High density polyethilene tank as an alternative techniqueto ageing wine using wood staves

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Abstract

The study of the evolution of wine components in four HDPE tanks for the five months of its aging was carried out, through three different types of HDPE and one kind of wood used: HDPE with low, medium and high permeability to oxygen and staves of French wood (*Quercus petraea*).

The HDPE tanks, which are permeable to oxygen, and the wooden staves that give their sensorial contribution, were used to simulate the wine ageing in barrels.

The tanks were compared to a steel tank to confront the evolution of the phenolic structure and color of wine during the months of aging.

The analysis of the wine was carried out on the colour, pigments and phenolic compounds (flavanols, flavonols, anthocyanins) by spectrophotometer; HPLC analyzes were also carried out to evaluate the content of catechins, procyanidins and anthocyanins monomers on the base wine.

The HDPE tanks could be a good alternative technology compared to the use of the barrels, which it allows to the wine to undergo a great aging and reducing the costs of aging in barrels, as the barrels have a higher cost than polyethylene tanks.

Probably, wine aging in HDPE tanks can complement the aging in barrique, in order to reduce the use of wood, as it is a limited resource on earth, while the polyethylene is recyclable.

Keywords

Wine – Ageing – HDPE – Barrels - Oxygen

Resumo

Foi realizado o estudo da evolução dos componentes do vinho em quatro tanques HDPE durante os primeiros cinco meses de envelhecimento, por meio de três tipos diferentes de HDPE e um tipo de madeira: HDPE com baixa, média e alta permeabilidade ao oxigênio e aduelas de madeira francesa.

Os tanques HDPE, que são permeáveis ao oxigênio, e as pranchas de madeira que dão sua contribuição sensorial, foram usadas para simular o envelhecimento do vinho em barris.

Os tanques foram coloque lado a lado por um tanque de aço para comparar a evolução da estrutura fenólica e da cor do vinho durante os cinco meses de envelhecimento.

Os tanques HDPE apresentaram uma maior evolução dos compostos fenólicos em comparação com o vinho armazenado em tanques de aço inoxidável, também esse efeito foi observado na evolução do vinho entre os próprios HDPEs, nos quais houve diferenças evidentes, dependentes do grau de permeabilidade.

As análises foram realizadas nos componentes fenólicos por espectrofotômetro, mostrando diferenças em relação a alguns parâmetros, a saber, a quantidade de não flavonóides, a quantidade total de compostos fenólicos, a cor das antocianinas devido à copigmentação, as antocianinas totais e o grau de ionização das antocianinas.

Além disso, os resultados mostraram que os tanques HDPE podem ser uma boa tecnologia alternativa em relação ao uso dos barris, o que permite que o vinho sofra um grande envelhecimento, garantindo alta qualidade e reduzindo os custos de envelhecimento em barris. Estudos futuros serão necessários em relação aos meses subsequentes ao envelhecimento do vinho para avaliar melhor a evolução do componente fenólico ao longo do tempo.

Provavelmente, o envelhecimento do vinho em tanques de HDPE pode complementar o envelhecimento em barrique, a fim de reduzir o uso de madeira, pois é um recurso limitado na terra. Os barris, quando terminam o trabalho para o envelhecimento do vinho, podem ser reutilizados para diferentes tipos de vinho (como o vinho do Porto) e para as aguardentes.

Palavras-chave

Vinho - Envelhecimento – HDPE – Barris – Oxigênio

Resumo alargado

Durante o processo de envelhecimento nos tanques HDPE, por meio de fenômenos físico-químicos, o vinho sofre alterações químicas significativas que destacam a entrada de oxigênio nos tanques e a extração de compostos de madeira. Esses fenômenos são influenciados por vários fatores, como a diferença na permeabilidade ao oxigênio dos tanques e as espécies de madeira utilizadas.

A tecnologia tradicional de envelhecimento do vinho utiliza barris de madeira e, como alternativas, podemos usar a micro-oxigenação e o uso de produtos alternativos de carvalho. A melhor qualidade do vinho é a obtida pelo envelhecimento em barril, mas é um processo desperdiçador em termos de tempo, dinheiro e ocupa grandes espaços na adega, além do fato de que a madeira nos últimos anos é um recurso natural limitado. Dado que hoje a mudança climática é uma emergência, é importante reduzir as emissões de gases de efeito estufa para limitar esse problema, evitando e/ou reduzindo o uso de barris de madeira cujo uso é de apenas alguns anos, por esse motivo os pesquisadores procuram de uma nova e mais sustentável tecnologia de envelhecimento, baseada no uso de menos gases de efeito estufa. Por outro lado, usando os tanques HDPE, é possível reduzir o espaço ocupado na adega, reduzir os custos da adega, pois esses tanques são reutilizáveis por vários anos. Durante o processo de envelhecimento, para torná-lo mais semelhante ao dos barris dentro desses tanques, são inseridos produtos alternativos de carvalho.

O estudo da evolução dos componentes do vinho em quatro tanques HDPE foi realizado durante os primeiros cinco meses de envelhecimento, através de três tipos diferentes de HDPE e um tipo de madeira usada: HDPE com baixa, média e alta permeabilidade a oxigênio e aduelas de madeira francesa.

Os tanques HDPE, permeáveis ao oxigênio, e as aduelas, devido à sua contribuição sensorial, foram utilizados para simular o envelhecimento do vinho em barril.

Os tanques foram flanqueados por um tanque de aço para comparar a evolução do vinho.

Os tanques HDPE mostraram uma maior evolução dos compostos fenólicos em comparação com o vinho preservado em aço, esse efeito também foi observado na evolução do vinho entre os próprios HDPEs, nos quais existem diferenças evidentes dependendo do grau de permeabilidade e pela presença de madeira.

As análises clássicas de vinho (pH, acidez volátil, acidez total, Etc.) foram realizadas durante o envelhecimento para avaliar a tendência e as análises sobre os componentes fenólicos importantes para a evolução do vinho.

Além disso, os resultados mostraram que os tanques HDPE podem ser uma boa alternativa ao uso do barril, permitindo que o vinho sofra um envelhecimento correto, garantindo uma boa qualidade e reduzindo os custos do envelhecimento. Estudos futuros serão necessários sobre os meses subsequentes ao envelhecimento do vinho para avaliarmos e compreendermos melhor a evolução do componente fenólico. Uma vez investigados, os tanques HDPE poderiam substituir gradualmente o uso de barris de madeira, reduzindo e o uso de madeira, pois é um recurso limitado na terra. Os barris, quando terminam o trabalho para o envelhecimento do vinho, podem ser reutilizados para diferentes tipos de vinho (como o vinho do Porto) e para as aguardentes.

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LIST OF ABBREVIATION

TPI= Total phenolic index

OIV= international Vine and Wine organizationMOX= Micro-oxygenation

MLF= malolactic fermentation AOP= alternative oak products

HDPE= high-density polyethylene

ISA= Instituto Superior de AgronomiaDO= Dissolved oxygen

TA= Total acidity

VA=Volatile acidity IC= colour intensityT= tonality

ANT= antocyanin

PIG= pigments

DP= degree of polymerization

PA=proantocyanidinds

TP= tannin power LP= low permeability

MO= medium permeabilityHP= high permeability

NTU= nephelometric

turbidity unit CC=

Copigmentation

A= absorbance

AU= absorbance unit

1 INTRODUCTION - LITERATURE REVIEW

1.1 Ageing wine

After the winemaking process (young wine) there follows an ageing period, during this time the wine undergoes important chemical modification so that its sensory property improves. These changes are due to several phenomena, caused by different ageing condition. The compounds' evolution in the wine depends not only from the ageing condition, but also the kind of wine play an important role (Garde-Cerdán and Ancín-Azpilicueta, 2006).

Regarding the ageing condition, the ageing time is a decisive factor. This factor tend to modify the sensory profile of wines by chemical reactions, that, depend on internal factors (concentration of phenolic and volatile compounds, additives, oxygen, pH, acidity, minerals, microorganisms) and external factors (temperature, bottle, humidity, luminosity) (Fulcrand *et al.*, 2006; Waterhouse and Laurie, 2006; Jaffré *et al.*, 2009; McRae *et al.*, 2012; Kreitman *et al.*, 2016;).

The sensory profile of aged wine change in term of astringency and colour. Wine's colour pass from an initial purple-red hue typical of young red wines to a brown-red hue, characteristic of aged wines (García-Estevez *et al,* 2015). There are different technologies to aged wine, such as barrels, stainless steel, wood, fiberglass, plastic tanks and others (Del Alamo-Sanza *et al.*, 2010).

1.1.1 Ageing in barrels

The ageing of wine in wood barrels has been practiced for many years (Del Alamo-Sanza and Nevares, 2014) and is traditionally used in winemaking to produce high quality wines (Sánchez-Gómez *et al.*, 2018) in most of the wine regions of the world (Del Alamo-Sanza and Nevares, 2014). Ageing wine in wood barrels is an important refinement phase that occurs before ageing in the bottle (Nevares and Del Álamo-Sanza, 2018). The compounds extracted by the wood and their interactions with the components of the wine have been extensively studied (Del Alamo-Sanza and Nevares, 2014).

During ageing time, the wine needs long periods of staying in oak barrels and this represents a high economic cost for companies that use this technique (Rubio-Bretón *et al.*, 2018).

Figure 1 Oak barrels

1.1.1.1 Barrels regulation

The definition of ageing in wooden barrels according to the OIV (OIV, 2019) is: ageing of a wine for an evolution through a natural process in small capacity wooden barrels during a set period of time, in compliance with the usual practices of each viticulture region.

The objectives of this oenological practice are:

- according to the type of wine, this practice try to improve its sensory characteristics through an oxidative and / or biological or diffusion process, in order to obtain a natural oxidative process of the wine;
- through continuous and controlled oxygenation and thanks to the progressive contribution of the substances left by the wood favour the natural physical and chemical mechanisms;
- obtain a total or partial physical-chemical stabilization of the wine

The prescriptions for this practice so that it is carried out in the best possible way are:

(I) it is recommended that the volume of the container be less / equal than to 600 liters, to have an efficient ageing;

(II) the botanical species also vary according to the area; locally, another botanical species other than oak can be used, namely *Castanea sativa*, but the most commonly used are: *Quercus petraea* (sessile oak), *Quercus robur* (pedunculate oak) and their hybrids and *Quercus alba* (American white oak). We recommend tracing the origin of the wood;

(III) for the manufacture of the container, only woods that maintain their natural structure will be used. To optimize the objectives, the cooperation techniques defined for the construction of new barrels will be used. The date of manufacture must be visibly engraved, and we also recommend traceability of the wood;

(IV) through the normal cooperation techniques, the inner surface of the containers can be regenerated. In this case, the date on which this is done must be visibly engraved;

(V) the modulation of oxygen supply to wine is dictated by environmental conditions (temperature, humidity and isolation);

(VI) the continuous control of the conditions of the barrels, their level of filling and the degree of modification of the sensory characteristics produced are recommended during the ageing process. The barrels are maintained in compliance with hygiene standards and are eliminated after a few years.

1.1.1.2 The evolution of wine in wood barrels

The ageing of red wines in wood barrels allows (Rubio-Bretón *et al*., 2018) the contact between wine and oxygen undergo to influence its composition (Sánchez-Gómez *et al*., 2018), this technique it is commonly used in cellars to increase the stability and complexity of wine (Rubio-Bretón *et al*., 2018).
During the wine ageing process some changes occur due to the contribution of compounds released from oak wood and to reactions involving phenolic and aromatic modifications, these evolutions in wine structures ensure that an organoleptic improvement of the product is obtained (Ribéreau-Gayon *et al.,* 2003; Rubio-Bretón *et al*., 2012).
The phenomena that take place in the wine during its ageing in cask involve the general evolution of the phenolic profile of the wine, these modifications are directly or indirectly determined by oxygen, such as the polymerization of tannins and anthocyanins (which improve the stability of the organoleptic characteristics of wine), consumption of free sulphur dioxide and oxidation of ethanol in acetaldehyde.
These phenomena involve changes in the compounds of the wine and in their interactions with the compounds that are extracted from the wood and being often guided by oxygen can be both beneficial and harmful. All this happens depending on the speed and uniformity of ageing, if occurs more slowly and uniformly, ensures that the reactions give the best results. Therefore, it is important to know the amount of oxygen that permeates in the wine barrel to monitor and manage this process, the mechanisms that govern it and the factors that influenceits permeation (Del Alamo-Sanza and Nevares, 2014).

1.1.1.3 Evolution of wine's characteristics

During ageing in barrels different phenomena occur, among the most important occurring there are the entry of oxygen, the complexation of proanthocyanidins with proteins, peptides and polysaccharides and their precipitation with the respective loss of some wine compounds (Del Álamo-Sanza and Nevares, 2018). The changes that take place in the wine, during its ageing in cask, involve its organoleptic characteristics which have been closely associated with the contribution of aromas and phenolic compounds coming from the barrel which improve its aromatic and taste profile (Hidalgo, 2003; Boulton, 2001). Flavanols and anthocyanins are the most abundant phenolic compounds in the skins of red grapes, while grape seeds are rich in flavan-3-oils and proanthocyanidins (Obreque- Slier *et al*., 2010; Obreque-Slier *et al*., 2012). As for the hydrolysable tannins and some low molecular weight non-flavonoid phenols, they are extracted from the wood of the barrels during ageing and for this reason they are present in wine (Lei *et al*., 2001; Obreque-Slier *et al*., 2009).

Copigmentation, condensation and polymerisation phenomena during ageing modify the phenolic composition of the wine, moreover some phenolics compounds are release to the wine, mainly ellagitannins (García-Puente Rivas *et al.*, 2006). The ellagitannins have some fundamental role for the regulation of oxidation, the acceleration of condensation between flavanols and between flavanols or tannins with anthocyanins. Furthermore, the ellagitannins can form other compounds such as flavano-ellagitannins or anthocyano-ellagitannins (Moreno and Peinado, 2012). Condensation could occur through acetaldehyde. From the condensation between acetaldehyde and pyruvate with anthocyanins the vitisins can be produced, the choose of yeast strains for winemaking is important for this process (Suarez and Morata, 2012). In red wines through the cycloaddition of pyruvate and acetaldehyde come to form one type of stable pigments that is the Vitisin A during fermentation and Vitisin B during wine ageing (Riberau-Gayon, 1970).

Other stable pigments can be formed through acetaldehyde that is the vinyl-pyrano-anthocyanins (portisins) and flavanol-anthocyanins ethyl-linked adducts (Morata *et al.*, 2016). The sodium bisulphite added to wine could bond the acetaldehyde, reducing the ability to produce stable pigments (Escott *et al*., 2018).

Acetaldehyde or ethanal is a metabolite present in wines during fermentation and wine oxidation, during fermentation is formed by different pathway. The first pathway is a part of the glycolysis from the pyruvate (Krivoruchkp and Nielsen, 2015) or coming from maloalcoholic fermentation (Suarez-Lepe *et al*., 2012). Finally, during the process of barrel ageing or when micro-oxygenation is applied, ethanal could be directly produced by the chemical peroxidation of ethanol (Bueno *et al*., 2018), but if there is an excess of molecular oxygen other aldehydes in wine can coming from amino acid (Grant-Preece *et al*., 2013).

