

# Characterisation on mineral composition of wine spirits produced by applying different ageing technologies

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Dissertation to obtain a Master's Degree in  
**Viticulture and Enology Engineering**

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## **Acknowledgements**

I would like to thank Professor Catarino for the great support she has given me for the construction of this thesis project, both for the period of analysis in the laboratory, thanks also to the support of the laboratory tutor Joanna, and for the period of thesis writing.

I would like to thank the University of Turin in Asti and the University of Lisbon because they have allowed me to grow both professionally and humanly, and have given me a unique university experience, thanks to the professionalism of the teaching staff.

I would like to thank my family, my parents Sergio and Giusi, my sister Marta, my grandparents and my aunts and uncles for the support they gave me during these two years spent between Asti and Lisbon. They have been a fixed point that has allowed me to move forward, both in happy and difficult times.

I would like to thank Francesco (Ciccio), we met in Asti, and in a short time complicity was created that allowed us to establish a strong friendship, which also led us to share a house in Lisbon and many experiences that have left a positive mark on me.

I would like to thank my lifelong friends, even if from a distance, they have always made me understand that they were there for any advice and support. Gabriele, like a brother, has inspired me throughout this journey, he has stimulated me with his desire to give his best in everything he does, he has been a friend and a point of reference throughout these years. Daniele, a sincere friend and companion along with Andrea and Omar of the experience in Lisbon, I am happy to have shared this period with him and other unforgettable moments. Fabio, Luciano, Edoardo, Giovanni, Davide and all the other friends are the foundations of my life, with whom I have shared many happy moments.

I would also like to thank my Italian colleagues who shared the university experience in Lisbon, especially the Sicilians Marco and Samuele, who welcomed me like a brother with Massimo's kitchen.

I would like to thank my colleagues from Asti, although we didn't see each other much because of the pandemic, we had a wonderful relationship, especially Michele, Piergiorgio, Nino, Alessandro, Simone and Luca, and my colleagues Rita, Franca, Valentina and Silvia. In particular, I would also like to thank my colleagues who, like me, came from Asti and shared the university experience in Lisbon, including Edoardo, Clara and Roberta.

## Abstract

The traditional ageing of wine spirits in wood barrels allows to obtain high quality products, with improved sensory characteristics. However, this technology presents several economical disadvantages, such as the high cost of new barrels, costs associated to the need of maintenance and replacement of the barrels, and the long period of time required for ageing. Furthermore, because wood is an exhaustible raw material, it also has sustainability drawbacks. Thus it is important to study in depth alternative ageing technologies, such as that using wood staves and micro-oxygenation in stainless steel tanks.

This study was carried out within the broader R&D project “Oxyrebrand - Oxidation Reactions: a key towards a novel and sustainable wine brandy ageing technology” (POCI-01-0145-FEDER-027819), which main objective is to develop a novel and sustainable wine spirit ageing technology based on the knowledge of underlying chemical mechanism.

This work aimed to evaluate the influence of the alternative ageing technology (wood staves combined with different levels of micro-oxygenation) in comparison with the traditional one (barrel ageing), using chestnut wood (*Castanea sativa* Mill.), on the mineral composition of the wine spirits. In addition the evolution of the physico-chemical characteristics of the corresponding wine spirits during bottle ageing was assessed.

The distillate and corresponding wine spirits (after 12 months of ageing) were characterized in terms of mineral composition (Al, As, Co, K, Li, Mg, Mn, Mo, Na, Ni, Rb, Sr, and Zn) by ICP-MS, and in their physico-chemical composition, namely alcoholic strength, acidity-related parameters and dry extract (after 6 months of bottle ageing).

In the experimental conditions of this study, no significant effect of the ageing technology was observed regarding the above mentioned analytical parameters, with exception for Sr element. Wood ageing increased the concentrations of several elements and resulted in the depletion of a few elements, being the final concentrations for all the elements quite low. Also for physico-chemical composition no significant differences were found between the different ageing modalities.

**Key-words:** mineral composition, physico-chemical composition, wine spirit, ageing, chestnut wood.

## Resumo

O envelhecimento de aguardente vínica em vasilha é uma prática tradicional que resulta na obtenção de produtos de elevada qualidade sensorial. No entanto, esta prática apresenta algumas desvantagens em termos de sustentabilidade, devido ao elevado custo das vasilhas, custos de substituição e manutenção das mesmas, requerendo um longo período para o envelhecimento, e ainda o facto de a madeira ser uma matéria-prima natural esgotável. Por esse motivo, é importante o estudo e desenvolvimento de tecnologias de envelhecimento alternativas, como por exemplo a que recorre à utilização de fragmentos de madeira combinada com micro-oxigenação, em depósitos de aço inoxidável.

O presente estudo foi desenvolvido no âmbito do projecto de I&D Oxyrebrand, “Oxyrebrand - Oxidation Reactions: a key towards a novel and sustainable wine brandy ageing technology” (POCI-01-0145-FEDER-027819), que tem por principal objectivo o desenvolvimento de uma inovadora e mais sustentável tecnologia de envelhecimento, com base no conhecimento dos mecanismos químicos subjacentes.

Foi avaliada a influência de uma tecnologia de envelhecimento alternativa (utilização de aduelas de madeira em combinação com diferentes níveis de micro-oxigenação), em comparação com a tecnologia de envelhecimento tradicional (envelhecimento em vasilha), utilizando madeira de castanheiro (*Castanea sativa* Mill.), na composição mineral das aguardentes vínicas. Adicionalmente, foi avaliada a composição físico-química das aguardentes resultantes do ensaio de envelhecimento, durante o estágio em garrafa.

