piceid	0.14 ± 0.01 **a**	0.16 ± 0.05 **a**	0.16 ± 0.01 **a**	0.14 ± 0.01 **a**	**ns**	0.14 ± 0.01 **a**	0.16 ± 0.03 **a**	**ns**
Σ Stilbens	**0.26 ± 0.07 a**	**0.27 ± 0.05 a**	**0.51 ± 0.29 b**	**0.52 ± 0.34 b**	*	**0.23 ± 0.03 a**	**0.54 ± 0.26 b**	*
Flavonols (mg QE/L)								
Myricetin-3-glc	4.40 ± 1.94 **a**	4.69 ± 0.56 **a**	6.32 ± 0.42 **b**	7.47 ± 1.10 **b**	*	5.93 ± 1.00 **a**	5.52 ± 2.19 **a**	**ns**
Myricetin-3-rha	nd **a**	nd **a**	nd **a**	nd **a**	**ns**	nd **a**	nd **a**	**ns**
Quercetin-3-glc	nd **a**	nd **a**	nd **a**	nd **a**	**ns**	nd **a**	nd **a**	**ns**
Quercetin-3-glcr	0.31 ± 0.10 **a**	0.47 ± 0.05 **b**	0.50 ± 0.17 **b**	0.63 ± 0.01 **c**	*	0.53 ± 0.13 **a**	0.43 ± 0.16 **a**	**ns**
Quercetin-3-galac	0.42 ± 0.23 **b**	0.45 ± 0.16 **b**	nd **a**	2.27 ± 0.36 **c**	*	0.72 ± 1.03 **a**	0.85 ± 0.88 **a**	**ns**
Laricitrin-3-glc	1.86 ± 0.52 **a**	2.28 ± 0.47 **a**	2.03 ± 0.46 **a**	2.02 ± 0.12 **a**	**ns**	2.03 ± 0.38 **a**	2.07 ± 0.46 **a**	**ns**
Quercetin-3-rha	0.43 ± 0.08 **a**	0.71 ± 0.14 **b**	0.39 ± 0.08 **a**	0.49 ± 0.07 **a**	*	0.48 ± 0.13 **a**	0.53 ± 0.18 **a**	**ns**
Syringetin-3-galac	2.38 ± 0.43 **a**	3.30 ± 0.66 **b**	1.99 ± 0.49 **a**	2.20 ± 0.19 **a**	*	2.43 ± 0.50 **a**	2.50 ± 0.84 **a**	**ns**
Laricitrin-3-rhamnose-7-tri-hydroxycinnamic acid	0.26 ± 0.09 **ab**	0.37 ± 0.08 **c**	0.18 ± 0.02 **a**	0.30 ± 0.08 **bc**	*	0.26 ± 0.08 **a**	0.29 ± 0.12 **a**	**ns**
Σ Flavonols	**10.06 ± 2.97 a**	**12.28 ± 1.18 b**	**11.41 ± 0.86 ab**	**15.37 ± 1.31 c**	*	**12.38 ± 1.82 a**	**12.18 ± 3.29 a**	**ns**
Flavan-3-ols (mg CE/L)								
Procyanidin B3	2.72 ± 1.47 **a**	6.69 ± 2.97 **b**	9.37 ± 2.36 **c**	8.09 ± 0.43 **bc**	*	6.30 ± 2.51 **a**	7.13 ± 3.84 **a**	**ns**
(+)-Catechin	6.91 ± 1.65 **b**	10.85 ± 7.99 **d**	4.55 ± 0.71 **a**	9.82 ± 4.84 **c**	*	6.91 ± 4.45 **a**	9.15 ± 5.48 **b**	*
Procyanidin B1	10.18 ± 6.76 **b**	7.40 ± 4.51 **a**	10.76 ± 0.60 **b**	10.55 ± 1.00 **b**	*	12.27 ± 2.44 **b**	7.18 ± 3.52 **a**	*
Procyanidin B4	9.04 ± 4.39 **b**	7.37 ± 1.48 **a**	9.10 ± 4.32 **b**	8.84 ± 0.93 **ab**	*	10.59 ± 2.57 **b**	6.57 ± 1.70 **a**	*

(-)-Epicatechin	5.11 ± 1.52 **a**	5.88 ± 0.72 **a**	10.59 ± 4.49 **b**	10.49 ± 1.62 **b**	*	8.92 ± 4.07 **a**	7.11 ± 2.69 **a**	**ns**
Procyanidin B2	3.69 ± 1.07 **a**	3.32 ± 1.76 **a**	4.80 ± 0.61 **b**	5.35 ± 0.59 **b**	*	3.84 ± 1.73 **a**	4.73 ± 0.44 **b**	*
Σ Flavan-3-ols	**37.65 ± 11.78 a**	**41.51 ± 7.00 a**	**49.16 ± 7.92 b**	**53.13 ± 5.65 b**	*	**48.84 ± 9.92 b**	**41.88 ± 8.96 a**	*
Anthocyanins (mg ME/L)								
Dp-3-glc	0.54 ± 0.41 **b**	1.71 ± 0.12 **c**	0.29 ± 0.03 **a**	0.36 ± 0.08 **ab**	*	0.78 ± 0.58 **a**	0.67 ± 0.70 **a**	**ns**
Cy-3-glc	nd **a**	nd **a**	nd **a**	nd **a**	**ns**	nd **a**	nd **a**	**ns**
Pt-3-glc	0.64 ± 0.41 **b**	2.22 ± 0.14 **c**	0.33 ± 0.03 **a**	0.56 ± 0.03 **ab**	*	1.00 ± 0.77 **a**	0.88 ± 0.87 **a**	**ns**
Pn-3-glc	0.16 ± 0.19 **b**	0.93 ± 0.03 **c**	0.07 ± 0.09 **a**	0.11 ± 0.13 **ab**	*	0.40 ± 0.33 **a**	0.23 ± 0.43 **a**	**ns**
Mv-3-glc	6.51 ± 3.84 **b**	18.56 ± 2.16 **c**	3.12 ± 0.24 **a**	3.76 ± 0.86 **a**	*	8.51 ± 6.25 **a**	7.46 ± 7.60 **a**	**ns**
Dp-3-acetylglc	3.62 ± 0.77 **ab**	4.21 ± 0.45 **b**	3.31 ± 0.60 **ab**	3.25 ± 0.85 **a**	*	3.49 ± 0.75 **a**	3.71 ± 0.75 **a**	**ns**
Pyrano-Mv-3-glc (Vitisin B)	1.85 ± 0.38 **b**	2.02 ± 0.28 **b**	1.45 ± 0.03 **a**	1.30 ± 0.18 **a**	*	1.66 ± 0.39 **a**	1.65 ± 0.39 **a**	**ns**
Mv-3-glc-8-ethyl-gallocat	0.97 ± 0.13 **c**	0.96 ± 0.03 **c**	0.57 ± 0.02 **a**	0.69 ± 0.05 **b**	*	0.82 ± 0.21 **a**	0.77 ± 0.18 **a**	**ns**
Mv-3-*p*-coumglc-(epi)cat	0.35 ± 0.06 **b**	0.43 ± 0.04 **c**	0.23 ± 0.07 **a**	0.33 ± 0.10 **b**	*	0.30 ± 0.11 **a**	0.37 ± 0.07 **a**	**ns**
Pt-3-acetylglc	nd **a**	nd **a**	nd **a**	nd **a**	**ns**	nd **a**	nd **a**	**ns**
Pyrano-Mv-3-acetylglc (acetyl-Vitisin B)	nd **a**	nd **a**	nd **a**	nd **a**	**ns**	nd **a**	nd **a**	**ns**
Pn-3-acetylglc	0.72 ± 0.28 **b**	1.43 ± 0.15 **c**	0.33 ± 0.07 **a**	0.42 ± 0.11 **a**	*	0.79 ± 0.44 **a**	0.66 ± 0.52 **a**	**ns**
Mv-3-acetylglc	2.83 ± 1.29 **b**	7.56 ± 0.59 **c**	1.69 ± 0.24 **a**	1.53 ± 0.35 **a**	*	3.69 ± 2.67 **a**	3.12 ± 2.71 **a**	**ns**
Pyrano-Mv-3-*p*-coumglc (*p*-coum-Vitisin B)	0.75 ± 0.23 **b**	0.94 ± 0.21 **c**	0.57 ± 0.05 **a**	0.59 ± 0.07 **a**	*	0.69 ± 0.19 **a**	0.74 ± 0.23 **a**	**ns**
Mv-3-caffeoylglc	nd **a**	nd **a**	nd **a**	nd **a**	**ns**	nd **a**	nd **a**	**ns**
Pt-3-*p*-coumglc	nd **a**	nd **a**	nd **a**	nd **a**	**ns**	nd **a**	nd **a**	**ns**
Mv-3-*p*-coumglc-8-ethyl-(epi)cat	0.23 ± 0.27 **c**	0.21 ± 0.03 **bc**	0.10 ± 0.11 **a**	0.16 ± 0.19 **b**	*	0.29 ± 0.12 **b**	0.06 ± 0.11 **a**	*
Pn-3-*p*-coumglc	nd **a**	nd **a**	nd **a**	nd **a**	**ns**	nd **a**	nd **a**	**ns**
Mv-3-*p*-coumglc	0.77 ± 0.59 **b**	1.74 ± 0.11 **c**	0.37 ± 0.08 **a**	0.46 ± 0.16 **a**	*	1.00 ± 0.60 **a**	0.67 ± 0.65 **a**	**ns**
Mv-3-glc-4-vinyl(epi)cat	0.10 ± 0.11 **c**	0.14 ± 0.01 **d**	nd **a**	0.07 ± 0.08 **b**	*	0.12 ± 0.08 **a**	0.04 ± 0.06 **a**	**ns**
Mv-3-glc-4-vinyl-phenol/Pn-3-glc-vinyl-guaiacol	0.30 ± 0.08 **a**	0.40 ± 0.14 **b**	0.29 ± 0.14 **a**	0.37 ± 0.15 **b**	*	0.27 ± 0.09 **a**	0.41 ± 0.12 **a**	**ns**
Σ Anthocyanins	**20.35 ± 8.79 b**	**43.48 ± 3.46 c**	**12.71 ± 0.47 a**	**13.96 ± 1.28 a**	*	**23.81 ± 12.67 a**	**21.44 ± 15.02 a**	**ns**

Phenolic compounds	*Leaf remval * Treatment with oak chips*								
	N	**N+chips**	**E**	**E+chips**	**E/W**	**E/W+chips**	**F**	**F+chips**	*Significance*
Phenolic acids and derivatives									
(mg GAE/L; mg CAE/L)									
Gallic acid	32.88 ± 2.65 **b**	26.86 ± 5.57 **ab**	31.32 ± 0.58 **b**	34.86 **b**	30.47 ± 5.07 **b**	21.76 ± 4.87 **a**	32.26 ± 2.77 **b**	31.20 ± 1.28 **b**	*
Caftaric acid	13.36 ± 0.09 **d**	10.50 ± 0.25 **c**	7.85 ± 0.11 **a**	10.56 ± 0.38 **c**	8.38 ± 0.67 **ab**	9.66 ± 1.16 **bc**	9.37 ± 1.57 **abc**	8.55 ± 0.41 **ab**	*
Caffeic acid	18.65 ± 4.43 **b**	16.37 ± 2.18 **ab**	14.05 ± 0.01 **a**	15.14 ± 0.23 **ab**	16.14 ± 0.44 **ab**	15.82 ± 0.89 **ab**	15.93 ± 0.58 **ab**	15.71 ± 0.89 **ab**	*
p-Coumaric acid	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	**ns**
Ferulic acid	5.16 ± 0.76 **a**	5.55 ± 0.55 **a**	5.49 ± 0.97 **a**	5.01 ± 0.17 **a**	5.08 ± 1.99 **a**	3.85 ± 0.24 **a**	4.76 ± 1.84 **a**	6.02 ± 0.06 **a**	**ns**
Σ Phenolic acids and derivatives	**70.06 ± 6.22 c**	**59.28 ± 2.59 ab**	**58.71 ± 0.51 ab**	**65.57 ± 0.03 bc**	**60.07 ± 7.28 ab**	**51.10 ± 4.36 a**	**62.32 ± 1.92 bc**	**61.49 ± 0.75 bc**	*
Stilbens (mg RE/L)									
cis-Piceid	0.07 **a**	0.18 ± 0.01 **b**	0.10 ± 0.04 **ab**	0.11 **ab**	0.10 ± 0.03 **ab**	0.59 ± 0.05 **c**	0.09 ± 0.01 **ab**	0.67 ± 0.09 **c**	*
trans-Piceid	0.14 **a**	0.15 ± 0.02 **a**	0.15 ± 0.02 **a**	0.18 ± 0.07 **a**	0.15 **a**	0.16 ± 0.01 **a**	0.13 ± 0.02 **a**	0.14 ± 0.01 **a**	**ns**
Σ Stilbens	**0.20 a**	**0.32 ± 0.01 b**	**0.25 ± 0.02 ab**	**0.29 ± 0.07 ab**	**0.25 ± 0.03 ab**	**0.76 ± 0.04 c**	**0.23 ± 0.03 ab**	**0.81 ± 0.09 c**	*
Flavonols (mg QE/L)									
Myricetin-3-glc	5.95 ± 1.27 **bcd**	2.85 ± 0.17 **a**	4.82 ± 0.93 **bc**	4.56 ± 0.03 **ab**	6.12 ± 0.05 **bcd**	6.52 ± 0.61 **cde**	6.81 ± 0.66 **de**	8.13 ± 1.21 **e**	*
Myricetin-3-rha	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	**ns**
Quercetin-3-glc	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	**ns**
Quercetin-3-glcr	0.38 ± 0.09 **b**	0.23 **a**	0.45 ± 0.07 **bc**	0.50 ± 0.04 **c**	0.64 ± 0.03 **d**	0.36 ± 0.07 **b**	0.64 **d**	0.62 ± 0.02 **d**	*
Quercetin-3-galac	0.23 ± 0.09 **ab**	0.61 ± 0.02 **b**	0.31 ± 0.01 **ab**	0.58 **b**	nd **a**	nd **a**	2.34 ± 0.52 **c**	2.19 ± 0.31 **c**	*
Laricitrin-3-glc	2.26 ± 0.40 **b**	1.46 ± 0.07 **a**	1.94 ± 0.43 **ab**	2.63 **b**	1.87 ± 0.68 **ab**	2.20 ± 0.22 **ab**	2.06 ± 0.07 **ab**	1.98 ± 0.18 **ab**	*
Quercetin-3-rha	0.48 ± 0.10 **ab**	0.38 **a**	0.63 ± 0.14 **bc**	0.80 ± 0.10 **c**	0.35 ± 0.04 **a**	0.44 ± 0.09 **ab**	0.45 **ab**	0.52 ± 0.10 **ab**	*
Syringetin-3-galac	2.72 ± 0.27 **ab**	2.03 ± 0.04 **ab**	2.80 ± 0.58 **b**	3.79 ± 0.05 **c**	2.09 ± 0.70 **ab**	1.89 ± 0.42 **a**	2.13 ± 0.08 **ab**	2.28 ± 0.29 **ab**	*
Laricitrin-3-rhamnose-7-tri-hydroxycinnamic acid	0.33 ± 0.10 **cd**	0.20 **ab**	0.31 ± 0.04 **bc**	0.44 ± 0.02 **d**	0.17 **a**	0.19 ± 0.03 **ab**	0.25 ± 0.06 **abc**	0.34 ± 0.07 **cd**	*
Σ Flavonols	**12.35 ± 2.32 bc**	**7.76 ± 0.18 a**	**11.26 ± 0.12 b**	**13.30 ± 0.14 bc**	**11.24 ± 1.43 b**	**11.59 ± 0.23 b**	**14.67 ± 0.20 cd**	**16.07 ± 1.78 d**	*
Flavan-3-ols (mg CE/L)									
Procyanidin B3	3.98 ± 0.35 **a**	1.46 ± 0.09 **a**	4.22 ± 1.34 **a**	9.15 ± 0.68 **b**	8.75 ± 1.73 **b**	9.99 ± 3.49 **b**	8.27 ± 0.13 **b**	7.91 ± 0.64 **b**	*
(+)-Catechin	5.53 ± 0.70 **b**	8.29 ± 0.18 **c**	3.93 ± 0.14 **a**	17.77 ± 0.14 **e**	4.17 ± 0.95 **a**	4.92 ± 0.16 **ab**	14.01 ± 0.12 **d**	5.63 ± 0.36 **b**	*
Procyanidin B1	15.97 ± 1.75 **c**	3.55 ± 0.23 **a**	11.26 ± 1.18 **b**	4.39 ± 0.07 **a**	10.89 ± 0.51 **b**	10.63 ± 0.87 **b**	10.94 ± 0.41 **b**	10.17 ± 1.50 **b**	*
Procyanidin B4	12.82 ± 0.77 **d**	5.25 ± 0.22 **a**	8.21 ± 1.93 **bc**	6.52 ± 0.02 **ab**	12.83 ± 0.28 **d**	5.36 ± 0.33 **a**	8.52 ± 1.40 **bc**	9.16 ± 0.50 **c**	*
(-)-Epicatechin	6.23 ± 1.35 **ab**	3.99 ± 0.31 **a**	6.02 ± 1.22 **ab**	5.74 ± 0.10 **ab**	12.83 ± 6.36 **c**	8.36 ± 0.03 **abc**	10.62 ± 2.16 **bc**	10.35 ± 1.79 **bc**	*

Procyanidin B2	2.91 ± 0.89 **a**	4.48 ± 0.38 **b**	1.81 ± 0.44 **a**	4.82 ± 0.06 **b**	5.04 ± 0.91 **b**	4.56 ± 0.23 **b**	5.63 ± 0.06 **b**	5.07 ± 0.85 **b**	*
Σ Flavan-3-ols	**47.43 ± 5.80 cd**	**27.87 ± 0.04 a**	**35.46 ± 0.93 ab**	**47.55 ± 0.25 cd**	**54.50 ± 7.81 de**	**43.82 ± 3.61 bc**	**57.98 ± 0.04 e**	**48.29 ± 1.33 cd**	*
Anthocyanins (mg ME/L)									
Dp-3-glc	0.86 ± 0.30 **b**	0.22 ± 0.04 **a**	1.62 ± 0.08 **c**	1.80 ± 0.07 **c**	0.31 ± 0.04 **a**	0.27 ± 0.01 **a**	0.34 ± 0.12 **a**	0.38 ± 0.06 **a**	*
Cy-3-glc	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	**ns**
Pt-3-glc	0.94 ± 0.37 **b**	0.34 ± 0.07 **a**	2.16 ± 0.20 **c**	2.28 ± 0.02 **c**	0.35 ± 0.01 **a**	0.32 ± 0.04 **a**	0.55 ± 0.02 **a**	0.57 ± 0.04 **a**	*
Pn-3-glc	0.31 ± 0.09 **c**	nd **a**	0.93 ± 0.04 **d**	0.93 ± 0.04 **d**	0.15 ± 0.03 **b**	nd **a**	0.22 **b**	nd **a**	*
Mv-3-glc	9.79 ± 0.96 **b**	3.23 ± 0.57 **a**	17.37 ± 2.83 **c**	19.75 ± 0.56 **c**	3.25 ± 0.09 **a**	2.98 ± 0.30 **a**	3.62 ± 1.21 **a**	3.90 ± 0.82 **a**	*
Dp-3-acetylglc	4.17 ± 0.73 **bc**	3.08 ± 0.25 **ab**	3.85 ± 0.29 **abc**	4.57 ± 0.06 **c**	3.28 ± 0.77 **ab**	3.33 ± 0.69 **abc**	2.65 ± 0.09 **a**	3.84 ± 0.87 **abc**	*
Pyrano-Mv-3-glc (Vitisin B)	2.12 ± 0.35 **cd**	1.57 ± 0.09 **ab**	1.81 ± 0.23 **bc**	2.24 ± 0.03 **d**	1.44 ± 0.03 **ab**	1.45 ± 0.05 **ab**	1.27 ± 0.19 **a**	1.32 ± 0.23 **a**	*
Mv-3-glc-8-ethyl-gallocat	1.06 ± 0.10 **d**	0.88 ± 0.08 **c**	0.94 ± 0.02 **cd**	0.98 ± 0.03 **cd**	0.59 ± 0.01 **ab**	0.54 ± 0.01 **a**	0.69 ± 0.07 **b**	0.70 ± 0.03 **b**	*
Mv-3-*p*-coumglc-(epi)cat	0.37 ± 0.07 **cde**	0.34 ± 0.08 **bcd**	0.41 ± 0.05 **de**	0.45 **e**	0.17 ± 0.04 **a**	0.29 ± 0.01 **bc**	0.25 ± 0.05 **ab**	0.41 ± 0.02 **de**	*
Pt-3-acetylglc	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	**ns**
Pyrano-Mv-3-acetylglc (acetyl-Vitisin B)	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	**ns**
Pn-3-acetylglc	0.95 ± 0.20 **b**	0.50 ± 0.05 **a**	1.36 ± 0.23 **c**	1.50 ± 0.02 **c**	0.38 ± 0.06 **a**	0.28 ± 0.03 **a**	0.47 ± 0.15 **a**	0.37 ± 0.07 **a**	*
Mv-3-acetylglc	3.86 ± 0.89 **b**	1.81 ± 0.11 **a**	7.62 ± 1.00 **c**	7.50 ± 0.15 **c**	1.55 ± 0.24 **a**	1.83 ± 0.17 **a**	1.72 ± 0.43 **a**	1.33 ± 0.16 **a**	*
Pyrano-Mv-3-*p*-coumglc (*p*-coum-Vitisin B)	0.92 ± 0.23 **c**	0.59 ± 0.08 **ab**	0.76 ± 0.01 **bc**	1.12 **d**	0.53 ± 0.03 **a**	0.61 ± 0.02 **ab**	0.53 ± 0.02 **a**	0.64 **ab**	*
Mv-3-caffeoylglc	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	**ns**
Pt-3-*p*-coumglc	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	**ns**
Mv-3-*p*-coumglc-8-ethyl-(epi)cat	0.46 ± 0.02 **d**	nd **a**	0.20 ± 0.03 **b**	0.23 ± 0.01 **b**	0.19 ± 0.04 **b**	nd **a**	0.32 ± 0.08 **c**	nd **a**	**ns**
Pn-3-*p*-coumglc	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	**ns**
Mv-3-*p*-coumglc	1.27 ± 0.25 **b**	0.28 **a**	1.76 ± 0.18 **c**	1.73 ± 0.05 **c**	0.40 ± 0.10 **a**	0.34 ± 0.08 **a**	0.57 ± 0.16 **a**	0.34 **a**	*
Mv-3-glc-4-vinyl(epi)cat	0.19 **c**	nd **a**	0.14 **b**	0.14 ± 0.01 **b**	nd **a**	nd **a**	0.15 **b**	nd **a**	*
Mv-3-glc-4-vinyl-phenol/ Pn-3-glc-vinyl-guaiacol	0.37 ± 0.05 **cd**	0.23 ± 0.02 **ab**	0.29 ± 0.08 **bc**	0.52 **f**	0.17 ± 0.01 **a**	0.42 ± 0.01 **de**	0.25 ± 0.02 **ab**	0.50 ± 0.02 **ef**	*
Σ Anthocyanins	**27.65 ± 4.10 b**	**13.06 ± 1.37 a**	**41.23 ± 3.82 c**	**45.73 ± 1.01 c**	**12.75 ± 0.11 a**	**12.68 ± 0.81 a**	**13.61 ± 2.06 a**	**14.31 ± 0.43 a**	*

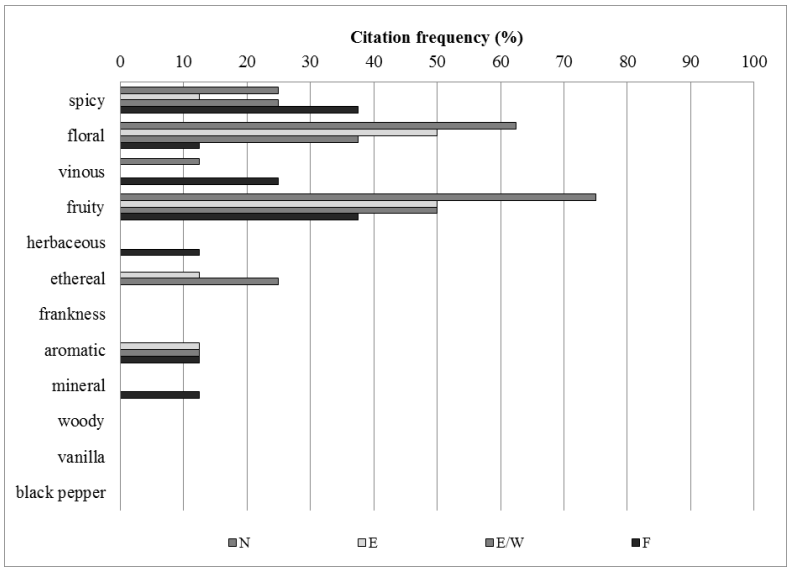
Table 56 – Single and interactive effects of leaf removal and treatment with oak chips on the phenolic profile of Nero di Troia wines analysed after 12 months after racking.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
nd: not detected; glc: glucoside, glcr: glucuronide, rha: rhamnoside, galac: galactoside, Dp: delphinidin, Cy: cyanidin, Pt: petunidin, Pn: peonidin, Mv: malvidin, acetylglc: acetylglucoside, *p*-coumglc: *p*-coumaroylglucoside, caffeoylglc: caffeoylglucoside, cat: catechin.
In row, as part of leaf removal or treatment with oak chips or combination of leaf removal-oak chips, different letters indicate significant differences ($p < 0.05$ - LSD test).