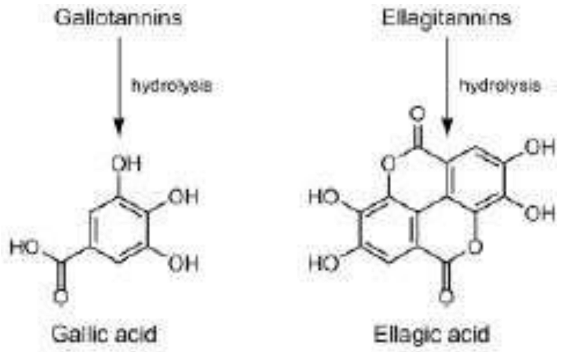


Figure 2 Gallotannins and Ellagittannins chemical structure (Ribéreau-Gayon, 2006)

1.1.1.4 Strengths and weaknesses of ageing wine in wood barrels

The ageing of the wine in barrels is a process that can be carry out for many types of wine, since it releases aromas and other compounds (such as aldehydes and polyphenols) in the wine to improve its quality, furthermore due to the evaporative losses of ethanol and water it contributes to the concentration of the wine and providing appropriate exposure to air allows for the occurrence of important chemical changes including colour stabilization (Singleton, 1974, 1995).

The wood of the oak barrel is a porous material, this mean that during ageing the wine undergoes various phenomena associated with the so-called "low oxidation conditions". Many specific compounds of oak wood are formed during the toasting, kind of the wood and cellar condition and are then given to the wine during ageing (Vivas and Glories, 1993).

During the ageing time in barrels the wine undergoes changes not only concerning the sensorial profile, achievinga reasonable aromatic complexity, but also the colour is stabilized. Furthermore, another effect is the spontaneous wine’s clarification (Jackson, 1994).

Several factors, including the quantity of potentially extractable compounds, the contact time between wine and wood and the composition of the wine, play an important role in the extraction of volatile compounds from barrels. However, these compounds extracted from the barrels undergo transformations that modify the concentration of these substances in the wine over time, one of these transformations is due to microbiological modifications (Spillman *et al*., 1998). Furthermore, wood and wine’s lees absorb the extracted compounds from the wine (Chassagne *et al*., 2005), this factor could also influence the volatile compounds of the wine. During the ageing time of the wine, especially in barrels that have already been used, there could be a significant problem that would be the formation of ethyl-phenols and these are undesirable compounds, because they give unpleasant odours to the wine, compromising its quality. These compounds are formed through certain yeasts present in contaminated wood (Brettanomyces Bruxellensis genera). These yeasts are able to decarboxylated cinnamatic acids and forming these ethyl-phenols in wines. This contamination of microbiological origin is one of the most serious problem during the ageing (Chatonnet *et al*., 1992).

Wine aged in barrels can be influenced on quality due to all these processes that give only an idea of the enormous complexity of the ageing factors (Garde-Cerdán and Ancín-Azpilicueta, 2006).

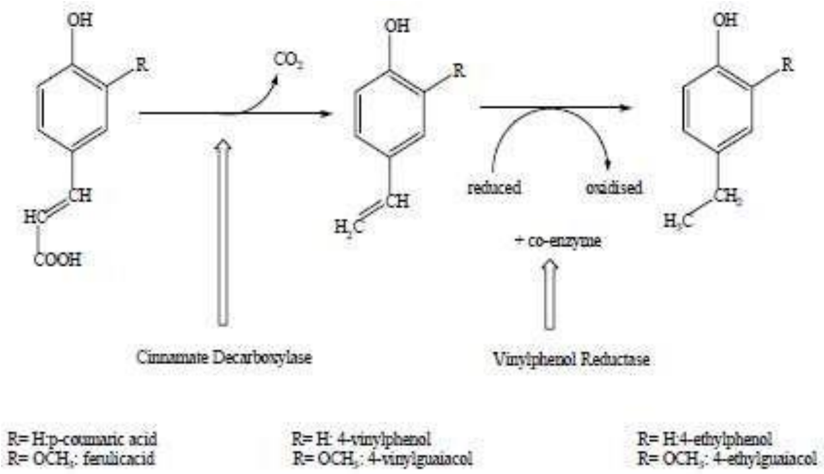


Figure 3 Biosynthesis of ethylphenols in wine by Brettanomyces Bruxellensis (Chatonnet et al., 1992).

1.1.2 The Micro-oxygenation

Micro-oxygenation (MOX small dosage of oxygen) is a technique that allows the management of oxygen in wine by continuously supplying it with a little and controlled quantity during its storage in stainless steel tanks, this technique has been commercially available since the mid-years 90s and can be applied in different

stages of the winemaking process (Pour -Nikfardjam and Dykes, 2003; Devatine *et al.*, 2007; Heras *et al., 2008)*.

Figure 4 Micro-oxygenator (Biondi Bartolini et al. 2008)

1.1.2.1 Micro-oxygenation regulation

The definition of this oenological practice in according to the OIV (OIV, 2019) is addition of oxygen or air to wine. The objectives of this oenological practice are:

(I) the use "micro-oxygenation" technologies on wines;

(II) to initiate oxidation phenomena with the aim of:

- encourage the production of acetaldehyde to contribute to the stabilization of colour and the maturation of red wines, this can react with flavanols and anthocyanins which leads to the formation of new, more stable and more intensely coloured pigments (hyperchromic and bathochromic effect) compared to native anthocyanins,

(III) to reduce the concentration of "volatile sulphur compounds";

(IV) to reduce the vegetable sensory characteristics linked to aromatic compounds;

(V) to facilitate fining of wines.

The prescriptions for this practice so that it is carried out in the best possible way are:

(I) the oxygen during the "micro-oxygenation" must not be accumulated in the wines during the treatment, therefore the speed with which the quantity of oxygen is added, using this technique, should be lower than that of the consumption of oxygen by the treated wine. This technique is preferable to use it when there is a high concentration of free anthocyanins in wines;

(II) in the "micro-oxygenation" technique the oxygen doses to be added to the wine are about 1-5 mg/l per month, this depends on the initial concentration of anthocyanin and polyphenols in addition to the concentration of free SO2. This technique leads to the stabilization of the colour and the improvement of the quality of a red wine during maturation;

(III) the use of micro-oxygenation is recommended at temperatures between 15 ° C and 22 ° C to avoid the accumulation of oxygen with a excessive oxidation. In order to define an optimal duration and temperature according to the desired aromatic profile the wine subjected to oxygenation should be tasted regularly, also to avoid the potential development of oxidative aromas;

(IV) the reduction of sulphite in wines containing excess sulphur dioxide should not be the goal of oxygenation.

(V) microbiological stability (especially with regard to *Brettanomyces bruxellensis*) should be monitored to avoid organoleptic deviations in wines.

1.1.2.2 Use of micro-oxygenation

The limited addition of oxygen in red wines has shown great benefits regarding the final product, applying it after the end of alcoholic fermentation, causing acceleration of the wine ageing (Perez-Magarino *et al*., 2007). The oxygenation of red wine has a fundamental role in the regulation of yeast activity and in ensuring that odours derived from the fermentation of sulphur and poultry are kept under control (Jones *et al*., 2004).

The use of micro-oxygenation technique has dominated for many years all over the world (Heras *et al*., 2008), and recent research has focused mainly on its effect on phenolic compounds in wine. The phenolic composition is directly influenced by the oxygen, furthermore the sensory properties (namely colour, aroma and astringency) are indirectly influenced by the oxygen. The micro-oxygenation technique developed by Ducournau and Laplace (Parish *et al*., 2000) simulate the oxygen transfer occurring in the barrel. This novel technology has been applied to optimize the evolution of the wine's compounds. This is report in several studies made on red wine (Gómez- Plaza and Cano-López, 2011) and in other wine-derived product such us wine vinegar (Guerrero *et al*., 2011), cider brandy (Rodríguez *et al*., 2013) and wine spirit (Canas *et al.,* 2019). The use of micro-oxygenation allows a more rapid loss of less stable monomeric anthocyanins, thus allowing to stabilize the colour of the wine and increase its density (Atanasova *et al*., 2002; McCord, 2003; Perez-Magarino *et al*., 2007). Few studies have been done on the effects micro-oxygenation has on sensory properties, flavours and aromas (Heras *et al*., 2008). The application of the micro-oxygenation technique is performed during the malolactic pre-fermentation phase (MLF) or after the MLF by adding excess sulphur dioxide. The fact that the application of micro-oxygenation is avoided once MLF is started can be traced back to the fact that lactic bacteria can consume the acetaldehyde produced by the self-oxidation of dihydroxyphenols (Cano-López *et al*., 2006), reducing the amount and making it less available for reactions between wine polyphenols and the formation of stable polymeric pigments (Nguyen *et al*., 2010).

The use of the micro-oxygenation technique can be perfectly combined with alternative oak products (AOP) to reduce both the time and costs of barrel ageing (Del Alamo Sanza et al., 2004; Oberholster *et al*., 2015). This combination used up to now (Navares *et al*., 2009; Gallego *et al*., 2012) appears to be essential for reproducing the behaviour of the barrels and therefore of its benefits quite faithfully (Del Alamo *et al*., 2010).

1.1.2.3 Application of micro-oxygenation and oxygen level

The critical points of the controlled introduction of oxygen in wine (MOX) are the oxygen dosage and the durationof its addition, these parameters correctly applied ensure that through this treatment very positive effects canbe obtained in the quality of the wine.

This can happen when oxygen management is specified for each type of wine, the type of alternative kind of wood product used (chips, cubes and staves, among others) and its botanical origin (Del Alamo *et al*. 2010). Thereare two methods on how active micro-oxygenation could be used:

- the fixed MOX dosage, which consists of the continuous supply of small dosages of oxygen;
- the fluctuating MOX dosage, through a dosage of oxygen adaptive to the dissolved oxygen (DO) level present in the wine in order to arrive at the desired quantity of DO in the product (setpoint) (Sánchez- Gómez *et al*; 2018).

All the demand for oxygen during the ageing process can be satisfied by using the method of dosing fluctuating MOX that must be maintained all the time in order to guarantee the best interaction of wood and wine. Precisely for this reason it is necessary to adjust the dosage by comparing the reading of each DO measurement with the DO level reference (Sánchez-Gómez *et al*; 2018).

1.1.3 The use of wood alternative product

The experimentation of alternative forms of barrel ageing has been going on for many years (Garde-Cerdán and Ancín-Azpilicueta, 2006), as a valid alternative to traditional ageing in barrels, the International Vine and Wine Organization (OIV) has identified the use of pieces of oak wood during winemaking as an authorized oenological practice and included in the International Oenological Code (Resolution OENO, 2001; Resolution OENO, 2005). This practice has been approved by the European Community (European Commission, 2005) and is subject to regulation (European Commission, 2006; European commission, 2009). Furthermore, the use of pieces of oak wood for ageing reduces the cost of the process compared to the classic ageing in barrel (Rubio-Bretón *et al*., 2018).

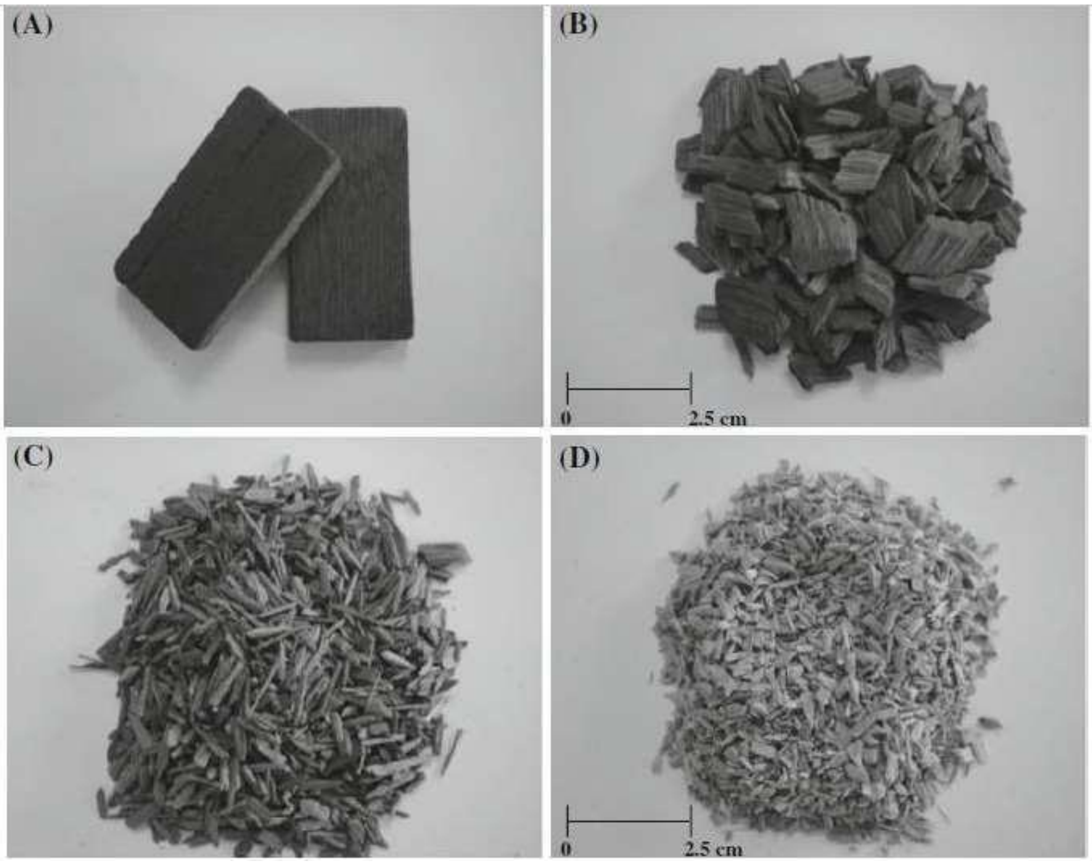


Figure 5 Different kind of alternative oak products (extracted from Jordão et al., 2012)

1.1.3.1 Product alternative at wood regulation

The definition according to the OIV (OIV 2015/01, II.3.5-16) is the usage of pieces of oak wood in winemaking.

The objective of this oenological practice is to introduce the characteristics of certain oak wood constituents into wine.

The prescriptions for this practice so that it is carried out in the best possible way are:

(I) the dimensions of the pieces of wood (from the *Quercus petrea*, *Castanea sativa*, etc.) must be adequate higher than 2 mm;

(II) the pieces of oak wood must not be charred but can be grilled or burned, even on the surface;

(III) it is at the discretion of the winemaker to decide the quantity of pieces of oak wood to be used in the wine;

(IV) the pieces of oak wood shall comply with the prescriptions of the International Oenological Codex.