O destilado utilizado no ensaio de envelhecimento e aguardentes envelhecidas correspondentes (ao fim 12 meses de envelhecimento) foram analisados em termos de composição mineral (Al, As, Co, K, Li, Mg, Mn, Mo, Na, Ni, Rb, Sr, and Zn) por ICP-MS, e composição físico-química geral, nomeadamente teor alcoólico, parâmetros relacionados com acidez, e extracto seco (ao fim de 6 meses de envelhecimento em garrafa).

Nas condições experimentais deste estudo, não foi observado efeito significativo da tecnologia de envelhecimento no que respeita às características analíticas anteriormente referidas, com excepção do elemento Sr. O envelhecimento pelo sistema tradicional e pelo sistema alternativo resultou no aumento de concentração de vários elementos minerais e decréscimo de outros, sendo no entanto de destacar as concentrações finais extremamente baixas. Também no que respeita à composição físico-química não foram observadas diferenças significativas entre as diferentes modalidades de envelhecimento.

**Palavras-chave:** composição mineral, composição físico-química, aguardente vínica, envelhecimento, madeira de castanheiro.

## Resumo alargado

A aguardente vínica é um produto vitivinícola derivado do vinho, com uma história secular, e tradição de produção pelos principais países europeus. Uma etapa fundamental da sua produção, com um efeito importantíssimo nas suas características sensoriais, é o processo de envelhecimento, tradicionalmente realizado em vasilhas de madeira. Este processo enriquece o produto nas suas diferentes dimensões sensoriais, tais como a cor e aroma. Nos últimos anos, contudo, este sistema de envelhecimento tem-se revelado menos sustentável, devido aos elevados custos associados à aquisição de vasilhas novas, sua manutenção, substituição e reciclagem, assim como com o facto de a madeira ser uma matéria-prima não inesgotável.

Por esta razão, têm sido procuradas tecnologias de envelhecimento alternativas, envolvendo a utilização de outros tipos de recipientes, por exemplo depósitos de aço-inoxidável, com utilização de fragmentos de madeira e em combinação com micro-oxigenação, numa tentativa de simular os fenómenos de extracção e permeação de oxigénio que acontecem nas vasilhas de madeira.

O presente estudo foi realizado no âmbito projecto de I&D Oxyrebrand, “Oxyrebrand - Oxidation Reactions: a key towards a novel and sustainable wine brandy ageing technology” (POCI-01-0145-FEDER-027819), visando o desenvolvimento de uma inovadora e mais sustentável tecnologia de envelhecimento, com base no conhecimento dos mecanismos químicos subjacentes.

Pretendeu-se avaliar a influência de uma tecnologia de envelhecimento alternativa, com recurso à utilização de aduelas de madeira em combinação com diferentes níveis de micro-oxigenação, em comparação com a tecnologia de envelhecimento tradicional (envelhecimento em vasilha), utilizando madeira de castanheiro (*Castanea sativa* Mill.), após o período de envelhecimento de 12 meses, na composição mineral das aguardentes vínicas obtidas. Adicionalmente, foi avaliada a composição físico-química das aguardentes resultantes do ensaio de envelhecimento, após 6 meses de envelhecimento em garrafa.

O destilado utilizado no ensaio de envelhecimento e aguardentes envelhecidas correspondentes foram analisados em termos de composição mineral (Al, As, Co, K, Li, Mg, Mn, Mo, Na, Ni, Rb, Sr, and Zn) por ICP-MS, e composição físico-química geral (teor alcoólico, parâmetros relacionados com acidez, e extracto seco) aplicando os protocolos analíticos descritos pela OIV.

Os elementos Al, As, Co, K, Li, Mg, Mn, Mo, Na, Ni, Rb, Sr e Zn foram quantificados no destilado (início do ensaio de envelhecimento) e nas aguardentes correspondentes às diferentes modalidades de envelhecimento, ao fim de 12 meses de estágio, através de um método semi-quantitativo por ICP-MS, no Laboratório de Análise Mineral do INIAV-Dois Portos. Não foram encontradas diferenças significativas entre as tecnologias de envelhecimento, excepto para o elemento Sr. O envelhecimento pelo sistema tradicional e pelo sistema alternativo resultou no aumento de concentração de vários elementos minerais e decréscimo de outros, sendo no entanto de destacar as concentrações finais extremamente baixas, na ordem de ng/L.

No que respeita à composição físico-química, foram avaliados o teor alcoólico, acidez total, acidez fixa, acidez volátil, pH e extracto seco total, aquando do engarrafamento e 6 após o mesmo. Também para estes parâmetros, não foram encontradas diferenças significativas entre as diferentes aguardentes, com valores próximos para todas as modalidades de envelhecimento. Por conseguinte, é importante que sejam realizados mais estudos sobre estas tecnologias a fim de se poder simular com precisão as tecnologias tradicionais através de tecnologias alternativas mais sustentáveis.

**Palavras-chave:** composição mineral, composição físico-química, aguardente vínica, envelhecimento, madeira de castanheiro.