	CI	T	dA(%)	% yellow	% red	% blue	dAl%	dAT%	dTAT%
				Leaf removal					
N	9.837 ± 1.170 **a**	0.686 ± 0.010 **a**	50.4 ± 1.2 **b**	34.4 ± 0.2 **a**	50.2 ± 0.6 **b**	15.4 ± 0.5 **a**	0.8 ± 0.1 **a**	10.2 ± 3.0 **a**	89.0 ± 3.0 **b**
E	13.255 ± 2.032 **b**	0.724 ± 0.019 **b**	47.8 ± 3.4 **a**	35.4 ± 0.6 **b**	49.0 ± 1.6 **a**	15.6 ± 2.0 **ab**	1.6 ± 0.7 **b**	18.0 ± 2.1 **b**	80.8 ± 3.2 **a**
E/W	9.307 ± 0.674 **a**	0.696 ± 0.013 **a**	48.1 ± 1.2 **ab**	34.1 ± 0.3 **a**	49.1 ± 0.6 **a**	16.8 ± 0.4 **c**	0.7 ± 0.1 **a**	8.9 ± 1.8 **a**	90.4 ± 2.0 **b**
F	9.613 ± 1.092 **a**	0.703 ± 0.037 **ab**	48.1 ± 3.3 **ab**	34.5 ± 0.7 **a**	49.1 ± 1.5 **a**	16.4 ± 0.9 **bc**	0.8 ± 0.1 **a**	9.1 ± 3.7 **a**	90.1 ± 3.8 **b**
Significance	*	*	*	*	*	*	*	*	*
				Treatment with oak chips					
No CHIPS	10.701 ± 2.556 **a**	0.696 ± 0.023 **a**	48.9 ± 2.8 **a**	34.4 ± 0.5 **a**	49.5 ± 1.3 **a**	16.1 ± 1.3 **a**	0.8 ± 0.3 **a**	11.6 ± 4.1 **a**	87.8 ± 4.2 **a**
+ CHIPS	10.305 ± 1.476 **a**	0.708 ± 0.027 **a**	48.3 ± 2.5 **a**	34.8 ± 0.8 **a**	49.2 ± 1.2 **a**	16.0 ± 1.2 **a**	1.1 ± 0.7 **a**	11.5 ± 5.2 **a**	87.4 ± 5.8 **a**
Significance	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
				Leaf removal * Treatment with oak chips					
N	10.354 ± 1.132 **a**	0.677 ± 0.006 **a**	51.5 ± 0.2 **c**	34.3 ± 0.2 **ab**	50.7 ± 0.1 **c**	14.9 ± 0.2 **ab**	0.8 ± 0.1 **a**	12.6 ± 1.5 **b**	86.6 ± 1.5 **b**
N+chips	9.320 ± 1.094 **a**	0.694 ± 0.003 **ab**	49.3 ± 0.1 **bc**	34.5 ± 0.1 **abc**	49.6 **abc**	15.9 ± 0.1 **bc**	0.9 ± 0.2 **ab**	7.7 ± 1.7 **a**	91.4 ± 1.9 **c**
E	14.208 ± 2.383 **c**	0.730 ± 0.022 **c**	46.0 ± 4.2 **a**	35.1 ± 0.4 **cd**	48.1 ± 1.9 **a**	16.8 ± 2.3 **c**	1.1 ± 0.5 **b**	17.2 ± 2.9 **c**	82.5 ± 4.0 **a**
E+chips	12.302 ± 1.238 **b**	0.717 ± 0.016 **bc**	49.7 ± 0.5 **bc**	35.7 ± 0.6 **d**	49.8 ± 0.2 **bc**	14.4 ± 0.4 **a**	2.2 ± 0.1 **c**	18.8 ± 0.1 **c**	79.2 **a**
E/W	9.045 ± 0.473 **a**	0.688 ± 0.002 **ab**	48.6 ± 0.5 **abc**	33.9 ± 0.2 **a**	49.3 ± 0.2 **abc**	16.7 ± 0.4 **c**	0.7 **a**	8.2 ± 0.5 **a**	91.2 ± 0.5 **c**
E/W+chips	9.570 ± 0.807 **a**	0.703 ± 0.015 **abc**	47.6 ± 1.5 **ab**	34.3 ± 0.2 **ab**	48.8 ± 0.7 **ab**	16.9 ± 0.5 **c**	0.6 ± 0.2 **a**	9.6 ± 2.5 **ab**	89.7 ± 2.8 **bc**
F	9.197 ± 1.500 **a**	0.688 ± 0.004 **ab**	49.4 ± 0.3 **bc**	34.2 ± 0.1 **ab**	49.7 ± 0.1 **bc**	16.1 ± 0.1 **bc**	0.8 **a**	8.4 ± 0.9 **a**	90.9 ± 1.0 **c**
F+chips	10.028 ± 0.273 **a**	0.718 ± 0.050 **bc**	46.7 ± 4.5 **ab**	34.7 ± 0.9 **bc**	48.5 ± 2.1 **ab**	16.8 ± 1.2 **c**	0.8 ± 0.2 **ab**	9.8 ± 5.5 **ab**	89.3 ± 5.6 **bc**
Significance	*	*	*	*	*	*	*	*	*

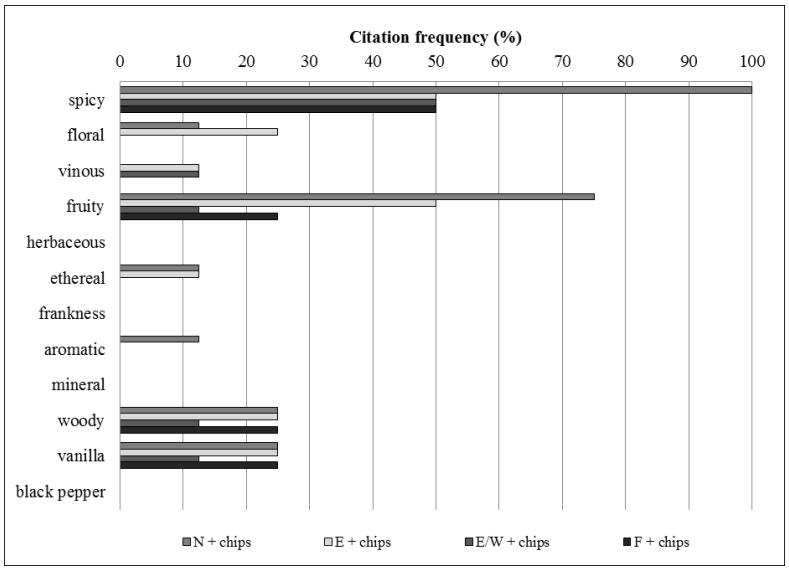
Table 57 – Single and interactive effects of leaf removal and treatment with oak chips on the colour parameters of Nero di Troia wines analysed 12 months after racking.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
CI: colour intensity; T: tonality; dA(%): percentage of red colour due to flavilium cation of free and combined anthocyanins; % yellow: percentage of yellow component; % red: percentage of red component; % blue: percentage of blue component; dAl%: percentage of absorbance at 520 nm due to monomeric anthocyanins; dAT%: percentage of absorbance at 520 nm due to polymeric pigments decolorized with SO_2; dTAT%: percentage of absorbance at 520 nm due to polymeric pigments not decolorized.
In column, as part of leaf removal or treatment with oak chips or combination of leaf removal-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).*: significant difference; ns: no significant difference.

	Leaf removal * Treatment with oak chips								
	N	**N+chips**	**E**	**E+chips**	**E/W**	**E/W+chips**	**F**	**F+chips**	*Significance*
					Visual descriptors				
Clearness	2.00 **a**	2.00 **a**	2.00 **a**	1.88 ± 0.35 **a**	2.00 **a**	2.00 **a**	1.81 ± 0.37 **a**	2.00 **a**	**ns**
Texture	2.38 ± 0.44 **a**	2.38 ± 0.44 **a**	2.38 ± 0.44 **a**	2.38 ± 0.44 **a**	2.44 ± 0.42 **a**	2.38 ± 0.48 **a**	2.25 ± 0.65 **a**	2.38 ± 0.48 **a**	**ns**
					Olfactory descriptors				
Olfactory intensity	2.13 ± 0.58 **a**	2.44 ± 0.56 **a**	2.44 ± 0.42 **a**	2.44 ± 0.90 **a**	2.25 ± 0.65 **a**	2.63 ± 0.48 **a**	2.25 ± 0.65 **a**	2.13 ± 0.85 **a**	**ns**
Olfactory complexity	1.88 ± 0.64 **a**	2.31 ± 0.59 **a**	2.00 ± 0.71 **a**	2.13 ± 0.83 **a**	2.13 ± 0.69 **a**	2.25 ± 0.50 **a**	2.19 ± 0.65 **a**	2.00 ± 0.82 **a**	**ns**
Olfactory quality	2.06 ± 0.32 **a**	2.56 ± 0.56 **a**	2.13 ± 0.83 **a**	2.25 ± 0.53 **a**	1.94 ± 0.82 **a**	2.50 ± 0.58 **a**	1.94 ± 0.78 **a**	2.25 ± 0.65 **a**	**ns**
					Gustatory-olfactory descriptors				
Sugars	0.00 **a**	0.00 **a**	0.00 **a**	0.00 **a**	0.00 **a**	0.00 **a**	0.00 **a**	0.00 **a**	**ns**
Alcohols	2.38 ± 0.52 **a**	2.31 ± 0.53 **a**	2.44 ± 0.78 **a**	2.44 ± 0.78 **a**	2.63 ± 0.44 **a**	2.50 ± 0.71 **a**	2.19 ± 1.19 **a**	2.63 ± 0.48 **a**	**ns**
Polyols	2.19 ± 0.26 **a**	2.13 ± 0.23 **a**	2.25 ± 0.38 **a**	1.94 ± 0.62 **a**	1.94 ± 0.42 **a**	2.13 ± 0.25 **a**	2.25 ± 0.38 **a**	2.13 ± 0.25 **a**	**ns**
Acids	2.06 ± 0.68 **a**	1.81 ± 0.53 **a**	1.88 ± 0.64 **a**	1.94 ± 0.56 **a**	2.13 ± 0.58 **a**	2.00 ± 0.41 **a**	2.44 ± 0.98 **a**	2.25 ± 0.65 **a**	**ns**
Tannins	2.56 ± 0.68 **ab**	2.94 ± 0.50 **b**	2.13 ± 0.74 **a**	2.50 ± 0.76 **b**	2.19 ± 0.84 **ab**	2.38 ± 0.95 **ab**	1.81 ± 0.88 **a**	2.38 ± 0.63 **ab**	*
Minerals	1.69 ± 0.65 **a**	1.94 ± 0.50 **ab**	1.88 ± 0.52 **ab**	1.88 ± 0.58 **ab**	2.00 ± 0.89 **ab**	2.13 ± 0.63 **ab**	2.38 ± 0.88 **b**	2.25 ± 0.50 **ab**	*
Structure	2.50 ± 0.46 **a**	2.50 ± 0.53 **a**	2.63 ± 0.44 **a**	2.56 ± 0.50 **a**	2.44 ± 0.73 **a**	2.63 ± 0.48 **a**	2.13 ± 0.83 **a**	2.75 ± 0.50 **a**	**ns**
Balance	2.38 ± 0.35 **b**	2.63 ± 0.52 **b**	2.50 ± 0.60 **b**	2.56 ± 0.56 **b**	2.19 ± 0.37 **ab**	2.38 ± 0.48 **b**	1.69 ± 0.70 **a**	2.38 ± 0.48 **b**	*
Gustatory-olfactory intensity	2.44 ± 0.32 **ab**	2.75 ± 0.46 **b**	2.50 ± 0.93 **ab**	2.56 ± 0.86 **b**	2.13 ± 0.69 **ab**	2.50 ± 0.71 **ab**	1.81 ± 0.92 **a**	2.38 ± 0.63 **ab**	*
Gustatory-olfactory persistence	2.50 ± 0.38 **ab**	2.75 ± 0.46 **b**	2.38 ± 0.92 **ab**	2.63 ± 0.74 **ab**	2.25 ± 0.71 **ab**	2.38 ± 0.95 **ab**	2.00 ± 0.71 **a**	2.38 ± 0.75 **ab**	*
Gustatory-olfactory quality	2.38 ± 0.35 **bc**	2.63 ± 0.52 **c**	2.25 ± 0.85 **bc**	2.31 ± 0.75 **bc**	1.88 ± 0.74 **ab**	2.38 ± 0.85 **bc**	1.38 ± 0.69 **a**	2.25 ± 0.65 **bc**	*
					Final considerations				
Evolutionary state	2.88 ± 0.35 **a**	3.13 ± 0.64 **a**	3.06 ± 0.78 **a**	2.69 ± 0.59 **a**	3.06 ± 0.56 **a**	3.13 ± 0.25 **a**	3.19 ± 0.53 **a**	3.25 ± 0.50 **a**	**ns**
Harmony	2.38 ± 0.44 **b**	2.63 ± 0.52 **b**	2.25 ± 0.65 **b**	2.56 ± 0.56 **b**	2.31 ± 0.46 **b**	2.50 ± 0.71 **b**	1.56 ± 0.73 **a**	2.38 ± 0.48 **b**	*

Table 58 – Interactive effects of leaf removal and treatment with oak chips on the sensory characteristics of Nero di Troia wines analysed 12 months after racking.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.
In column, as part of leaf removal or treatment with oak chips or combination of leaf removal-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference; ns: no significant difference.

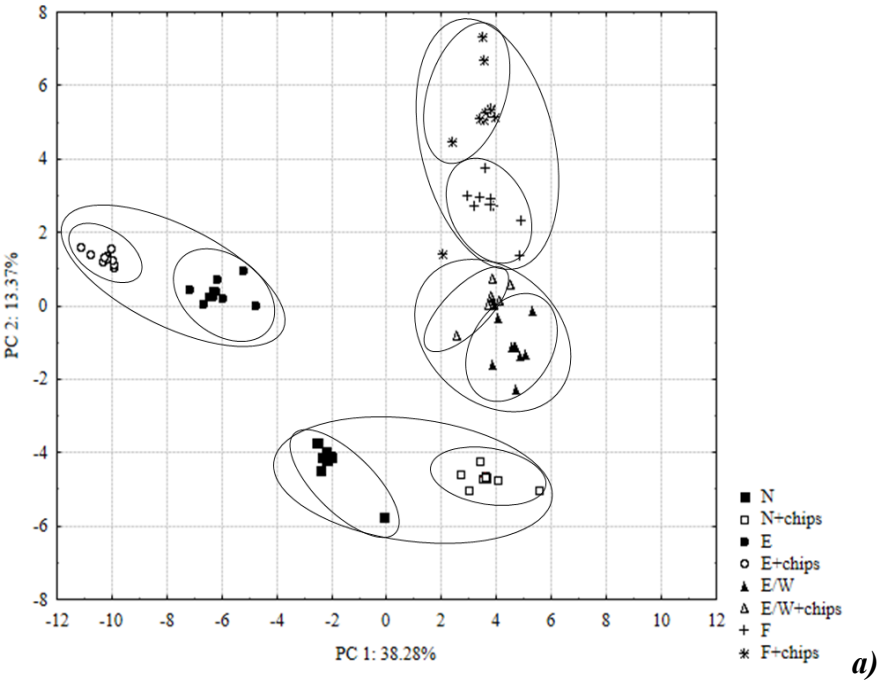


a)

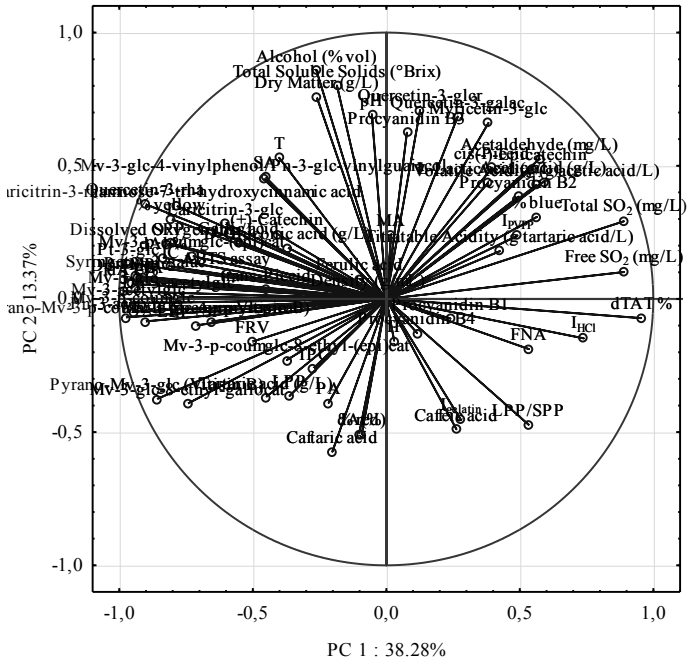


b)

Figure 20 - Citation frequency (%) of Nero di Troia wines not treated (*a*) and treated with oak chips (*b*) aged for 12 months.



a)



b)

Figure 21 - PCA scatter plot for projection on the factor plane of: **a)** Nero di Troia wine samples after 12 months from the racking and **b)** analytical results.
N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and in pre-harvest also along the east side.

7.6 Evaluation of single and interactive effects of the winemaking technologies and treatment with oak chips on colour, phenolic composition and sensory profile of Nero di Troia wines

The main oenological parameters of the wines after 12 months of aging are listed in Table 59. The main effects were those related to the wine-making technology. Even after one year of aging wines produced through cryomaceration still showed the highest alcohol strength while extended macerated wines exhibited the lowest alcohol contents. Extended macerated wines also had the lowest dry matter, while wines obtained through traditional procedure or addition of pectolytic enzymes exhibited the highest values of this parameter. The cryomacerated wines showed the lowest volatile acidity value and the highest free and total sulphur dioxide concentrations as a consequence of the lower oxidation phenomena occurred in these wines. Carillo *et al.* (2011) highlighted that wines produced by cryomaceration show higher stability to oxidation than those obtained through the traditional red-wine winemaking. The prolonged macerated wines exhibited the highest volatile acidity value and the lowest free/total SO_2 ratio, probably as a consequence of ethanol oxidation. As already observed at racking, even after 12 months the titratable acidity was significantly lower in the wines produced through cryomaceration and through extended maceration. The titratable acidity values in all wines decreased with respect to the racking, as consequence of the precipitation of potassium hydrogen tartrate (Ribéreau-Gayon *et al*., 2004). Density and acetaldehyde content did not show significant differences among the winemaking procedures. The traditional wines exhibited the highest values of dissolved oxygen content and the redox potential.

Concerning the single effects of the treatment with oak chips, the only parameter that showed significant differences was the dissolved oxygen content, which was lower in the wines subjected to the treatment with oak chips. As already explained in the previous paragraphs, the decrease of dissolved oxygen may be correlated to the capacity of ellagic tannins (released from wood) to absorb the oxygen and protect wine phenolics from oxidation (Vivas and Glories, 1996; Guerra *et al*., 1996).

Concerning the interactive effects, they determined significant differences on all the main oenological parameters except for density and acetaldehyde. The highest alcohol strength and the lowest titratable acidity were detected in wines obtained through cryomaceration

(independently on the treatment with oak chips) while the traditional wines (untreated and treated with oak chips) showed the highest dry matter, titratable acidity, dissolved oxygen content (together with the cryomacerated wines), and redox potential, and the lowest pH. The wines obtained through prolonged maceration exhibited the lowest alcohol content, dry matter, titratable acidity and redox potential, and the highest volatile acidity.

Compared to the racking (Tables 38 and 39; paragraph 7.3), in all the wines: ethanol content decreased and volatile acidity increased due to the acetic acid bacteria metabolism; titatrable acidity decreased and pH increased due to the malo-lactic conversion by lactic acid bacteria; total soluble solids slight decreased; free and total sulphur dioxide content decreased as consequence of oxidation; acetaldehyde decreased as consequence of its participation in polymerization reactions involving the wine phenolic compounds (Liu and Pilone, 2000); dissolved oxygen and red-ox potential decreased as a consequence of the in-bottle aging.

The single and combined effects of the winemaking technology and the treatment with oak wood chips on the organic acids composition of Nero di Troia wines were reported in Table 60. Concerning the single effect of winemaking technology, the only significant difference among samples was detected for the acetic acid content, which exhibited the lowest and the highest values in cryomacerated and extended macerated wines, respectively, in agreement with the data of the volatile acidity. Regarding the single effects of the treatment with oak chips, the highest L-lactic acid contents were detected in the treated wines.

After 12 months of aging, the organic acid profiles strongly changed with respect to racking (Table 40, paragraph 7.3). First of all, slight decreases of the tartaric acid content, in particular in the cryomacerated wines, were observed. This data was probably to the precipitation of the crystallized tartrates. Furthermore, the L-malic fell under the detection limit due to its conversion into L-lactic acid (Ribéreau-Gayon *et al*., 2000). The concentration of citric acid was about halved compared to that of the racking, essentially due to its oxidation and the synthesis of acetic acid and diacetyl (Shimazu *et al*., 1985). Indeed, both malic and citric acids are fermented by lactic acid bacteria after the major growth phase, when the bacteria enter the stationary phase (Jackson, 2008). The acetic acid content increased, in agreement with the increase of the volatile acidity of the wines, due to its production by acetic acid bacteria and/or lactic acid bacteria. Acetic acid can be produced by the metabolism of citric, malic, tartaric, and gluconic acids, as well as

hexoses, pentoses, and glycerol (Jackson, 2008). The consumption of gluconic acid, similarly to that explained for acetic acid, could be due the metabolism of acetic acid bacteria and/or lactic acid bacteria. Indeed, gluconic acid is also metabolized by lactic acid bacteria, with the exception of the pediococci (Jackson, 2008). The disappearance of pyruvic acid in all the wines, after 12 months of aging, may be due to its reaction with free sulphur dioxide (Ribéreau-Gayon *et al*., 2000) or with anthocyanins to form cycloaddition products called pyranoanthocyanins (Jackson, 2008; Morata *et al*., 2007).

Tables from 61 to 63 report the results concerning the single and interactive effects of the winemaking technology and treatment with oak chips on the phenolic composition, antioxidant activity, monomeric and polymeric pigments distribution, structure indices, and phenolic profile of the wines 12 months after racking. Concerning the single effects of winemaking on the phenolic classes (Table 61): wines obtained through cryomaceration and addition of enzymes exhibited the highest contents of total anthocyanins, anthocyanins sensitive to SO_2, and total phenolics; wines obtained through cryomaceration also showed the highest concentrations of total flavonoids, flavonoids different from anthocyanins, flavans reactive with vanillin, and proanthocyanidins. With reference to the single effects of the oak chips, the highest concentrations of anthocyanins sensitive to SO_2 (+24%), monomeric anthocyanins (+19%), total flavonoids (+6.6%), flavonoids different from anthocyanins (+8%), and total phenolics (+4.5%) were detected in the treated wines (data between brackets are referred to the concentrations respect to those detected in the control wines). As already discussed, these phenomena can be explained by the antioxidant action of ellagic tannins, which are oxygen scavenger, thus protecting wine phenolics (in particular anthocyanins and flavans) from oxidation (Vivas and Glories, 1996; Guerra *et al*., 1996).

Concerning the interactive effects of winemaking and oak chips: the highest concentrations of total anthocyanins were detected in cryomacerated wines; the highest anthocyanins sensitive to SO_2 were detected in wines treated with chips and obtained through cryomaceration, addition of enzymes, and extended maceration; the highest monomeric anthocyanins in wines produced through addition of enzymes; the highest concentrate contents of flavonoids different from anthocyanins, flavans reactive with vanillin, and proanthocyanidins were detected in wines treated with chips and obtained by cryomacerated grapes. Winemaking procedures and oak chips didn't have significant effects on the antioxidant capacity of the wines.

According to the results of Table 62, the winemaking procedures exerted significant singles effects on all the listed parameters. The distribution of anthocyanins among monomeric and polymeric forms suffered several changes during the 12 months of in-bottle aging. The wines obtained through addition of enzymes exhibited the highest content of monomeric and small polymeric pigments, and the lowest large-to-small polymeric pigments ratio in comparison with the other technologies. The wines obtained through the traditional vinification, instead, showed the lowest values of absorbance due to monomeric pigments, and the highest values of polymeric pigments (small and large) and of large-to-small polymeric pigments ratio, thus indicating a higher degree of phenolic polymerization. Concerning the structure indices, a decrease of the gelatin index and an increase of HCl index were observed in all the wines, especially in wines elaborated with addition of enzymes, which showed the lowest value of gelatin index, while extended macerated wines showed the lowest HCl index value. The PVPP index decreased in all the wines with respect to the racking, and its lowest values were detected in wines obtained by traditional vinification. The oak chips produced significant single effects only on small polymeric pigments and HCl index. Oak-treated samples exhibited a greater value of absorbance due to the unstable small polymeric pigments and a lower degree of tannins polymerization (low HCl index). These data were similar to those found in Nero di Troia wines treated with oak chips before or after malolactic fermentation (paragraph 7.4; Table 48). As already explained, both HCl and PVPP indices were already high (average by 35.7 and 69.2) at racking, thus indicating a high degree of polymerization of tannins and anthocyans, respectively. No effect was observed on the gelatin index. These data further indicated that the contact with oak chips, and thus the extraction of ellagitannins, did not contribute to the increase of anthocyanin polymerization, in disagreement with the study of Piracci *et al*. (2001), Del Alamo Sanza and Nevares Domínguez (2006), and with the data discussed in paragraphs 7.2.1, 7.2.2 and 7.2.3 for Aglianico, Montepulciano, and Nero di Troia wines. As already stated, this lack in the polymerization of anthocyans could be due the unbalanced tannins-to-anthocyans ratio (T/A) found in the studied wines. The tannin-to-anthocyanin ratio is an index used by several authors to indicate the wine quality (Ribéreau-Gayon *et al*., 2000; Salinas, 2003). The best T/A ratio for wines that are going to be submitted to oak barrel aging are between 1 and 4 (Hidalgo, 2003; Zamora, 2003; Ribéreau-Gayon *et al*., 2004). In the Nero di Troia wines discussed in the present paragraph, at racking, this ratio was about 7.6 (traditional vinification), 7.1 (cryomaceration), 7.3 (maceration with enzymes), and 9.8 (extended maceration), which

are most similar to that found in Nero di Troia wines in the paragraph 7.4 (about 9). Concerning the combined effects on pigments and structure indices, also in this case different trends were observed. Between the wines produced by traditional winemaking technology, those treated with oak chips exhibited the lowest absorbance due to monomeric pigments, LPP/SPP ratio, gelatin and HCl indexes and the highest values of small and large polymeric pigments, and of PVPP index. Even in cryomacerated wines, the treatment with oak chips comported an increase of small polymeric pigments and of PVPP index, whereas large polymeric pigments, large-to-small ratio, and HCl index were lower than those detected in the control wines. In wines treated with pectolytic enzymes and those produced through extended maceration, the contact with oak chips led to a higher gelatin index, and lower HCl and PVPP indices than the corresponding controls.

Concerning the single effects of winemaking technologies on the phenolic profile of Nero di Troia wines (Table 63), the cryomacerated wines showed the highest concentrations of phenolic acids, stilbens, flavan-3-ols (with those obtained through addition of enzymes and extended maceration), and anthocyanins (with the wines obtained through addition of enzymes). The wines obtained through addition of enzymes exhibited the highest contents of flavonols.