1.1.3.2 Technique and kind of wood

This alternative ageing technology consists of adding pieces of oak wood to the wine to give it a woody aroma and flavour. During this process the wine can also be micro-oxygenated, allowing reactions to take place between the compounds of the wine and those coming from pieces of oak wood, thus simulating the traditional ageing in barrel (Garde-Cerdán and Ancín-Azpilicueta, 2006). Pieces of oak wood can be influenced by many factors(fragment size, origin of oak wood, toasting level, production process, dose, contact time with wine, etc.), which makes their effects very much variables on wine quality, there is a wide range of commercial products on the market (Chattonnet, 2007; Verdier *et al*., 2007; Tavares, 2018). The different types of wood on the market come from different botanical species for example *Castanea Sativa, Robinia pseudoacacia, Cerasus avium, Fraxinus excelsior*.

The oak fragments available on the market vary according to the shape and size: dust, shavings, medium-sizedpieces (chips, cubes or beans, blocks or segments) or larger pieces (replicas, staves) to be inserted in the tanks. The dimensions of these particles must be such that at least 95% in weight be retained by the screen of 2 mm(9 mesh) (Resolution OENO, 2005).
The fragment size changes according to the type of wood shape:

- Chips +/- 1.9 x 1.2 x 0.3 cm
- Beans 1 cm^3
- Segments 5 to 8 cm
- Replicas 45 x 2.5 x 1 cm
- Staves 91 x 4-6 x 1 cm

Furthermore, these products are made with oak of different origins (American, French, Spanish, Hungarian, etc.), with different toasting processes (direct fire, convection by hot air or infrared radiation) and different toasting levels (light, medium, medium plus, or high). The effects of oak pieces on wine have been widely studied (Rubio- Bretón *et al*.; 2018).

1.1.3.3 Application of wood alternative product

The large contact surface of these materials with the wine permit a faster extraction of the compounds than the barrels (Rubio-Bretón *et al*.; 2018).
The contact time between the pieces of oak wood and the wine depends on the type of wood, on the size of the fragment, on the dose and on the sensory profile to be obtained in the wines, on the basis of these parameters the time of contact between wine and wood that can vary from a few days to several weeks and even months (Rubio-Bretón *et al*.; 2018).

During the ageing of red wines, the optimal time of contact between the oak fragments and the wine is about two / three months, this period leads to an improvement of the volatile and phenolic compounds coming from the wood influencing the evolution of the colour of the wine. For the sensory compounds, besides the contact time, the kind of wood in all its parts is also important (shape, size, botanic origin, toasting level, etc.) (De Coninck*et al*., 2006).

The use of pieces of oak wood may be an appropriate option for red wines intended for short periods of ageing (Rubio-Bretón *et al*.; 2018).

1.1.4 Alternatives techniques to ageing wine (Wood and barrel alternative)

The high cost of barrels for wine ageing has led to the design of alternative solutions based on the active and passive incorporation of oxygen into the wine and on the use of alternatives technology (stainless steel tanks with staves, cubes, shavings, etc.), to simulate the changes observed during the ageing of the barrel at low cost (Del Alamo-Sanza *et al*., 2015).

About the incorporation of active oxygen, beyond the classical ranking with huge an uncontrolled doses of oxygen incorporate, the best technology, moreover the one most commonly used, is the micro-oxygenation system (Moutounet *et al*., 1996), while as regards an economical way of incorporating passive oxygen it has been suggested use of porous plastic materials called high-density polyethylene (HDPE) in different parts of thewinemaking process (Paul and Kelly, 2005; Schmidtke *et al*., 2011). Furthermore, the use of HDPE has been considered as potentially economical alternatives technology to traditional ageing in barrel, either alone or in combination with wood alternatives (chips, cubes, staves) (Flecknoe-Brown, 2002; Paul and Kelly, 2005).

1.1.5 High density polyethylene (HDPE)

High density polyethylene (HDPE) is a thermoplastic polymer belonging to the Polyolefin family. It is obtained from the polymerization of ethylene and is one of the most processed and used polymers, constituting the largestfraction of polymers worldwide. The technical characteristics strongly depend on its molecular weight, the crystallinity rate and the molecular weight distribution.

In recent years, especially for small and medium-sized cellars that use tanks from 2hl to 22hl, there have been increases in the use of plastic tanks (HDPE). The increase in the use of these tanks is also since the problems arising from the plastic for storing wine (odour transfer, use of suitable plastic, etc.) have been solved. These reservoirs are porous and can have a controlled oxygen permeability due to the material with which they are produced (polymers), which makes their use more attractive for cellars (Del Alamo *et al*., 2010).

Figure 6 HDPE permeable tank

1.1.5.1 Features and application of High-density polyethylene

They are currently used for the fermentation, preserve and ageing wines (Nguyen *et al*., 2010). HDPE permeable tanks provide an alternative technology of incorporating oxygen into wines, as they can be made with different levels of controlled oxygen permeability (Del Alamo- Sanza *et al*., 2015).
The plastic tanks are used for the preservation and ageing of wines, because thanks to their polymer structure and the addition of pieces of wood inside it is possible to simulate the ageing of wood in barrels. These tanks have various advantages that lead the winemaker to use them in the cellar, first of all it is a cheaper ageing technology than barrels, it takes up less space in the cellar because it can be easily stacked in 5 heights and is very versatile because there are so many in the market various size and shape in accordance with the needs ofthe cellar. One of the most important advantages is the fact that the tanks allow the winemaker to control the amount of oxygen that the wine receives (Del Alamo *et al*., 2010).
Inside the HDPE tanks it is allowed to add the kind of wood most suitable for the wine to be made, furthermore the oenologist can decide to mix various kind of wood based on their origin and their toasting level, in a way to develop an adequate ageing process for each type of wine, guaranteeing individuality and potential. To manage the ageing process, it is necessary to know the oxygen transfer rate by having a self-micro-oxygenator system similar to that which occurs in barrels (Del Alamo *et al*., 2010).
The use of HDPE is a valid alternative for ageing compared to the use of wooden barrels.

At the best of our knowledge, there are not a lot of studies concerning the polyphenolic and aromatic evolutionof wines and the possible consequences related to preservation inside of these plastic tanks.

1.2 The phenolic compounds

Polyphenols are secondary metabolites of plants widely distributed in beverages and plant-derived foods (Laqui-Estaña *et al.,* 2019). During the winemaking process and ageing, oxygen plays an important role in the structure of wine, mainly due to the evolution of phenolic compounds (Du Toit *et al.*, 2006; Chiciuc *et al.*, 2010; Anli and Cavuldak, 2012).

Polyphenols play an important role during ageing. During the ageing time the red wines undergoes modification the visual evolution and gustative quality. The extraction of polyphenols from the grapes must be as complete and accurate as possible in order to guarantee the intensity of the colour and stability during ageing (Bautista- Ortin *et al.*, 2007). The polyphenols responsible for these characteristics are the anthocyanins and tannins, directly responsible for the colour and the mouth of the red wine (Bautista-Ortin *et al.*, 2016).

The concentration of phenolic compounds in wine could change due on several factors such as grape variety (genetic variability), region of origin (environmental variability, including kind of soil, climatic condition, solar radiation, altitude) and ageing (temporal variability and kind of ageing) (Monagas *et al.*, 2006). In order to obtain a red wine with an optimal quality is required a period of ageing (Ferreira *et al.*, 2014). These phenolic compounds contribute on the quality of wines for sensorial characteristics and for antioxidant capacity (Agazzi *et al.*, 2018).

The colour, astringency, bitterness and structure of the wine are influenced by flavonoid compounds. They are flavonols, condensed tannins and anthocyanins, they are a group of phenolic compounds derived from the structure of flavones. Flavonoids are derived from grape skins, seeds and fats and are compounds that are attributed to the main antioxidant activity in wines. The flavonoid content is found by subtracting the content of non-flavonoids found from the total phenol content.

The class of flavonoids includes catechin, epicatechin, flavonols, anthocyanins and condensed tannins, whereas that of non-flavonoids includes phenolic acids, benzoic and cinnamic acids and their derivatives (examples hydroxybenzoic acids and hydroxycinnamic acids), stilbenes and other volatile phenols.

Non-flavonoid compounds through intra and intermolecular reactions tend to stabilize and improve the colour of red wines, even if they are not coloured. Volatile phenolic acids can also serve the taste of wine and also exhibit biological activities such as stilbene. (Moreno-Arribas and Polo, 2009).

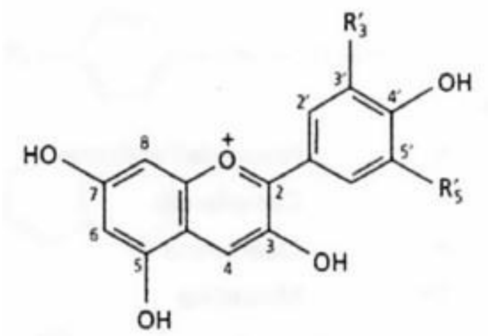


Figure 7 Anthocyanin chemical structure (Ribéreau-Gayon, 2006)

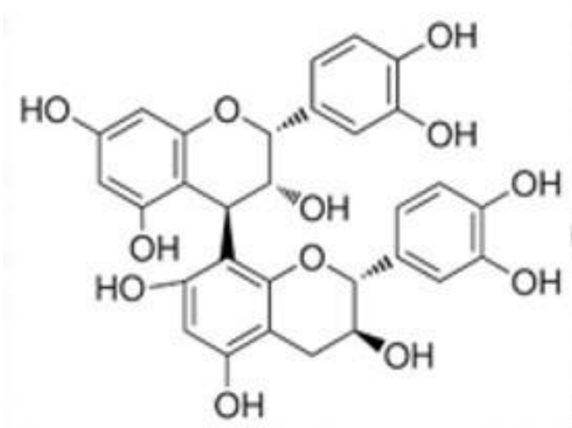


Figure 8 Dimer Procyanidin (flavan-3-ols) chemical structure (Ribéreau- Gayon, 2006)

Where:

- R’3 OH & R’5 H = Cyanidin
- R’3 OCH3 & R’5 H = Peonidin
- R’3 OH & R’5 OH = Delphinidin
- R’3 OH & R’5 OCH = Petunidin
- R’3 OCH3 & R’5 OCH = Malvidin

1.2.1 Anthocyanins and flavanols

Anthocyanins are the main compounds involved in the colour of black grapes and those responsible for the pigmentation of different parts of flowering plants (flowers, fruit, leaves, stems and roots and storage organs). Anthocyanins are extracted in the must during the winemaking, therefore being responsible for the colour of the wine (Alcalde-Eon *et al.*, 2006).

The colour of the young red wine is mainly given by monomer anthocyanins extracted from the skin of the red Grape (Degenhardt *et al.*, 2000; Jensen *et al.*, 2008). In addition, the colour can improve its expression by the self-association and copigmentation reactions, which would be the intramolecular or intermolecular interaction between anthocyanins, anthocyanins and phenolic compounds, like flavonols, and between anthocyanins and other organic chemical substances (Gonzalez-Manzano *et al.*, 2008; Cavalcanti *et al.*, 2011).

Tannins undergo the process of condensation with anthocyanins, this physico-chemical association involves the anthocyanins and the colourless pigment (tannin), despite the fact that tannin is not directly responsible for colour plays a key role in stabilizing and enhancing the colour of wine (Lambert *et.al.*, 2011). The formation of new compounds take place due to the direct bonds between anthocyanins and proanthocyanidins, or between anthocyanins and proanthocyanidins trough ethyl bridge and flavanyl pyranoanthocyanins (He *et al.*, 2012).

The maceration of the grapes ensures that the anthocyanins and tannins are extracted from the skins to spread into the must, the tannins are also extracted from the seeds (Busse-Valverde *et al.*, 2011). These

polyphenols are contained in the vacuoles inside the cells, whereas the large tannins end up binding to the cell walls failing to be extracted (Amrani Joutei and Glories, 1995; Geny *et al.*, 2003).
The quality of the wine is influenced by the chromatic characteristics that are very important factors, together with the taste and flavours, and all together represent the sensorial properties of a wine. Only 0.5% of the components in wine is responsible for the colour of red and white wines, the rest almost 99.5% is colourless. In red wines the main source of pigmentation is anthocyanins. Thanks to the complexes between anthocyanins and colourless cofactors, through the copigmentation reaction, the visible colour is enhanced thus contributing to the red colour of the wine.
We can divide the "colour characteristics" of a wine by:

- Luminosity: varies inversely with wine colour intensity and depends on transmittance. This test combinesthree optical densities and shades to compare the relationship between red and yellow wavelengths.

-Chromaticity: depends on the dominant wavelength (which distinguishes shade/hue) and purity. (OIV, 2009). The luminosity and chromaticity are the chromatic characteristics of a wine, they are related to: tonality, colour intensity, total phenolic index (TPI), ionization index and total anthocyanin content in wine. Climatic conditions (rain, sun, cold and hot temperatures, etc.), vineyard management and soil characteristics influence the brightness of the wine. The several oenological treatments and characteristics such as: acidity and pH, oxidationconditions of phenolic compounds, etc... influence the chromaticity of the wine (Ubigli, 2004).

1.2.2 The extraction of phenolic compounds

The methods of extraction of phenolic compounds have been studied thoroughly in every point of view, since these compounds have a high influence on the quality of the red wine. In order to extract the phenolic compounds from the skin through vacuole, walls cell and are bounded with the polysaccharide and protein, during the maceration process, the cellular walls must be broken to simplify the spread of the vacuole's contents in the must. So, the content of the phenolic compounds in the red wines depend from the concentration of these pigments present in the grape's skin at harvest time, on the vinification techniques used and on the ease of extraction in maceration (Busse-Valverde *et al.*, 2010). As Rolle *et al.* (2012) demonstrated to estimate the concentration of anthocyanins which are extracted and spread in the wine is not enough to know the amount of anthocyanins present in the skins of the grape, all this is due to the fact that the barrier effect caused by the cell walls detain part of the anthocyanins present in the grape's skins (Ortega-Regules *et al.*, 2006; Rolle *et al.*, 2009). Also, the correlation between grape tannins and tannins that are extracted in the wines is not satisfactory (Harbertson *et al.*, 2002; Adams and Scholz, 2007; Busse-Valverde *et al.*, 2010) and the quantities found are often much lower than expected (Busse-Valverde *etal.*, 2012). Moreover, some studies have shown that tannins in addition to being bonded by cell walls in grape's skins also have interactions during the vinification always between tannin and cell wall, this phenomenon can be explained from the cell walls constituted mainly of proteins and polysaccharides that contain hydroxyl groups, as well as oxygen atoms and glycogen these have both the ability to form hydrogen bonds and to form interactions hydrophobic with some molecules (Le Bourvellec *et al.*, 2004; McManus *et al.*, 1985), including some phenolic compounds. The extractable tannins during the fermentation process trough maceration are substantially reduced by these interactions, due to a large part can be adsorbed by the cell walls that remain suspended in the must during the vinification and finally they precipitate during stabilization (Bautista-Ortin *et al.*, 2015).