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## 1. General introduction and objectives of the work

Aged wine spirit (WS) is one of the well known alcoholic beverages in the world, from the point of view of production, trade and consumption. Its production originates in the traditional European wine-growing countries, where it had, in addition to an important historical role, also a fundamental socio-economic role (Canas, 2017). Among the European countries famous for its production we find France with its regions of Armagnac (15<sup>th</sup> century) and that of Cognac (16<sup>th</sup> century), with the production of aged WS among the best-selling and best known (Canas, 2017). In Italy, WS is commonly called wine spirit or Italian brandy and originates in the eighth century (Papo, 1987). With its Lourinhã region, Portugal is one of the most famous producing countries on the international scene. The historical references regarding the production of this country date back to the early 1900s, while in 1992 the area was defined as a denomination for the production of aged WS (Canas, 2017).

According to the traditional process of WS production, after the vinification of the grapes, the distillation of the wine takes place. Distillation is an operation that allows to separate two or more substances that are part of a mixture, working on the difference in the boiling points of the same substances, or on their difference in volatility (Sinnott et al., 2005). Its importance, towards the composition of the WS, depends on the fact that this distillation is carried out under the correct conditions (Avakiants, 1992; Orriols et al., 2008). Besides wine characteristics, there are different aspects concerning the distillation operation that allow a higher quality of the distillate, among these first of all the periodic maintenance of the distiller, then a correct separation of the various parts deriving from the first distillation, the head and tail of the first distillate. Precisely for this last function of the process, higher quality is allowed in the product, this because between the parts of the first distillate, such as the tail, they allow the appearance of unpleasant aromas that worsen the quality of the WS, therefore the fact of cutting these parts enriches quality the WS, without neglecting the fact of the elimination of methanol through the elimination of the head as well (Avakiants, 1992; Caldeira et al., 2006; Faisca et al., 2012).

An important phase to give quality to the WS product is that of ageing. The European legislation establishes, according to the EU Reg. N° 787/2019, that the wine spirit can be aged for at least six months in wooden containers with a capacity of less than 1000 L or one year in wooden containers with a capacity greater than 1000 L. On the other hand, in the case of WS with Denomination of Origin, the minimum maturation time complies with European legislation or is even more restrictive. Among these Appellations there is the example of ageing of at least one year for Armagnac and two years for Lourinhã and Cognac.

The aged wine distillate has unique characteristics, concerning color, aroma and taste. The ageing technologies to which the distillate must be subjected foresee a period of several years, in order to favor a better extraction of compounds from the wood and a series of reactions that are triggered. Traditionally, in the production technology, the ageing of the wine distillate is carried out in wooden barrels. In this part of the process various physico-chemical phenomena take place that enrich the product, including extraction reactions and various reactions that occur between the wood and the distillate itself. Among the various changes there is that in terms of color, for a product that has more green/yellow, golden and amber reflections. After ageing the aged WS is refined according to various operations, such as that of blending, dilution with demineralized water, this is made to adjust its alcoholic content to the value defined for the trade in each Designation of Origin and before bottling takes place the filtration.

However, traditional technology has drawbacks in its application in the production process. First of all for the cost of the barrels, because after a certain number of uses they must be changed, then they take up a lot of space in the cellar, allowing the evaporation of ethanol and therefore its loss through the pores of the wood. Another problem is the sustainability of the product itself, as it is an exhaustible raw material. For these reasons, in recent years, new technologies have been investigated and experimented to optimize the production process, in order to achieve similar characteristics through the use of new containers and wood fragments, with the use of micro-oxygenation (MOX). The objective is to guarantee similar level of quality, through higher speed, given by the greater control of the process and the lower cost of the material. In fact, using a steel container allows saving in not having to sanitize or buy back the wooden barrels, replacing the latter with fragments, placed in a quantity proportional to the wood present in the barrel to simulate the contact that would be created in a barrel.

This study was carried out within the broader R&D project “Oxyrebrand - Oxidation Reactions: a key towards a novel and sustainable wine brandy ageing technology” (POCI-01-0145-FEDER-027819), which main objective is to develop a novel and sustainable WS ageing technology based on the knowledge of underlying chemical mechanism.

The study developed for this master thesis aims to evaluate, at the end of a period of 12 months, the influence of an alternative ageing technology (wood staves combined with different levels of MOX and nitrogen) in comparison with the traditional ageing technology (barrel ageing), in both cases using chestnut wood, on the mineral elements composition of the WS. In addition the evolution of the physico-chemical characteristics of the corresponding wine spirits during bottle ageing was assessed.

## 2. Literature review

### 2.1 Wine spirit

According to the International Organization of Vine and Wine (OIV), WS is defined as a "*spirit drink obtained from the distillation of wine, fortified wine or wine with added wine distillate, or from redistillation of a wine distillate whose final product preserves the aroma and flavor of its raw materials*". These conditions have been established according to the regulations of the European Union (Reg. UE 2019/787). This regulation establishes and guarantees the definition, description, presentation, labeling and protection of geographical indications of spirit drinks. Furthermore, this document also provides guidelines on the requirements that the WS must present, although there are more restrictive conditions that are imposed by some particular producing regions. Among the requirements they must present are a minimum alcoholic strength of 37.5% vol., a maximum methanol content of 200 g/hL of 100% vol. alcohol, a level of volatile substances equal to or greater than 125 g/hL of 100% vol. alcohol, caramel can be added only to adapt the color, alcohol cannot be added and moreover it cannot be flavored, not excluding traditional production methods. Furthermore, WS can be sweetened, to improve its taste, with the addition of sweetening products, which can have a maximum of 20 g/L, expressed as invert sugar (EU regulation 787/2019).