Concerning the effects of time on the specific phenolic contents, the flavan-3-ols content remained almost unchanged with respect to the racking, except for the wines produced through the traditional winemaking, which instead exhibited a decrease by 16%. The total anthocyans content drastically decreased in all the samples (average by about -71.6%), nonetheless all the non-traditional technologies exhibited the highest amount of free forms with respect to the traditional wines, whereas the polymeric forms were present in the following descending order: cryomacerated > added with pectolytic enzymes > traditional > extended macerated wines. The highest contents of monomeric and polymeric anthocyans detected in cryomacerated wines improved their colour in comparison with the other technologies, although also enzymatic macerated wines exhibited a high content in stable and unstable pigment forms. Polymeric pigments, indeed, improve wine colour stability with respect to monomeric anthocyanins because they had the saturated C ring and, thus, they are more resistant to oxidation, pH and sulphur dioxide bleaching (Margalit, 2004; Somers and Evans, 1977; Timberlake and Bridle, 1976).

Oak chips exerted few significant single effects on the phenolic profile. In particular, the treated wines had the highest concentrations of the flavanols catechin and procyanidins B1,

B2, B3, and B4 and of the anthocyanins cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvinidin-3-glucoside, delphinidin-3-acetylglucoside, malvidin-3-acetylglucoside, peonidin-3-*p*-coumaroylglucoside, and malvidin-*p*-coumaroylglucoside. The contact with oak chips and, the subsequent extraction of ellagic tannins, allowed the enhanced protection against oxidative phenomenon on tannins and anthocyanins. These findings were in agreement with the results observed by Vivas and Glories (1996), Guerra *et al*. (1996), Obradovic (2006), and Roure and Anderson (2006). The interactive effects of the winemaking technologies and oak chips treatment strongly depended on the vinification procedure applied. The wines produced through cryomaceration exhibited the highest concentrations of stilbens and, if treated with oak chips, the highest concentrations of phenolic acids, flavanols, and anthocyanins. The wines produced though addition of pectolytic enzymes had the highest content of flavonols.

The colour parameters listed in Table 64 highlighted that the wines obtained though cryomaceration and addition of pectolytic enzymes had the highest colour intensity as a consequence of the higher anthocyanins content. These results were supported by the findings of Puertas *et al*. (2013), who highlighted that dry ice maceration was an alternative procedure to obtain highly coloured wines, and by Romero-Cascales *et al*. (2008) and Revilla and González-San José (2003), who found that the enzyme-treated wines showed, after 12 months, a higher colour intensity, and that all the red wines treated enzymatically presented better chromatic characteristics than the corresponding controls, respectively. The prolonged macerated wines exhibited lower colour intensity, as a result of the lower anthocyanin content. The prolonged macerated wines also exhibited the highest hue, due to the highest values of the yellow and blue components and the lowest values of the red component. These results were corroborated by Bautista-Ortin *et al.* (2004), Gomez-Plaza *et al.* (2001), and Sipiora and Granda (1998), who found that increased maceration time up to 30 days can reduce wine colour. The decomposition of absorbance at 520 nm in the values due to the three pigments components (monomeric, polymeric sensitive and polymeric resistant) showed that in the traditional wines the contribute of the resistant polymeric pigments was higher than those due to both monomeric and unstable polymeric pigments. These data were in agreement with those concerning the distribution of anthocyanins among monomeric and polymeric pigments, and with the HPLC-DAD-ESI-MS/MS results, that revealed a higher amount in polymeric forms with respect to the total anthocyan content (about 27.7 % vs. 26.7 %, 24.1 %, and

20.2 %, respectively detected in cryomacerated, treated with enzymes, and prolonged macerated wines). Among the wines, those obtained through cryomaceration showed the highest content in both monomeric and polymeric anthocyanins, data that explain their better colour. The oak chips treatment didn't exert significant effects on the state of polymerization of wine pigments. The only statically significant differences were found in tonality, % of yellow component and dAl%, which exhibited the highest values in oak-treated samples, and % of red component, which was the lowest in oak-treated wines.

Winemaking technologies and treatment with oak chips didn't exert significant effects on visual descriptors, but affected olfactory complexity and quality, which were higher in traditional wines treated with chips and in prolonged macerated wines treated or not with chips, and minerality, gustatory-olfactory quality, and harmony, which were higher in prolonged macerated wines treated or not with chips (Table 65). Figure 22*a* highlights that the four vinification procedures had different effects on the perception of selected flavours. In particular, the wines produced through the traditional winemaking showed a flavour profile characterised by spicy, floral, vinous, fruity, ethereal, frankness, aromatic, and woody notes. Cryomaceration determined the disappearance of spicy, woody, floral, and aromatic flavours, the decrease of the fruity notes, and the increase of the ethereal character. The enzymatic and prolonged macerations, instead, improved the flavour by increasing spicy, floral, fruity, and frankness aromas. The contact with oak chips improved the olfactory quality in all the wines, making them more harmonious, through the attenuation of the floral and fruity notes, and the accentuation and/or the appearance of spicy, woody, and vanilla flavours (Figure 22*b*), confirming, once again, the findings obtained by Guchu *et al*. (2006) and Gómez García-Carpintero *et al*. (2011; 2012).

The Principal Component analysis (PCA) was applied to the whole standardize data set and the obtained results are reported in Figures 23*a* and *b*. The first two principal components (PCs) accounted only for about 62% of the total variance, and showed the principal groupings. As it can be seen, the aging process standardised the effects of the winemaking technology and reduced the differences among the different wines. The effects of the treatment with chips were higher in the wines obtained through vinification procedures different from the traditional ones.

	Alcohol (% vol)	Density (g/mL)	Dry Matter (g/L)	Vol. Ac.	Titr. Ac.	pH	Free SO_2 (mg/L)	Total SO_2 (mg/L)	TSS (°Brix)	Dissolved O_2 (mg/L)	E_H (mV)	Acetaldehyde (mg/L)
Winemaking technology												
T	12.90 ± 0.15 **ab**	0.994 **a**	27.7 ± 0.8 **b**	0.50 ± 0.19 **b**	5.81 ± 0.19 **b**	3.71 ± 0.03 **a**	17.6 ± 1.1 **a**	23.0 ± 1.9 **a**	7.5 ± 0.1 **b**	1.52 ± 0.39 **b**	49.3 ± 10.2 **b**	1.53 ± 0.21 **a**
C	13.05 ± 0.20 **b**	0.994 ± 0.001 **a**	27.2 ± 1.0 **ab**	0.29 ± 0.04 **a**	5.30 ± 0.05 **a**	3.79 ± 0.03 **b**	20.0 ± 2.4 **b**	26.0 ± 2.4 **b**	7.5 ± 0.1 **b**	1.44 ± 0.20 **b**	31.5 ± 8.7 **a**	1.70 ± 0.34 **a**
E	12.77 ± 0.23 **ab**	0.994 ± 0.001 **a**	27.4 ± 0.5 **b**	0.51 ± 0.17 **b**	5.74 ± 0.11 **b**	3.73 ± 0.04 **ab**	17.6 ± 1.8 **a**	23.2 ± 1.5 **ab**	7.5 ± 0.1 **b**	1.16 ± 0.27 **ab**	33.5 ± 8.2 **a**	1.48 ± 0.19 **a**
PM	12.66 ± 0.12 **a**	0.994 ± 0.001 **a**	26.0 ± 0.8 **a**	0.74 ± 0.10 **c**	5.37 ± 0.15 **a**	3.73 ± 0.05 **ab**	17.8 ± 2.1 **ab**	24.4 ± 3.3 **ab**	7.3 ± 0.1 **a**	0.83 ± 0.22 **a**	29.6 ± 9.4 **a**	1.40 ± 0.24 **a**
Significance	*	**ns**	*	*	*	*	*	*	*	*	*	**ns**
Treatment with oak chips												
No CHIPS	12.83 ± 0.27 **a**	0.994 ± 0.001 **a**	27.2 ± 1.2 **a**	0.46 ± 0.21 **a**	5.56 ± 0.30 **a**	3.74 ± 0.05 **a**	18.2 ± 2.4 **a**	23.8 ± 2.8 **a**	7.5 ± 0.1 **a**	1.47 ± 0.39 **b**	41.3 ± 14.3 **a**	1.48 ± 0.24 **a**
+ CHIPS	12.88 ± 0.15 **a**	0.994 ± 0.001 **a**	27.2 ± 0.8 **a**	0.56 ± 0.19 **a**	5.65 ± 0.24 **a**	3.73 ± 0.05 **a**	18.1 ± 1.3 **a**	24.1 ± 2.0 **a**	7.4 ± 0.2 **a**	1.12 ± 0.33 **a**	35.9 ± 10.6 **a**	1.57 ± 0.25 **a**
Significance	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	*	**ns**	**ns**
Winemaking technology * Treatment with oak chips												
T	12.86 ± 0.18 **ab**	0.994 **a**	27.8 ± 1.1 **b**	0.44 ± 0.15 **abcd**	5.78 ± 0.28 **c**	3.71 ± 0.03 **a**	17.2 ± 1.5 **a**	22.4 ± 2.3 **a**	7.5 ± 0.1 **b**	1.69 ± 0.47 **d**	53.3 ± 11.9 **d**	1.48 ± 0.24 **a**
T+chips	12.94 ± 0.13 **ab**	0.994 **a**	27.7 ± 0.5 **b**	0.56 ± 0.23 **bcde**	5.85 ± 0.02 **c**	3.72 ± 0.04 **a**	18.0 ± 0.5 **a**	23.6 ± 1.4 **ab**	7.6 ± 0.1 **b**	1.36 ± 0.27 **bcd**	45.4 ± 7.7 **cd**	1.58 ± 0.19 **a**
C	13.07 ± 0.33 **b**	0.994 **a**	27.4 ± 1.6 **ab**	0.26 ± 0.03 **a**	5.27 ± 0.02 **a**	3.80 ± 0.03 **b**	22.0 ± 0.6 **b**	28.0 ± 1.1 **c**	7.6 **b**	1.60 ± 0.04 **cd**	38.1 ± 7.4 **abc**	1.63 ± 0.39 **a**
C+chips	13.02 ± 0.13 **b**	0.994 ± 0.001 **a**	27.1 ± 0. **ab**	0.32 **ab**	5.33 ± 0.06 **a**	3.79 ± 0.05 **ab**	18.0 ± 0.6 **a**	24.0 **abc**	7.5 ± 0.1 **b**	1.28 ± 0.13 **abcd**	24.9 ± 0.4 **ab**	1.85 ± 0.07 **a**
E	12.81 ± 0.40 **ab**	0.994 ± 0.001 **a**	27.2 ± 0.4 **ab**	0.37 ± 0.06 **abc**	5.70 ± 0.13 **bc**	3.74 ± 0.03 **ab**	17.6 ± 2.3 **a**	23.6 ± 1.7 **ab**	7.6 ± 0.1 **b**	1.36 ± 0.21 **bcd**	40.1 ± 2.7 **bcd**	1.55 ± 0.07 **a**
E+chips	12.73 ± 0.04 **ab**	0.994 ± 0.001 **a**	27.6 ± 0.6 **ab**	0.65 ± 0.02 **cde**	5.78 ± 0.11 **bc**	3.72 ± 0.06 **ab**	17.6 ± 2.3 **a**	22.8 ± 1.7 **ab**	7.4 ± 0.1 **b**	0.95 ± 0.03 **ab**	27.0 ± 4.9 **ab**	1.40 ± 0.28 **a**
PM	12.56 ± 0.06 **a**	0.994 ± 0.001 **a**	25.8 ± 0.8 **a**	0.79 ± 0.15 **e**	5.28 ± 0.08 **a**	3.73 ± 0.06 **ab**	16.8 ± 1.1 **a**	22.4 ± 2.3 **ab**	7.4 ± 0.1 **b**	1.00 ± 0.11 **abc**	22.0 ± 0.5 **a**	1.35 ± 0.21 **a**
PM+chips	12.76 ± 0.01 **ab**	0.994 ± 0.001 **a**	26.3 ± 0.9 **ab**	0.69 ± 0.03 **de**	5.46 ± 0.17 **ab**	3.73 ± 0.06 **ab**	18.8 ± 2.8 **ab**	26.4 ± 3.4 **bc**	7.2 ± 0.1 **a**	0.67 ± 0.13 **a**	37.2 ± 5.7 **abc**	1.45 ± 0.35 **a**
Significance	*	**ns**	*	*	*	*	*	*	*	*	*	**ns**

Table 59 – Single and interactive effects of the winemaking technologies and treatment with oak chips on the physicochemical characteristics of Nero di Troia wines, 12 months after racking.
T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: prolonged maceration.
Vol. Ac.: volatile acidity (g acetic acid/L); Titr. Ac.: titratable acidity (g tartaric acid/L); TSS: total soluble solids (°Brix).
In column, as part of winemaking technologies or treatment with oak chips or combination of technology-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference; ns: no significant difference.

	Tartaric acid	L-Malic acid	L-Lactic acid	Acetic acid	Citric acid	Pyruvic acid	D-Gluconic acid
Winemaking technology							
T	2.10 ± 0.22 **a**	nd **a**	0.71 ± 0.64 **a**	0.39 ± 0.17 **ab**	0.13 ± 0.01 **a**	nd **a**	nd **a**
C	1.92 ± 0.20 **a**	nd **a**	0.70 ± 0.69 **a**	0.32 ± 0.14 **a**	0.13 ± 0.02 **a**	nd **a**	nd **a**
E	2.16 ± 0.14 **a**	nd **a**	0.71 ± 0.70 **a**	0.36 ± 0.13 **ab**	0.13 ± 0.02 **a**	nd **a**	nd **a**
PM	1.99 ± 0.17 **a**	nd **a**	1.34 ± 0.08 **a**	0.54 ± 0.01 **b**	0.12 ± 0.01 **a**	nd **a**	nd **a**
Significance	**ns**	**ns**	**ns**	*	**ns**	**ns**	**ns**
Treatment with oak chips							
No CHIPS	2.07 ± 0.18 **a**	nd **a**	0.58 ± 0.62 **a**	0.36 ± 0.17 **a**	0.12 ± 0.01 **a**	nd **a**	nd **a**
+ CHIPS	2.05 ± 0.23 **a**	nd **a**	1.09 ± 0.51 **b**	0.44 ± 0.12 **a**	0.13 ± 0.01 **a**	nd **a**	nd **a**
Significance	**ns**	**ns**	*	**ns**	**ns**	**ns**	**ns**
Winemaking technology * Treatment with oak chips							
T	2.07 ± 0.28 **a**	nd **a**	0.69 ± 0.68 **ab**	0.40 ± 0.18 **ab**	0.13 ± 0.01 **ab**	nd **a**	nd **a**
T+chips	2.13 ± 0.18 **a**	nd **a**	0.73 ± 0.70 **ab**	0.38 ± 0.18 **ab**	0.13 ± 0.01 **ab**	nd **a**	nd **a**
C	1.99 ± 0.05 **a**	nd **a**	0.10 ± 0.02 **a**	0.20 ± 0.01 **a**	0.12 ± 0.01 **ab**	nd **a**	nd **a**
C+chips	1.85 ± 0.31 **a**	nd **a**	1.30 **b**	0.44 ± 0.01 **ab**	0.14 ± 0.01 **b**	nd **a**	nd **a**
E	2.13 ± 0.11 **a**	nd **a**	0.11 ± 0.02 **a**	0.25 **a**	0.13 ± 0.02 **ab**	nd **a**	nd **a**
E+chips	2.20 ± 0.21 **a**	nd **a**	1.32 ± 0.01 **b**	0.47 ± 0.01 **ab**	0.13 ± 0.01 **ab**	nd **a**	nd **a**
PM	2.07 ± 0.06 **a**	nd **a**	1.32 ± 0.11 **b**	0.54 **b**	0.11 ± 0.01 **a**	nd **a**	nd **a**
PM+chips	1.92 ± 0.25 **a**	nd **a**	1.36 ± 0.08 **b**	0.54 ± 0.01 **b**	0.12 **ab**	nd **a**	nd **a**
Significance	**ns**	**ns**	*	*	*	**ns**	**ns**

Table 60 – Single and interactive effects of winemaking technologies and treatment with oak chips on the organic acids content of Nero di Troia wines 12 months after racking (data are expressed as g per L of wine).
nd: not detected.
T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: prolonged maceration.
In column, as part of winemaking technologies or treatment with oak chips or combination of technology-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference; ns: no significant difference.

	TA	SA	MA	TF	FNA	FRV	PA	TPC	ABTS assay
					Winemaking technology				
T	128 ± 17 **a**	68 ± 10 **a**	30 ± 6 **a**	1779 ± 149 **a**	1593 ± 144 **a**	1214 ± 177 **a**	3828 ± 153 **b**	2897 ± 218 **a**	22.35 ± 3.85 **a**
C	173 ± 18 **b**	95 ± 19 **b**	34 ± 3 **b**	2067 ± 65 **c**	1815 ± 85 **c**	1360 ± 58 **c**	4316 ± 244 **c**	3184 ± 188 **b**	23.41 ± 3.35 **a**
E	162 ± 4 **b**	94 ± 12 **b**	40 ± 11 **c**	1904 ± 44 **b**	1668 ± 46 **b**	1312 ± 40 **bc**	3995 ± 193 **b**	3176 ± 105 **b**	22.77 ± 3.26 **a**
PM	142 ± 4 **a**	97 ± 9 **b**	34 ± 10 **b**	1861 ± 64 **b**	1654 ± 65 **ab**	1259 ± 49 **ab**	3581 ± 131 **a**	2832 ± 239 **a**	22.28 ± 3.37 **a**
Significance	*	*	*	*	*	*	*	*	**ns**
					Treatment with oak chips				
No CHIPS	150 ± 29 **a**	75 ± 14 **a**	31 ± 4 **a**	1829 ± 151 **a**	1611 ± 125 **a**	1280 ± 134 **a**	3864 ± 259 **a**	2931 ± 205 **a**	22.46 ± 3.67 **a**
+ CHIPS	149 ± 13 **a**	93 ± 17 **b**	37 ± 10 **b**	1957 ± 99 **b**	1741 ± 87 **b**	1287 ± 93 **a**	3981 ± 362 **a**	3064 ± 270 **b**	22.81 ± 3.35 **a**
Significance	**ns**	*	*	*	*	**ns**	**ns**	*	**ns**
					Winemaking technology * Treatment with oak chips				
T	121 ± 23 **a**	61 ± 10 **a**	32 ± 3 **ab**	1662 ± 110 **a**	1485 ± 119 **a**	1251 ± 250 **ab**	3908 ± 137 **cde**	2833 ± 237 **a**	21.47 ± 3.58 **a**
T+chips	134 ± 7 **a**	74 ± 4 **b**	27 ± 7 **a**	1897 ± 55 **bc**	1701 ± 54 **bc**	1178 ± 48 **a**	3748 ± 137 **bc**	2960 ± 185 **abc**	23.22 ± 4.06 **a**
C	183 ± 23 **c**	79 ± 9 **bcd**	32 ± 2 **ab**	2017 ± 49 **de**	1751 ± 73 **c**	1326 ± 45 **bc**	4142 ± 122 **e**	3065 ± 91 **bcd**	23.77 ± 4.08 **a**
C+chips	163 ± 3 **b**	110 ± 10 **e**	37 ± 2 **bc**	2117 ± 25 **e**	1879 ± 20 **d**	1394 ± 51 **c**	4490 ± 208 **f**	3304 ± 188 **e**	23.06 ± 2.78 **a**
E	164 ± 4 **b**	84 ± 1 **c**	31 ± 4 **ab**	1875 ± 43 **bc**	1636 ± 37 **b**	1311 ± 41 **bc**	3888 ± 160 **bcd**	3108 ± 65 **cde**	22.66 ± 3.96 **a**
E+chips	159 ± 2 **b**	105 ± 4 **e**	50 ± 5 **d**	1932 ± 25 **cd**	1701 ± 27 **bc**	1313 ± 42 **bc**	4102 ± 181 **de**	3244 ± 94 **de**	22.88 ± 2.77 **a**
PM	142 ± 6 **a**	89 ± 1 **d**	27 ± 4 **a**	1818 ± 49 **b**	1612 ± 58 **b**	1238 ± 32 **ab**	3501 ± 122 **a**	2814 ± 96 **a**	22.91 ± 3.60 **a**
PM+chips	143 ± 3 **a**	104 **e**	42 ± 6 **c**	1904 ± 49 **bc**	1696 ± 46 **bc**	1280 ± 56 **abc**	3661 ± 93 **ab**	2851 ± 340 **ab**	21.65 ± 3.34 **a**
Significance	*	*	*	*	*	*	*	*	**ns**

Table 61 – Single and interactive effects of winemaking technologies and treatment with oak chips on the phenolic composition and antioxidant activity of Nero di Troia wines 12 months after racking.

TA: total anthocyanins (as mg malvidin-3-glucoside/L); SA: anthocyanins sensitive to SO_2 (as mg malvidin-3-glucoside/L); MA: monomeric anthocyanins (as malvidin-3-glucoside mg/L); TF: total flavonoids (as mg (+)-catechin/L); FNA: flavonoids different from anthocyanins (as mg (+)-catechin/L); FRV: flavans reactive with vanillin (as mg (+)-catechin/L); PA: proanthocyanidins (as mg cyanidin chloride/L); TPC: total phenolic compounds (as mg gallic acid/L); ABTS assay: antioxidant activity by ABTS assay (as mmol Trolox eq/L).

T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: prolonged maceration.

In column, as part of winemaking technologies or treatment with oak chips or combination of technology-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).

*: significant difference; ns: no significant difference.

	MP	SPP	LPP	LPP/SPP	$I_{gelatin}$	I_{HCl}	I_{PVPP}
				Winemaking technology			
T	0.32 ± 0.07 **a**	0.32 ± 0.04 **b**	0.68 ± 0.05 **b**	2.17 ± 0.25 **b**	28.9 ± 6.3 **b**	52.9 ± 2.6 **d**	54.6 ± 13.7 **a**
C	0.38 ± 0.02 **ab**	0.37 ± 0.02 **c**	0.72 ± 0.07 **b**	1.94 ± 0.24 **ab**	27.6 ± 3.7 **b**	49.6 ± 5.1 **c**	62.9 ± 5.8 **b**
E	0.40 ± 0.05 **b**	0.36 ± 0.01 **c**	0.67 ± 0.07 **ab**	1.88 ± 0.18 **a**	18.7 ± 5.0 **a**	47.2 ± 4.7 **b**	57.8 ± 7.6 **ab**
PM	0.35 ± 0.03 **ab**	0.28 ± 0.01 **a**	0.60 ± 0.05 **a**	2.12 ± 0.14 **ab**	26.2 ± 4.6 **b**	38.8 ± 2.8 **a**	64.8 ± 7.4 **b**
Significance	*	*	*	*	*	*	*
				Treatment with oak chips			
No CHIPS	0.36 ± 0.07 **a**	0.32 ± 0.04 **a**	0.67 ± 0.07 **a**	2.12 ± 0.26 **a**	25.9 ± 7.8 **a**	51.1 ± 5.7 **b**	57.2 ± 13.1 **a**
+ CHIPS	0.33 ± 0.05 **a**	0.34 ± 0.04 **b**	0.67 ± 0.07 **a**	2.00 ± 0.21 **a**	25.7 ± 4.2 **a**	45.4 ± 5.7 **a**	60.8 ± 7.9 **a**
Significance	**ns**	*	**ns**	**ns**	**ns**	*	**ns**
				Winemaking technology * Treatment with oak chips			
T	0.33 ± 0.09 **ab**	0.30 ± 0.03 **a**	0.66 ± 0.03 **abc**	2.25 ± 0.25 **b**	31.9 ± 6.2 **c**	54.6 ± 2.1 **f**	46.1 ± 12.9 **a**
T+chips	0.30 ± 0.04 **a**	0.34 ± 0.02 **b**	0.71 ± 0.05 **c**	2.10 ± 0.26 **ab**	25.0 ± 4.7 **b**	51.1 ± 1.6 **d**	63.2 ± 8.5 **c**
C	0.39 ± 0.01 **ab**	0.35 ± 0.01 **bc**	0.73 ± 0.11 **c**	2.06 ± 0.30 **ab**	28.7 ± 4.9 **bc**	54.2 ± 1.8 **ef**	59.6 ± 6.0 **bc**
C+chips	0.37 ± 0.02 **ab**	0.38 ± 0.01 **c**	0.70 ± 0.02 **bc**	1.83 ± 0.09 **a**	26.5 ± 2.7 **bc**	45.1 ± 1.0 **c**	66.2 ± 3.9 **c**
E	0.41 ± 0.04 **b**	0.36 **bc**	0.67 ± 0.08 **abc**	1.88 ± 0.22 **a**	13.4 ± 1.5 **a**	51.5 ± 0.5 **de**	63.8 ± 4.7 **c**
E+chips	0.38 ± 0.06 **ab**	0.36 ± 0.02 **bc**	0.67 ± 0.07 **abc**	1.88 ± 0.19 **a**	22.1 ± 1.5 **b**	43.0 ± 0.4 **bc**	51.9 ± 4.0 **ab**
PM	0.35 ± 0.03 **ab**	0.29 **a**	0.62 ± 0.07 **ab**	2.15 ± 0.21 **ab**	23.3 ± 1.1 **b**	40.6 ± 0.7 **b**	70.1 ± 4.9 **c**
PM+chips	0.34 ± 0.03 **ab**	0.28 ± 0.01 **a**	0.59 ± 0.04 **a**	2.09 ± 0.04 **ab**	29.2 ± 5.1 **bc**	37.0 ± 3.0 **a**	59.5 ± 5.4 **bc**
Significance	*	*	*	*	*	*	*

Table 62 – Single and interactive effects of winemaking technologies and treatment with oak chips on the monomeric and polymeric pigments, and structure indices of Nero di Troia wines 12 months after racking.
MP: absorbance at 520 nm due to monomeric pigments; SPP: absorbance at 520 nm due to small polymeric pigments; LPP: absorbance at 520 nm due to large polymeric pigments; LPP/SPP: ratio between large and small polymeric pigments; $I_{gelatin}$: gelatin index; I_{HCl}: hydrochloric acid index; I_{PVPP}: PVPP index.
T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: prolonged maceration.
In column, as part of winemaking technologies or treatment with oak chips or combination of technology-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference; ns: no significant difference.