About whether anthocyanins and tannins affect their reciprocal extractability, studies showed that through eliminating the seeds (one of the source of tannins) during the winemaking process there is a reduction in the concentrationof proanthocyanidins up to 40% compared to a control wine on the contrary the amount of anthocyanins was not affected, means that one of the major sources of tannins extraction is the seed (Bautista-Ortin *et al.,* 2014).

1.2.3 The evolution of the colour in the wine

The colour of the wine gives different information including its age and storage conditions, the colour is one of the main characteristics perceived by consumers that could influence its acceptance. Anthocyanins provide the characteristic red-purple hue of young red wines, they are the pigments responsible for the colour of the grape, which are extracted and spread in the must during vinification (Boido *et al.*, 2006).

The concentration of monomeric anthocyanins, especially the acylated anthocyanin, in red wines decreases after their extraction during fermentation and decreases steadily during the maturation and ageing of the wine. At this stage there are several mechanisms concerning the concentration of anthocyanins which begin to decrease, among which we have in the first phase of vinification the adsorption by the yeast (Wrolstad *et al.*, 2005; Gomez- Miguez *et al.*, 2007; Jackson, 2008). Subsequently, anthocyanins may disappear due to reactions of condensation, polymerization, oxidation and precipitation with proteins (Boido *et al.*, 2006), polysaccharides or condensed tannins. Furthermore, there is progressive and irreversible formation of stable and much more complex anthocyanin pigments, among these we have the various pyroanthocyanins, the polymeric anthocyanins with direct or mediated binding from the aldehydes, as well as their further derivatives. All these reactions from the degradation of anthocyanin at their condensation and polymerization provide different nuances to the wine lead to significant changes in the anthocyanin wine profile (Robinson *et al.*, 1966; Brouillard *et al*., 2003; Monagas *et al.*, 2005, Wrolstad *et al.*, 2005; Ribereau-Gayon *et al.*, 2006; Jackson, 2008; He *et al.*, 2012). Through these reactions the colour of the wine evolves to the shades of the orange-brick (Boido *et al.*, 2006) typical of the aged wines. Furthermore, the ageing contributes to changes in the sensation of the perception of the wine in the mouth and in all the organoleptic properties of the red wines (Robinson *et al.*, 1966; Brouillard *et al.*, 2003; Monagas *et al.*, 2005, Wrolstad *et al.*, 2005; Ribereau-Gayon *et al.*, 2006; Jackson, 2008; He *et al.*, 2012). The anthocyanin families' role during the stages of ageing is not yet well defined. The principal method for wine ageing is in oak barrels (Boido *et al.*, 2006).

2 AIM OF THE WORK

The purpose of the work is to evaluate the evolution and aging of wine between a stainless steel tank and four tanks in permeable high-density polyethylene (HDPE), which differ in their different permeability to oxygen, in particular HDPE with low permeability, with medium permeability and with high permeability.
To give the flavours and characteristics of the wood, French wooden staves (*Quercus petraea*) have been put in three low, medium and high density tanks, I will also study the evolution of another permeable tank in HDPE with medium permeability without staves. The tanks and wooden staves for processing are offered by the company OENOVATION.
During this work, there will perform a series of analyzes on wine at the Ferreira Lapa laboratory of the Instituto Superior de Agronomia. The analyzes, carried out in the four permeable tanks in HDPE and on the control, are chemical-physical and spectrophotometric determination on the phenolic compounds of the wine.

3 MATERIAL AND METHODS

3.1 Materials

3.1.1 Wine

The wine used for the thesis project was a blend of four red varieties (Syrah, Touriga Nacional, Cabernet Sauvignon and Trincadeira) from the Instituto Superior de Agronomia vineyard of the 2020 vintage.
The 15/03/2021 the wine is put inside the HDPE tanks and into the control in stainstell tank.
Before inserting the wine into their respective tanks, the polyethilene tanks were washed according to the protocol, which indicates a first wash with 3% soda with cold water for 15 minutes, a second wash with peracetic acid for 15 minutes and a third wash with lots of water.
The sampling frequency was every 15th of the month.

3.1.2 Tanks and wood

The tanks used for the thesis project were 1 stainless steel tank and 4 HDPE permeable tanks with three different oxygen permeability (low, medium, high) and 100 litres volume each one.
The expected permeability in the tanks were:

- 25 to 40 mg/l/years for low permeability tank;
- 35 to 70 mg/l/years for medium permeability tank;
- 65 to 90 mg/l/years for high permeability tank.
  These tanks can be used in the long period until they suffer possible damage or deterioration.
  The 15/03/2021 the staves, with the wine, are put inside the HDPE tanks and into the control

in stainstell tank.

The staves used for the thesis were of medium toasted French oak (*Quercus petraea*), used for each type of tank with different permeability and a medium permeability tank without staves, the staves were eleven for each tank, four of 95.1 cm in length and 2,4 cm of large and seven of 47,2 cm of lenght and 2,5 cm of large, all the staves had a height of 1 cm. The total volume of the staves in each tank was 1705 cm^3, while the area of the staves for each tank was 5325 cm^2.

In all the thesis in May added 1 gr/hl of potassium metabisulphite.

Figure 9 HDPE permeable tanks and oak staves

3.2 Methods

3.2.1 Classical analysis

The most important physical chemical parameters were determined, according to the methods of O.I.V. such as Total Acidity (TA), Volatile Acidity (VA), Alcoholic Strength, pH, Total and Free SO2 and Reducing Sugars.
Listed below we have the methods used for classic chemical analyses with a brief description and their importance.

- TA: Method OIV-MA-AS313-01

The total acidity is defined by the OIV as the sum of titratable acids up to pH 7.0 without considering SO_2 and CO_2. (OIV, 2016).
Following the OIV method, 50 ml of wine was placed in a under vacuum flask. The vacuum was applied using a water pump while it was being continuously shaken to eliminate carbon dioxide. In a becker 25 ml of boiled water, 1 ml of bromothymol blue solution (indicator of titration) and 5 ml of wine were combined. For the titration was added 0.1 mol/L sodium hydroxide solution until the colour changed to blue - green. Three replicas were made for each sample for each month.

- VA: Method OIV-MA-AS313-02

For dried red wines the maximum acceptable limits of the OIV are less than or equal to 20 meq / L. The quantification of volatile acidity is based on the separation of volatile acids by steam distillation followed by a titration using standard sodium hydroxide preceded by the removal of carbon dioxide from the wine. The evaporation temperature of volatile acids is much lower than that of water and alcohol.

Following the OIV method, 50 ml of wine was placed in a under vacuum flask. The vacuum was applied using a water pump while it was being continuously shaken to eliminate carbon dioxide.
In a flask containing about 0.5 g of tartaric acid are placed 20 ml of wine that has been released to carbon dioxide this is boiled for an extended period to collect the totality of the volatile acid in a solution to the holder with sodium hydroxide (NaOH) with phenolphthalein as an indicator.
Sodium hydroxide neutralizes collected volatile acids and when the solution returns neutral, the indicator (phenolphthalein) will signal that all acids have been neutralized and the endpoint has been reached. The addition of starch and sulfuric acid after it will consider sulfur added to the wine. Four drops of diluted hydrochloric acid, 2 ml of starch solution and some potassium iodide crystals have been added. The free sulfur dioxide was then titled with the Iodine Solution. The sodium tetraborate solution was then added until the pink colouring reappeared. The combined sulfur dioxide was then re-tinted with the iodine solution. Three replicas were made for each sample for each month.

- Alcohol Strength: Method OIV-MA-AS312-01A

Ethanol has a depressive effect on the boiling point of the wine, so the alcoholic content of the wine is related to the temperature difference between the boiling point of the wine and the boiling point of distilled water, this is the principle on which it is based method.

According to the OIV method, the boiling point of the water is first determined by filling the ebulliometer with distilled water, bringing it to the boiling point. This temperature is recorded as a temperature at 0.0% alcohol. Filling the boiling chamber with 50 ml of wine we determine the boiling point of the wine sample, filling the capacitor with cold water (this prevents the evaporation of alcohol) and boiling the solution. There is a thermometer in the ebulliometer and once the temperature is stable it is recorded. Through a wheel of degreeof ebulliometer we determine the alcoholic gradation of the wine, in the wheel are located the boiling point ofthe distilled water and the wine sample and through these is read the alcoholic content (volume / volume).

- pH: Method OIV-MA-AS313-15.

pH is a measure of acids found in wine (e.g. tartaric acid). These acids dissociatein hydrogen ions and anions. A pH scale, for a watery state, ranging from 1 to 14 was created to overcome small concentrations of hydrogen ions, for wines usually the pH varies from 3.0 to 4.0. pH has an impact on the taste and aroma of wine and can also result in the interaction of phenolic compounds (impact on the colour of the wine), grape maturity, wine stability for storage, influence fermentation, tartar solubility, microbial activity and the determination of molecular SO_2 which is the only effective form against microorganisms. Protection against microorganisms is not so strong with a high pH value (lower pH, higher FREE SO_2) (Darias *et al.*, 2003).

A pH-meter can be used to measure pH in grape must and wine, the OIV method is based on the potential difference between two electrodes immersed in the liquid under consideration. The pH-meter calculates the activity of hydrogen ions in pH units by measuring the difference in electrical potential between a pH electrode and an electrode reference connected to a power meter. Low pH values correspond to a higher concentration of these free anions. Three replicas were made for each sample for each month.

- Total and Free SO2: Method OIV-MA-AS323-04A.

Free and total sulphur dioxide is determined by potentiometric titration with iodide/iodate. The free sulphur dioxide is determined after the addition of 1/3 sulfuric acid while the total sulphur dioxide is determined after alkaline hydrolysis (NaOH) lasting 5 minutes and adding 10% sulfuric acid. The potentiometric titration, with iodide/iodate, was carried out with a double platinumelectrode and an LED indicator that detects the electric current inside the solution, the user controls the flow of this solution drop by drop, the end of the measurement is determined once all the LEDs are on and fixed for a few seconds, this happens as soon as the oxidizing solution of iodide / iodate is in excess. Three replicas were made for each sample for each month.

- ***Reducing Substances: Method OIV-MA-AS311-01A.***

The reducing substances detected with this method include all the sugars that have ketone and aldehyde functions and are determined by their reducing action onan alkaline solution of a copper salt. The excess copper ion concentration is determined by iodometry. To eliminate the interference of other reducing compounds, the wine is treated with neutral lead acetate, thus making a previous clarification.

Due to their influence on the microbiology, taste and flavours of wine, the determination of these parameters in wine is of extreme importance. These characteristics are essential to indicate the stability of the wine in termsof microbiological and / or oxidative reactions or if any deterioration of the same is occurring. Furthermore, they explain the response of the wine to the addition of the products (Ribéreau-Gayon *et al*., 2006).

3.2.2 Wine's chromatic characterization and phenolic composition

The wines' phenolic composition analyses were performed to better understand how phenolic compound influenced the evolution of wine.

The chromatic characteristics of red and rosé wines, conventionally, are described by the intensity of colour and hue, in accordance with the method of work adopted with the relative procedure.

Wine producers use colour as a selling point for their wines, being an important factor for consumers (OIV, 2009). According to the method proposed by Somers and Evans (1977) through the use of the spectrophotometer method (method OIV-MA-AS2-07B, type IV method) the chromatic characteristics of the wine were determined. Considering the values of absorbance (or optical density) at different wavelengths (280 nm, 420 nm, 520 nm and 620 nm), various parameters were analysed.

Below are all the procedures and formulas used for the calculation.

3.2.2.1 Determination of the colour intensity (IC) (OIV, 2009)

Note the absorbance (optical density) at 420, 520 and 620 nm, in 1 mm cell. The IC has been calculated according to the Equation :

$$\boldsymbol{IC}\ (\boldsymbol{u.a.}) = (A420 \times k) + (A520 \times k) + (A620 \times k)$$

Where: A420 = absorbance at 420 nm of the wine; A520 = absorbance at 520 nm of the wine; A620 = absorbance at 620 nmof the wine; *K = correction factor = 10 (according the optical length size); u.a. = absorbance unit*

The analysis has been performed in triplicates.

3.2.2.2 Determination of the Tonality (T) (OIV, 2009)

Note the absorbance (optical density) at 420, 520 and 620 nm, in 1 mm cell. The S has been calculated according to the Equation:

$$\boldsymbol{S}\ (\boldsymbol{u.a.}) = (A420 \times k) / (A520 \times k)$$

Where: A420 = absorbance at 420 nm of the wine; A520 = absorbance at 520 nm of the wine; K = correction factor = 10(according to the optical length size); u.a. = absorbance unit.

The analysis has been performed in triplicates.

3.2.2.3 CIElab analysis (OIV 2006)

The second method used in the present work for the determination of chromatic characteristics was the CIE Lab method, which allows us to determine different characteristics of a wine, such as clarity, chroma and hue (OIV 2006). This method is important because allows us to calculate the total colorimetric difference and the difference in hue between two samples.

This analytic method starts clarifying the wine by centrifugation at 3500 rpm for ten minutes, then a measurement of absorbance in a range between 380 and 780 nm is applied. In this way the device gives us back three values called "L", "a" and "b" for each sample.

Following the official protocol, with the "L", "a" and "b" coordinates is possible to calculate the clarity, the chroma (C) and the hue (H) with the following calculations:

Clarity= L

C= (a2 + b2) 1/2

H= tg (b/a)

3.2.2.4 Colour due to Copigmentation (Boulton R., 2001)

About 80% of the grape anthocyanins at the pH of the wine are colourless and their predominant form is hydrated. These pigments are stabilized by mechanisms. The stabilizing effect occurs when the anthocyanin chromophore is complexed by the compounds present in the wine, which may be: one of the acyclic aromatic groups (intramolecular copigmentation), another anthocyanin molecule (self-association) or other molecules (copigmentation intermolecular).

The anthocyanin auto-association and the pigmentation lead to the stabilization of the colour, this is due to the displacement towards flavylium form of the hydration equilibrium after the pigment fixation or from the pigment-copigment complexes due to the displacement of the quinonic bases.

Through the method of Boulton it is possible to estimate colour of wine to copigmentation (Boulton R., 2001).The procedure is described below:

1st step: 10 ml of wine and 0.1 ml of acetaldehyde were placed in a tube (12.6% v / v).

2nd step: after 45 minutes the spectrophotometer reading at 520 nm (A^a520) of absorbance was carried out inthe 1 mm cell

3rd step: 1 ml of the solution formed in step 1 is diluted in proportion 1/25 with a hydroalcoholic solutioncorrected to the pH of the wine.

4th step: after 45 minutes the spectrophotometer reading at 520 nm (A^b520) of absorbance was performed inthe 10 mm cell.