It is important that the grapes, from which the wine to be distilled for the production of WS derives, have a high production yield (Belchior et al., 2015). This is normally correlated with grapes presenting a high fixed acidity, a low sugar content, in order to obtain a wine with a not too alcoholic content, more suitable for the product to be obtained. It is also important that they do not have sanitary problems, caused by microbiological contamination, this is important because during the vinification the use of sulfur dioxide (SO<sub>2</sub>) must be avoided, in order not to find it later in the WS since there would be residues of SO<sub>2</sub> that could lead to the formation of sulfur compounds such as mercaptans, compounds that are carriers of unpleasant aromas (Belchior et al., 2015).

The legislation "EU regulation 787/2019" also establishes regulations concerning the ageing time, in which the WS can be aged for at least six months in wooden containers with a capacity of less than 1000 L or one year in wooden containers with a capacity greater than 1000 L, with more severe restrictions regarding some Designations of Origin (DO), such as the one concerning, among other things, the DO of the WS of this project, that is to say that of the Lourinhã, in which the minimum ageing time is two years.

Figure 1 shows the map of Portugal with the various production areas with the various DO, in which is highlighted the DO Lourinhã.



**Fig 1. Protected Designations of Origin for WS in Portugal, where the Lourinhã area has the exclusivity of the DOC for WS.** Source: IVV 2019.

## **2.2 Distillation process**

Distillation is a crucial operation, which must take place with the right attention and precautions, to obtain a qualitative WS and to bring a good quality product to ageing. Distillation is a technique that allows the separation of two or more substances that are part of a mixture, working on the difference in the boiling points of the same substances, or on their difference in volatility (Sinnott et al., 2005). The final product is the distillate that derives from the vapors that condense to produce the same, which will have different characteristics compared to the starting product (Berk, 2018). Distillation such as alcoholic fermentation, have left important traces in the history of the culture and tradition of the various civilizations that have followed one another (Russell et al., 2003).

Different origins are attributed to this technology, for some it derives from both the Far East and the Mediterranean area, according to others from ancient China (Russell et al., 2003; Plouvier et al., 2008). The first alembic, a key instrument of distillation, was created by the Arabs, who then spread to Europe for the production of essences and spirits between the

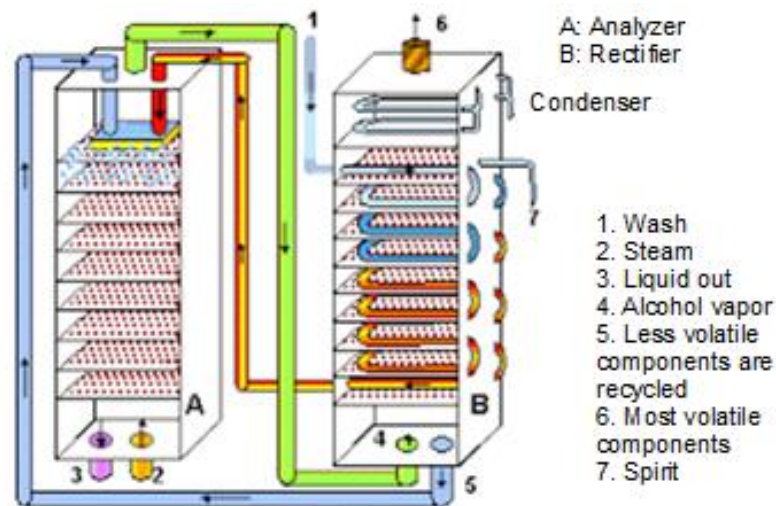
eighth and fifteenth centuries. The name was changed by the Arabs themselves to "ambic" from the previous Greek name of "ambix", later called "Al ambic". This instrument originally had few attached instruments, in fact it consisted of a closed container that was heated, connected to a condenser and then to a container to receive the final distillate (Plouvier, 2008). Initially medical and mystical properties were attributed to the production of this process, in fact the distillate was immediately called "aqua vitae", or water of life from the Latin. The Portuguese name instead "Aguardente" seems to derive from the Latin designation "aquam ardentem" often used by alchemists (Belchior et al., 2015). The wine spirit derives from the distillation of wine, this must be obtained from grapes with high fixed acidity, low concentration of sugars and without problems from a health point of view, this is due to the fact that the vinification must be obtained without the use of sulfur dioxide (SO<sub>2</sub>), in order not to risk finding it in the WS or that there may be compounds with sulfur origins, in both cases the risk is to have a drink with unpleasant aromas with the consequence of having a poor quality. These grapes can only come from varieties that have a higher yield, because the volume that can be obtained are lower than those of the wine itself (Belchior et al., 2015).

There are two different distillation methods for making spirit. One is the batch method, in which a particular distiller is used, called "Charantais". In this type of alembic a double distillation is carried out to increase the alcohol content and consequently to have a better quality of the same WS. In this system, two fractions of the distillate are separated, called "heads" and "tails", due to the presence of methanol and other unwanted substances, and then the "heart" fraction is taken to the second distillation, where, however, a further separation of the fractions of head and tail to avoid unpleasant odors, which would naturally worsen the product (Belchior et al., 2015). Figure 2 shows the structure of the still with discontinuous distillation and each of its components.