Phenolic compounds	*Winemaking technology*					*Treatment with oak chips*		
	T	**C**	**E**	**PM**	*Significance*	**No CHIPS**	**+ CHIPS**	*Significance*
Phenolic acids and derivatives								
(mg GAE/L; mg CAE/L)								
Gallic acid	44.77 ± 0.88 **a**	48.24 ± 1.17 **b**	44.14 ± 0.57 **a**	44.90 ± 2.32 **a**	*	45.30 ± 2.04 **a**	45.73 ± 2.22 **a**	**ns**
2-S-Glutathionyl-Caftaric acid	0.33 ± 0.06 **a**	0.35 ± 0.05 **a**	0.28 ± 0.03 **a**	0.29 ± 0.04 **a**	**ns**	0.30 ± 0.05 **a**	0.33 ± 0.06 **a**	**ns**
Caftaric acid + Caffeic acid	19.43 ± 0.96 **a**	21.03 ± 0.31 **b**	19.86 ± 0.32 **ab**	20.90 ± 1.30 **b**	*	20.50 ± 1.24 **a**	20.11 ± 0.79 **a**	**ns**
p-Coumaric acid	8.21 ± 0.13 **a**	9.19 ± 0.14 **c**	8.64 ± 0.25 **b**	8.31 ± 0.51 **ab**	*	8.72 ± 0.49 **a**	8.45 ± 0.46 **a**	**ns**
Ferulic acid	7.42 ± 0.06 **c**	7.33 ± 0.14 **c**	6.94 ± 0.21 **b**	6.57 ± 0.41 **a**	*	7.18 ± 0.36 **a**	6.95 ± 0.45 **a**	**ns**
Σ Phenolic acids and derivatives	**80.16 ± 0.88 a**	**86.14 ± 1.46 b**	**79.86 ± 0.29 a**	**80.96 ± 4.55 a**	*	**81.99 ± 3.50 a**	**81.57 ± 3.55 a**	**ns**
Stilbens (mg RE/L)								
cis-Piceid	2.39 ± 0.37 **b**	2.89 ± 0.63 **c**	2.58 ± 0.68 **bc**	0.48 ± 0.05 **a**	*	2.40 ± 1.23 **b**	1.77 ± 0.84 **a**	**ns**
trans-Piceid	3.60 ± 0.39 **b**	3.93 ± 0.14 **b**	3.61 ± 0.34 **b**	3.07 ± 0.37 **a**	*	3.72 ± 0.32 **a**	3.38 ± 0.48 **a**	**ns**
Σ Stilbens	**5.99 ± 0.73 b**	**6.82 ± 0.73 c**	**6.19 ± 0.89 bc**	**3.56 ± 0.42 a**	*	**6.13 ± 1.50 b**	**5.15 ± 1.26 a**	**ns**
Flavonols (mg QE/L)								
Myricetin-3-glc	1.36 ± 0.16 **ab**	1.45 ± 0.07 **b**	1.31 ± 0.02 **ab**	1.21 ± 0.07 **a**	*	1.34 ± 0.10 **a**	1.33 ± 0.14 **a**	**ns**
Myricetin-3-glcr	2.69 ± 0.23 **b**	3.23 ± 0.19 **c**	2.92 ± 0.08 **b**	2.39 ± 0.09 **a**	*	2.78 ± 0.28 **a**	2.83 ± 0.43 **a**	**ns**
Myricetin-3-galac	8.10 ± 0.37 **b**	6.96 ± 0.39 **a**	9.72 ± 0.25 **c**	9.21 ± 0.54 **c**	*	8.53 ± 1.39 **a**	8.46 ± 0.96 **a**	**ns**
Quercetin-3-glc	1.56 ± 0.10 **a**	1.50 ± 0.04 **a**	1.55 ± 0.14 **a**	1.46 ± 0.15 **a**	**ns**	1.56 ± 0.08 **a**	1.48 ± 0.13 **a**	**ns**
Quercetin-3-glcr	9.59 ± 1.15 **b**	10.66 ± 0.31 **b**	10.63 ± 0.30 **b**	8.36 ± 0.62 **a**	*	9.92 ± 1.10 **a**	9.70 ± 1.27 **a**	**ns**
Quercetin-3-galac	0.92 ± 0.20 **b**	0.52 ± 0.02 **a**	1.53 ± 0.13 **c**	2.09 ± 0.27 **d**	*	1.36 ± 0.72 **b**	1.17 ± 0.57 **a**	**ns**
Laricitrin-3-glc	3.22 ± 0.47 **a**	3.14 ± 0.03 **a**	3.81 ± 0.12 **b**	3.23 ± 0.32 **a**	*	3.46 ± 0.43 **a**	3.24 ± 0.30 **a**	**ns**
Quercetin-3-rha	7.84 ± 0.95 **ab**	8.76 ± 0.16 **b**	8.51 ± 0.61 **b**	7.10 ± 0.36 **a**	*	8.24 ± 0.87 **a**	7.86 ± 0.84 **a**	**ns**
Isorhamnetin-3-glc	3.58 ± 0.27 **a**	3.75 ± 0.21 **ab**	4.07 ± 0.17 **b**	3.71 ± 0.51 **ab**	*	3.92 ± 0.29 **a**	3.64 ± 0.34 **a**	**ns**
Syringetin-3-galac	6.74 ± 0.77 **a**	7.81 ± 0.57 **b**	7.55 ± 0.11 **ab**	6.86 ± 0.53 **a**	*	7.14 ± 0.62 **a**	7.34 ± 0.77 **a**	**ns**
Laricitrin-3-rhamnose-7-tri-hydroxycinnamic acid	3.28 ± 0.64 **a**	3.42 ± 0.11 **a**	4.42 ± 0.48 **b**	3.78 ± 0.05 **a**	*	3.88 ± 0.74 **a**	3.58 ± 0.37 **a**	**ns**
Σ Flavonols	**48.89 ± 4.70 a**	**51.19 ± 1.45 ab**	**56.03 ± 1.59 b**	**49.40 ± 3.22 a**	*	**52.13 ± 4.57 a**	**50.62 ± 3.44 a**	**ns**
Flavan-3-ols (mg CE/L)								
(-)-(Epi)Gallocatechin	25.71 ± 2.56 **a**	29.00 ± 2.06 **b**	28.99 ± 1.68 **b**	30.37 ± 0.91 **b**	*	27.12 ± 2.43 **a**	29.92 ± 1.59 **b**	**ns**
Procyanidin B3	52.80 ± 5.85 **a**	63.21 ± 6.99 **b**	62.26 ± 3.19 **b**	65.15 ± 2.53 **b**	*	58.26 ± 6.84 **a**	63.45 ± 5.69 **b**	*

(+)-Catechin	65.23 ± 2.74 **a**	72.70 ± 5.22 **b**	70.34 ± 1.82 **ab**	74.54 ± 3.77 **b**	*	68.84 ± 4.73 **a**	72.56 ± 4.47 **b**	*
Procyanidin B1	40.71 ± 6.96 **a**	44.80 ± 6.03 **ab**	44.74 ± 6.02 **ab**	49.39 ± 5.46 **b**	*	41.74 ± 4.91 **a**	48.08 ± 6.26 **b**	*
Procyanidin B4	55.72 ± 2.85 **a**	65.66 ± 5.81 **b**	65.94 ± 4.30 **b**	71.77 ± 1.73 **c**	*	62.22 ± 6.31 **a**	67.33 ± 6.96 **b**	*
(-)-Epicatechin	102.28 ± 7.33 **a**	112.29 ± 3.65 **b**	106.73 ± 2.88 **ab**	105.98 ± 5.21 **ab**	*	106.31 ± 5.68 **a**	107.33 ± 6.36 **a**	**ns**
Procyanidin B2	18.03 ± 1.78 **a**	20.02 ± 2.31 **ab**	20.06 ± 2.15 **ab**	22.32 ± 3.56 **b**	*	18.40 ± 1.48 **a**	21.81 ± 2.73 **b**	*
Σ Flavan-3-ols	**360.48 ± 25.22 a**	**407.68 ± 30.92 b**	**399.07 ± 21.49 b**	**419.52 ± 16.34 b**	*	**382.89 ± 27.77 a**	**410.48 ± 30.14 b**	*
Anthocyanins (mg ME/L)								
Dp-3-glc	0.58 ± 0.09 **a**	0.96 ± 0.37 **bc**	1.02 ± 0.21 **c**	0.88 ± 0.09 **b**	*	0.72 ± 0.12 **a**	0.99 ± 0.31 **b**	**ns**
Cy-3-glc	0.26 ± 0.05 **a**	0.32 ± 0.07 **b**	0.36 ± 0.03 **b**	0.35 ± 0.03 **b**	*	0.30 ± 0.05 **a**	0.35 ± 0.05 **b**	*
Pt-3-glc	0.45 ± 0.07 **a**	0.76 ± 0.35 **b**	0.78 ± 0.22 **b**	0.80 ± 0.12 **b**	*	0.57 ± 0.12 **a**	0.83 ± 0.27 **b**	*
Pn-3-glc	0.35 ± 0.07 **a**	0.56 ± 0.23 **b**	0.58 ± 0.16 **b**	0.56 ± 0.07 **b**	*	0.43 ± 0.06 **a**	0.59 ± 0.20 **b**	*
Mv-3-glc	4.48 ± 0.95 **a**	7.80 ± 3.53 **b**	8.54 ± 2.39 **c**	8.04 ± 1.09 **bc**	*	5.86 ± 1.09 **a**	8.57 ± 3.02 **b**	*
Dp-3-acetylglc	0.46 ± 0.07 **b**	0.47 ± 0.07 **b**	0.42 ± 0.09 **b**	0.29 ± 0.06 **a**	*	0.46 ± 0.08 **b**	0.36 ± 0.09 **a**	*
Pyrano-Mv-3-glc (Vitisin B)	2.42 ± 0.47 **b**	3.20 ± 0.22 **c**	2.64 ± 0.26 **b**	1.57 ± 0.17 **a**	*	2.60 ± 0.70 **a**	2.31 ± 0.63 **a**	**ns**
Carboxypyrano-Mv-3-acetylglc (acetyl-Vitisin A)	1.31 ± 0.35 **b**	1.72 ± 0.03 **c**	1.37 ± 0.05 **b**	0.92 ± 0.06 **a**	*	1.39 ± 0.36 **a**	1.28 ± 0.32 **a**	**ns**
Cy-3-acetylglc	nd **a**	nd **a**	nd **a**	nd **a**	**ns**	nd **a**	nd **a**	**ns**
Pt-3-acetylglc	2.32 ± 0.46 **a**	2.57 ± 0.21 **a**	2.47 ± 0.23 **a**	2.20 ± 0.15 **a**	**ns**	2.41 ± 0.36 **a**	2.37 ± 0.24 **a**	**ns**
Pyrano-Mv-3-acetylglc (acetyl-Vitisin B)	1.92 ± 0.18 **a**	2.25 ± 0.21 **b**	2.14 ± 0.08 **ab**	1.89 ± 0.10 **a**	*	2.04 ± 0.24 **a**	2.06 ± 0.19 **a**	**ns**
Pn-3-acetylglc	3.46 ± 0.37 **a**	3.89 ± 0.41 **a**	3.75 ± 0.04 **a**	3.51 ± 0.18 **a**	**ns**	3.59 ± 0.27 **a**	3.72 ± 0.37 **a**	**ns**
Mv-3-acetylglc	7.88 ± 0.98 **a**	10.27 ± 1.44 **b**	10.01 ± 0.87 **b**	9.57 ± 0.44 **b**	*	8.81 ± 0.97 **a**	10.06 ± 1.37 **b**	*
Pyrano-Mv-3-*p*-coumglc (*p*-coum-Vitisin B)	3.49 ± 0.40 **b**	3.98 ± 0.17 **c**	3.77 ± 0.28 **bc**	2.99 ± 0.20 **a**	*	3.60 ± 0.52 **a**	3.52 ± 0.42 **a**	**ns**
Mv-3-caffeoylglc	2.64 ± 0.20 **a**	2.69 ± 0.22 **a**	2.63 ± 0.10 **a**	2.40 ± 0.10 **a**	**ns**	2.62 ± 0.14 **a**	2.56 ± 0.23 **a**	**ns**
Pt-3-*p*-coumglc	1.45 ± 0.21 **a**	1.59 ± 0.11 **a**	1.52 ± 0.26 **a**	1.49 ± 0.18 **a**	**ns**	1.51 ± 0.19 **a**	1.51 ± 0.19 **a**	**ns**
Mv-3-*p*-coumglc-8-ethyl-(epi)cat	0.58 ± 0.09 **a**	0.80 ± 0.08 **b**	0.73 ± 0.03 **b**	0.58 ± 0.04 **a**	*	0.67 ± 0.13 **a**	0.67 ± 0.11 **a**	**ns**
Pn-3-*p*-coumglc	0.38 ± 0.04 **a**	0.48 ± 0.06 **b**	0.47 ± 0.05 **b**	0.40 ± 0.03 **a**	*	0.41 ± 0.04 **a**	0.46 ± 0.07 **b**	*
Mv-3-*p*-coumglc	1.06 ± 0.12 **a**	1.69 ± 0.39 **b**	1.74 ± 0.43 **b**	1.55 ± 0.12 **b**	*	1.34 ± 0.30 **a**	1.67 ± 0.41 **b**	*
Mv-3-glc-4-vinyl(epi)cat	0.15 ± 0.02 **a**	0.18 ± 0.02 **b**	0.15 ± 0.02 **ab**	0.16 **ab**	*	0.17 ± 0.02 **a**	0.16 ± 0.02 **a**	**ns**
Σ Anthocyanins	**35.65 ± 4.33 a**	**46.18 ± 6.24 b**	**45.10 ± 3.68 b**	**40.14 ± 1.28 a**	*	**39.49 ± 3.50 a**	**44.05 ± 6.90 b**	*

Phenolic compounds	*Winemaking technology * Treatment with oak chips*								
	T	**T+chips**	**C**	**C+chips**	**E**	**E+chips**	**PM**	**PM+chips**	*Significance*
Phenolic acids and derivatives									
(mg GAE/L; mg CAE/L)									
Gallic acid	44.30 ± 1.02 **ab**	45.24 ± 0.61 **ab**	47.31 ± 0.14 **bc**	49.17 ± 0.80 **c**	43.65 ± 0.08 **a**	44.63 ± 0.07 **ab**	45.93 ± 3.45 **ab**	43.87 ± 0.30 **a**	*
2-S-Glutathionyl-Caftaric acid	0.32 ± 0.07 **abv**	0.34 ± 0.07 **ab**	0.31 **ab**	0.38 ± 0.06 **b**	0.25 ± 0.02 **a**	0.30 ± 0.01 **ab**	0.31 ± 0.06 **ab**	0.27 ± 0.02 **ab**	*
Caftaric acid + Caffeic acid	19.26 ± 1.25 **a**	19.60 ± 1.05 **a**	20.97 ± 0.14 **ab**	21.09 ± 0.51 **ab**	20.09 **ab**	19.62 ± 0.27 **a**	21.67 ± 1.62 **b**	20.12 ± 0.09 **ab**	*
p-Coumaric acid	8.18 ± 0.12 **a**	8.25 ± 0.18 **a**	9.25 ± 0.06 **d**	9.13 ± 0.21 **cd**	8.86 ± 0.03 **bcd**	8.42 ± 0.03 **ab**	8.61 ± 0.67 **abc**	8.02 ± 0.04 **a**	*
Ferulic acid	7.44 ± 0.10 **c**	7.40 ± 0.01 **c**	7.40 ± 0.01 **c**	7.26 ± 0.19 **bc**	7.11 ± 0.07 **bc**	6.78 ± 0.14 **ab**	6.76 ± 0.56 **ab**	6.37 ± 0.18 **a**	*
Σ Phenolic acids and derivatives	**79.48 ± 0.18 a**	**80.83 ± 0.68 ab**	**85.25 ± 0.35 bc**	**87.02 ± 1.77 c**	**79.96 ± 0.14 ab**	**79.75 ± 0.43 a**	**83.27 ± 6.36 abc**	**78.66 ± 0.62 a**	*
Stilbens (mg RE/L)									
cis-Piceid	2.48 ± 0.46 **b**	2.30 ± 0.42 **b**	3.44 ± 0.04 **c**	2.34 ± 0.03 **b**	3.17 ± 0.03 **c**	1.99 **b**	0.52 ± 0.03 **a**	0.45 **a**	*
trans-Piceid	3.82 ± 0.16 **b**	3.37 ± 0.49 **ab**	4.00 ± 0.15 **b**	3.86 ± 0.11 **b**	3.75 ± 0.10 **b**	3.47 ± 0.50 **ab**	3.32 ± 0.38 **ab**	2.82 ± 0.15 **a**	*
Σ Stilbens	**6.31 ± 0.62 bc**	**5.67 ± 0.90 b**	**7.43 ± 0.20 d**	**6.20 ± 0.14 bc**	**6.92 ± 0.14 cd**	**5.47 ± 0.50 b**	**3.84 ± 0.41 a**	**3.27 ± 0.15 a**	*
Flavonols (mg QE/L)									
Myricetin-3-glc	1.36 ± 0.23 **ab**	1.36 ± 0.15 **ab**	1.40 ± 0.03 **b**	1.49 ± 0.08 **b**	1.32 ± 0.02 **ab**	1.31 ± 0.01 **ab**	1.26 ± 0.05 **ab**	1.16 **a**	*
Myricetin-3-glcr	2.71 ± 0.34 **bc**	2.68 ± 0.20 **abc**	3.08 ± 0.05 **de**	3.39 ± 0.10 **e**	2.90 ± 0.03 **cd**	2.93 ± 0.13 **cd**	2.45 ± 0.08 **ab**	2.32 **a**	*
Myricetin-3-galac	8.09 ± 0.63 **b**	8.11 ± 0.09 **b**	6.65 ± 0.07 **a**	7.27 ± 0.26 **a**	9.80 ± 0.05 **d**	9.65 ± 0.41 **d**	9.59 ± 0.50 **cd**	8.83 ± 0.17 **bc**	*
Quercetin-3-glc	1.58 ± 0.16 **a**	1.55 ± 0.05 **a**	1.51 ± 0.04 **a**	1.49 ± 0.05 **a**	1.57 ± 0.01 **a**	1.54 ± 0.23 **a**	1.57 ± 0.11 **a**	1.35 ± 0.04 **a**	**ns**
Quercetin-3-glcr	9.58 ± 1.78 **abc**	9.59 ± 0.90 **abc**	10.41 ± 0.03 **bc**	10.90 ± 0.23 **c**	10.87 ± 0.01 **c**	10.39 ± 0.20 **bc**	8.81 ± 0.58 **ab**	7.90 ± 0.08 **a**	*
Quercetin-3-galac	0.98 ± 0.14 **b**	0.87 ± 0.31 **b**	0.53 ± 0.02 **a**	0.52 ± 0.03 **a**	1.61 ± 0.12 **cd**	1.44 ± 0.10 **c**	2.31 ± 0.11 **e**	1.86 ± 0.04 **d**	*
Laricitrin-3-glc	3.37 ± 0.75 **abc**	3.08 ± 0.12 **ab**	3.13 ± 0.03 **ab**	3.15 ± 0.04 **ab**	3.91 ± 0.02 **c**	3.71 ± 0.09 **bc**	3.45 ± 0.35 **abc**	3.02 ± 0.01 **a**	*
Quercetin-3-rha	8.16 ± 1.41 **abc**	7.51 ± 0.52 **abc**	8.65 ± 0.14 **bc**	8.87 ± 0.08 **c**	8.84 ± 0.68 **c**	8.17 ± 0.43 **abc**	7.32 ± 0.34 **ab**	6.89 ± 0.29 **a**	*
Isorhamnetin-3-glc	3.56 ± 0.30 **ab**	3.61 ± 0.35 **abc**	3.85 ± 0.11 **abc**	3.65 ± 0.28 **abc**	4.20 ± 0.16 **c**	3.95 **abc**	4.07 ± 0.10 **bc**	3.35 ± 0.49 **a**	*
Syringetin-3-galac	6.63 ± 1.13 **a**	6.84 ± 0.70 **a**	7.32 ± 0.16 **ab**	8.29 ± 0.10 **b**	7.48 ± 0.06 **ab**	7.63 ± 0.10 **ab**	7.12 ± 0.75 **ab**	6.60 ± 0.06 **a**	*
Laricitrin-3-rhamnose-7-tri-hydroxycinnamic acid	3.41 ± 1.08 **a**	3.15 ± 0.02 **a**	3.48 ± 0.05 **a**	3.37 ± 0.15 **a**	4.84 ± 0.07 **b**	4.01 ± 0.07 **ab**	3.78 ± 0.02 **a**	3.78 ± 0.08 **a**	*
Σ Flavonols	**49.45 ± 7.67 ab**	**48.33 ± 2.47 ab**	**50.00 ± 0.15 ab**	**52.38 ± 0.81 abc**	**57.34 ± 0.73 c**	**54.73 ± 0.54 bc**	**51.75 ± 2.99 abc**	**47.05 ± 0.26 a**	*
Flavan-3-ols (mg CE/L)									
(-)-(Epi)Gallocatechin	23.64 ± 0.84 **a**	27.77 ± 1.37 **bc**	27.41 ± 0.26 **b**	30.60 ± 1.58 **d**	27.59 ± 0.37 **b**	30.39 ± 0.67 **d**	29.82 ± 1.00 **cd**	30.92 ± 0.52 **d**	*
Procyanidin B3	50.21 ± 7.77 **a**	55.38 ± 3.95 **ab**	57.51 ± 1.74 **abc**	68.91 ± 3.71 **d**	59.56 ± 1.02 **bc**	64.96 ± 0.56 **cd**	65.75 ± 4.14 **cd**	64.54 ± 0.77 **cd**	*
(+)-Catechin	63.56 ± 2.91 **a**	66.91 ± 1.70 **ab**	68.94 ± 0.14 **abc**	76.46 ± 5.02 **d**	68.79 ± 0.44 **abc**	71.89 ± 0.34 **bcd**	74.09 ± 6.09 **bcd**	75.00 ± 2.19 **cd**	*
Procyanidin B1	42.80 ± 11.30 **ab**	38.61 ± 0.46 **a**	39.58 ± 0.35 **a**	50.01 ± 0.16 **bc**	39.75 ± 0.56 **a**	49.74 ± 2.92 **bc**	44.84 ± 1.37 **abc**	53.94 ± 2.16 **c**	*

Procyanidin B4	54.86 ± 4.12 **a**	56.58 ± 2.11 **ab**	60.93 ± 0.19 **bc**	70.40 ± 3.38 **d**	62.27 ± 0.26 **c**	69.61 ± 1.20 **d**	70.82 ± 1.49 **d**	72.71 ± 1.77 **d**	*
(-)-Epicatechin	104.16 ± 11.48 **ab**	100.40 ± 3.91 **a**	109.14 ± 0.24 **ab**	115.44 ± 0.60 **b**	104.34 ± 1.12 **ab**	109.12 ± 0.83 **ab**	107.60 ± 7.49 **ab**	104.36 ± 3.86 **ab**	*
Procyanidin B2	16.90 ± 2.03 **a**	19.17 ± 0.48 **ab**	18.67 ± 0.52 **ab**	21.37 ± 2.91 **bc**	18.27 ± 0.52 **ab**	21.85 ± 0.88 **bc**	19.77 ± 1.49 **ab**	24.87 ± 3.11 **c**	*
Σ Flavan-3-ols	**356.14 ± 40.46 a**	**364.82 ± 13.98 a**	**382.18 ± 2.43 ab**	**433.18 ± 16.15 c**	**380.57 ± 1.52 ab**	**417.57 ± 3.84 bc**	**412.69 ± 23.08 bc**	**426.35 ± 9.04 c**	*
Anthocyanins (mg ME/L)									
Dp-3-glc	0.60 ± 0.10 **a**	0.55 ± 0.11 **a**	0.64 ± 0.01 **a**	1.28 ± 0.06 **c**	0.85 ± 0.02 **ab**	1.20 ± 0.02 **c**	0.81 ± 0.01 **a**	0.95 ± 0.02 **b**	*
Cy-3-glc	0.25 ± 0.06 **a**	0.28 ± 0.04 **ab**	0.26 **a**	0.38 ± 0.01 **c**	0.34 **bc**	0.39 ± 0.01 **c**	0.34 ± 0.04 **bc**	0.36 ± 0.02 **c**	*
Pt-3-glc	0.49 ± 0.01 **ab**	0.41 ± 0.09 **a**	0.46 ± 0.04 **ab**	1.06 ± 0.07 **e**	0.59 ± 0.01 **bc**	0.97 ± 0.01 **de**	0.72 ± 0.13 **c**	0.88 ± 0.01 **d**	*
Pn-3-glc	0.41 ± 0.02 **bc**	0.29 ± 0.04 **a**	0.36 ± 0.02 **b**	0.75 ± 0.01 **f**	0.45 **cd**	0.72 ± 0.02 **f**	0.50 ± 0.06 **d**	0.61 ± 0.02 **e**	*
Mv-3-glc	5.08 ± 0.61 **b**	3.88 ± 0.94 **a**	4.74 ± 0.08 **ab**	10.85 ± 0.16 **e**	6.47 ± 0.04 **c**	10.60 ± 0.02 **e**	7.13 ± 0.52 **c**	8.94 **d**	*
Dp-3-acetylglc	0.46 ± 0.07 **cd**	0.46 ± 0.10 **cd**	0.52 ± 0.01 **d**	0.41 ± 0.02 **bc**	0.50 ± 0.01 **cd**	0.34 ± 0.02 **ab**	0.34 ± 0.04 **b**	0.24 **a**	*
Pyrano-Mv-3-glc (Vitisin B)	2.45 ± 0.63 **b**	2.39 ± 0.50 **b**	3.39 ± 0.01 **c**	3.01 ± 0.05 **bc**	2.86 ± 0.03 **bc**	2.41 ± 0.02 **b**	1.71 ± 0.08 **a**	1.43 ± 0.05 **a**	*
Carboxypyrano-Mv-3-acetylglc (acetyl-Vitisin A)	1.41 ± 0.53 **bc**	1.22 ± 0.23 **ab**	1.75 ± 0.01 **c**	1.70 ± 0.02 **c**	1.41 ± 0.01 **bc**	1.33 ± 0.02 **abc**	0.97 ± 0.05 **ab**	0.88 ± 0.01 **a**	*
Cy-3-acetylglc	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	**ns**
Pt-3-acetylglc	2.20 ± 0.61 **a**	2.45 ± 0.45 **a**	2.58 ± 0.34 **a**	2.57 ± 0.10 **a**	2.67 ± 0.01 **a**	2.27 ± 0.08 **a**	2.20 ± 0.26 **a**	2.19 ± 0.04 **a**	**ns**
Pyrano-Mv-3-acetylglc (acetyl-Vitisin B)	1.85 ± 0.25 **ab**	1.99 ± 0.14 **abc**	2.28 ± 0.37 **c**	2.23 ± 0.02 **bc**	2.09 ± 0.09 **abc**	2.19 ± 0.02 **abc**	1.95 ± 0.12 **abc**	1.82 ± 0.01 **a**	*
Pn-3-acetylglc	3.41 ± 0.55 **a**	3.50 ± 0.30 **a**	3.58 ± 0.21 **a**	4.21 ± 0.28 **b**	3.75 ± 0.07 **ab**	3.75 ± 0.03 **ab**	3.61 ± 0.21 **ab**	3.41 ± 0.12 **a**	*
Mv-3-acetylglc	7.62 ± 1.54 **a**	8.14 ± 0.47 **ab**	9.02 ± 0.04 **abc**	11.52 ± 0.09 **e**	9.26 ± 0.09 **bc**	10.76 ± 0.07 **de**	9.33 ± 0.53 **bc**	9.81 ± 0.26 **cd**	*
Pyrano-Mv-3-*p*-coumglc (*p*-coum-Vitisin B)	3.29 ± 0.49 **abc**	3.70 ± 0.29 **cd**	4.06 ± 0.25 **d**	3.90 ± 0.02 **d**	3.97 ± 0.21 **d**	3.57 ± 0.17 **bcd**	3.07 ± 0.30 **ab**	2.91 ± 0.04 **a**	*
Mv-3-caffeoylglc	2.65 ± 0.15 **a**	2.63 ± 0.31 **a**	2.69 ± 0.17 **a**	2.69 ± 0.34 **a**	2.66 ± 0.10 **a**	2.60 ± 0.12 **a**	2.46 ± 0.08 **a**	2.33 ± 0.07 **a**	**ns**
Pt-3-*p*-coumglc	1.50 ± 0.30 **a**	1.40 ± 0.17 **a**	1.55 ± 0.16 **a**	1.64 ± 0.01 **a**	1.37 ± 0.26 **a**	1.67 ± 0.19 **a**	1.63 ± 0.05 **a**	1.35 ± 0.12 **a**	**ns**
Mv-3-*p*-coumglc-8-ethyl-(epi)cat	0.55 ± 0.12 **a**	0.61 ± 0.08 **abc**	0.82 ± 0.12 **d**	0.78 ± 0.07 **cd**	0.73 ± 0.06 **bcd**	0.73 ± 0.02 **bcd**	0.60 ± 0.04 **ab**	0.55 ± 0.04 **a**	*
Pn-3-*p*-coumglc	0.38 ± 0.06 **a**	0.39 ± 0.04 **a**	0.44 ± 0.05 **abc**	0.53 ± 0.05 **c**	0.43 **ab**	0.51 ± 0.03 **bc**	0.38 **a**	0.42 ± 0.02 **ab**	**ns**
Mv-3-*p*-coumglc	1.04 ± 0.15 **a**	1.07 ± 0.14 **a**	1.51 ± 0.56 **ab**	1.87 ± 0.10 **bc**	1.38 ± 0.19 **ab**	2.10 ± 0.08 **c**	1.45 ± 0.07 **ab**	1.65 ± 0.03 **bc**	*
Mv-3-glc-4-vinyl(epi)cat	0.16 ± 0.03 **ab**	0.14 ± 0.02 **a**	0.20 ± 0.01 **b**	0.17 ± 0.03 **ab**	0.17 ± 0.02 **ab**	0.14 **a**	0.16 **ab**	0.17 **ab**	*
Σ Anthocyanins	**35.81 ± 6.23 ab**	**35.48 ± 4.17 a**	**40.85 ± 1.15 ab**	**51.52 ± 1.22 d**	**41.93 ± 0.69 bc**	**48.26 ± 0.35 cd**	**39.38 ± 1.51 ab**	**40.91 ± 0.53 ab**	*