Once the 2 absorbances are obtained it is possible to calculate the degree of copigmentation.The copigmentation has been calculated according to the Equations :

$$CC\ (u.a.) = A^a520 - A^b520$$

$$CC\ (\%) = \{(A^a520 - A^b520)/A^a520\} * 100$$

CC can be expressed in mg/l of malvidin 3-glucoside, according to the Equation :

$$\textbf{\textit{CC (mg/l)}} = \text{CC (u.a.)} * 20{,}3$$

Where: CC = copigmentation; u.a. = absorbance unit; A^a520 *= absorbance at 520 nm of the wine (1st sample);* A^b520 *= absorbance at 520 nm of the wine (2nd sample); 20,3 = convertion factor mg/l.*

The analysis has been performed in triplicates.

3.2.2.5 The total anthocyanin content (Somers and Evans, 1997)

The total anthocyanin content includes colourless anthocyanins and ionized anthocyanins, responsible for colour. This determination of the parameters is extremely important, considering that the anthocyanins are the pigments linked to the red colour of wines (Ribéreau-Gayon *et al.*, 2006). The analysis of the total anthocyanin content is carried out by measuring the difference in absorbance of the wine sample with the introduction of hydrochloric acid (HCl) and sulphur dioxide (SO_2).

1st step: 5 ml of sodium metabisulfite solution were added to the sample

2nd step: mix thoroughly by inversion and record the absorbance at 520 nm after 1 minute, in a 1 mm cell.

3rd step: 10 ml of HCl 1M were added with 100 ml of wine.

4th step: measure the absorbance at 520 nm in a 10 mm cell (corrected for the dilution used, ie × 101) after 3-4hours

The total anthocyanin content has been calculated according to the Equation:

$$\boldsymbol{ANT\_tot}\ (\textbf{\textit{mg/l}}) = 20 \times [(A''520 \times k') - (5/3 \times (A'520 \times k))]$$

where: A'520 = absorbance at 520 nm of a solution made of wine in presence of SO_2*; in other words, it is the absorbance at 520 nm after bleaching all free pigments with* SO_2*; A''520 = absorbance at 520 nm of a solution made of wine in presence of HCl; in other words, it is the absorbance at 520 nm after shifting all free pigments to the coloured flavylium form; k = correction/dilution factor = 10; k' = correction/dilution factor = 101; 5/3 = arbitrary factor in relation to polymeric pigments; 20 = conversion factor mg/l.*

The analysis has been performed in triplicates.

3.2.2.6 The coloured anthocyanin content (Somers and Evans, 1997)

Coloured anthocyanins (also known as ionized anthocyanins) are those in the form of flavylium cation, which form is characterized by a red colour and is the anthocyanin structure most present in acid solutions with pH conditions around 3. Measurement that is carried out refers to the concentration of coloured anthocyanins among the total anthocyanin content.

The coloured anthocyanin content has been calculated according to the Equation :

$$\boldsymbol{ANT\_col}\ (\textbf{\textit{mg/l}}) = 20 \times [(A520 \times k) - (A'520 \times k)]$$

where: A520 = absorbance at 520 nm of the wine; A'520 = absorbance at 520 nm of a solution made of wine in presence ofSO_2; in other words, it is the absorbance at 520 nm after bleaching all free pigments with SO_2; k = correction/dilution factor = 10; 20 = conversion factor mg/l.

The analysis has been performed in triplicates.

3.2.2.7 The ionization index (Somers and Evans, 1997)

Ionized anthocyanins are those strictly responsible for the colour of wine, so this measure expresses the percentage of ionized anthocyanins compared to the total quantity. It was calculated by measuring the absorbance of the wine sample with the presence of HCl and SO_2 solution.

The ionization index has been calculated according to the Equation :

$$\boldsymbol{Ionization\ index}\ (\%) = (\mathbf{A520\ x\ k}) - (\mathbf{A'520}\ \boldsymbol{x}\ \boldsymbol{k})\ (\boldsymbol{A''}\ \mathbf{520}\ \boldsymbol{x}\ \boldsymbol{k'}) - (\mathbf{5/3}\ \boldsymbol{x}\ (\boldsymbol{A'}\ \mathbf{520}\ \boldsymbol{x}\ \boldsymbol{k}))\ \text{x}\ 100$$

where: A520 = absorbance at 520 nm of the wine; A'520 = absorbance at 520 nm of a solution made of wine in presence ofSO_2; in other words, it is the absorbance at 520 nm after bleaching all free pigments with SO_2; A''520 = absorbance at 520 nm of a solution made of wine in presence of HCl; in other words, it is the absorbance at 520 nm after shifting all free pigments to the coloured flavylium form; k = correction/dilution factor = 10; k' = correction/dilution factor = 101; 5/3= arbitrary factor in relation to polymeric pigments;.

The analysis has been performed in triplicates.

3.2.2.8 The total pigments content (Somers and Evans, 1997)

This parameter expresses the concentration of a wide range of molecules, such as phenolic compounds (ie flavonoids) and anthocyanins and, moreover, refers to the substances resulting from the polymerization of the different phenolic compounds. We use this method to measure polymerized and unpolymerized pigments using absorbance.

The total pigments content has been calculated according to the Equation:

$$\boldsymbol{PIG\_tot}\ (\boldsymbol{u.a.}) = \text{A''520 x k'}$$

where: A''520 = absorbance at 520 nm of a solution made of wine in presence of HCl; in other words, it is the absorbance at 520 nm after shifting all free pigments to the coloured flavylium form; k' = correction/dilution factor = 101;

The analysis has been performed in triplicates.

3.2.2.9 The polymerized pigments content (Somers and Evans, 1977)

The polymerized pigment is the estimation of anthocyanins combined with flavanols representing the redcolour.

The polymerized pigments content has been calculated according to the Equation:

$$\boldsymbol{PIG\_pol}\ (\boldsymbol{u.a.}) = \text{A'520 x k}$$

where: A'520 = absorbance at 520 nm of a solution made of wine in presence of SO_2; in other words, it is the absorbance at 520 nm after bleaching all free pigments with SO_2; k = correction/dilution factor = 10;

The analysis has been performed in triplicates.

3.2.2.10 The polymerization index (Somers and Evans, 1997)

The polymerization index has been calculated according to the Equation:

$$\boldsymbol{Polymerization\ index}\ (\%) = [(A''520 \times k') / (A'520 \times k)] \times 100$$

where: A'520 = absorbance at 520 nm of a solution made of wine in presence of SO_2; in other words, it is the absorbance at 520 nm after bleaching all free pigments with SO_2; A''520 = absorbance at 520 nm of a solution made of wine in presence of HCl; in other words, it is the absorbance at 520 nm after shifting all free pigments to the coloured flavylium form; k = correction/dilution factor = 10; k' = correction/dilution factor = 101.

The analysis has been performed in triplicates.

3.2.2.11 The total phenols content (Somers and Evans, 1997)

In all the thesis it is very important to analyse the total phenolic content. This group of compounds can be divided into two categories: flavonoids and non-flavonoids, which are a large group of different chemical compounds that influence taste, colour and sensation in the mouth. Red wines for more than 80% of their total phenolic content are characterized by flavonoids (Jackson, 2014).

Total phenols, non- flavonoids and flavonoids value is detected in absorbance unity, will be also expressed in mg/l of gallic acid. The curve has been calculated according to the Equation :

$$\boldsymbol{y} = 0.038\boldsymbol{x} - 0.0344 \qquad \text{Therefore: } \boldsymbol{x} = (0.0344 + \boldsymbol{y})/0.038$$

Where: y = absorbances values; x = value expressed in mg/l

Through the method of Somers and Evans (1977) it is possible to estimate the total phenolic compounds. The procedure is described below:

1st step: 1 mL of wine was diluted with water in a 100 mL flask.

2nd step: Measure absorbance of the wine diluted at 280 nm in 10 mm cell by the spectrophotometer. Compounds with a benzene ring (common to all phenolic compounds) are absorbed with a high absorbancecapacity of 280.

The total phenols content has been calculated according to the Equation:

$$\boldsymbol{PHEN\_tot}\ (\boldsymbol{u.a.}) = A280 \times k''$$

where: *A280 = absorbance of the wine at 280 nm; k'' = corretion factor = 100*

The analysis has been performed in triplicates.

3.2.2.12 Quantification of flavonoid phenols and non-flavonoid phenols (Kramling and Singleton, 1969)

The Kramling and Singleton method (1969) allows to obtain an estimate of non-flavonoids. The procedure is described below:

1st step: 10 ml of wine are pipetted into a large tube followed by 10 ml of 1: 4 conc. HCl.

2nd step: 5 ml of formaldehyde standard solution containing 8 mg/ml added.

3rd step: the test tube was spread with nitrogen and plugged.

4th step: after 72 hours, they were measured on the spectrometer.

- ***The non-flavonoids content***

This determination is based on the absorbency measurement at 280 nm wavelength of the sample before and after the precipitation of the flavonoids through a reaction with formaldehyde under specific conditions of lowpH and room temperature.

The non-flavonoids content has been calculated according to the Equation:

$$\boldsymbol{Non\_flav}\ (\boldsymbol{u.a.}) = A280 \times k$$

where: *A280 = absorbance of the wine at 280 nm; k'' = corretion factor = 100*

The analysis has been performed in triplicates.

- ***The flavonoids content***

It is the result of the difference between the total phenols and the non- flavonoids content.

The ionization index has been calculated according to the Equation:

$$\boldsymbol{Flavonoids}\ (\boldsymbol{u.a.}) = (PHEN\_tot) - (Non\_flav)$$

The analysis has been performed in triplicates.

3.2.2.13 Tannin power analysis (Freitas and Mateus, 2001)

To evaluate the astringency (more precisely its level) of a wine a very important index is used which is the tannin power.

Freitas and Mateus (2001) have determined the tannin power of wines with a procedure described based on the concept that: "The molecular structure of procyanidin contains different groups such as the aromatic rings and the carbon-hydrogen skeleton of the pyranic ring which supply many sites of a hydrophobic nature capable of interacting with proteins "(Kaushal, 2014).

The procedure is described below:

1st step: the wine sample has been diluted 1/50 with a wine model solution (hydro alcoholic solution:12 % (v/v); tartaric acid: 5 g/l; pH: 3.2) previously filtrated (0.45 µm). 4 ml of the diluted solution have been placed on a turbidity meter paper and the turbidity has been determined by using a nephelometer (HACH 2100 N). The obtained value will be designated as **d0**.

2nd step: after the measurement, 300 µl of a BSA solution (Bovine Serum Albumin 0.8 g/l) have been added to 8 ml of the solution prepared in the 1st step, agitating using a vortex. The solution has been stored in a dark placeat ambient temperature for 45 minutes. Once ready, the turbidity of the solution has been determined by using a nephelometer (HACH 2100 N). The obtained value will be designated as **d**.

The tannin power of the wine sample has been calculated according to the Equation :

$$\boldsymbol{Tannin\ power}\ (\boldsymbol{NTUml}) = (\boldsymbol{d} - \boldsymbol{d0}) / \boldsymbol{0.08}$$

The analysis has been performed in triplicates.

4 RESULTS AND DISCUSSION

4.1 HDPE influence in the ageing wine

The wine studied for this thesis has been analysed for pH, total and volatile acidity, density, alcohol, sugars, SO_2 levels, total phenols, flavonoids, non-flavonoids, tannin and colour in its various characteristics. In the tables and in the graphs below are reported the evolution of the wine inside the HDPE and in the controlin steel.

4.2 Physical-Chemical analyses

To determine the basic parameters of the wine and therefore its correct ageing, the main basic analyses were performed. The analyses carried out pre-ageing in the wine are the following (tab 1) and give us a first entity of the wine.

Table 1 Initial parameters of the wine

t0 : initial time of the experiment	
pH	3.76
Total acidity (g/l tartaric acid)	7.2
Alcohol streght (% vol)	14.9%
Residual sugar (g/l)	1.8
SO_2 free (mg/l)	30
SO_2 tot (mg/l)	70
Volatil Acidity (g/l acetic acid)	0.28

In table 1 we can see, from the results obtained, the wine in question has a fairly high pH with a fairly good total acidity, expressed in g/l of tartaric acid, a good alcohol content, an adequate quantity of SO_2 and a low quantity of reducing sugars.

The reducing substances include all the sugars exhibiting ketone and aldehyde functions and are determined by their reducing action on an alkaline solution of a copper salt (OIV, 2009). So, ketone and aldehyde compounds other than sugar, which is present in wine, can influence this measurement as it may be in competition.

The amount of volatile acidity could be attributed to the maturity of the grapes before the harvest or to problems during the fermentation.

The results obtained from the general physico-chemical analyses carried out during the second month ageing on the wine considered for ageing in HDPE with different levels of oxygen permeation (low, medium and high) and with wood staves are shown in the table 2. In this table we can see the parameter as regards total and free SO_2 (expressed in mg/l) and volatile acidity (expressed in g/l of acetic acid) in the second month of aging.

Table 2 The parameters of the wines ageing in different tank, on the second months storage.

Month	April				
	Test	L	M	H	M.C.
SO_2 free (mg/l)	22	23	26	22	22
SO_2 tot (mg/l)	75	67	64	69	64
Volatil Acidity (g/l acetic acid)	0.283	0.321	0.280	0.322	0.312

Test – stainless steel; L. – *low permeability tank with French oak****; M. –*** *medium permeability tank with French oak****; H –*** *high permeability tank with French oak****; M.C.. –*** *medium permeability tank without staves*

The total content of sulphur dioxide in wine has decreased over the months (Table 2). This is probably due to the reactions with phenolic compounds and contact with oxygen. The good initial alcohol content of the wine has a good microbiological protection.

The volatile acidity, expressed as acetic acid in g/l in table 2, is a little bit elevated in HDPE this probably due to an entrance of oxygen through the permeable material. The legal limits for volatile acidity depend on the country and the style of the wine. According to Fugelsang, in the United States, the legal limit is 1.4 g/l for table reds, 1.2 g/l for table whites, 1.7 g/l for dessert reds and 1.5 g/l for dessert the whites. These limitations change when looking at the state of California where 1.2 g/l is the legal limit for table reds and 1.1 g/l for table whites. Finally, in the European Union it is 1.2 g/l for table reds and 1.08 g/l for table whites, even concentrations are subject to change depending on the country (Fugelsang, 1997; Neeley, 2004).

4.3 Spectrophotometric analysis of the wine

As regards the spectrophotometric analysis of phenolic compounds in wine at time 0, the entity of the wine had to be determined in order to assess its suitability for good aging. In 2019 Piga G. evaluated the evolution of phenolic compounds in a red wine with aging in polyethylene tanks and the presence of wooden staves, then a comparison was made to evaluate any differences in the entity of the wine.