**Fig 2. Discontinuous Alembic Still.** Source: <https://cognacecotognata.wordpress.com/2012/01/27/cognac-la-distillazione-lalambicco-1-parte/>

Unlike this method, the second, the continuous distillation method produces a uniform distillate, in this case in fact the separation of the heads or tails does not occur. This is because in this process there is a continuous feeding of wine and therefore there is a higher productivity, which depends on the continuous acquisition of the distillate, with consequent equilibrium of the system (Belchior et al., 2015). Figure 3 displays the continuous still distillation and each of its components.



**Fig 3. Continuous Still Distillation.** Source: <https://atinnkeeperslodge.wordpress.com>

The distillation apparatus are therefore equipped with rectifiers or have rectifying capitals, also introduced in the twentieth century, with the aim of obtaining purer distillates. This adjustment, as already mentioned, can be discontinuous or continuous (Castroreale, 2017).

In discontinuous rectification, the passage from one distillation to the other occurs through the filling of the boiler, since it is necessary to interrupt the distillation between the two. The device that is usually used is composed of a concentration column with 40-50 plates and with the following connected devices:

- Boiler: which is the instrument where the liquid to be distilled boils and the vapors come out;
- Distillation, concentration and rectification column: it is connected to the boiler, as already mentioned it has a variable number of reflux or retrogradation dishes (from 24 to 50, generally between 40 and 50), where the vapors arrive in the condensed liquid present in the dishes of the column. The flow that is created in this case, therefore between condensation and vaporization, is continuous, constant;

- Partial vapor condenser: this is connected to the top of the previous column system, which ensures the reflux in the column (it is important that this reflux occurs, because it allows the liquid of a part of the condensed vapors to be re-admitted to the lower plate of the column above);

- Refrigerant: connected to the condenser, here the most volatile substances and the final distillate condense, which is then collected in a flow tube that has taps, "multiple taps", where the different fractions, the Head, the Tail and the Heart .

With this system there is the formation of an ascending and descending current, which are respectively that of the vapors and the other of the condensate, this allows the enrichment of the vapors of substances and volatiles and also allows the separation of the various phases and substances present in the hydroalcoholic mixture (Castroreale, 2017).

The best quality distillate, the part of the alcoholic liquid obtained from the first phase of distillation, is then redistilled to recover the alcohol contained in them. The final reflux tube gives me both the alcohol content (% vol), to understand the quality of the distillate, and the separation of Head, Tail and Heart based on the values released. The drawback of batch rectification is that it does not completely eliminate the top fraction of the distillate, especially for substances such as ethyl isobutyrate which is retained more by alcohol (Castroreale, 2017).

Instead, the continuous rectification has improved this distillation process through columns that make up the main system. One column is that of distillation, concentration and rectification, which has about 40-50 dishes divided into two sections, with the aim of going to exhaust and concentrate the alcohol, also going to exhaust the water or Tail products by eliminating them. This column allows to recover the fraction of the Heart, with its compounds and the alcohol attached to it at an high alcoholic strenght. The other column, on the other hand, is the purifying column, mainly used to eliminate the components of the head, has about 24 plates, also divided into two sections (Castroreale, 2017).

The system is called continuous, or without interruption of the process as in the discontinuous one seen above, where the separation of the phlegm took place or in any case where a recharging cycle always had to be carried out. This may have other tools attached: a distillation column to exhaust the alcohol of the mixture to be distilled; a concentration column, where the alcohol concentration and alcohol content increase; the purification column, allows the elimination of the fractions of Head and Tail; the rectification column eliminates residual impurities; a demethylating column to remove methanol ( $\text{CH}_3\text{OH}$ ). There are plants that even have only a large column, with continuous feeding, exhaustive for all the operations related to the process (Catroreale, 2017).

## 2.3 Ageing process

The ageing process is a crucial phase of the WS production. In this phase the wine distillate takes the title of aged, where it improves from a sensorial, physico-chemical, and therefore qualitative point of view, reaching fullness and completeness (Anjos et al., 2020).

The reactions that occur during this process are physico-chemical, these are extraction and oxidation phenomas, which mainly concern the interactions between the distillate and the woody substances with a low molecular weight (Anjos et al., 2020). From the point of view of chemical and sensory changes, both under a quantitative and qualitative aspect for the volatile and non-volatile components of the product, changes occur in both color, aroma and taste (Anjos et al., 2020).

According to European legislation (EU Regulation 2019/787), the WS must be aged for at least one year in contact with wood, or only six months, but in this case in wooden containers with a capacity of less than 1000 L. There are wine distillates that have the certification relating to their geographical denomination, so in this case the ageing period is at least one year for types such as Armagnac, two years for Cognac and Lourinhã (Canas, 2017). Figure 4 displays different WS from different ageing times, for this reason each one has a different and typical colour linked to the ageing time.



**Fig 4. Different WS derived from different types of ageing times.** Source: <https://projects.iniav.pt/oxyrebrand/index.php/en/wine-spirit-and-its-ageing/the-ageing-process>.

### 2.3.1 Barrel ageing

In traditional production, ageing generally takes place in wooden barrels (mainly oak or chestnut), during which there are several modifications that improve the WS (Canas et al., 2012).