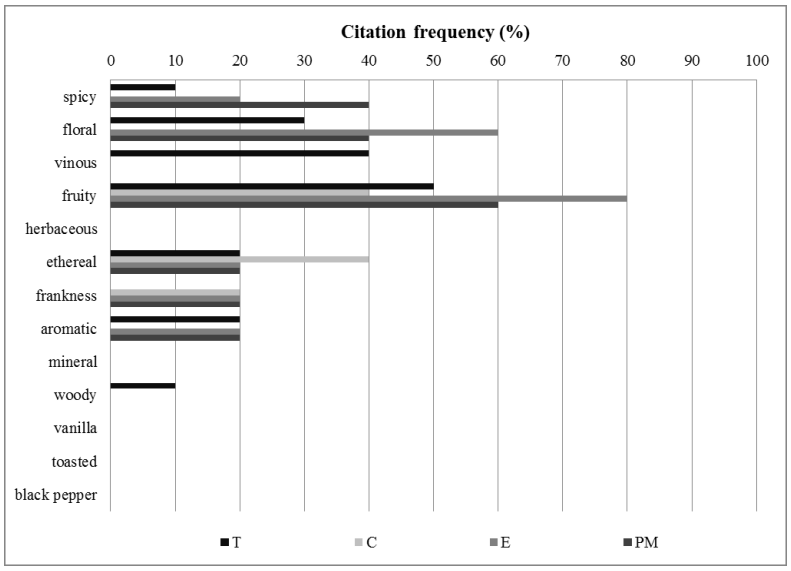
Table 63 – Single and interactive effects of winemaking technologies and treatment with oak chips on the phenolic profile of Nero di Troia wines 12 months after racking.
nd: not detected; T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: prolonged maceration.
glc: glucoside, glcr: glucuronide, rha: rhamnoside, galac: galactoside, Dp: delphinidin, Cy: cyanidin, Pt: petunidin, Pn: peonidin, Mv: malvidin, acetylglc: acetylglucoside, *p*-coumglc: *p*-coumaroylglucoside, caffeoylglc: caffeoylglucoside, cat: catechin.
In row, as part of winemaking technologies or treatment with oak chips or combination of technology-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).

	CI	T	dA(%)	% yellow	% red	% blue	dAl%	dAT%	dTAT%
				Winemaking technology					
T	8.813 ± 0.471 **b**	0.681 ± 0.011 **a**	51.6 ± 1.6 **b**	34.6 ± 0.1 **a**	50.8 ± 0.8 **b**	14.5 ± 0.8 **ab**	1.3 ± 0.3 **a**	29.0 ± 3.5 **a**	70.4 ± 3.5 **c**
C	9.602 ± 0.098 **c**	0.676 ± 0.011 **a**	52.3 ± 0.4 **b**	34.6 ± 0.4 **a**	51.2 ± 0.2 **b**	14.2 ± 0.2 **ab**	1.2 ± 0.2 **ab**	30.7 ± 2.5 **ab**	68.1 ± 2.7 **bc**
E	9.453 ± 0.089 **c**	0.682 ± 0.028 **a**	52.5 ± 1.9 **b**	35.0 ± 0.8 **b**	51.3 ± 1.0 **b**	13.7 ± 0.2 **a**	1.7 ± 0.5 **c**	32.6 ± 1.2 **b**	65.7 ± 1.8 **ab**
PM	8.467 ± 0.124 **a**	0.719 ± 0.019 **b**	48.8 ± 1.4 **a**	35.5 ± 0.5 **c**	49.4 ± 0.7 **a**	15.0 ± 0.3 **b**	1.6 ± 0.5 **bc**	34.5 ± 0.2 **b**	63.9 ± 0.6 **a**
Significance	*	*	*	*	*	*	*	*	*
				Treatment with oak chips					
No CHIPS	8.932 ± 0.619 **a**	0.679 ± 0.018 **a**	51.9 ± 1.9 **b**	34.6 ± 0.3 **a**	51.0 ± 1.0 **b**	14.4 ± 0.8 **a**	1.3 ± 0.3 **a**	30.5 ± 3.5 **a**	68.9 ± 3.7 **a**
+ CHIPS	9.127 ± 0.432 **a**	0.696 ± 0.024 **b**	50.9 ± 1.9 **a**	35.1 ± 0.6 **b**	50.5 ± 1.0 **a**	14.4 ± 0.7 **a**	1.5 ± 0.5 **b**	31.9 ± 2.9 **a**	66.5 ± 3.4 **a**
Significance	**ns**	*	*	*	*	**ns**	*	**ns**	**ns**
				Winemaking technology * Treatment with oak chips					
T	8.587 ± 0.602 **ab**	0.685 ± 0.013 **bc**	51.4 ± 1.9 **bcd**	34.7 **c**	50.7 ± 1.0 **bcd**	14.6 ± 1.0 **ab**	1.5 ± 0.3 **a**	28.4 ± 4.8 **a**	71.3 ± 4.1 **b**
T+chips	9.040 ± 0.141 **bc**	0.678 ± 0.008 **bc**	51.9 ± 1.5 **bc**	34.5 ± 0.1 **b**	51.0 ± 0.8 **bc**	14.5 ± 0.9 **ab**	1.1 ± 0.3 **a**	29.4 ± 2.9 **a**	69.5 ± 3.2 **b**
C	9.517 **cd**	0.666 **ab**	52.6 **cd**	34.2 **a**	51.4 **cd**	14.4 **ab**	1.0 **a**	28.7 ± 0.4 **ab**	70.2 ± 0.3 **b**
C+chips	9.687 ± 0.004 **d**	0.685 ± 0.001 **c**	51.9 ± 0.2 **bcd**	34.9 **d**	51.0 ± 0.1 **bcd**	14.1 ± 0.1 **ab**	1.4 **a**	32.6 ± 2.0 **abc**	66.0 ± 2.0 **ab**
E	9.530 ± 0.002 **cd**	0.658 **a**	54.1 **d**	34.3 **a**	52.2 **d**	13.5 **a**	1.2 **a**	31.7 ± 0.7 **abc**	67.2 ± 0.6 **ab**
E+chips	9.376 ± 0.004 **cd**	0.706 ± 0.005 **d**	50.9 ± 0.2 **bc**	35.6 ± 0.1 **e**	50.5 ± 0.1 **bc**	13.9 **ab**	2.1 **b**	33.6 ± 0.6 **abc**	64.2 ± 0.4 **a**
PM	8.440 ± 0.071 **a**	0.703 ± 0.003 **d**	49.8 ± 0.4 **ab**	35.1 **d**	49.9 ± 0.2 **ab**	15.0 ± 0.2 **b**	1.2 ± 0.2 **a**	34.4 **bc**	64.4 ± 0.2 **a**
PM+chips	8.494 ± 0.195 **a**	0.734 ± 0.010 **e**	47.9 ± 1.2 **a**	35.9 ± 0.1 **f**	49.0 ± 0.6 **a**	15.1 ± 0.5 **b**	1.9 **b**	34.6 ± 0.3 **c**	63.4 ± 0.2 **a**
Significance	*	*	*	*	*	*	*	*	*

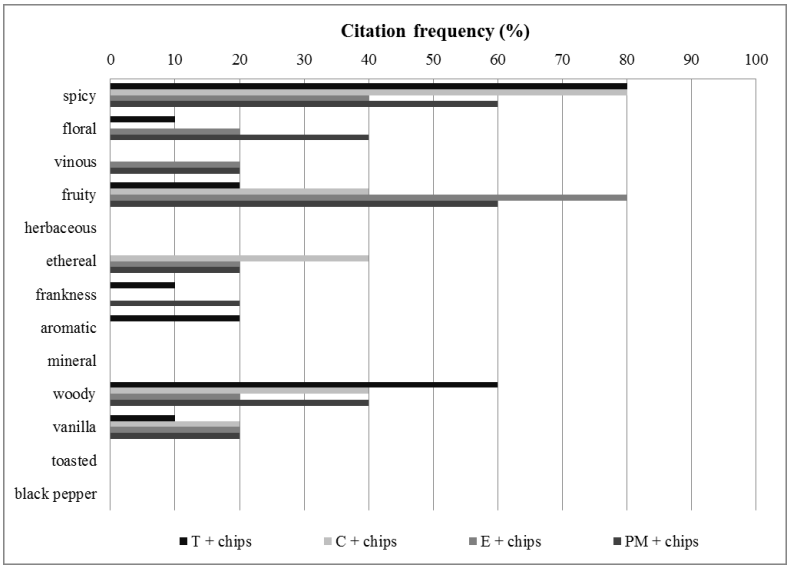
Table 64 – Single and interactive effects of winemaking technologies and treatment with oak chips on the colour parameters of Nero di Troia wines 12 months after racking.
CI: colour intensity; T: tonality; dA(%): percentage of red colour due to flavilium cation of free and combined anthocyanins; % yellow: percentage of yellow component; % red: percentage of red component; % blue: percentage of blue component; dAl%: percentage of absorbance at 520 nm due to monomeric anthocyanins; dAT%: percentage of absorbance at 520 nm due to polymeric pigments decolorized with SO_2; dTAT%: percentage of absorbance at 520 nm due to polymeric pigments not decolorized.
T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: prolonged maceration.
In column, as part of winemaking technologies or treatment with oak chips or combination of technology-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference; ns: no significant difference.

*Winemaking technology * Treatment with oak chips*									
Sensory descriptors	**T**	**T+chips**	**C**	**C+chips**	**E**	**E+chips**	**PM**	**PM+chips**	*Significance*
Visual descriptors									
Clearness	1.7 ± 0.7 **a**	1.8 ± 0.4 **a**	2.0 **a**	1.8 ± 0.4 **a**	1.8 ± 0.4 **a**	2.0 **a**	1.8 ± 0.4 **a**	1.8 ± 0.4 **a**	**ns**
Texture	2.2 ± 0.4 **a**	2.2 ± 0.5 **a**	2.1 ± 0.5 **a**	2.2 ± 0.4 **a**	2.2 ± 0.4 **a**	2.2 ± 0.4 **a**	2.2 ± 0.4 **a**	2.2 ± 0.4 **a**	**ns**
Olfactory descriptors									
Olfactory intensity	2.3 ± 0.6 **a**	2.2 ± 0.6 **a**	1.8 ± 0.6 **a**	1.9 ± 0.5 **a**	2.4 ± 0.4 **a**	2.1 ± 0.7 **a**	2.2 ± 0.3 **a**	2.3 ± 0.4 **a**	**ns**
Olfactory complexity	1.9 ± 0.6 **ab**	2.2 ± 0.6 **b**	1.5 ± 0.5 **a**	1.9 ± 0.7 **ab**	2.2 ± 0.4 **ab**	2.0 ± 0.7 **ab**	2.4 ± 0.4 **b**	2.4 ± 0.4 **b**	*
Olfactory quality	1.8 ± 0.6 **ab**	2.4 ± 0.5 **c**	1.3 ± 0.3 **a**	1.9 ± 0.2 **abc**	2.2 ± 0.3 **bc**	2.1 ± 0.7 **bc**	2.4 ± 0.4 **c**	2.4 ± 0.4 **c**	*
Gustatory-olfactory descriptors									
Sugars	0.0 **a**	0.0 **a**	0.0 **a**	0.0 **a**	0.0 **a**	0.0 **a**	0.0 **a**	0.0 **a**	**ns**
Alcohols	2.4 ± 0.5 **a**	2.3 ± 0.6 **a**	2.1 ± 0.7 **a**	2.3 ± 0.4 **a**	2.3 ± 0.4 **a**	2.2 ± 0.4 **a**	2.3 ± 0.6 **a**	2.4 ± 0.4 **a**	**ns**
Polyols	1.5 ± 0.5 **a**	1.7 ± 0.5 **a**	1.4 ± 0.5 **a**	1.5 ± 0.5 **a**	1.7 ± 0.4 **a**	1.8 ± 0.4 **a**	2.0 ± 0.7 **a**	2.0 ± 0.7 **a**	**ns**
Acids	2.8 ± 0.8 **a**	2.6 ± 0.5 **a**	2.6 ± 0.4 **a**	2.7 ± 0.4 **a**	2.7 ± 0.3 **a**	2.2 ± 0.6 **a**	2.4 ± 0.4 **a**	2.2 ± 0.3 **a**	**ns**
Tannins	2.2 ± 0.6 **a**	2.6 ± 1.0 **a**	2.4 ± 1.2 **a**	2.5 ± 0.5 **a**	2.2 ± 0.8 **a**	2.2 ± 0.6 **a**	2.3 ± 0.8 **a**	2.0 ± 0.6 **a**	**ns**
Minerals	2.0 ± 0.4 **a**	2.2 ± 0.5 **ab**	2.3 ± 0.4 **ab**	2.4 ± 0.4 **ab**	2.6 ± 0.4 **b**	2.0 ± 0.4 **a**	2.3 ± 0.4 **ab**	2.3 ± 0.7 **ab**	*
Structure	2.5 ± 0.5 **a**	2.5 ± 0.6 **a**	2.3 ± 0.4 **a**	2.4 ± 0.4 **a**	2.3 ± 0.4 **a**	2.4 ± 0.5 **a**	2.4 ± 0.4 **a**	2.3 ± 0.4 **a**	**ns**
Balance	1.4 ± 0.8 **ab**	1.7 ± 1.0 **ab**	0.9 ± 0.5 **a**	1.4 ± 0.9 **ab**	1.7 ± 0.4 **ab**	2.1 ± 0.2 **ab**	2.2 ± 0.6 **b**	1.9 ± 0.2 **b**	*
Gustatory-olfactory intensity	2.4 ± 0.9 **a**	2.4 ± 1.1 **a**	1.9 ± 0.7 **a**	2.2 ± 0.6 **a**	2.2 ± 0.4 **a**	2.1 ± 0.9 **a**	2.4 ± 0.4 **a**	2.4 ± 0.4 **a**	**ns**
Gustatory-olfactory persistence	2.5 ± 0.9 **a**	2.4 ± 0.9 **a**	2.2 ± 0.4 **a**	2.2 ± 0.6 **a**	2.2 ± 0.4 **a**	2.3 ± 0.6 **a**	2.4 ± 0.4 **a**	2.5 ± 0.5 **a**	**ns**
Gustatory-olfactory quality	1.4 ± 0.6 **a**	1.8 ± 0.5 **ab**	1.5 ± 0.4 **ab**	1.8 ± 0.4 **abc**	2.0 **bcd**	1.9 ± 0.4 **bcd**	2.4 ± 0.4 **cd**	2.5 ± 0.6 **d**	*
Final considerations									
Evolutionary state	2.5 ± 0.9 **a**	2.0 ± 0.3 **a**	2.1 ± 0.8 **a**	2.3 ± 0.6 **a**	2.5 ± 0.6 **a**	2.3 ± 0.5 **a**	2.5 ± 0.6 **a**	2.3 ± 0.5 **a**	**ns**
Harmony	1.4 ± 0.8 **ab**	1.7 ± 0.9 **abc**	1.2 ± 0.8 **a**	1.5 ± 0.9 **abc**	1.6 ± 0.9 **abc**	2.0 **abc**	2.2 ± 0.4 **bc**	2.4 ± 0.5 **c**	*

Table 65 – Interactive effects of winemaking technologies and treatment with oak chips on the sensory characteristics of Nero di Troia wines 12 months after racking.
T: traditional red-winemaking; C: cryo-maceration; E: winemaking with addition of pectolytic enzymes; PM: prolonged maceration.
In row, within the combination of technology-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference; ns: no significant difference.

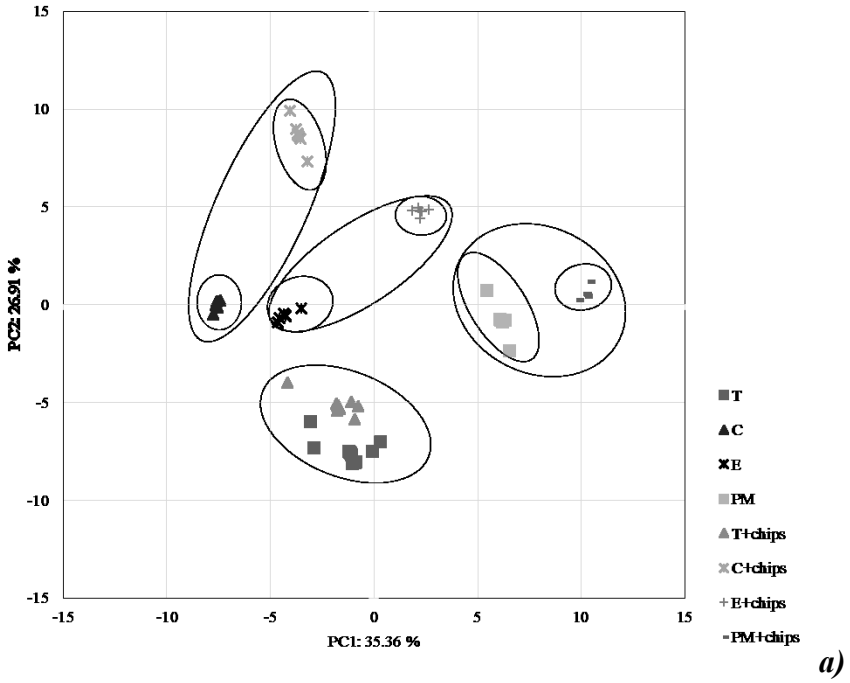


a)

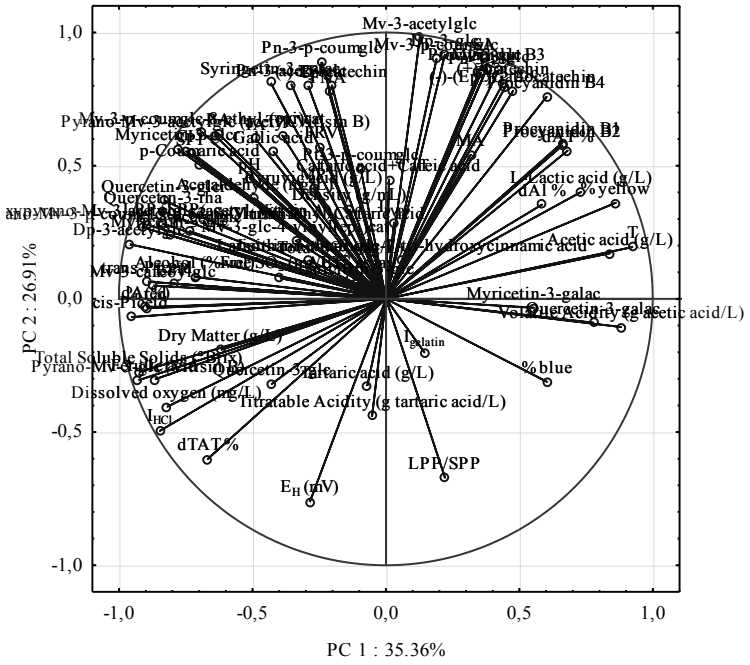


b)

Figure 22 - Citation frequency (%) of the flavours of Nero di Troia wines not treated (*a*) and treated with French oak chips (*b*) 12 months after racking.



a)



b)

Figure 23 - PCA scatter plot for projection on the factor plane of: **a)** Nero di Troia wine samples after 12 months from the first racking and **b)** analytical results.

7.7 Evaluation of single and interactive effects of the treatment with oak chips and different types of closure on Nero di Troia wine quality

The main physicochemical characteristics of the Nero di Troia wines, at the first racking and after 12 months of aging, are listed in Table 66. At racking, they exhibited: medium alcohol content and dry matter; low values of volatile acidity, free and total SO_2; high titratable acidity; medium acetaldehyde concentration deriving from the yeast metabolism; concentrations of total soluble solids that indicate the correct progress of fermentation. After 12 months, both the type of closure and the treatment with oak chips did not exert significant single effects on the oenological parameters but only a significant interactive effect on the acetaldehyde contents, which was the highest in S300 samples and the lowest in S100 and S700+chips. Concerning the evolution of the oenological parameters with the aging time, ethanol content decreased and volatile acidity increased due to the acetic acid bacteria metabolism. Titratable acidity decreased and pH increased due to the malo-lactic conversion by lactic acid bacteria. Free and total sulphur dioxide content slightly decreased because of oxidation, and acetaldehyde decreased as a consequence of its participation in polymerization reactions involving anthocyanins and/or flavanols (Fulcrand *et al*., 1996; Saucier *et al*., 1997).

The graphs concerning the evolution of dissolved oxygen (Figures 24 *a* and *c*) and red-ox potential (Figures 24 *b* and *d*) during 12 months of aging highlight that, before bottling, when wines were submitted to several rackings from a stainless steel tank to another, the dissolved oxygen and, thus, the redox potential increased. In fact, racking is known to encourage the incorporation of atmospheric oxygen into the wine, with the consequent increase of the dissolved oxygen (Ribéreau-Gayon *et al*., 2004; Schneider, 2005; Vidal and Moutounet, 2006). From bottling, dissolved oxygen decreased until concentrations similar or slightly lower than those detected at the first racking in the case of the untreated wines or until values higher than those detected at the first racking in the case of the wines treated with oak chips. Even the redox potential decreased from to 12 months of aging, reaching values 3-4 times lower than those of the first racking. Similar to what was observed by Gao *et al*. (2015), a rapid drop of the dissolved oxygen content occurred within the first three months after bottling, in the present study both dissolved oxygen concentration and redox potential rapidly fell to low levels within the first four months. This behaviour was explained by Dimkou *et al*. (2011), who found a high rate of oxygen consumption in the

considered period because the capacity of wine to consume oxygen is not compensate by the input rate of the oxygen inside the bottle. At around seven months, consumption and input rebalanced. It is interesting to observe that after 12 months, the dissolved oxygen content was higher in the wines sealed with stoppers having medium (S300) or high (S700) oxygen transmission rates than in the bottles closed with low OTR stoppers (S100).

Table 67 shows the organic acids composition of Nero di Troia wines. At the first racking, they were in a decreasing order: tartaric > malic > citric > acetic > D-gluconic > lactic > pyruvic. After 12 months from the first racking (nine months of bottling), the composition in organic acids strongly changed, becoming the following: tartaric > L-lactic > acetic > citric > L-malic, pyruvic, and D-gluconic. No significant effects were exerted by the chips and the stoppers on the organic acids composition.

Tables from 68 to 70 showed the phenolic composition, the distribution of monomeric and polymeric pigments among, the structure indices, and the phenolic profile of Nero di Troia wines, at the first racking and after 12 months of aging. The data concerning phenolic classes at the first racking are index of a red wine with an intermediate phenolic concentrations. After 12 months of aging (i.e. nine months of bottle storage), the wines in bottles closed with the highest OTR stoppers exhibited the lowest concentrations of almost all the phenolic classes, except for monomeric anthocyanins. Furthermore, also the antioxidant activity did not show significant difference among the samples. The loss of anhocyanins was in agreement with the findings of Wirth *et al*. (2010), who reported that the increase of OTR values can noticeably increase the rate of anthocyanins and monomer flavan-3-ol degradation, the rate of the SO_2 consumption, and chromaticity. The single effects of oak chips was exerted on total, sensitive and monomeric anthocyanins (-13.7%, -19.4%, and -41.4%, respectively), total flavonoids (-4.6%), flavanols (-11.1%) and proanthocyanidins (also termed condensed tannins; -6.5%). No differences in flavonoids different from anthocyanins, total phenolic compounds, and antioxidant activity were highlighted The loss of anthocyanins, in this case, validated the results already observed in Aglianico, Montepulciano, and Nero di Troia wines (sections 7.2.1, 7.2.2, and 7.2.3) and were in agreement with the studies performed by Del Alamo Sanza and Nevares Domínguez (2006), and Del Alamo Sanza *et al.* (2004) on Cigales appellation of origin red wine. The combination of the treatment with oak chips with the subsequent nano-oxygenation through the stoppers favoured the polymerization reactions among

anthocyanins and tannins, and this phenomenon was more intense especially in S300 samples (medium OTR).