Table 3 Analysis of Piga G. wine control of the 2019's thesis

	t0
	Control
IC (UA)	7.90
TC (UA)	0.66
TOTAL ANT (mg/L)	381.97
ANT COLOR (mg/L)	31.67
IONIZATION INDEX (%)	8.28%
TOTAL PIGMENTS (UA)	23.40
POLIMERIZATI PIGMENTS (UA)	2.58
POLIMERIZATION INDEX (%)	11.05%
TOTAL PHENOLS (mg/L)	1303.54
NO FLAVONOID (UA)	158.45
FLAVONOID (UA)	1164.41
TANNIN POWER (NTU/ml)	187.75

Table 4 Analysis of the control wine 2021

Control	t0
IC (UA)	10
TC (UA)	0.67
Total Ant (mg/l)	267.15
Ant Color (mg/l)	36.21
Ionization Index (%)	14.72
Total pigments (UA)	19.51
Polimerizati pigments (UA)	3.74
Polimerization index (%)	19.12
Total phenols (mg/l)	1295
No Flavonoid (UA)	167
Flavonoid (UA)	1127.92
Tannin Power (NTU/ml)	436.71

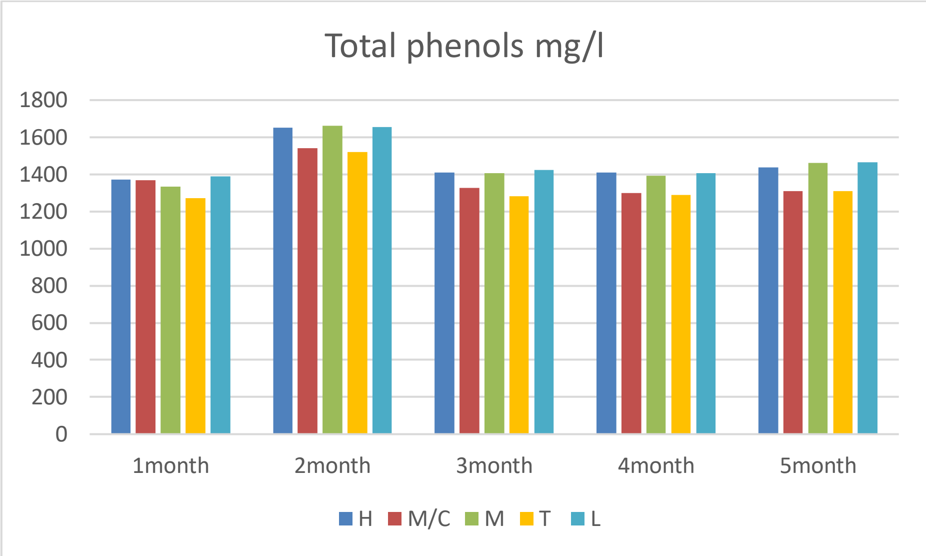
Wines obtained from the same varieties clearly show similar data, but not the same as what changes is the vintage factor, but as we can see the quantity of phenolic compounds in general but in more detail for example total phenols, flavonoids, total anthocyanins have good values for excellent aging.

4.4 Evolution of the phenolic compounds

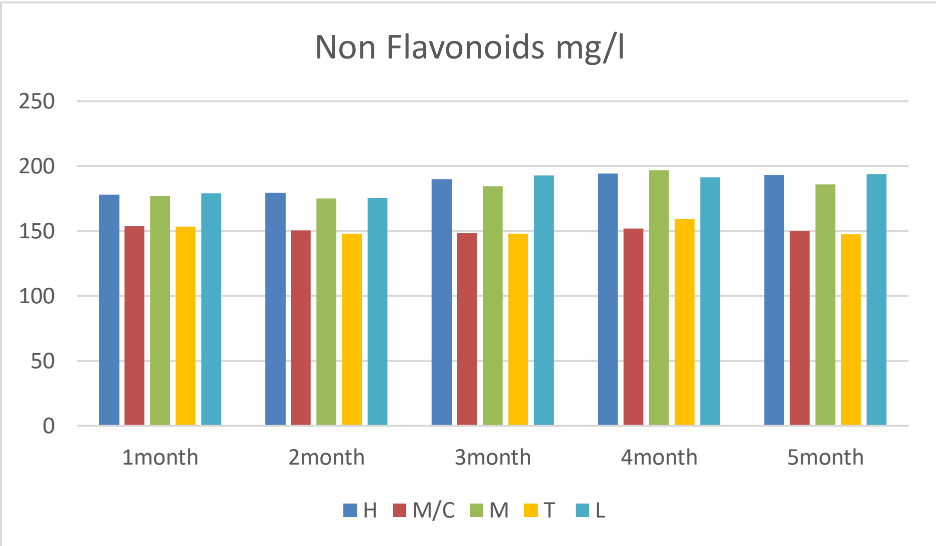
For all the studied wine we had evolutions regarding total phenols, flavonoid phenols, non-flavonoid phenols, tannin power, polymeric and total pigment parameters.

The graphs of the no-flavonoids (fig. 10), of the total phenols in (fig. 10), of the flavonoids (fig. 11), of tannin power (fig. 11), pigment polymerization index (fig. 12) and of the total and polymeric pigments (fig. 12).

In the following figures we can see the evolution of the wines in the various HDPE in the 5 months of ageing between the control (environment in reduction) and the various permeability of HDPE (environment with oxidations subsidiaries).



***Wines aged in: Test** – stainless steel; **L.** – low permeability tank with French oak; **M.** – medium permeability tank with French oak; **H.** – high permeability tank with French oak; **M.C.** – medium permeability tank without french oak*



***Wines aged in: Test** – stainless steel; **L.** – low permeability tank with French oak; **M.** – medium permeability tank with French oak; **H.** – high permeability tank with French oak; **M.C.** – medium permeability tank without french oak*

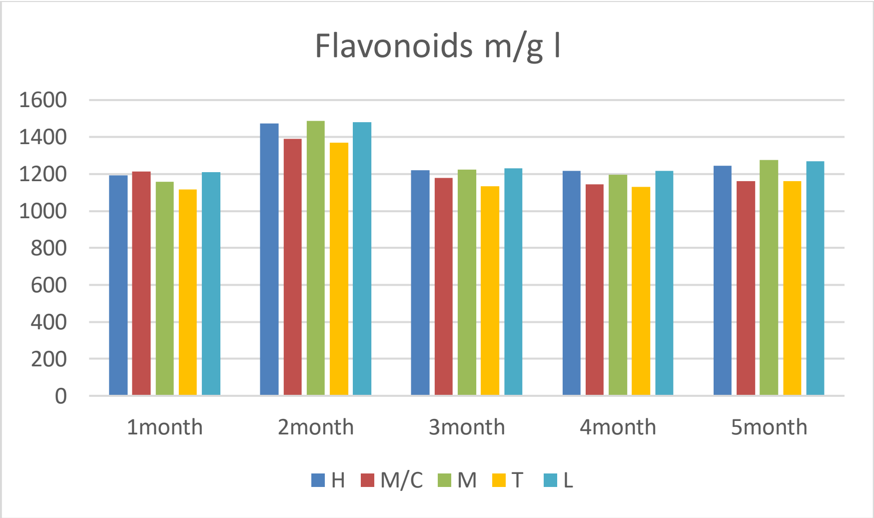
Figure 10 Total phenols and no-flavonoid phenols parameters of the wines ageing in different tanks, from 5 months ageing

For the total of the phenols expressed in mg/l we can note, in figure 10, an evolution of them during the ageing months with differences depending on the HDPE in which is contain.

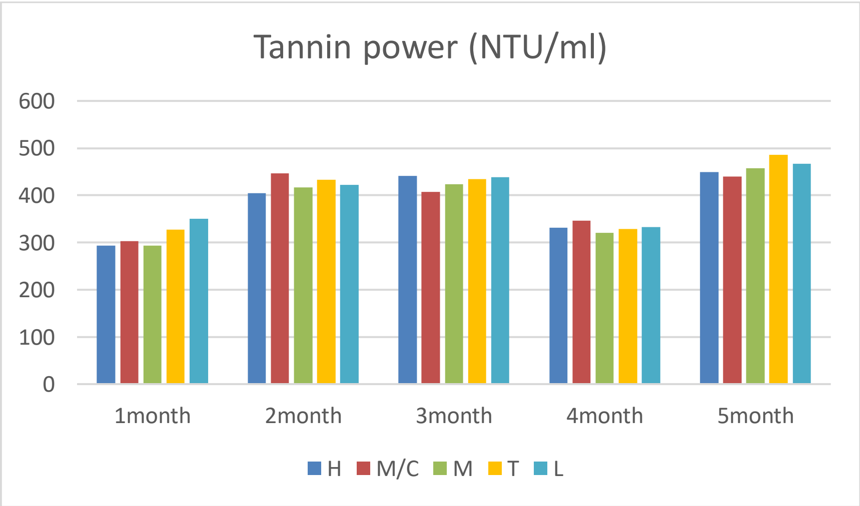
During the ageing months and for HDPE we have an initial decrease of these compounds, mainly due to precipitation, oxidation and complexations with other compounds, but in May (2 month of aging) the quantity of total phenols increase probably for the extraction of phenolic compounds from the wood.

The maceration period of this wine was about 15 days, allowing the phenolic acids to be transferred also from the pulp, as well as from the skins, this may have contributed to increasing the quantity of no-flavonoid phenols in this wine. For the quantity of no-flavonoids, we can see from the figure 10 that in the months of May, June and July there was a slight increase, with oscillations, of these compounds due to their extraction from the wood. The component of no-flavonoid compounds shows an increase in the component in HDPE due to the presence of wood in them, on the contrary in control (woodless wine) a decrease due to precipitation can be noted or complexations.

The kinetics of polyphenol extraction does not seem to follow a linear increase or decrease (Karvela et al., 2008), these evolutions could be explained by the fact that during the conservation period a complex balance is reached between phenolic substances extracted from wood and colouring material precipitated in red wine.



Wines aged in: Test*– stainless steel;* ***L*** *– low permeability tank with French oak;* ***M.*** *– medium permeability tank with French oak;* ***H.*** *– high permeability tank with French oak;* ***M.C.*** *– medium permeability tank without oak*

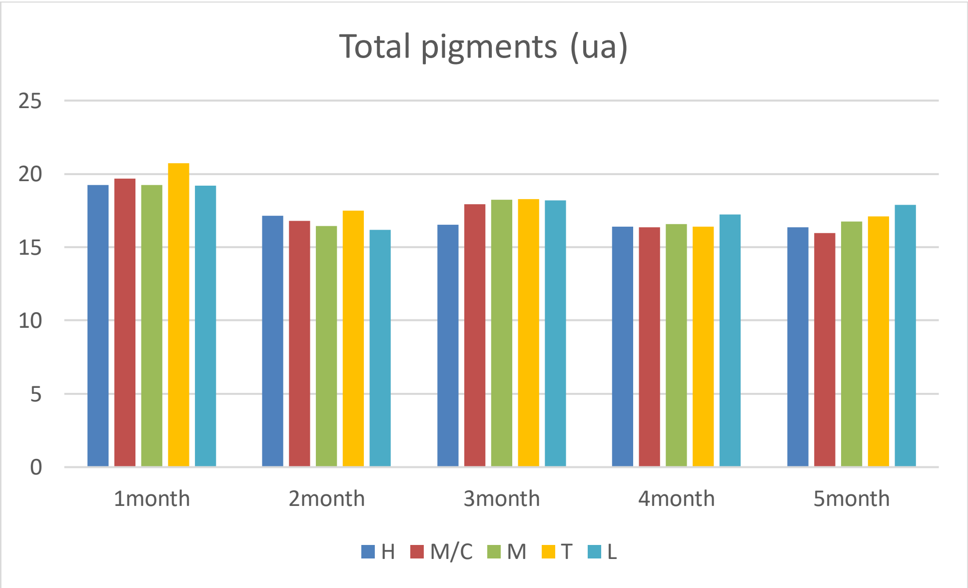


Wines aged in: Test*– stainless steel;* ***L*** *– low permeability tank with French oak;* ***M.*** *– medium permeability tank with French oak;* ***H.*** *– high permeability tank with French oak;* ***M.C.*** *– medium permeability tank without oak*

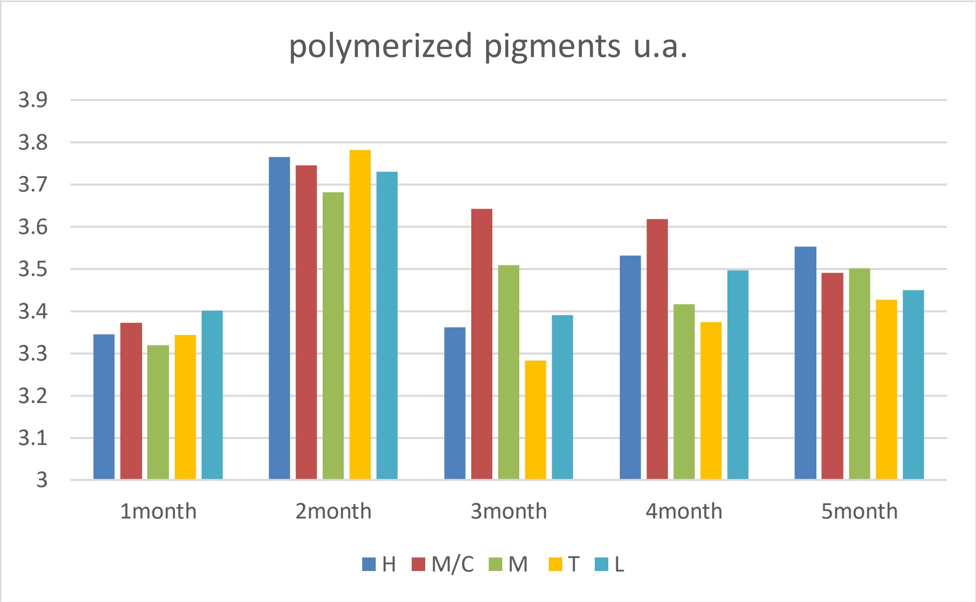
Figure 11 Flavonoid phenols and tannin power parameters of the wines ageing in different tank, from 5 months storage

For the flavonoid (fig. 11), during the 5 months of storage an oscillation of the values occurred in all the tanks that contained the wine. This is due to the transfer of the compounds from the wood to the wine, in fact the quantity of flavonoids is increased in all the wines in contact with wood (Jindra and Gallender, 1987). Furthermore, in the HDPE we can notice a greater increase in the flavonoid component due to the presence of the wood from which the wood tannins are extracted. This result would make us predict a greater concentration in the tannin content, which is consistent with the increase in the tannin power parameter as shown in figure 11 the amount of tannin power increased significantly in all the samples in the second month, remained stable in the third month and a little decrease during the 4month but during the 5 month the value was stable.