The barrels not only have the function of container, but are active vessels that release chemical compounds that improve the physico-chemical and sensory properties of the product they contain (Carpena et al., 2020). They allow multiple reactions that explain the color, provide tannins and also allow the product to be oxygenated. Wooden barrels release a set of compounds and mineral elements, with an improvement in the organoleptic properties of the WS (Carpena et al., 2020). This will add to the wine distillate greater complexity, flavor and longevity. This step is essential to complete the manufacturing process. The phenomenon of oxidation of WS is an important phase during ageing and this occurs through the passage of oxygen between the pores, the wooden slaves and the bunghole of the barrel (Belchior et al., 1982).

The Figure 5 displays typical barrels that are used for the traditional ageing process.



**Fig 5. Image depicting barrels used for traditional ageing.** Source: <https://projects.iniav.pt/oxyrebrand/index.php/en/wine-spirit-and-its-ageing/the-ageing-process>

### 2.3.2 Alternative ageing technology

The aforementioned traditional technology has costs for the capital invested in the purchase and management of the barrels. Furthermore, the barrels take up a lot of space in the cellar, they do not have an unlimited duration and through their use, during the ageing period, a loss of product due to its evaporation cannot be avoided (Canas et al., 2012). As for the sustainability of the product, the demand for barrels is always higher than the supply, so costs have increased due to the limited availability of the wood raw material (Canas et al., 2012).

For some time, however, studies have been launched in order to remedy the traditional technology that has these disadvantages, replacing it with more sustainable alternative technologies (Anjos et al., 2020). In this regard, the research has mainly focused on the use as an addition of woody material in the distillate, with subsequent conservation of the same reasonably no longer in wooden barrels, but in stainless steel tanks, as in the case for other alcoholic beverages (Anjos et al., 2020). These alternative technologies at the first make the ageing process more sustainable. It simplify the ageing process, while ensuring that the compounds of woody origin are released into the WS, with the addition of pieces of wood to the drink stored in stainless steel tanks, or other material. This allows a shortening of the ageing period, while improving the quality of the final product. This alternative ageing aims to reproduce the physico-chemical processes involved in the traditional ageing of alcoholic beverages, in our case of WS. In this context, not only the wood species are determining factors, but also the use of oxygen is of considerable importance. To assure this ageing process there is therefore the MOX technique, which mimics the oxygenation phenomena that occurs naturally inside the wooden barrel, the pores and the bunghole with the passage of oxygen (Gómez-Plaza et al., 2011; del Alamo-Sanza and Nevares, 2014). Some studies indicate that this technique produce good results regarding the phenolic composition and color of WS (Anjos et al., 2020). It has been reported that the MOX process carried out in stainless steel tanks has replicated the same properties of traditional technology in excellent conditions, the direct supply of oxygen allowing to speed up ageing, with a greater dissolution of oxygen (Canas et al., 2019). MOX combined with the use of wood staves increase the pool of phenolic components and single components with low molecular weight (such as syringic acid, ellagic acid, vanillin and others) (Canas et al., 2019).

Canas et al. (2009) compared alternative systems for the ageing of wine spirits, considering oxygenation and wood shape effects. Wooden barrels (traditional system) and small volume stainless-steel tanks with wood tablets, with and without oxygenation (alternative systems) were used for ageing. In both cases Portuguese chestnut (*Castanea sativa Mill.*), high level toasting, was used. The quantity of pieces of wood has been calculated in order to reproduce the surface/volume ratio of the barrel.. The oxygenation (4.5 mg of oxygen/L) was carried out by applying air with a special device in the medium height of the tanks, after 60, 90, 120, 180 and 360 days of ageing. Under these experimental conditions, the results obtained showed how the ageing technology affects both the color of the WS and their chemical composition. In a completed study of Canas et al. 2016, the WS aged with micro-oxygenation showed not significant effect of the wooden barrels and staves on the consumption of oxygen with MOX, but related to the redox status of WS. The results of Canas et al. 2009 also showed that the WS aged in cask had a lower chromatic evolution than those aged with an alternative method.

Furthermore, the effects of oxidation on the traditional method showed greater degradation of the dry extract, or of the non-phenolic compounds, and of the phenolic compounds, such as syringaldehyde, syringic acid and ellagic acid.

The shape of the wood plays a significant role in the dissolved oxygen of the WS. WS traditionally aged in wooden barrels showed a lower dissolved oxygen level than WS aged with alternative technology (with MOX and wood chips in stainless steel tanks). Another difference that can be highlighted between traditional and alternative ageing, in these conditions, is the heat treatment that the wood undergoes during roasting, and therefore also the behavior of the resulting compounds. This difference derives from the fact that in the case of the alternative method the pieces of wood, at the same level of toasting as the wooden barrels, have a higher degree of toasting over the entire surface of the wood itself. This is due to the fact that the barrel, unlike the pieces of wood, undergoes a different roasting process and also the different shape leads to a consequence of a different effect. The consequence therefore leads to a lower content of extractable low molecular weight compounds, caused by the greater intensity of the heat treatment on the different shape of the pieces of wood (Canas et al., 2009). Nevertheless, the redox status of the WS seems to be the most important factor (Canas et al., 2016).