According to the data listed in Table 69, the distribution among monomeric, small polymeric (bleachable), and large polymeric (not bleachable) pigments of Nero di Troia wines suffered several changes during the considered aging time, with a decrease of the contribution due to monomeric pigments and the rise of the contribution of the small and large polymeric pigments (especially this latter). In particular, the highest increase of the polymerization degree occurred in bottles closed with the medium (S300) and high permeable (S700) closures. Data also confirmed by the highest PVPP indices of the same samples. This means that the highest oxygen exposure of the wines due to the higher oxygen nano-permeation through the S300 and S700 stoppers promoted the polymerization reactions among flavan-3-ols and anthocyanins thus stabilising the colour of red wines (Singleton and Trousdale, 1992; Es-Safi *et al*., 1999; Monagas *et al*., 2005). In addition, the wines aged in bottles sealed with the highest OTR showed the highest HCl index value, thus indicating also a greater tannin polymerization state. No significant differences, instead, were observed in gelatin index among the investigated samples. The oak chips treatment produced the increase of the state of polymerization of tannins and anthocyanins, as already highlighted in sections 7.2.1, 7.2.2, and 7.2.3 for Aglianico, Montepulciano, and Nero di Troia wines (2012 campaign), and by Piracci *et al*. (2001). The treatment with oak chips exerted its significant single effects on monomeric anthocyanins (-30%), large polymeric pigments (+13%), large polymeric-to-small polymeric ratio (+15%), the gelatin index (-19%), and HCl index (+19%).

Concerning the interactive effects on pigments and structure indices, different trends were observed. Among the wines in bottles closed with high OTR devices, monomeric anthocyanins, small polymeric anthocyanins, and their ratio exhibited similar values in wines untreated or treated with chips, while the treatment with chips caused the decrease of the gelatin index and the increase of the large polymeric pigments and of the HCl and PVPP indices. In wine bottles closed with the intermediate OTR devices, the contact with oak chips determined the decrease of monomeric anthocyanins, and the increase of large polymeric pigments, large-to-small polymeric pigment ratio, and of the 3 structure indices. Among the wines in bottles closed with low OTR devices, monomeric anthocyanins, small polymeric anthocyanins, and gelatin index were lower in the samples treated with chips,

while large polymeric pigments, the large-to-small polymeric pigment ratio, and the HCl index were higher.

The phenolic profile of the wines at the first racking was characterized by the following compounds (Table 70): gallic acid (phenolic acids); *cis*- and *trans*-piceid (stilbens); myricetin-3-galactoside and quercetin-3-glucuronide (flavonols); epicatechin, catechin, procyanidins B1, B3, and B4 (flavan-3-ols); malvidin-3-glucoside and malvidin-3-acetylglucoside (anthocyanins). After 12 months, the phenolic concentrations decreased, especially those of anthocyanins (-77.6% for S300, -74.6% for S700, -71.4% for S100), flavonols (-43.0% for S300, -38.0% for S700, -33.0% for S100), and flavan-3-ols (-17.0% for S300, -8.0% for S700, -6.0% for S100). For almost all the phenolic families, the decrease of their contents was in the following decreasing order: medium > high > low OTR closures. Although the contents of both monomeric and more stable anthocyanin forms were the lowest in wines sealed with S300 closures, the stable forms contributed to the total amounts of anthocyanins of these wines in the highest percentages. In particular, the contribution of the free forms to the total anthocyanins was in a decreasing order: S300 < S700 < S100 wines; while the contribution of the stable forms was in an increasing order: S300 > S700 > S100 samples. The lowest ratio between free and more stable forms in wines aged in bottle with medium or high OTR closures improved their colour stability (Margalit, 2004; Somers and Evans, 1977; Timberlake and Bridle, 1976). Regarding the single effects of the type of closure on the phenolic profile of Nero di Troia wines (Table 70), the wines of bottles closed with the low OTR stoppers showed the highest concentrations of stilbens, flavonols, flavan-3-ols (with those bottled with high OTR stoppers), and anthocyanins, while the wines of bottles closed with the high OTR stoppers showed the highest concentrations of phenolic acids.

Oak chips exerted several significant single effects on the phenolic profile. In particular, the treated wines exhibited the lowest contents of stilbens, flavonols, flavan-3-ols, and anthocyanins. Even if the concentration of anthocyanins was lowest with respect to the controls, the polymeric-to-total anthocyanins ratio was higher in comparison with the untreated samples (26.4% vs. 24.1%) and this phenomenon led to a higher colour stability.

Concerning the combined effects of the treatment with oak chips and the types of closures, the wines stored in bottles where the nano-oxygenation process took place faster (i.e. with medium and high OTR closures) showed the decrease of flavan-3-ols and anthocyanins,

and the increase of the pigments polymerization (greater ratio between stable and total anthocyanin forms).

The colour parameters of Nero di Troia wines are shown in Table 71. The only chromatic feature that suffered the influence of the post-bottling oxygen exposure was the colour intensity. As anticipated, the highest ratio between monomeric and more stable pigments in wines aged in bottle with a medium or high oxygen transfer rate improved their colour intensity. As stated by Wirth *et al*. (2010), the higher values of colour intensity reflected the formation of different pigments as a result of oxygen exposure. Moreover, the mild oxidative conditions (nano-oxygenation) provided by medium and high OTR closures improved the oxidative conversion of anthocyanins into other pigments, having more orangish hues and resistance to SO_2 bleaching with respect to the starting grape anthocyanins. The oak chips treatment determined a decrease of the colour intensity and of the contribute of absorbance at 520 nm due to monomeric pigments, as a consequence of the loss of anthocyanins in oak-treated wines.

Concerning the evolution of the colour characteristics during aging (Table 71), colour intensity, % of red component, % of absorbance due to bleachable pigments decreased as consequence of the loss of anthocyanins and their participation in polymerization reactions, while % of yellow and blue components increased, as a consequence of the progressive formation of more stable pigments, such as orange pyranoanthocyanins (Cheynier *et al*., 2006) and/or co-pigmented complexes, which are purple and bluish in colour (Boulton, 2001). The single effects of the type of closure were exerted only on the colour intensity, which was the lowest in the wines with S100 closures. The single effects of chips were exerted on the colour intensity and on the percentage of absorbance at 520 nm due to monomeric anthocyanins, which were the lowest in the treated wines.

The different types of stopper and the treatment with oak chips did significantly affect olfactory quality, balance, gustatory-olfactory quality, and harmony, which were the highest in wines treated with chips and stored in bottles closed with low OTR devices (Table 72). According to Figure 25*a*, the untreated wines stored in bottles sealed with low OTR closures showed a flavour profile characterised by floral, vinous, fruity, and ethereal notes. The employment of stoppers with medium OTR values determined the appearance of spicy and aromatic flavours, the greater intensity of fruity attribute (with the wines bottled with high OTR stoppers), and the decrease of the vinous aroma. The high OTR

closures, instead, determined the appearance of aromatic and frankness flavours, the increase of fruity notes, and the decrease of floral, vinous, and ethereal notes.

The contact with oak chips improved the olfactory quality in all the wines, making them more balanced and harmonious, through the attenuation of the floral and fruity notes, and the accentuation and/or the appearance of spicy, woody, and vanilla flavours (Figure 25*b*), thus confirming, once again, the findings of Guchu *et al*. (2006) and Gómez García-Carpintero *et al*. (2011; 2012). An another interesting aspect is that the attenuation of floral, vinous, and fruity attributes was more marked in samples treated with oak chips and submitted to medium and high oxygen exposure after bottling, and, among these samples, those ones subjected to the medium oxygen exposure exhibited the highest attenuation.

The Principal Component analysis (PCA) was applied to the whole standardize data set and the obtained results are reported in Figure 26. The first two principal components (PCs) accounted only for about 59% of the total variance and showed the principal groupings. As it can be seen, the untreated wines in bottles closed with the different OTR devices, placed in the area of the planes characterized by negative values of the first principal components and positive values of the second principal components, were not well distinguishable from each other, while when treated with the oak chips treatment, the wines were clearly classified in groups homogeneous for type of closure.

	Alcohol (% vol)	Density (g/mL)	Dry Matter (g/L)	Volatile Acidity (g acetic acid/L)	Titratable Acidity (g tartaric acid/L)	pH	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Total Soluble Solids (°Brix)	Acetaldehyde (mg/L)
					At the first racking					
	13.00 ± 0.10	0.994	29.4 ± 0.2	0.25 ± 0.04	6.50 ± 0.03	3.48 ± 0.02	20.0 ± 1.1	26.4 ± 1.1	7.6	44.50 ± 0.71
					After 12 months					
					Type of closure					
S100	12.69 ± 0.11 **a**	0.994 **a**	26.4 ± 0.8 **a**	0.66 ± 0.04 **a**	5.72 ± 0.29 **a**	3.69 ± 0.07 **a**	18.0 ± 1.5 **a**	23.4 ± 1.8 **a**	7.4 ± 0.1 **a**	2.28 ± 0.38 **a**
S300	12.60 ± 0.11 **a**	0.993 ± 0.001 **a**	26.8 ± 0.3 **a**	0.69 ± 0.03 **a**	5.85 ± 0.11 **a**	3.69 ± 0.07 **a**	20.6 ± 1.0 **a**	26.0 ± 0.8 **a**	7.4 ± 0.1 **a**	2.60 ± 0.29 **a**
S700	12.60 ± 0.20 **a**	0.993 ± 0.001 **a**	26.0 ± 1.2 **a**	0.67 ± 0.06 **a**	5.96 ± 0.23 **a**	3.70 ± 0.06 **a**	17.8 ± 1.9 **a**	24.4 ± 3.0 **a**	7.4 **a**	2.30 ± 0.29 **a**
Significance	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
					Treatment with oak chips					
No CHIPS	12.61 ± 0.19 **a**	0.994 **a**	26.4 ± 0.4 **a**	0.68 ± 0.05 **a**	5.88 ± 0.22 **a**	3.70 ± 0.06 **a**	18.7 ± 2.1 **a**	24.5 ± 2.4 **a**	7.4 **a**	2.52 ± 0.33 **a**
+ CHIPS	12.65 ± 0.05 **a**	0.993 ± 0.001 **a**	26.4 ± 1.2 **a**	0.66 ± 0.04 **a**	5.81 ± 0.26 **a**	3.69 ± 0.07 **a**	18.9 ± 1.9 **a**	24.7 ± 2.1 **a**	7.4 ± 0.1 **a**	2.27 ± 0.31 **a**
Significance	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
					Type of closure * Treatment with oak chips					
S100	12.74 ± 0.16 **a**	0.994 ± 0.001 **a**	26.5 ± 0.8 **a**	0.65 ± 0.07 **a**	5.90 ± 0.36 **a**	3.70 ± 0.06 **a**	18.4 ± 1.1 **a**	24.0 ± 2.3 **a**	7.4 **a**	2.15 ± 0.21 **a**
S100+chips	12.64 ± 0.05 **a**	0.994 **a**	26.3 ± 1.2 **a**	0.67 ± 0.03 **a**	5.54 ± 0.02 **a**	3.69 ± 0.11 **a**	17.6 ± 2.3 **a**	22.8 ± 1.7 **a**	7.4 ± 0.1 **a**	2.40 ± 0.57 **bcd**
S300	12.55 ± 0.16 **a**	0.994 **a**	26.6 **a**	0.71 ± 0.01 **a**	5.84 ± 0.02 **a**	3.69 ± 0.09 **a**	20.4 ± 1.7 **a**	25.6 **a**	7.4 ± 0.1 **a**	2.85 ± 0.07 **d**
S300+chips	12.65 ± 0.03 **a**	0.993 ± 0.002 **a**	27.1 ± 0.2 **a**	0.66 ± 0.02 **a**	5.87 ± 0.19 **a**	3.70 ± 0.08 **a**	20.8 **a**	26.4 ± 1.1 **a**	7.5 **a**	2.35 ± 0.07 **b**
S700	12.55 ± 0.30 **a**	0.994 ± 0.001 **a**	26.1 ± 0.3 **a**	0.69 ± 0.06 **a**	5.90 ± 0.32 **a**	3.72 ± 0.06 **a**	17.2 ± 2.8 **a**	24.0 ± 4.5 **a**	7.4 **a**	2.55 ± 0.07 **c**
S700+chips	12.66 ± 0.10 **a**	0.993 ± 0.001 **a**	25.8 ± 2.0 **a**	0.65 ± 0.07 **a**	6.03 ± 0.21 **a**	3.68 ± 0.08 **a**	18.4 ± 1.1 **a**	24.8 ± 2.3 **a**	7.5 ± 0.1 **a**	2.05 ± 0.07 **a**
Significance	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	*

Table 66 – Physicochemical characteristics of Nero di Troia wines at the first racking and after 12 months of aging.
S100: Nomacorc *Select Series*™ 100 co-extruded closure; S300: Nomacorc *Select Series*™ 300 co-extruded closure; S700: Nomacorc *Select Series*™ 700 co-extruded closure.
In column, as part of type of closure or treatment with oak chips or combination of type of closure-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference.
ns: no significant difference.

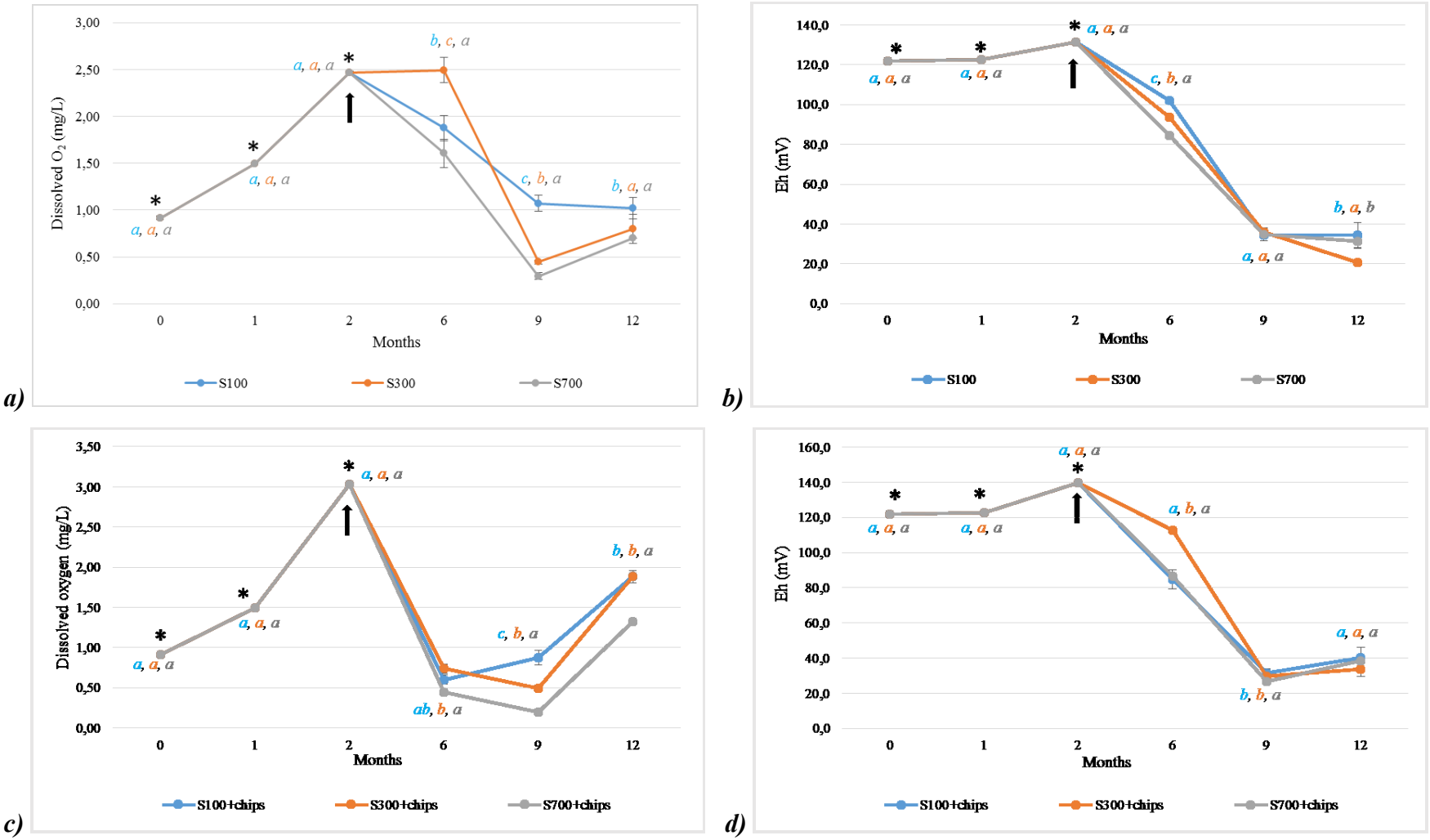


Figure 24 – Evolution of the dissolved oxygen and the red-ox potential (E_h) of Nero di Troia wines during bottle aging according to the different type of closure and the application of the treatment with French oak chips. Error bars represent standard deviations (n = 3). The asterisks indicate the execution of the rackings while the black arrows indicate the time corresponding to bottling. In the graph, within the same time of sampling, different letters correspond to significative differences ($p < 0.05$ - LSD test).
S100: Nomacorc *Select Series*™ 100 co-extruded closure; S300: Nomacorc *Select Series*™ 300 co-extruded closure; S700: Nomacorc *Select Series*™ 700 co-extruded closure.

	Tartaric acid	L-Malic acid	L-Lactic acid	Acetic acid	Citric acid	Pyruvic acid	D-Gluconic acid
				At the first racking			
	2.32 ± 0.26	1.76 ± 0.07	0.07 ± 0.01	0.20 ± 0.01	0.25 ± 0.01	0.03	0.10
				After 12 months			
				Type of closure			
S100	2.32 ± 0.05 **a**	nd **a**	1.23 ± 0.03 **a**	0.53 ± 0.02 **a**	0.14 ± 0.01 **a**	nd **a**	nd **a**
S300	2.29 ± 0.14 **a**	nd **a**	1.24 ± 0.02 **a**	0.50 ± 0.01 **a**	0.15 ± 0.02 **a**	nd **a**	nd **a**
S700	2.31 ± 0.09 **a**	nd **a**	1.29 ± 0.04 **a**	0.51 ± 0.01 **a**	0.14 ± 0.02 **a**	nd **a**	nd **a**
Significance	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
				Treatment with oak chips			
No CHIPS	2.26 ± 0.10 **a**	nd **a**	1.24 ± 0.04 **a**	0.51 ± 0.01 **a**	0.15 ± 0.01 **a**	nd **a**	nd **a**
+ CHIPS	2.35 ± 0.06 **a**	nd **a**	1.26 ± 0.04 **a**	0.52 ± 0.02 **a**	0.14 ± 0.02 **a**	nd **a**	nd **a**
Significance	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
				Type of closure * Treatment with oak chips			
S100	2.35 ± 0.06 **b**	nd **a**	1.20 ± 0.01 **a**	0.52 ± 0.02 **a**	0.14 ± 0.01 **a**	nd **a**	nd **a**
S100+chips	2.29 **ab**	nd **a**	1.26 ± 0.01 **a**	0.54 ± 0.01 **a**	0.15 ± 0.02 **a**	nd **a**	nd **a**
S300	2.17 ± 0.04 **a**	nd **a**	1.23 ± 0.03 **a**	0.51 ± 0.01 **a**	0.15 ± 0.01 **a**	nd **a**	nd **a**
S300+chips	2.41 ± 0.02 **b**	nd **a**	1.25 ± 0.02 **a**	0.49 **a**	0.15 ± 0.02 **a**	nd **a**	nd **a**
S700	2.26 ± 0.11 **ab**	nd **a**	1.29 ± 0.03 **a**	0.51 **a**	0.15 **a**	nd **a**	nd **a**
S700+chips	2.36 ± 0.05 **b**	nd **a**	1.28 ± 0.07 **a**	0.52 ± 0.01 **a**	0.13 ± 0.02 **a**	nd **a**	nd **a**
Significance	*	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**

Table 67 – Organic acids content of Nero di Troia wines at the first racking and after 12 months of aging (data are expressed as g per L of wine).
nd: not detected.
S100: Nomacorc *Select Series*™ 100 co-extruded closure; S300: Nomacorc *Select Series*™ 300 co-extruded closure; S700: Nomacorc *Select Series*™ 700 co-extruded closure.
In column, as part of type of closure or treatment with oak chips or combination of type of closure-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference.
ns: no significant difference.

	TA	SA	MA	TF	FNA	FRV	PA	TPC	ABTS assay
					At the first racking				
	357 ± 17	477 ± 41	135 ± 19	2368 ± 143	1848 ± 133	2278 ± 300	4610 ± 57	3506 ± 75	20.03 ± 1.75
					After 12 months				
					Type of closure				
S100	152 ± 7 **b**	96 ± 10 **ab**	33 ± 6 **a**	1925 ± 32 **ab**	1704 ± 38 **ab**	1291 ± 124 **b**	3992 ± 208 **b**	3053 ± 173 **b**	23.82 ± 4.70 **a**
S300	157 ± 9 **b**	112 ± 14 **b**	36 ± 7 **a**	1961 ± 89 **b**	1732 ± 83 **b**	1241 ± 125 **ab**	4102 ± 110 **b**	2902 ± 198 **a**	21.75 ± 2.57 **a**
S700	140 ± 23 **a**	84 ± 24 **a**	36 ± 10 **a**	1847 ± 134 **a**	1642 ± 101 **a**	1171 ± 188 **a**	3658 ± 258 **a**	2839 ± 213 **a**	21.45 ± 3.07 **a**
Significance	*	*	**ns**	*	*	*	*	*	**ns**
					Treatment with oak chips				
No CHIPS	161 ± 6 **b**	108 ± 12 **b**	41 ± 6 **b**	1956 ± 62 **b**	1722 ± 56 **a**	1307 ± 147 **b**	4049 ± 159 **b**	2909 ± 216 **a**	22.27 ± 3.21 **a**
+ CHIPS	139 ± 15 **a**	87 ± 21 **a**	29 ± 4 **a**	1866 ± 116 **a**	1664 ± 98 **a**	1162 ± 125 **a**	3786 ± 304 **a**	2954 ± 208 **a**	22.32 ± 3.99 **a**
Significance	*	*	*	*	**ns**	*	*	**ns**	**ns**
					Type of closure * Treatment with oak chips				
S100	157 ± 4 **cd**	99 ± 15 **bc**	36 ± 4 **bc**	1918 ± 43 **b**	1690 ± 48 **b**	1371 ± 90 **c**	4156 ± 93 **c**	2981 ± 206 **bc**	23.66 ± 3.22 **a**
S100+chips	146 ± 4 **b**	94 ± 6 **b**	28 ± 6 **ab**	1932 ± 25 **b**	1719 ± 27 **b**	1211 ± 102 **b**	3828 ± 139 **b**	3124 ± 105 **c**	23.95 ± 5.99 **a**
S300	163 ± 7 **d**	121 ± 7 **c**	42 ± 8 **cd**	1989 ± 89 **b**	1751 ± 80 **b**	1238 ± 162 **bc**	4102 ± 167 **c**	2843 ± 203 **ab**	21.60 ± 2.95 **a**
S300+chips	151 ± 4 **bc**	104 ± 16 **bc**	32 ± 2 **ab**	1932 ± 98 **b**	1712 ± 98 **b**	1243 ± 91 **bc**	4102 ± 46 **c**	2961 ± 192 **abc**	21.91 ± 2.41 **a**
S700	161 ± 6 **d**	105 **bc**	44 ± 5 **d**	1961 ± 43 **b**	1726 ± 34 **b**	1311 ± 168 **bc**	3888 ± 69 **b**	2902 ± 253 **abc**	21.79 ± 3.64 **a**
S700+chips	119 ± 4 **a**	63 ± 3 **a**	27 ± 4 **a**	1733 ± 65 **a**	1559 ± 61 **a**	1032 ± 56 **a**	3427 ± 53 **a**	2777 ± 163 **a**	21.11 ± 2.69 **a**
Significance	*	*	*	*	*	*	*	*	**ns**

Table 68 – Phenolic composition and antioxidant activity of Nero di Troia wines at the first racking and after 12 months of aging.
TA: total anthocyanins (as mg malvidin-3-glucoside/L); SA: anthocyanins sensitive to SO_2 (as mg malvidin-3-glucoside/L); MA: monomeric anthocyanins (as malvidin-3-glucoside mg/L); TF: total flavonoids (as mg (+)-catechin/L); FNA: flavonoids different from anthocyanins (as mg (+)-catechin/L); FRV: flavans reactive with vanillin (as mg (+)-catechin/L); PA: proanthocyanidins (as mg cyanidin chloride/L); TPC: total phenolic compounds (as mg gallic acid/L); ABTS assay: antioxidant activity by ABTS assay (as mmol Trolox eq/L).
S100: Nomacorc *Select Series*™ 100 co-extruded closure; S300: Nomacorc *Select Series*™ 300 co-extruded closure; S700: Nomacorc *Select Series*™ 700 co-extruded closure.
In column, as part of type of closure or treatment with oak chips or combination of type of closure-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference; ns: no significant difference.