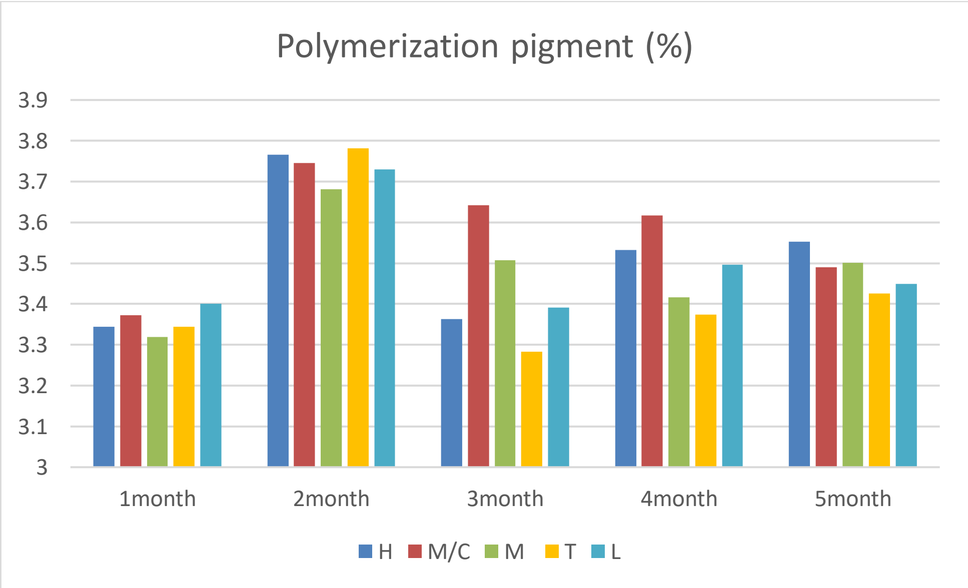
The ellagitannins extracted from the wood are in continuous transformation giving rise to other compounds such as ethyl derivatives and flavano-ellagitannins (Jourdes et al., 2011), moreover, a similar behaviour is expected by gallotannins. The polyphenols extracted from the wood during ageing can take part in this process also increasing the final level of tannin power, which leads to an influence of the sensorial profile of the wine, changing its bitterness and astringency.



Wines aged in: Test*– stainless steel;* ***L*** *– low permeability tank with French oak;* ***M.*** *– medium permeability tank with French oak;* ***H.*** *– high permeability tank with French oak;* ***M.C.*** *– medium permeability tank without oak*



Wines aged in: Test*– stainless steel;* ***L*** *– low permeability tank with French oak;* ***M.*** *– medium permeability tank with French oak;* ***H.*** *– high permeability tank with French oak;* ***M.C.*** *– medium permeability tank without oak*



Wines aged in: Test *– stainless steel;* ***L.*** *– low permeability tank with French oak;* ***M.*** *– medium permeability tank with French oak;* ***H.*** *– high permeability tank with French oak;* ***M.C.*** *– medium permeability tank without frenchoak*

Figure 12 Total pigments, polymeric pigments and pigment polymerized index parameters of the wines ageing in different tank, from 5 months storage

Among all the wines studied, there were appreciable differences in the total pigments (fig. 12), which may indicate that there is a slight stabilization of the colour of the wine due at the decrease in total pigments (fig. 12), at the decrease in polymeric pigments (fig. 12) and at the increase of the pigment polymerization index (fig. 12).

The results of the polymeric pigments showed a light increase in all aged wines.

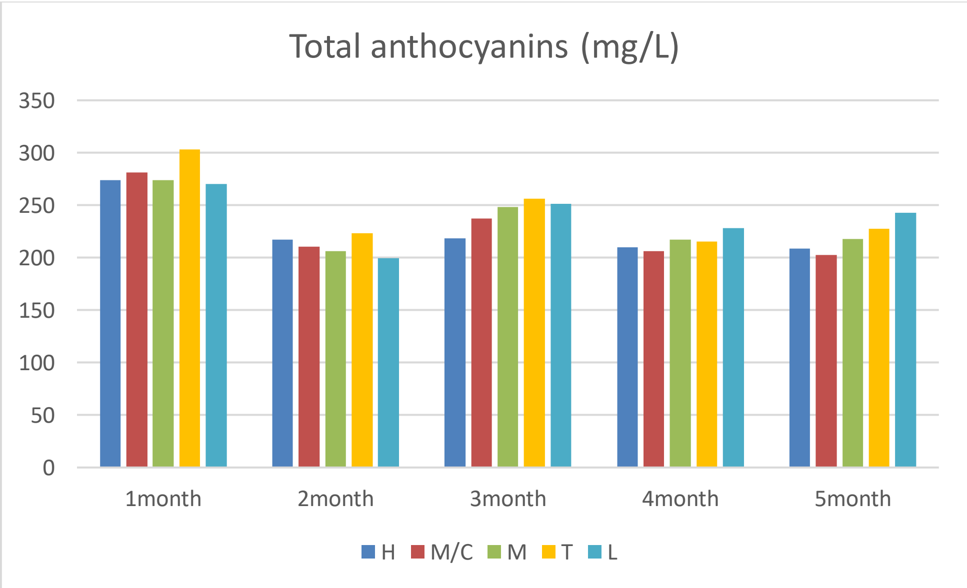
A difference also lies of the polymerization pigment index (fig. 12) depending on the kind of tank and wood used in the various months.

The stabilization of the polymeric fraction of the pigments and the increase of the pigment polymerization index, as shown in figure 12 differences between the wine that has not aged in contact with wood (control) and the wine in the HDPE polymeric tanks (with wood), since in the wines in contact with the wood is believed to be due to the reaction of anthocyanins with proanthocyanidins, both for direct polymerization and for acetaldehyde bridges.

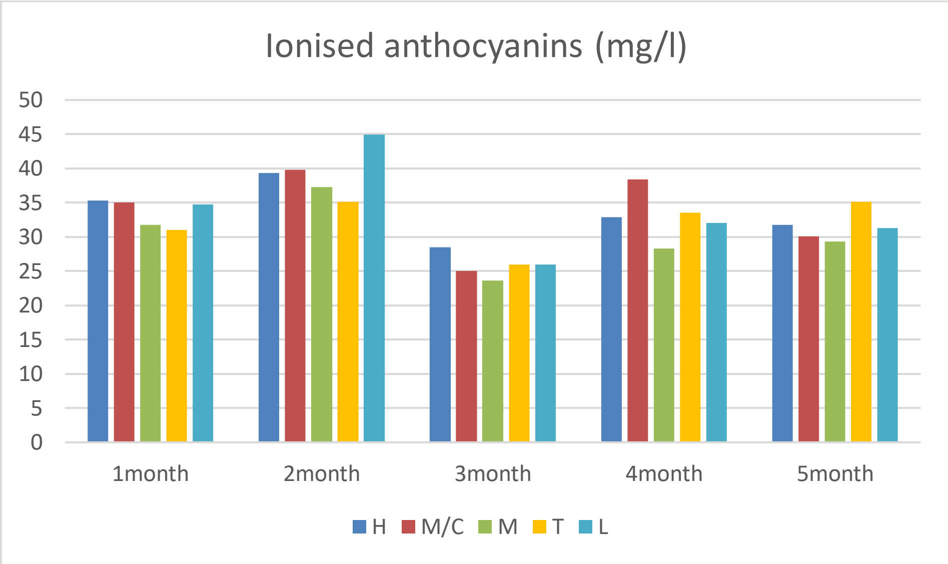
In the figure 12 we can see a costant decrease of the total pigments, a little increase of the polymeric pigments with an increase of the pigment polymerization index, this makes us understand that there is a polymerization of the pigments in the wine that will lead to its stability.

4.5 Evolution of the colour component

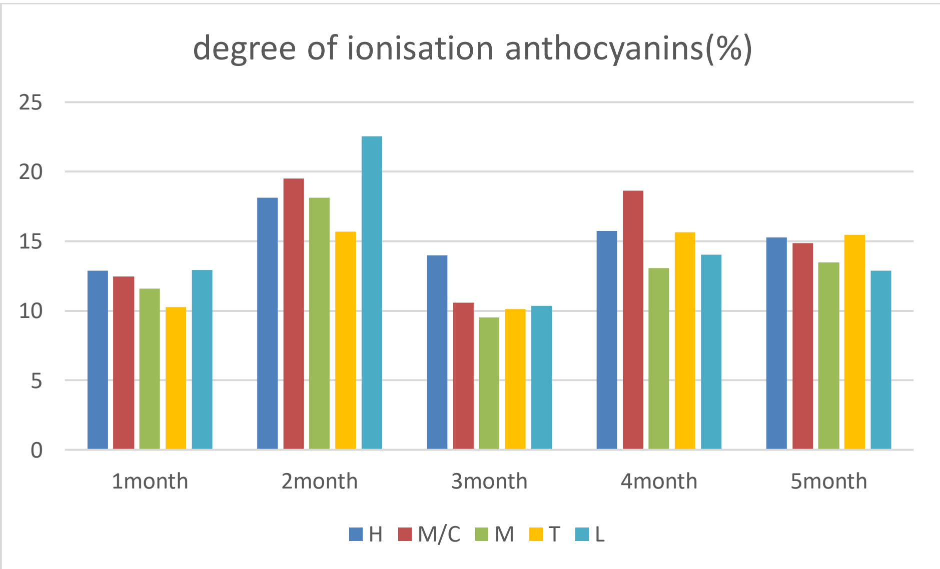
Below are the graphs of total anthocyanins and of coloured anthocyanins and of degree of ionization of anthocyanins (fig. 13), of colour intensity and of tonality (fig. 14), of the clarity, tonality and the angle of hue (fig.15), of copigmentation (fig.16).



Wines aged in: Test*– stainless steel;* ***L*** *– low permeability tank with French oak;* ***M.*** *– medium permeability tank with French oak;* ***H.*** *– high permeability tank with French oak;* ***M.C.*** *– medium permeability tank without oak*



Wines aged in: Test*– stainless steel;* ***L*** *– low permeability tank with French oak;* ***M.*** *– medium permeability tank with French oak;* ***H.*** *– high permeability tank with French oak;* ***M.C.*** *– medium permeability tank without oak*



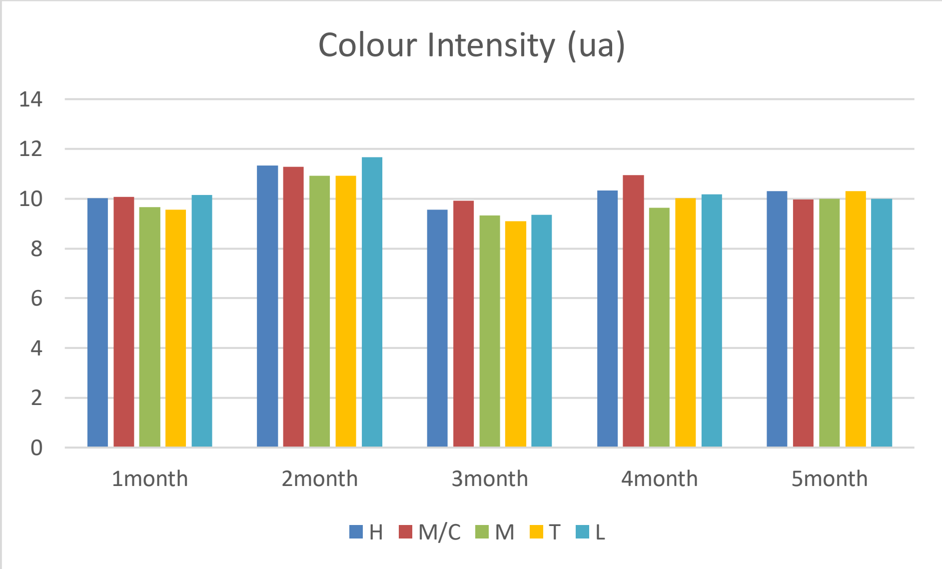
Wines aged in: Test *– stainless steel;* ***L.*** *– low permeability tank with French oak;* ***M.*** *– medium permeability tank with French oak;* ***H.*** *– high permeability tank with French oak;* ***M.C.*** *– medium permeability tank without french oak*

Figure 13 Total anthocyanins, coloured anthocyanins and degree of ionization of anthocyanins parameters of the wines ageing in different tank, from 5 months storage

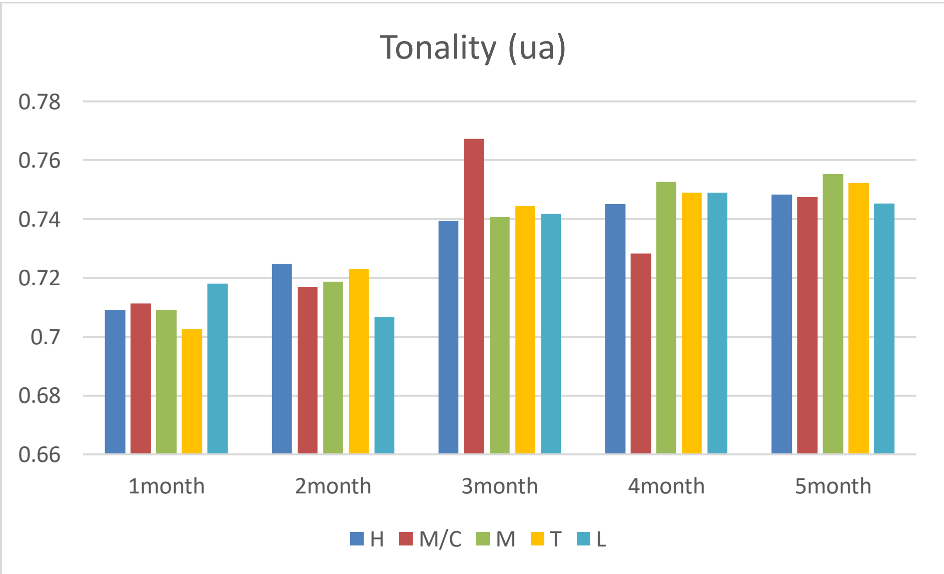
During the 5 months of the experiment the amount of total anthocyanins (fig. 13) showed a decrease in all the tanks more or less linear caused by polymerizations and precipitation of the colouring matter. Within the various tanks the effect on coloured anthocyanins, which are the predominant pigments in young red wines, has little fluctuations with an increase and a subsequent decrease, but stay stable in the months.

The little decrease of the coloured anthocyanins (fig. 13) parameter can be explained by the fact that the

anthocyanin profile changes from monomeric pigments to polymers, due to their interaction ((-) - epicathequin and (+) - cathequin) with colourless phenolic compounds (Liao et al., 1992).
The degree of ionization of the anthocyanins (fig. 13) had an increase during the months and then stabilized between, due to the fact that the total anthocyanins decrease for precipitation and the coloured anthocyanins stay stable during the months.