#### ***2.4 Role of the wood***

For several centuries wood has been used for food usage and to make the barrels, used as containers for beverages. Since the quality it brought to the product was discovered, it was used not only for wine, but also for other beverages already in use at the time, such as beer, whiskey and other types of spirits and liqueurs (Carpena et al., 2020). Mainly, chemical modifications occur due to contact with wood, which are the exchange of wood compounds, in particular ellagitannins, between the wood and the product present inside the barrel (Carpena et al., 2020). In general, these physico-chemical processes, which take place between the WS and the wood, derive from these different phenomena (Canas, 2017):

- extraction of wood constituents including mineral elements
- decomposition of wood biopolymers, including lignin, hemicellulose and cellulose, by subsequent release of these derivative compounds in the distillate;
- chemical reactions of mineral elements (extractable wood compounds);
- chemical reactions of distilled compounds only;
- chemical reactions between extractable wood compounds and distilled compounds;

- evaporation of volatile compounds and concentration of volatile and non-volatile compounds;
- formation of hydrogen bonds between ethanol and water;

Among the phenomena described, for the improvement of the overall quality of the distillate for the chemical composition, as for the sensory composition, the most important process is that of the release of phenolic compounds, of those with low molecular weight, and of tannins, therefore of compounds removable wood. As already mentioned, the oxidation phenomena involving these compounds and those of the distillate are also important. This phenomenon is caused by the diffusion of oxygen through the wooden slaves. Precisely for these fundamental processes, it is important to underline the importance of some factors, including:

- the type of botanical species used and the intrinsic characteristics in the technology used for the formation of the barrels, such as seasoning, maturation and heat treatment of the barrel, and finally also the size of the barrel is very important;
- the conditions of temperature, air circulation and relative humidity in the cellar;
- the operations that are performed during the ageing process itself.

Among these operations, for example, there is the filling of the barrels with the same distillate (topping up operation), this happens because during this period the distillate evaporates from the barrel. Furthermore, another operation can be the addition of water to lower the alcohol content, or the agitation to which it is stimulated to homogenize and to improve the extraction of wood compounds. A good phenolic extraction also improves the final sensory properties, in fact it has been shown that there is a close correlation between phenolic composition and color. Not only that, but this aspect also affects the aroma and flavor (Canas, 2017).

Among the most used botanical species for beverages ageing are oak (*Quercus robur L.*, *Quercus petraea*, *Quercus alba*) and chestnut (*Castanea sativa Mill.*). Their wood for the construction of the barrels was already used from the beginning for its resistance, flexibility and in part, also for its impermeability, although the latter concept is a little different for its wood essences (Carpena et al., 2020). Oak is the most used wood with chestnut, but there are further studies that also report woods suitable for these functions, such as cherry or acacia. Obviously, all distillates can be aged with the use of other materials, such as concrete or stainless-steel. However, the use of these materials without the use of wood does not bring the same characteristics in composition of elements and unique sensoriality to the product, which instead is given by ageing in wood.

The toasting process to which the wood is subjected modifies both the quantity and the quality of the extractable substances that it can release (Chatonnet et al., 1999). Toasting can be

carried out under different conditions of time and heat intensity, which can be low, medium and high. These various levels of toasting yield different volatile and semi-volatile compounds depending on the thermal degradation that the wood has undergone. In fact, as the toasting increases, it is noticed how even the flavor of the wood approaches a smoky flavor (Chatonnet et al., 1999; Carpena et al., 2020). A thermodegradation occurs for compounds such as carbohydrates, namely cellulose and hemicelluloses (obtaining furanic aldehydes), for lignin (phenolic aldehydes and volatile phenols), and through the phenomenon of dehydration, brought about by high temperatures, there is the formation of lactones (Chira & Teissedre, 2013; Carpena et al., 2020).

The heat treatment, the toasting, carried out on oak and chestnut wood is of considerable importance for the pool of extractable compounds that can then release into the WS when it comes into contact (Canas, 2017). This treatment is part of the work to be done for the manufacture of the barrel or the preparation of the wood fragments (Canas, 2017). There are several techniques to carry it out, the French technique uses fire, while the American one uses heated steam to bend the staves and then the fire (Chatonnet et al., 1989). In Europe, the barrels are heated over a fire and through various techniques of spraying and plugging with water the staves are folded, a fold that can create the concave shape for the barrel, without it breaking (Canas et al., 2007). After these steps, the barrel is placed on a fire to allow the surface to be heated and to cause significant toasting. This step is essential to modify the structure, physical properties and chemical composition of the wood, which give important characteristics to the WS (Hale et al., 1999; Acuña et al., 2014).

The toasting level can be classified into three different types, light, medium or heavy. These three levels depend on the relationship between the temperature used and the time taken, also depending on the type of wood used (Canas et al., 2000; Canas et al., 2007; Canas 2017). Intermediate toasting levels can also be used, including "medium plus" toasting. Different studies on the effect of toasting on WS reveal that for the same ageing time, the enrichment of wood compounds and therefore color and other more advanced sensory properties are induced by heavy toasting, and secondarily by medium toasting (Caldeira et al., 2006; Canas, 2017).

An increase in the content of phenolic aldehydes in WS was found with an increase in the toasting intensity of the barrel: about 18 mg/L, 30 mg/L and 58 mg/L under the influence of light, medium and heavy toasting, respectively (Artajona et al., 1991). In addition, an increase in the content of ellagic acid (approximately 15 mg/L and 60 mg/L), gallic acid (approximately 4 mg/L and 9 mg/L) and vanillin (approximately 0.5 mg/L and 1 mg/L) in WS aged for a period of two years in new oak barrels with light and heavy toasting levels, more marked with the

second (Rabier & Moutounet, 1991). As regards the vanillin content, an increasing vanillin content was found with the increase in the toasting intensity, with a similar behavior also found for syringaldehyde, with an increase in concentration in parallel with the increase in the toasting level. Levels of compounds such as coniferaldehyde and synapaldehyde were found with a large increase between light and medium toasting and a slight decrease with heavy toasting (Puech et al., 1992).