	MP	SPP	LPP	LPP/SPP	$I_{gelatin}$	I_{HCl}	I_{PVPP}
				At the first racking			
	0.70 ± 0.03	0.21 ± 0.01	0.18 ± 0.03	0.86 ± 0.11	49.5 ± 5.5	34.5 ± 5.2	71.8 ± 2.0
				After 12 months			
				Type of closure			
S100	0.36 ± 0.12 **a**	0.32 ± 0.03 **a**	0.63 ± 0.05 **a**	1.99 ± 0.38 **a**	17.1 ± 5.0 **a**	55.1 ± 10.0 **c**	57.0 ± 5.7 **a**
S300	0.38 ± 0.08 **a**	0.36 ± 0.02 **b**	0.69 ± 0.08 **b**	1.91 ± 0.16 **a**	20.3 ± 2.5 **a**	44.1 ± 2.7 **a**	66.8 ± 6.7 **b**
S700	0.35 ± 0.05 **a**	0.35 ± 0.02 **b**	0.69 ± 0.04 **b**	1.96 ± 0.18 **a**	19.7 ± 4.6 **a**	45.9 ± 1.2 **b**	66.1 ± 5.1 **b**
Significance	**ns**	*	*	**ns**	**ns**	*	*
				Treatment with oak chips			
No CHIPS	0.43 ± 0.06 **b**	0.35 ± 0.01 **a**	0.63 ± 0.05 **a**	1.82 ± 0.13 **a**	21.1 ± 2.8 **b**	44.2 ± 2.0 **a**	61.7 ± 5.6 **a**
+ CHIPS	0.30 ± 0.05 **a**	0.34 ± 0.04 **a**	0.71 ± 0.06 **b**	2.10 ± 0.26 **b**	17.0 ± 4.4 **a**	52.6 ± 8.8 **b**	64.3 ± 8.7 **a**
Significance	*	**ns**	*	*	*	*	**ns**
				Type of closure * Treatment with oak chips			
S100	0.47 ± 0.02 **c**	0.34 **b**	0.59 ± 0.02 **a**	1.72 ± 0.06 **a**	20.3 ± 3.1 **bc**	46.0 ± 0.2 **bc**	57.6 ± 4.9 **a**
S100+chips	0.26 ± 0.03 **a**	0.29 ± 0.03 **a**	0.66 ± 0.05 **b**	2.27 ± 0.38 **b**	12.3 ± 1.7 **a**	64.3 ± 0.7 **d**	56.4 ± 7.1 **a**
S300	0.45 ± 0.01 **c**	0.34 **b**	0.62 ± 0.02 **ab**	1.80 ± 0.08 **a**	18.9 **bc**	41.7 ± 0.4 **a**	64.2 ± 8.2 **ab**
S300+chips	0.32 ± 0.06 **ab**	0.37 ± 0.01 **b**	0.76 ± 0.05 **c**	2.03 ± 0.12 **ab**	21.2 ± 3.0 **c**	46.5 ± 1.0 **c**	68.7 ± 5.7 **b**
S700	0.37 ± 0.07 **b**	0.35 ± 0.02 **b**	0.68 ± 0.05 **b**	1.93 ± 0.16 **ab**	23.5 ± 2.0 **c**	44.9 ± 0.7 **b**	63.9 ± 0.6 **ab**
S700+chips	0.33 ± 0.01 **b**	0.36 ± 0.02 **b**	0.71 ± 0.04 **bc**	2.00 ± 0.23 **ab**	15.9 ± 2.4 **ab**	46.9 ± 0.4 **c**	69.1 ± 7.4 **b**
Significance	*	*	*	*	*	*	*

Table 69 – Monomeric and polymeric pigments and structure indices of Nero di Troia wines at the first racking and after 12 months of aging.
MP: absorbance at 520 nm due to monomeric pigments; SPP: absorbance at 520 nm due to small polymeric pigments; LPP: absorbance at 520 nm due to large polymeric pigments; LPP/SPP: ratio between large and small polymeric pigments; $I_{gelatin}$: gelatin index; I_{HCl}: hydrochloric acid index; I_{PVPP}: PVPP index.
S100: Nomacorc *Select Series*™ 100 co-extruded closure; S300: Nomacorc *Select Series*™ 300 co-extruded closure; S700: Nomacorc *Select Series*™ 700 co-extruded closure.
In column, as part of type of closure or treatment with oak chips or combination of type of closure-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference.
ns: no significant difference.

Phenolic compounds	*At the first racking*	*After 12 months*						
		Type of closure				*Treatment with oak chips*		
		S100	**S300**	**S700**	*Significance*	**No CHIPS**	**+ CHIPS**	*Significance*
Phenolic acids and derivatives (mg GAE/L; mg CAE/L)								
Gallic acid	45.91 ± 0.50	43.65 ± 0.50 **a**	43.78 ± 0.30 **a**	44.85 ± 0.86 **b**	*	44.28 ± 1.07 **a**	43.91 ± 0.34 **a**	**ns**
2-S-Glutathionyl-Caftaric acid	0.22 ± 0.02	0.24 ± 0.02 **a**	0.31 ± 0.09 **b**	0.34 ± 0.03 **b**	*	0.28 ± 0.05 **a**	0.32 ± 0.08 **b**	*
Caftaric acid + Caffeic acid	19.94 ± 0.05	20.84 ± 0.13 **ab**	20.74 ± 0.03 **a**	21.08 ± 0.27 **b**	*	20.93 ± 0.26 **a**	20.85 ± 0.18 **a**	**ns**
p-Coumaric acid	8.29 ± 0.06	8.77 ± 0.07 **ab**	8.59 ± 0.20 **a**	8.89 ± 0.18 **b**	*	8.84 ± 0.15 **b**	8.65 ± 0.19 **a**	*
Ferulic acid	8.40 ± 0.04	7.34 ± 0.13 **a**	7.32 ± 0.17 **a**	7.37 ± 0.26 **a**	**ns**	7.43 ± 0.17 **a**	7.26 ± 0.16 **a**	**ns**
Σ Phenolic acids and derivatives	**82.76 ± 0.48**	**80.85 ± 0.46 a**	**80.74 ± 0.25 a**	**82.54 ± 1.43 b**	*	**81.75 ± 1.54 a**	**81.00 ± 0.55 a**	**ns**
Stilbens (mg RE/L)								
cis-Piceid	2.89	0.70 ± 0.06 **c**	0.52 ± 0.27 **a**	0.66 ± 0.02 **b**	*	0.72 ± 0.05 **b**	0.54 ± 0.19 **a**	*
trans-Piceid	3.68 ± 0.53	3.35 ± 0.13 **b**	2.87 ± 0.66 **a**	2.91 ± 0.07 **a**	*	3.25 ± 0.25 **b**	2.84 ± 0.48 **a**	*
Σ Stilbens	**6.57 ± 0.53**	**4.05 ± 0.14 b**	**3.39 ± 0.93 a**	**3.57 ± 0.06 a**	*	**3.96 ± 0.30 b**	**3.37 ± 0.65 a**	*
Flavonols (mg QE/L)								
Myricetin-3-glc	2.40 ± 0.03	1.37 ± 0.06 **b**	1.16 ± 0.20 **a**	1.30 ± 0.09 **b**	*	1.34 ± 0.07 **b**	1.21 ± 0.19 **a**	*
Myricetin-3-glcr	4.67 ± 0.03	3.04 ± 0.05 **c**	2.53 ± 0.47 **a**	2.85 ± 0.08 **b**	*	2.96 ± 0.07 **b**	2.66 ± 0.42 **a**	*
Myricetin-3-galac	17.98 ± 0.02	9.54 ± 0.17 **c**	8.25 ± 1.27 **a**	8.87 ± 0.27 **b**	*	9.28 ± 0.32 **b**	8.49 ± 1.10 **a**	*
Quercetin-3-glc	2.03 ± 0.09	1.60 ± 0.19 **a**	1.57 ± 0.32 **a**	1.42 ± 0.04 **a**	**ns**	1.65 ± 0.21 **b**	1.41 ± 0.14 **a**	*
Quercetin-3-glcr	11.88 ± 0.05	10.20 ± 0.16 **c**	8.81 ± 1.70 **a**	9.47 ± 0.22 **b**	*	10.08 ± 0.36 **b**	8.91 ± 1.27 **a**	*
Quercetin-3-galac	7.45 ± 0.03	0.89 ± 0.07 **a**	0.88 ± 0.13 **a**	0.88 ± 0.06 **a**	**ns**	0.94 ± 0.05 **b**	0.83 ± 0.08 **a**	*
Laricitrin-3-glc	4.47 ± 0.01	3.75 ± 0.08 **c**	3.19 ± 0.61 **a**	3.38 ± 0.03 **b**	*	3.60 ± 0.16 **b**	3.28 ± 0.52 **a**	*
Quercetin-3-rha	8.56 ± 0.45	9.31 ± 1.12 **a**	7.14 ± 2.34 **a**	8.06 ± 1.31 **a**	**ns**	8.84 ± 1.32 **a**	7.51 ± 2.04 **a**	**ns**
Isorhamnetin-3-glc	6.16 ± 0.11	4.07 ± 0.22 **b**	3.65 ± 0.50 **a**	4.00 ± 0.25 **b**	*	4.14 ± 0.19 **b**	3.67 ± 0.35 **a**	*
Syringetin-3-galac	9.80 ± 0.02	7.93 ± 0.11 **c**	6.90 ± 1.11 **a**	7.55 ± 0.11 **b**	*	7.79 ± 0.21 **b**	7.13 ± 0.94 **a**	*
Laricitrin-3-rhamnose-7-tri-hydroxycinnamic acid	7.68 ± 0.11	4.16 ± 0.16 **c**	3.31 ± 1.41 **a**	3.74 ± 0.15 **b**	*	4.07 ± 0.42 **b**	3.40 ± 1.03 **a**	*
Σ Flavonols	**83.09 ± 0.94**	**55.88 ± 1.23 c**	**47.41 ± 9.86 a**	**51.52 ± 1.86 b**	*	**54.69 ± 2.82 b**	**48.52 ± 7.70 a**	*
Flavan-3-ols (mg CE/L)								
(-)-(Epi)Gallocatechin	30.37 ± 0.42	28.69 ± 1.00 **ab**	28.05 ± 3.41 **a**	29.87 ± 0.85 **b**	*	29.74 ± 1.65 **b**	28.00 ± 2.20 **a**	*
Procyanidin B3	59.54 ± 10.01	61.79 ± 2.45 **c**	50.86 ± 10.70 **a**	59.87 ± 2.00 **b**	*	60.41 ± 0.94 **b**	54.60 ± 10.40 **a**	*
(+)-Catechin	76.86 ± 0.79	71.29 ± 3.07 **b**	63.69 ± 8.98 **a**	69.85 ± 3.89 **b**	*	71.15 ± 2.03 **b**	65.41 ± 8.09 **a**	*

Procyanidin B1	56.62 ± 1.30	44.52 ± 2.77 **b**	37.13 ± 5.63 **a**	43.45 ± 2.01 **b**	*	43.04 ± 1.94 **a**	40.36 ± 6.59 **a**	**ns**
Procyanidin B4	64.82 ± 7.89	68.51 ± 2.60 **c**	57.56 ± 8.58 **a**	64.97 ± 2.98 **b**	*	66.07 ± 1.03 **b**	61.29 ± 9.42 **a**	*
(-)-Epicatechin	116.19 ± 1.50	105.68 ± 0.89 **b**	96.45 ± 10.14 **a**	105.01 ± 2.93 **b**	*	105.68 ± 1.98 **b**	99.08 ± 8.92 **a**	*
Procyanidin B2	19.38 ± 0.73	19.30 ± 1.11 **b**	16.32 ± 4.82 **a**	18.86 ± 0.93 **b**	*	19.38 ± 0.97 **b**	16.94 ± 3.85 **a**	*
Σ Flavan-3-ols	**423.77 ± 18.45**	**399.78 ± 12.48 b**	**350.05 ± 51.99 a**	**391.89 ± 12.99 b**	*	**395.46 ± 6.34 b**	**365.69 ± 48.85 a**	*
Anthocyanins (mg ME/L)								
Dp-3-glc	4.05 ± 0.03	0.94 ± 0.16 **c**	0.55 ± 0.40 **a**	0.67 ± 0.09 **b**	*	0.82 ± 0.07 **b**	0.62 ± 0.39 **a**	*
Cy-3-glc	0.83 ± 0.02	0.40 ± 0.04 **c**	0.28 ± 0.12 **a**	0.32 ± 0.03 **b**	*	0.36 ± 0.02 **b**	0.30 ± 0.11 **a**	*
Pt-3-glc	6.23	0.79 ± 0.13 **c**	0.45 ± 0.32 **a**	0.57 ± 0.07 **b**	*	0.68 ± 0.05 **b**	0.53 ± 0.32 **a**	*
Pn-3-glc	3.78 ± 0.01	0.53 ± 0.08 **b**	0.33 ± 0.23 **a**	0.36 ± 0.05 **a**	*	0.47 ± 0.05 **b**	0.35 ± 0.21 **a**	*
Mv-3-glc	59.94 ± 0.29	7.62 ± 1.31 **c**	4.29 ± 3.15 **a**	5.46 ± 0.66 **b**	*	6.51 ± 0.45 **b**	5.07 ± 3.22 **a**	*
Dp-3-acetylglc	1.78 ± 0.03	0.32 ± 0.04 **b**	0.28 ± 0.02 **a**	0.31 ± 0.02 **b**	*	0.31 ± 0.04 **a**	0.30 ± 0.02 **a**	**ns**
Pyrano-Mv-3-glc (Vitisin B)	1.16 ± 0.01	2.00 ± 0.15 **c**	1.88 ± 0.12 **a**	1.95 ± 0.04 **b**	*	2.01 ± 0.10 **b**	1.88 ± 0.10 **a**	*
Carboxypyrano-Mv-3-acetylglc (acetyl-Vitisin A)	nd	1.16 ± 0.08 **c**	1.01 ± 0.04 **a**	1.09 ± 0.02 **b**	*	1.11 ± 0.09 **b**	1.06 ± 0.06 **a**	*
Cy-3-acetylglc	1.21	nd **a**	nd **a**	nd **a**	**ns**	nd **a**	nd **a**	**ns**
Pt-3-acetylglc	4.57 ± 0.08	2.19 ± 0.33 **c**	1.73 ± 0.11 **a**	1.95 ± 0.14 **b**	*	2.05 ± 0.33 **b**	1.86 ± 0.18 **a**	*
Pyrano-Mv-3-acetylglc (acetyl-Vitisin B)	3.47 ± 0.02	2.33 ± 0.21 **a**	2.15 ± 0.46 **a**	2.37 ± 0.15 **a**	**ns**	2.40 ± 0.24 **b**	2.18 ± 0.33 **a**	*
Pn-3-acetylglc	9.13 ± 0.21	4.09 ± 0.26 **b**	3.36 ± 0.53 **a**	3.62 ± 0.24 **a**	*	3.86 ± 0.24 **a**	3.52 ± 0.58 **a**	**ns**
Mv-3-acetylglc	34.40 ± 0.35	10.23 ± 0.40 **c**	8.12 ± 2.19 **a**	9.37 ± 0.27 **b**	*	9.83 ± 0.26 **b**	8.65 ± 1.98 **a**	*
Pyrano-Mv-3-*p*-coumglc (*p*-coum-Vitisin B)	2.82 ± 0.05	3.71 ± 0.23 **b**	3.26 ± 0.39 **a**	3.68 ± 0.26 **b**	*	3.65 ± 0.24 **a**	3.45 ± 0.43 **a**	**ns**
Mv-3-caffeoylglc	2.24 ± 0.05	2.75 ± 0.09 **b**	2.60 ± 0.23 **a**	2.87 ± 0.10 **b**	*	2.83 ± 0.12 **b**	2.65 ± 0.20 **a**	*
Pt-3-*p*-coumglc	4.17 ± 0.09	1.71 ± 0.07 **b**	1.53 ± 0.12 **a**	1.71 ± 0.13 **b**	*	1.69 ± 0.11 **a**	1.61 ± 0.15 **a**	**ns**
Mv-3-*p*-coumglc-8-ethyl-(epi)cat	nd	0.72 ± 0.06 **c**	0.57 ± 0.06 **a**	0.65 ± 0.04 **b**	*	0.65 ± 0.03 **a**	0.64 ± 0.11 **a**	**ns**
Pn-3-*p*-coumglc	3.73 ± 0.37	0.45 ± 0.03 **a**	0.43 ± 0.11 **a**	0.43 ± 0.06 **a**	**ns**	0.46 ± 0.05 **a**	0.42 ± 0.08 **a**	**ns**
Mv-3-*p*-coumglc	9.46 ± 0.38	1.70 ± 0.16 **b**	1.23 ± 0.52 **a**	1.36 ± 0.13 **a**	*	1.56 ± 0.13 **b**	1.30 ± 0.48 **a**	*
Mv-3-glc-4-vinyl(epi)cat	nd	0.15 ± 0.01 **a**	0.17 ± 0.02 **a**	0.15 ± 0.01 **a**	**ns**	0.16 ± 0.02 **a**	0.15 ± 0.01 **a**	**ns**
Σ Anthocyanins	**152.98 ± 0.39**	**43.79 ± 1.77 c**	**34.25 ± 8.95 a**	**38.90 ± 1.37 b**	*	**41.42 ± 1.13 b**	**36.54 ± 8.48 a**	*

Phenolic compounds	*At the first racking*	*After 12 months*						
		*Type of closure * Treatment with oak chips*						
		S100	**S100+chips**	**S300**	**S300+chips**	**S700**	**S700+chips**	*Significance*
Phenolic acids and derivatives (mg GAE/L; mg CAE/L)								
Gallic acid	45.91 ± 0.50	43.67 ± 0.79 **a**	43.64 ± 0.37 **a**	43.70 ± 0.45 **a**	43.86 ± 0.22 **a**	45.47 ± 0.79 **b**	44.23 ± 0.19 **ab**	*
2-S-Glutathionyl-Caftaric acid	0.22 ± 0.02	0.25 ± 0.02 **a**	0.23 ± 0.02 **a**	0.24 ± 0.01 **a**	0.39 ± 0.05 **b**	0.34 ± 0.03 **b**	0.35 ± 0.04 **b**	*
Caftaric acid + Caffeic acid	19.94 ± 0.05	20.95 ± 0.01 **a**	20.73 ± 0.03 **a**	20.74 **a**	20.73 ± 0.06 **a**	21.09 ± 0.47 **a**	21.08 ± 0.01 **a**	**ns**
p-Coumaric acid	8.29 ± 0.06	8.83 **b**	8.71 ± 0.03 **ab**	8.77 ± 0.01 **b**	8.42 ± 0.04 **a**	8.94 ± 0.29 **b**	8.83 ± 0.06 **b**	*
Ferulic acid	8.40 ± 0.04	7.29 ± 0.08 **a**	7.39 ± 0.20 **a**	7.44 ± 0.14 **a**	7.19 ± 0.05 **a**	7.54 ± 0.24 **a**	7.21 ± 0.20 **a**	**ns**
Σ Phenolic acids and derivatives	**82.76 ± 0.48**	**80.99 ± 0.74 a**	**80.70 ± 0.08 a**	**80.90 ± 0.30 a**	**80.59 ± 0.01 a**	**83.38 ± 1.82 b**	**81.70 ± 0.09 ab**	*
Stilbens (mg RE/L)								
cis-Piceid	2.89	0.75 ± 0.01 **c**	0.65 ± 0.02 **b**	0.75 **c**	0.29 ± 0.01 **a**	0.65 ± 0.02 **b**	0.67 **b**	*
trans-Piceid	3.68 ± 0.53	3.35 ± 0.20 **c**	3.36 ± 0.11 **c**	3.44 ± 0.02 **c**	2.30 ± 0.09 **a**	2.96 ± 0.01 **b**	2.85 ± 0.07 **b**	*
Σ Stilbens	**6.57 ± 0.53**	**4.09 ± 0.21 c**	**4.01 ± 0.08 c**	**4.19 ± 0.02 c**	**2.59 ± 0.07 a**	**3.61 b**	**3.53 ± 0.08 b**	*
Flavonols (mg QE/L)								
Myricetin-3-glc	2.40 ± 0.03	1.38 ± 0.05 **b**	1.36 ± 0.09 **b**	1.33 ± 0.03 **b**	0.99 ± 0.04 **a**	1.31 ± 0.13 **b**	1.29 ± 0.08 **b**	*
Myricetin-3-glcr	4.67 ± 0.03	3.03 ± 0.02 **cd**	3.05 ± 0.08 **d**	2.94 ± 0.02 **cd**	2.13 ± 0.01 **a**	2.91 ± 0.09 **bc**	2.79 **b**	*
Myricetin-3-galac	17.98 ± 0.02	9.53 ± 0.02 **d**	9.55 ± 0.28 **d**	9.35 ± 0.01 **cd**	7.15 ± 0.03 **a**	8.96 ± 0.42 **bc**	8.77 ± 0.03 **b**	*
Quercetin-3-glc	2.03 ± 0.09	1.67 ± 0.22 **bc**	1.54 ± 0.20 **abc**	1.84 ± 0.12 **c**	1.31 ± 0.03 **a**	1.45 ± 0.05 **ab**	1.40 ± 0.01 **ab**	*
Quercetin-3-glcr	11.88 ± 0.05	10.32 ± 0.11 **d**	10.09 ± 0.11 **d**	10.28 ± 0.13 **d**	7.34 ± 0.02 **a**	9.64 ± 0.17 **c**	9.31 ± 0.10 **b**	*
Quercetin-3-galac	7.45 ± 0.03	0.94 ± 0.04 **b**	0.85 ± 0.08 **ab**	0.99 ± 0.05 **b**	0.78 ± 0.05 **a**	0.89 ± 0.05 **ab**	0.87 ± 0.10 **ab**	*
Laricitrin-3-glc	4.47 ± 0.01	3.69 ± 0.02 **c**	3.82 ± 0.02 **d**	3.72 **c**	2.67 ± 0.06 **a**	3.39 ± 0.04 **b**	3.37 ± 0.01 **b**	*
Quercetin-3-rha	8.56 ± 0.45	9.89 ± 0.29 **b**	8.74 ± 1.54 **b**	9.00 ± 1.61 **b**	5.28 ± 0.05 **a**	7.62 ± 0.87 **ab**	8.49 ± 1.91 **b**	*
Isorhamnetin-3-glc	6.16 ± 0.11	4.23 ± 0.20 **b**	3.91 ± 0.03 **b**	4.08 ± 0.14 **b**	3.23 ± 0.11 **a**	4.12 ± 0.33 **b**	3.88 ± 0.16 **b**	*
Syringetin-3-galac	9.80 ± 0.02	7.97 ± 0.03 **c**	7.90 ± 0.18 **c**	7.86 ± 0.07 **c**	5.94 ± 0.10 **a**	7.55 ± 0.17 **b**	7.55 ± 0.10 **b**	*
Laricitrin-3-rhamnose-7-tri-hydroxycinnamic acid	7.68 ± 0.11	4.07 ± 0.18 **cd**	4.26 ± 0.01 **d**	4.53 ± 0.08 **e**	2.09 ± 0.01 **a**	3.63 ± 0.14 **b**	3.85 **bc**	*
Σ Flavonols	**83.09 ± 0.94**	**56.69 ± 0.25 c**	**55.07 ± 1.36 bc**	**55.91 ± 1.58 c**	**38.91 ± 0.04 a**	**51.47 ± 2.33 b**	**51.57 ± 2.23 b**	*
Flavan-3-ols (mg CE/L)								
(-)-(Epi)Gallocatechin	30.37 ± 0.42	27.87 ± 0.55 **b**	29.51 ± 0.12 **bc**	30.90 ± 1.59 **c**	25.21 ± 0.26 **a**	30.45 ± 0.45 **c**	29.30 ± 0.81 **bc**	*
Procyanidin B3	59.54 ± 10.01	59.68 ± 0.42 **bc**	63.89 ± 0.45 **d**	60.07 ± 0.16 **bc**	41.65 ± 2.13 **a**	61.48 ± 0.78 **cd**	58.27 ± 1.00 **b**	*
(+)-Catechin	76.86 ± 0.79	68.79 ± 0.66 **c**	73.80 ± 1.64 **e**	71.47 ± 0.02 **d**	55.91 ± 0.44 **a**	73.19 ± 0.65 **de**	66.51 ± 0.68 **b**	*
Procyanidin B1	56.62 ± 1.30	42.84 ± 3.25 **b**	46.21 ± 1.00 **b**	41.93 ± 0.87 **b**	32.32 ± 1.38 **a**	44.35 ± 1.23 **b**	42.55 ± 2.70 **b**	*
Procyanidin B4	64.82 ± 7.89	66.30 ± 0.07 **bc**	70.72 ± 0.82 **d**	64.93 ± 0.32 **bc**	50.19 ± 1.90 **a**	66.98 ± 0.92 **cd**	62.97 ± 3.14 **b**	*

(-)-Epicatechin	116.19 ± 1.50	105.02 ± 0.69 **b**	106.34 ± 0.40 **b**	105.18 ± 1.77 **b**	87.72 ± 0.51 **a**	106.84 ± 3.45 **b**	103.17 ± 0.61 **b**	*
Procyanidin B2	19.38 ± 0.73	18.63 ± 0.95 **b**	19.97 ± 0.98 **b**	20.41 ± 0.50 **b**	12.23 ± 1.59 **a**	19.09 ± 0.36 **b**	18.63 ± 1.50 **b**	*
Σ Flavan-3-ols	**423.77 ± 18.45**	**389.13 ± 3.59 bc**	**410.43 ± 0.97 e**	**394.88 ± 1.85 cd**	**305.23 ± 8.21 a**	**402.38 ± 2.82 de**	**381.40 ± 7.60 b**	*
Anthocyanins (mg ME/L)								
Dp-3-glc	4.05 ± 0.03	0.81 ± 0.03 **d**	1.08 ± 0.01 **f**	0.90 ± 0.03 **e**	0.20 ± 0.01 **a**	0.75 ± 0.01 **c**	0.59 ± 0.01 **b**	*
Cy-3-glc	0.83 ± 0.02	0.36 **c**	0.43 **e**	0.38 ± 0.01 **d**	0.18 ± 0.01 **a**	0.35 ± 0.01 **c**	0.29 **b**	*
Pt-3-glc	6.23	0.68 ± 0.04 **d**	0.90 **e**	0.73 ± 0.02 **d**	0.17 **a**	0.63 ± 0.02 **c**	0.51 ± 0.01 **b**	*
Pn-3-glc	3.78 ± 0.01	0.47 ± 0.01 **cd**	0.60 ± 0.05 **e**	0.52 ± 0.01 **d**	0.13 **a**	0.41 ± 0.01 **c**	0.32 ± 0.02 **b**	*
Mv-3-glc	59.94 ± 0.29	6.49 ± 0.12 **d**	8.75 ± 0.03 **f**	7.02 ± 0.08 **e**	1.56 ± 0.04 **a**	6.03 ± 0.05 **c**	4.89 ± 0.01 **b**	*
Dp-3-acetylglc	1.78 ± 0.03	0.35 **c**	0.29 ± 0.01 **ab**	0.27 ± 0.02 **a**	0.29 ± 0.01 **ab**	0.31 ± 0.02 **ab**	0.32 ± 0.02 **bc**	*
Pyrano-Mv-3-glc (Vitisin B)	1.16 ± 0.01	2.13 ± 0.02 **e**	1.87 **b**	1.98 ± 0.01 **d**	1.77 ± 0.03 **a**	1.92 ± 0.01 **c**	1.98 ± 0.01 **d**	*
Carboxypyrano-Mv-3-acetylglc (acetyl-Vitisin A)	nd	1.22 ± 0.04 **c**	1.09 ± 0.03 **b**	1.04 ± 0.04 **ab**	0.99 ± 0.03 **a**	1.07 ± 0.02 **b**	1.10 ± 0.01 **b**	*
Cy-3-acetylglc	1.21	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	nd **a**	**ns**
Pt-3-acetylglc	4.57 ± 0.08	2.46 ± 0.02 **c**	1.91 ± 0.12 **b**	1.81 ± 0.01 **ab**	1.65 ± 0.11 **a**	1.88 ± 0.19 **ab**	2.02 ± 0.06 **b**	*
Pyrano-Mv-3-acetylglc (acetyl-Vitisin B)	3.47 ± 0.02	2.17 ± 0.14 **b**	2.50 ± 0.07 **b**	2.52 ± 0.30 **b**	1.79 ± 0.09 **a**	2.50 ± 0.06 **b**	2.24 ± 0.01 **b**	*
Pn-3-acetylglc	9.13 ± 0.21	4.07 ± 0.24 **b**	4.11 ± 0.37 **b**	3.81 ± 0.26 **b**	2.92 ± 0.01 **a**	3.72 ± 0.14 **b**	3.53 ± 0.33 **ab**	*
Mv-3-acetylglc	34.40 ± 0.35	9.90 ± 0.21 **c**	10.56 ± 0.03 **d**	10.02 ± 0.33 **c**	6.23 ± 0.14 **a**	9.59 ± 0.05 **c**	9.15 ± 0.12 **b**	*
Pyrano-Mv-3-*p*-coumglc (*p*-coum-Vitisin B)	2.82 ± 0.05	3.87 ± 0.23 **b**	3.56 ± 0.09 **b**	3.58 ± 0.23 **b**	2.95 ± 0.03 **a**	3.51 ± 0.19 **b**	3.85 ± 0.21 **b**	*
Mv-3-caffeoylglc	2.24 ± 0.05	2.77 ± 0.15 **b**	2.73 ± 0.01 **b**	2.80 ± 0.03 **b**	2.40 ± 0.03 **a**	2.92 ± 0.13 **b**	2.82 ± 0.05 **b**	*
Pt-3-*p*-coumglc	4.17 ± 0.09	1.67 ± 0.06 **b**	1.75 ± 0.05 **b**	1.61 ± 0.11 **ab**	1.46 ± 0.11 **a**	1.81 ± 0.05 **b**	1.62 ± 0.10 **ab**	*
Mv-3-*p*-coumglc-8-ethyl-(epi)cat	nd	0.67 ± 0.02 **b**	0.77 **c**	0.61 ± 0.02 **b**	0.52 ± 0.03 **a**	0.67 ± 0.02 **b**	0.64 ± 0.05 **b**	*
Pn-3-*p*-coumglc	3.73 ± 0.37	0.45 ± 0.02 **abc**	0.45 ± 0.05 **abc**	0.53 ± 0.02 **c**	0.34 **a**	0.41 ± 0.02 **ab**	0.46 ± 0.10 **bc**	*
Mv-3-*p*-coumglc	9.46 ± 0.38	1.58 ± 0.07 **cd**	1.82 ± 0.12 **d**	1.68 ± 0.01 **d**	0.78 ± 0.02 **a**	1.41 ± 0.03 **bc**	1.31 ± 0.20 **b**	*
Mv-3-glc-4-vinyl(epi)cat	nd	0.15 ± 0.02 **ab**	0.15 ± 0.01 **a**	0.18 **b**	0.15 ± 0.01 **ab**	0.14 ± 0.01 **a**	0.16 **ab**	*
Σ Anthocyanins	**152.98 ± 0.39**	**42.26 ± 0.01 d**	**45.33 ± 0.06 e**	**41.99 ± 0.64 d**	**26.51 ± 0.04 a**	**40.02 ± 0.08 c**	**37.78 ± 0.80 b**	*