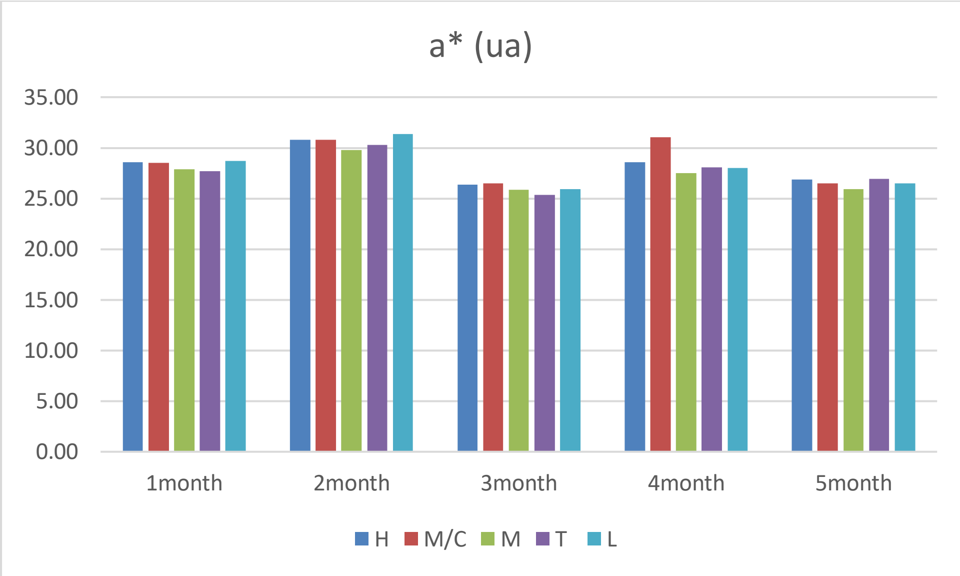
Wines aged in: Test*– stainless steel;* ***L*** *– low permeability tank with French oak;* ***M.*** *– medium permeability tank with French oak;* ***H.*** *– high permeability tank with French oak;* ***M.C.*** *– medium permeability tank without oak*



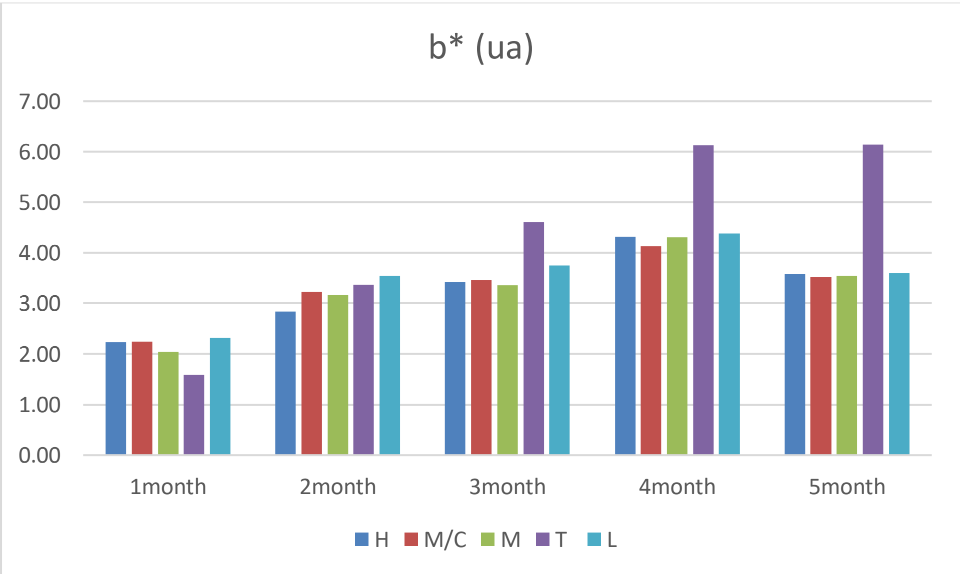
Wines aged in: Test*– stainless steel;* ***L*** *– low permeability tank with French oak;* ***M.*** *– medium permeability tank with French oak;* ***H.*** *– high permeability tank with French oak;* ***M.C.*** *– medium permeability tank without oak*

Figure 14 Colour intensity, colour tonality parameters of the wines ageing in different tank, from 5 months storage

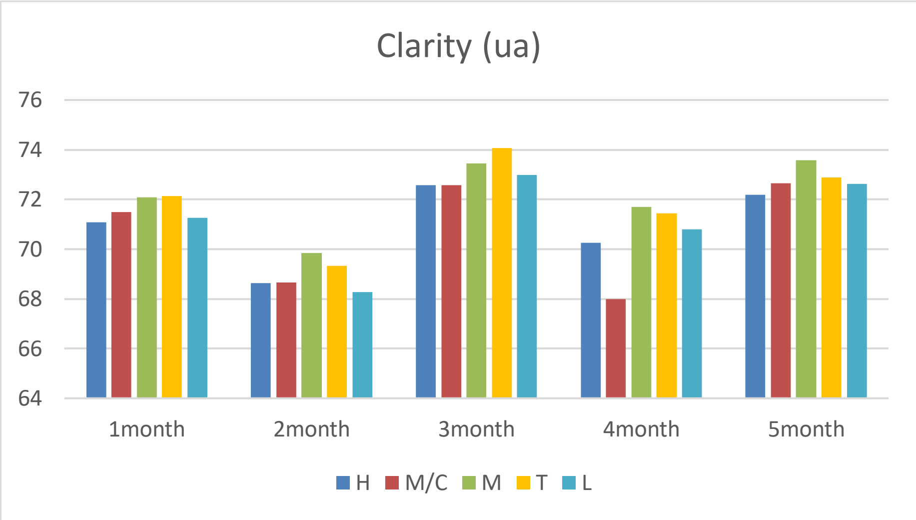
The intensity of the colour (fig. 14) during the experiment showed little oscillations during the months of aging. A parameter that allows us to characterize the colour is the tonality (fig. 14), this parameter has shown differences between all the wines with a sequential increase, which means that the wine has evolved slightly for a predominant yellow colour on a red colour.



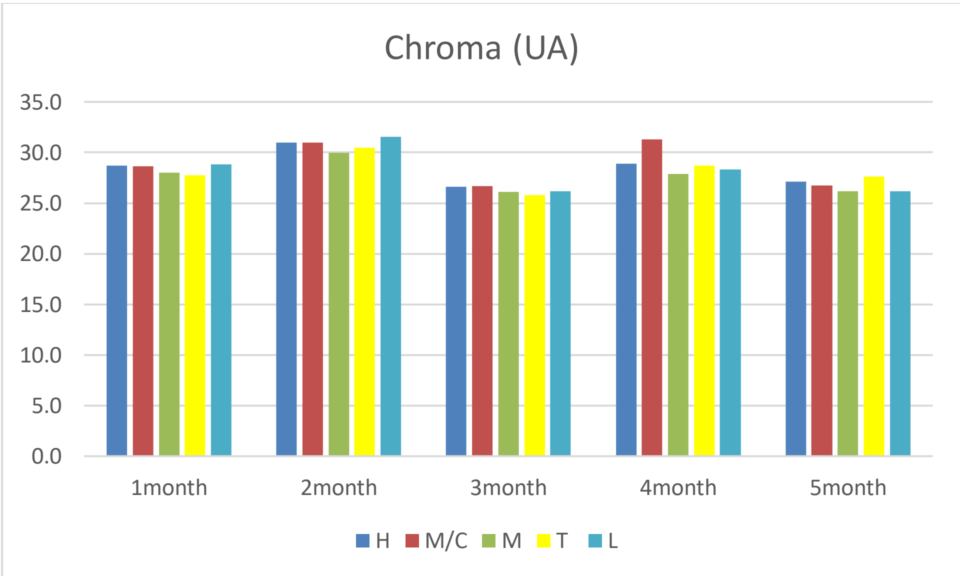
***Wines aged in: Test** – stainless steel; **L.** – low permeability tank with French oak; **M.** – medium permeability tank with French oak; **H.** – high permeability tank with French oak; **M.C.** – medium permeability tank without french oak*



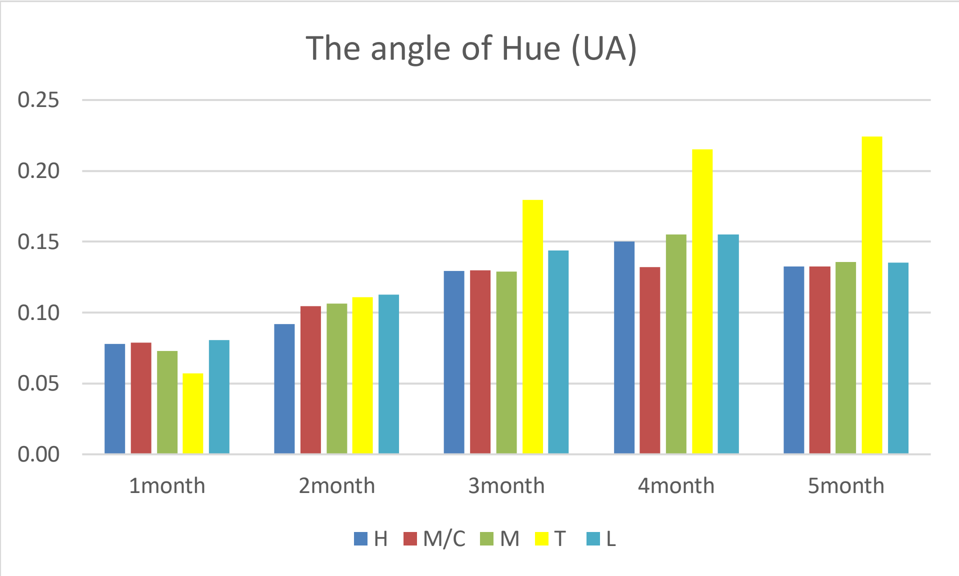
***Wines aged in: Test** – stainless steel; **L.** – low permeability tank with French oak; **M.** – medium permeability tank with French oak; **H.** – high permeability tank with French oak; **M.C.** – medium permeability tank without french oak*



Wines aged in: Test *– stainless steel;* ***L.*** *– low permeability tank with French oak;* ***M.*** *– medium permeability tank with French oak;* ***H.*** *– high permeability tank with French oak;* ***M.C.*** *– medium permeability tank without french oak*

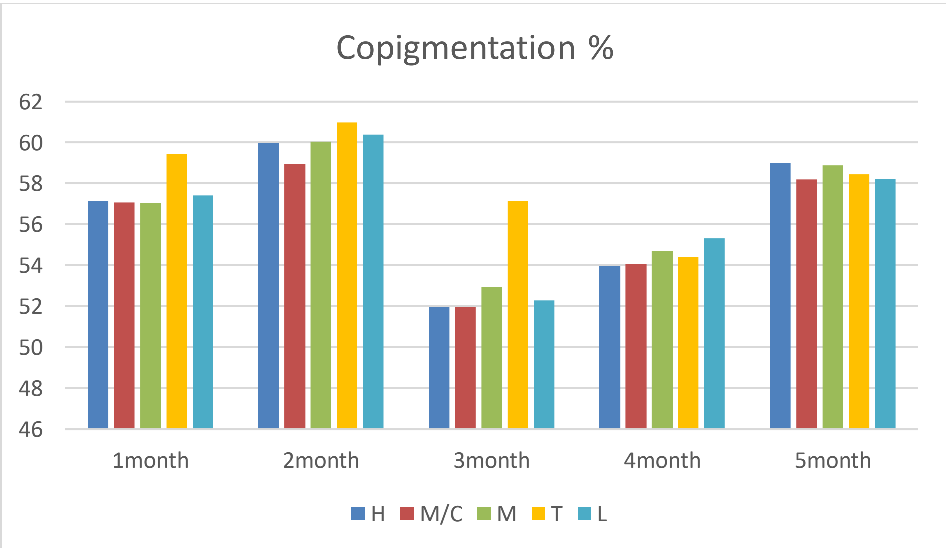


Wines aged in: Test *– stainless steel;* ***L.*** *– low permeability tank with French oak;* ***M.*** *– medium permeability tank with French oak;* ***H.*** *– high permeability tank with French oak;* ***M.C.*** *– medium permeability tank without french oak*



***Wines aged in: Test** – stainless steel; **L.** – low permeability tank with French oak; **M.** – medium permeability tank with French oak; **H.** – high permeability tank with French oak; **M.C.** – medium permeability tank without french oak*

Figure 15 a, b*, Clarity, chroma and the angle of hue (CIElab) parameters of the wines ageing in different tank, from 5 months storage*



***Wines aged in: Test**– stainless steel; **L.**– low permeability tank with French oak; **M.** – medium permeability tank with French oak; **H.** – high permeability tank with French oak; **M.C.** – medium permeability tank without french oak*

Figure 16 Copigmentation parameter of the wines ageing in different tank, from 5 months storage

As for the Cielab parameters (figure 15), the clarity shows an initial decrease and a subsequent increase in all the theses, while the chroma shows not many differences in all the results of the thesis, but the hue angle graph shows that during aging the test thesis increases its initial value. Furthermore b* parameter resulted higher in stainless steel and, as consequence, the hue of the same samples showed higher values.

In the figure 16 we can see the trend of the percentage of the colour (%) obtained from the copigmented anthocyanins, after the second month there is a highly decrease, then the value remained almost constant in the following months.

The decrease in the colour percentage of the copigmented anthocyanins it is due to the fact that during aging co-pigments, considered as anthocyanins storage, begin to decline. This event is probably due to oxygen and reactions that occur in aging that leads them to form polymer pigments, for this reason we can see an higher value for the thesis with a tank with an high permeability to the oxygen and for the test.

5 CONCLUSION

The aim of this work was the characterization of the phenolic composition of wines aged in four HDPE tanks with different permeability, through monthly chemical analyzes on aged wines.

There are already studies conducted in ISA on the evolution of the phenolic composition of wines in HDPE tanks (Piga G., 2019).

The study was based on a stainless steel still and four HDPE tanks with different permeability and with French staves inside and allowed to see the main differences between HDPE and control over the months, which was possible thanks to the excel analysis. . from the data obtained from the chemical analysis. The wine examined presented excellent parameters for good aging, as regards the spectrophotometric analysis on total phenols the results are interesting because in HDPE with high, medium and low permeability with French oak staves there is a slight increase during the months of aging, due to the presence of wood and the polymerization of phenolic compounds, excellent for stabilizing the color of the wine. As for the amount of no-flavonoids it increases only in HDPE tanks with wood and decreases slightly in the control, while the amount of flavonoids increases in all tanks but more in the tanks with wood, similarly to the increase in tannic power and as already said to the content of total phenols; in fact it is noted that in the analysis of the polymerization index there is an increase in the values in all the theses but less in the test.

As for the colored anthocyanins, the quantity remains almost stable with a slight decrease in June, but this is normal as with the stabilization of the wine, that is during the aging, a large part of colored anthocyanins precipitate or complex, while the other parameters follow the normal aging of the wine, it is also noted that the intensity and tonality of the color we have in the test shows a slightly higher value than the Flexcube or in the case of the analysis concerning co-pigmentation we see how the final values show a slight increase compared to the initial ones.

As for the Cielab parameters, they show interesting values that justify the evolution of the wine color, for example the final parameters of all the theses in the clarity graph show higher values than at the beginning, in the case of the chroma graph the parameters show approximately constant values, while the results obtained in the angle of hue graph show an interesting increase on all theses.

In conclusion, the study based on the evolution of phenolic compounds using Hdpe tanks has led to good results, given that the wine has excellent final results and organoleptically does not show any alterations.

The results shown are not definitive, as the study is longer in order to fully understand the use of these tanks to obtain quality wines.

It is interesting to do a sensory analysis for a relationship with the chemical variation, also carrying out statistical analyzes. Therefore, it is necessary to continue work on this topic.

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