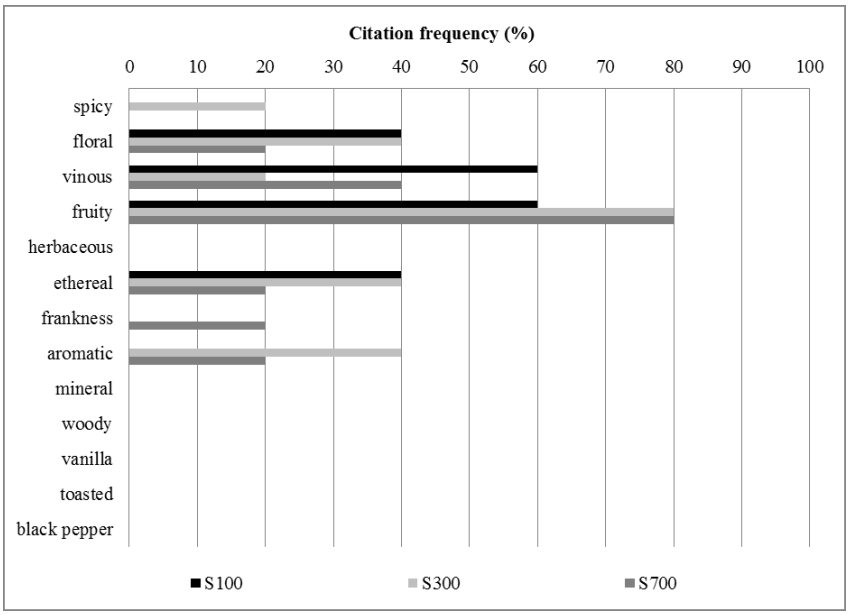
Table 70 – Phenolic profile of Nero di Troia wines at the first racking and after 12 months of aging.
nd: not detected.
S100: Nomacorc *Select Series*™ 100 co-extruded closure; S300: Nomacorc *Select Series*™ 300 co-extruded closure; S700: Nomacorc *Select Series*™ 700 co-extruded closure.
glc: glucoside, glcr: glucuronide, rha: rhamnoside, galac: galactoside, Dp: delphinidin, Cy: cyanidin, Pt: petunidin, Pn: peonidin, Mv: malvidin, acetylglc: acetylglucoside, *p*-coumglc: *p*-coumaroylglucoside, caffeoylglc: caffeoylglucoside, cat: catechin.
In row, among the same time of aging, within the type of closure or treatment with oak chips or combination of closure-oak chips, different letters indicate significant differences ($p < 0.05$ - LSD test).

	CI	T	dA(%)	% yellow	% red	% blue	dAl%	dAT%	dTAT%
					At the first racking				
	11.485 ± 0.001	0.540	63.8	31.3	58.0	10.7	5.3 ± 1.3	50.9 ± 7.9	43.8 ± 9.1
					After 12 months				
					Type of closure				
S100	8.986 ± 0.863 **a**	0.707 ± 0.013 **a**	49.2 ± 1.2 **a**	35.1 ± 0.3 **a**	49.6 ± 0.6 **a**	15.3 ± 0.5 **a**	1.6 ± 0.2 **a**	24.9 ± 8.0 **a**	73.7 ± 8.3 **a**
S300	9.572 ± 0.076 **b**	0.704 ± 0.016 **a**	49.7 ± 1.5 **a**	35.1 ± 0.3 **a**	49.8 ± 0.7 **a**	15.1 ± 0.5 **a**	1.3 ± 0.3 **a**	30.7 ± 5.0 **a**	68.1 ± 4.9 **a**
S700	9.582 ± 0.103 **b**	0.698 ± 0.019 **a**	50.0 ± 1.6 **a**	34.9 ± 0.4 **a**	50.0 ± 0.8 **a**	15.1 ± 0.4 **a**	1.3 ± 0.3 **a**	27.2 ± 3.2 **a**	71.6 ± 3.2 **a**
Significance	*****	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
					Treatment with oak chips				
No CHIPS	9.669 ± 0.072 **b**	0.702 ± 0.015 **a**	49.9 ± 1.2 **a**	35.0 ± 0.4 **a**	49.9 ± 0.6 **a**	15.0 ± 0.4 **a**	1.6 ± 0.2 **b**	29.6 ± 3.7 **a**	68.9 ± 3.8 **a**
+ CHIPS	9.091 ± 0.661 **a**	0.704 ± 0.017 **a**	49.4 ± 1.5 **a**	35.0 ± 0.3 **a**	49.7 ± 0.7 **a**	15.3 ± 0.4 **a**	1.3 ± 0.2 **a**	25.5 ± 7.0 **a**	73.4 ± 7.0 **a**
Significance	*****	**ns**	**ns**	**ns**	**ns**	**ns**	*****	**ns**	**ns**
					Type of closure * Treatment with oak chips				
S100	9.732 ± 0.028 **c**	0.705 ± 0.001 **a**	49.5 ± 0.6 **a**	35.1 ± 0.3 **a**	49.8 ± 0.3 **a**	15.1 ± 0.6 **a**	1.7 ± 0.3 **b**	31.7 ± 1.9 **b**	66.6 ± 1.6 **a**
S100+chips	8.240 ± 0.070 **a**	0.709 ± 0.022 **a**	49.0 ± 1.9 **a**	35.1 ± 0.4 **a**	49.5 ± 0.9 **a**	15.4 ± 0.5 **a**	1.5 **ab**	18.0 ± 1.9 **a**	80.8 ± 1.9 **b**
S300	9.619 ± 0.060 **bc**	0.702 ± 0.019 **a**	50.0 ± 1.7 **a**	35.1 ± 0.4 **a**	50.0 ± 0.8 **a**	14.9 ± 0.5 **a**	1.5 ± 0.2 **ab**	31.1 ± 1.9 **b**	67.5 ± 1.5 **a**
S300+chips	9.526 ± 0.071 **b**	0.706 ± 0.020 **a**	49.3 ± 1.7 **a**	35.0 ± 0.4 **a**	49.6 ± 0.9 **a**	15.3 ± 0.5 **a**	1.2 ± 0.1 **ab**	30.2 ± 8.4 **b**	68.6 ± 8.2 **a**
S700	9.657 ± 0.090 **bc**	0.698 ± 0.026 **a**	50.0 ± 2.0 **a**	34.9 ± 0.6 **a**	50.0 ± 1.0 **a**	15.1 ± 0.4 **a**	1.5 ± 0.2 **ab**	26.1 ± 5.0 **ab**	72.6 ± 5.1 **ab**
S700+chips	9.508 ± 0.043 **b**	0.698 ± 0.020 **a**	49.9 ± 1.9 **a**	34.9 ± 0.3 **a**	49.9 ± 0.9 **a**	15.2 ± 0.6 **a**	1.1 ± 0.2 **a**	28.3 ± 0.4 **b**	70.7 ± 0.7 **a**
Significance	*****	**ns**	**ns**	**ns**	**ns**	**ns**	*****	*****	*****

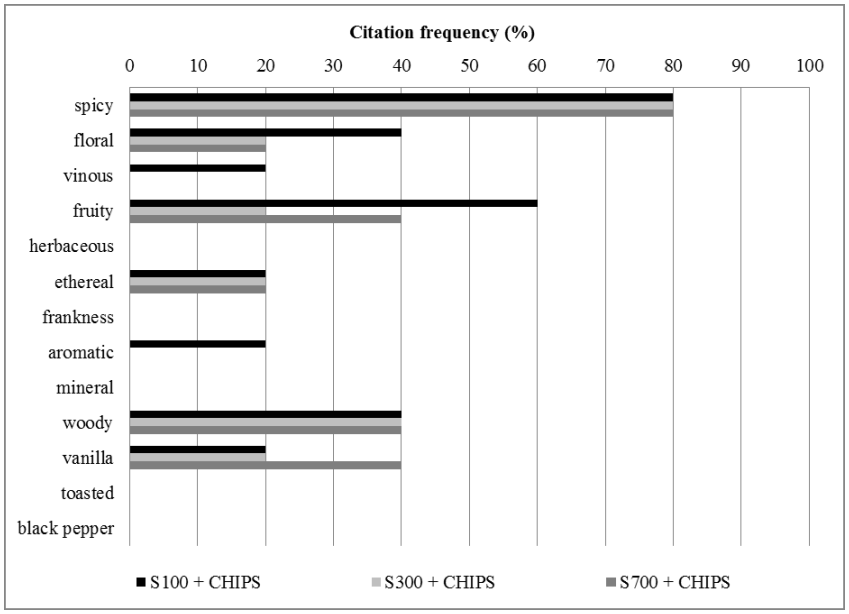
Table 71 – Colour parameters of Nero di Troia wines at the first racking and after 12 months of aging.
CI: colour intensity; T: tonality; dA(%): percentage of red colour due to flavilium cation of free and combined anthocyanins; % yellow: percentage of yellow component; % red: percentage of red component; % blue: percentage of blue component; dAl%: percentage of absorbance at 520 nm due to monomeric anthocyanins; dAT%: percentage of absorbance at 520 nm due to polymeric pigments decolorized with SO_2; dTAT%: percentage of absorbance at 520 nm due to polymeric pigments not decolorized.
S100: Nomacorc *Select Series*™ 100 co-extruded closure; S300: Nomacorc *Select Series*™ 300 co-extruded closure; S700: Nomacorc *Select Series*™ 700 co-extruded closure.
In column, as part of type of closure or treatment with oak chips or combination of type of closure-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference; ns: no significant difference.

*Type of closure * Treatment with oak chips*							
Sensory descriptors	**S100**	**S100+chips**	**S300**	**S300+chips**	**S700**	**S700+chips**	*Significance*
Visual descriptors							
Clearness	1.8 ± 0.4 **a**	1.8 ± 0.4 **a**	1.8 ± 0.4 **a**	2.0 **a**	2.0 **a**	1.8 ± 0.4 **a**	**ns**
Texture	2.2 ± 0.4 **a**	2.2 ± 0.4 **a**	2.2 ± 0.4 **a**	2.3 ± 0.4 **a**	2.3 ± 0.4 **a**	2.2 ± 0.4 **a**	**ns**
Olfactory descriptors							
Olfactory intensity	1.9 ± 0.7 **a**	2.2 ± 0.3 **a**	2.3 ± 0.4 **a**	2.4 ± 0.7 **a**	1.7 ± 0.6 **a**	2.3 ± 0.6 **a**	**ns**
Olfactory complexity	2.1 ± 0.7 **a**	2.6 ± 0.4 **a**	2.4 ± 1.0 **a**	2.5 ± 1.0 **a**	1.9 ± 0.7 **a**	2.5 ± 0.8 **a**	**ns**
Olfactory quality	1.9 ± 0.2 **a**	2.6 ± 0.2 **b**	2.0 ± 0.4 **a**	2.2 ± 0.6 **ab**	2.2 ± 0.4 **ab**	2.3 ± 0.4 **ab**	*
Gustatory-olfactory descriptors							
Sugars	0.0 **a**	0.0 **a**	0.0 **a**	0.0 **a**	0.0 **a**	0.0 **a**	**ns**
Alcohols	2.2 ± 0.4 **a**	2.5 ± 0.4 **a**	2.4 ± 0.4 **a**	2.4 ± 0.4 **a**	2.3 ± 0.4 **a**	2.2 ± 0.4 **a**	**ns**
Polyols	1.6 ± 0.5 **a**	2.1 ± 0.7 **a**	2.0 **a**	1.6 ± 1.0 **a**	2.0 ± 0.4 **a**	1.7 ± 0.4 **a**	**ns**
Acids	1.8 ± 0.3 **a**	1.8 ± 0.6 **a**	2.2 ± 0.4 **a**	1.9 ± 0.4 **a**	2.0 ± 0.6 **a**	2.0 ± 0.4 **a**	**ns**
Tannins	2.3 ± 0.4 **a**	2.5 ± 0.7 **a**	2.3 ± 0.6 **a**	2.6 ± 0.7 **a**	2.2 ± 0.4 **a**	2.7 ± 0.4 **a**	**ns**
Minerals	1.5 ± 0.4 **a**	1.5 ± 0.4 **a**	1.9 ± 0.5 **a**	1.6 ± 0.2 **a**	1.7 ± 0.4 **a**	1.8 ± 0.4 **a**	**ns**
Structure	2.5 ± 0.5 **a**	2.6 ± 0.4 **a**	2.4 ± 0.7 **a**	2.3 ± 0.4 **a**	2.6 ± 0.4 **a**	2.2 ± 0.4 **a**	**ns**
Balance	1.8 ± 0.6 **ab**	2.6 ± 0.5 **c**	1.5 ± 0.4 **a**	2.3 ± 0.6 **bc**	1.9 ± 0.5 **ab**	1.9 ± 0.3 **ab**	*
Gustatory-olfactory intensity	2.3 ± 0.4 **a**	2.6 ± 0.4 **a**	2.1 ± 0.5 **a**	2.3 ± 0.6 **a**	2.1 ± 0.2 **a**	2.5 ± 0.4 **a**	**ns**
Gustatory-olfactory persistence	2.2 ± 0.6 **a**	2.4 ± 0.5 **a**	2.0 ± 0.6 **a**	2.3 ± 0.6 **a**	2.4 ± 0.4 **a**	2.3 ± 0.4 **a**	**ns**
Gustatory-olfactory quality	1.8 ± 0.4 **ab**	2.3 ± 0.8 **b**	1.6 ± 0.4 **a**	1.9 ± 0.7 **ab**	1.9 ± 0.2 **ab**	1.9 ± 0.5 **ab**	*
Final considerations							
Evolutionary state	2.1 ± 0.3 **a**	2.1 ± 0.3 **a**	1.9 ± 0.3 **a**	2.3 ± 0.3 **a**	2.3 ± 0.5 **a**	2.3 ± 0.3 **a**	**ns**
Harmony	2.0 ± 0.4 **a**	2.9 ± 0.3 **b**	2.0 **a**	1.9 ± 0.3 **a**	1.7 ± 1.0 **a**	2.1 ± 0.3 **a**	*

Table 72 – Intercative effects of the type of closure and treatment with oak chips on the sensory characteristics of Nero di Troia wines 12 months after the first racking.
S100: Nomacorc *Select Series*™ 100 co-extruded closure; S300: Nomacorc *Select Series*™ 300 co-extruded closure; S700: Nomacorc *Select Series*™ 700 co-extruded closure.
In column, as part of type of closure or treatment with oak chips or combination of type of closure-oak chips, different letters correspond to significant differences ($p < 0.05$ - LSD test).
*: significant difference; ns: no significant difference.

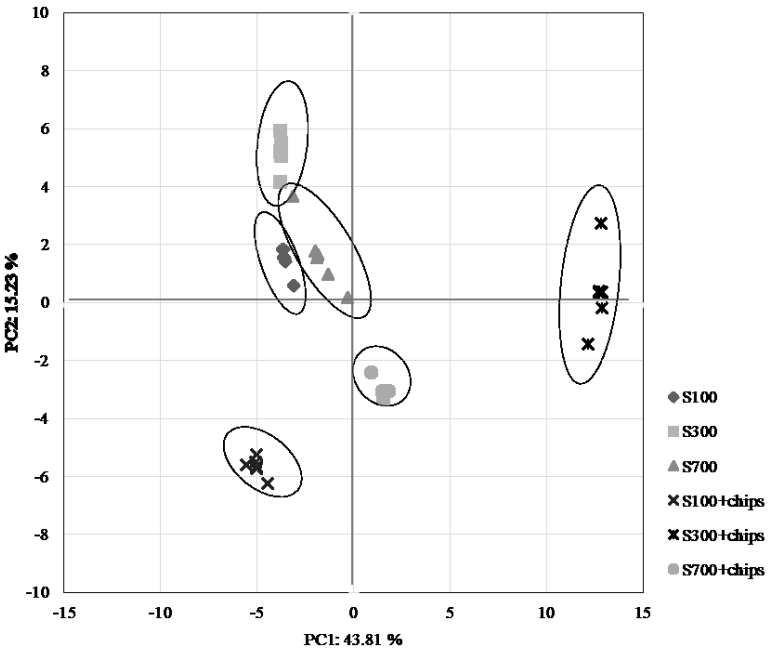


a)

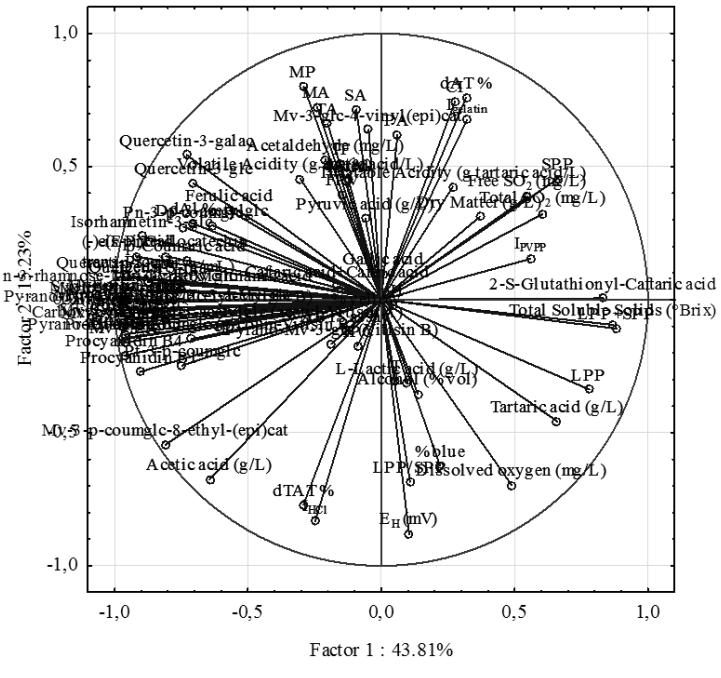


b)

Figure 25 - Citation frequency (%) of Nero di Troia wines not treated (*a*) and treated with French oak chips (*b*) 12 months after the first racking.



a)



b)

Figure 26 - PCA scatter plot for projection on the factor plane of: **a)** Nero di Troia wine samples after 12 months from the first racking and **b)** analytical results.

Chapter 8

Conclusions

The discussion of the results showed that the management of viticultural practices, vinification procedures, aging systems, and packaging materials is a tool useful to both improve the quality of red wine and diversify this product.

Application of defoliation at veraison

The effects of this practice depended not only on the amounts of removed and retained leaves but also by the vine side (east/west) to which leaf removal was exerted, since these variables affected photosynthetic photon flux and temperature in the cluster area. The effects were more evident in grapes than in wines due to the standardising action of the winemaking. The better results were observed by the wines produced from vines defoliated along the east side of the canopy, which exhibited the better chromatic characteristics, due to the highest content in both monomeric and more stable pigments, and the higher balance and harmony. The wines obtained from almost completely defoliated vines, instead, were less harmonius and balanced because of their lower astringency, gustatory intensity and quality, and more minerality.

Vinification procedures: traditional, cryomaceration, addition of pectoltic enzymes, and extended maceration

Among the considered vinification technologies, cryomaceration, addition of pectolytic enzymes and extended maceration protected the wines against oxidation phenomena better than the traditional red winemaking. In particular, cyomacerated wines exhibited the highest oxidation stability but showed the worst sensory characteristics. Cyomacerated together with those treated with pectolytic enzymes showed the best chromaticity due to the highest concentrations of free and more stable anthocyanins. The extended maceration negatively affected colour but, as well as the addition of pectolytic enzymes, improved the sensory profile by increasing spicy, floral, fruity, and frankness aromas. For these reasons, the addition of pectolytic enzymes seemed to be the best vinification procedures.

Aging techniques: treatment with oak chips and micro-oxigenation

The effects of micro-oxygenation and treatment with oak chips strongly depended on cultivar and chemical composition of the starting wine. The treatment with oak chips determined an immediate increase of the degree of anthocyanins and tannins polymerization in Aglianico wines and an increase in the degree of pigments polymerization (with the improvement of the colour stability) and an antioxidant effect (probably due to the ellagitannins deriving from wood one month after the treatment) in

Montepulciano and Nero di Troia wines. The sensory impact of wood chips was evident in all the wines, increasing the perception of spicy, woody, vanilla, and black pepper flavours and of the crimson colour. The increase of astringency in wines treated with chips caused a worsening of the organoleptic profile of Aglianico sample, due to its "tannic character", and the improvement of Montepulciano wines. In Nero di Troia wines, micro-oxygenation did not determine significant differences respect to the control in terms of phenolic profile and colour stabilization but exerted a significant improvement of the sensory characteristics by reducing astringency, and increasing olfactory intensity, gustatory complexity, roundness, fruity and spicy attributes.

Timing of the treatment with oak chips

The application of the treatment with oak chips before or after malolactic fermentation modified some of the quality parameters of Nero di Troia wines. The treatment did not allow the increase of the polymerization of anthocyanins and flavanols, as instead observed in Aglianico, Montepulciano, and Nero di Troia wines produced during 2012 campaign, because of the different phenolic composition (especially of tannins-to-anthocyanins ratio) found in the studied wines. Nevertheless, it determined a slight oxidation stability with respect to the controls due to the oxygen scavenger capacity of ellagitannins derived from the oak wood. The contact with oak chips determined in all the treated wines the decrease of floral and fruity notes, the increase of the spicy and woody flavours, and the attenuation of vinous character. The Nero di Troia wines treated before malolactic fermentation showed softer oak and spicy characters, more fruity notes, and less ethereal character than the wines treated after malolactic fermentation.

Use of synthetic stoppers having different oxygen transmission rates

It significantly affected the Nero di Troia wines quality. In particular, the aging in bottles with a medium and high oxygen exposure led to a greater polymerization among anthocyanins and flavanols, thus stabilizing wine colour, with respect to the closures having low oxygen permeability. Among the samples, those stored in bottle and subjected to a medium and high post-bottling oxygen exposure revealed an increase of fruity, spicy, and aromatic attributes, and a reduction of the vinous notes.

Interactive effects of defoliation and treatment with oak chips

In wines obtained from shaded grapes, the contact with oak chips led to the increase of polymerization reactions among anthocyanins and tannins, while in those ones derived

from defoliated grapes, an antioxidant effect due to the ellagitannins released from oak wood was highlighted. The contact with oak chips determined a greater astringency in the wines from shaded grapes and from grapes submitted to the mildest defoliation, and the attenuation of floral notes and the increase of spicy, woody and vanilla attributes in all the oak-treated samples.

Interactive effects of vinification procedures and treatment with oak chips

Similarly to what observed in Nero di Troia wines treated with oak chips before or after malolactic fermentation, the oak-treatment did not increase in anthocyanins and flavanols polymerization. Nevertheless, the extraction of ellagitannins from oak chips during the contact time allowed to enhance the oxidation stability of the wines obtained by non-traditional technologies, especially those produced by cryomaceration. The contact with oak chips improved the olfactory quality of all the wines, making them more harmonious, through the attenuation of the floral and fruity notes, and the accentuation and/or the appearance of spicy, woody, and vanilla flavours. In particular, among all the wines those produced by extended maceration exhibited the best sensory features.

Interactive effects of treatment with oak chips and types of closure

The combination of the oak chips treatment with the subsequent nano-oxygenation process through the stoppers determined the loss of anthocyanins and favoured polymerization reactions among anthocyanins and tannins. This phenomenon was more intense especially in bottles closed with medium OTR devices. The contact with oak chips improved the olfactory quality in all the wines, making them more balanced and harmonious, through the attenuation of the floral and fruity notes (especially in those ones submitted to medium and high post-bottling oxygen exposure), and the accentuation and/or the appearance of spicy, woody, and vanilla flavours.

In conclusion, through the correct management of some several procedures, both in field and in winery, it was possible to significantly modify physical, chemical, and sensory characteristics of grapes and wines.

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