The higher the degree of toasting of the wood, the more the color of the WS evolves, so there is a contribution of greater intensity and saturation, with significant increases between levels (Canas et al., 2000). These chromatic characteristics make WS aged in heavy toasting barrels appear older than that aged in medium and light toasting barrels, this has also been observed with the use of chestnut wood (Canas, 2017). These results are related to the extraction of phenolic compounds extracted from wood (Chira & Teissedre, 2015). The oxidative phenomena of the ageing process are also responsible for the color, the higher the level of toasting and the greater there is oxygen permeability in the wood, with a greater extension of the oxidation reactions (Hale et al., 1999; Acuña et al., 2014; Canas, 2017). This phenomenon is controlled in alternative ageing with MOX.

During ageing in wood there is potential extraction of mineral elements from the wood (Litchev, 1989; Cernîsev, 2017). The contact between the WS and the wood, during this period, causes this extraction (Canas et al., 2020). This process is of fundamental importance because among the mineral elements, iron and copper can play an important role as catalysts of oxidation reactions occurring during this process (Kaya et al., 2017; Pilet et al., 2019; Canas et al., 2020).

#### 2.4.1 Types of wood: oak and chestnut

In this study, as in the project in which this thesis is affiliated, ageing on chestnut wood will be analyzed for both traditional and alternative ageing technologies. However, traditionally the most used type of wood for WS ageing is oak (also considered in the broaden project).

The botanical species of the chestnut wood is *Castanea sativa* Mill. This wood has been widely used in the Mediterranean area, due to its affordability and availability. It is characterized by greater porosity than oak, but provides high amounts of polyphenols to wine and distillate (De Rosso et al., 2009). It has been reported that chestnut wood shows higher levels of gallic acid, levels 15 times higher than native oak and 30 times higher than commercial oak samples (Madrera et al., 2010). Also as regards the levels of phenolic aldehydes (synapaldehyde, coniferaldehyde and syringaldehyde), these were higher in the chestnut (subjected to 60 min of toasting at 185°C), with values similar to those present in commercial oaks and higher than

native oak for the same toasting (Madrera et al., 2010). It was observed that the WS aged in chestnut wood had a more evolved color than the WS aged in oak wood, presenting a greater intensity of color (it has a lower "L \*"), of higher shades of red (a \*) and yellow (b \*), with higher saturation (C \*) which makes it look older than the latter (Canas, 2017).

The botanical species of oak most used for the production of barrels and for the ageing process are three: *Quercus robur* L. or *pedunculata*, has a good quality and comes mainly from the French region of Limousin and from Balkan Europe; *Quercus petraea* or *sessilis*, among the oak species, is the most valuable, for two reasons, the fiber and the compounds, such as the tertiary aromas it releases. The regions of French origin are Allier, Argonne, Bourgogne, Nevers and Vosge, but it can also come from Hungary and the Caucasus; finally, there is the *Quercus alba* L. which has an American origin (Canas, 2017).

These two types of wood, oak and chestnut, have a higher content of volatile compounds and fatty acids, compared to other types of wood (Canas, 2017).

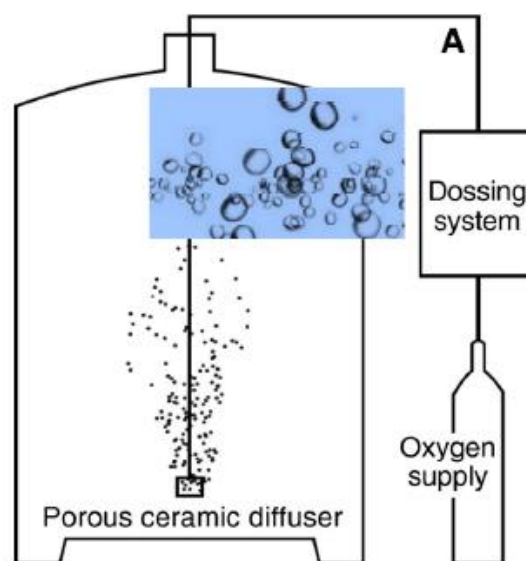
## **2.5 Micro-oxygenation treatment**

The phenomena that occur during the ageing period are controlled by the wood species used, the toasting, and the oxygen, therefore also the characteristics that will then be acquired by the aged WS (Canas et al., 2019; Granja-Soares et al., 2020). Chestnut wood, one of the most popular species for the ageing of these distillates, stood out for its high quality and faster ageing that gives the distillate, due to specific chemical and anatomical characteristics of the same wood species (Canas et al., 2019; García-Moreno et al., 2020). Even the supply of oxygen, in traditional technology, comes from the wood that is used (Del Álamo-Sanza et al., 2017).

In this scenario, however, to face the new challenges in terms of environmental sustainability and solve the problems concerning the waste of wood, an exhaustible raw material, the time and the high economic costs, alternative technologies have been tested, which mimic oxygenation of WS during ageing through MOX, since it is no longer a question of the use of barrels but of the use of wooden slaves in stainless steel containers, in order to replicate the transfer that would naturally occur in the barrel (Canas et al., 2019; Anjos et al., 2020; Granja-Soares et al., 2020). The technique consists in the calculated addition of alimentary oxygen to replicate traditional ageing (Gómez-Plaza & Cano-López, 2011).

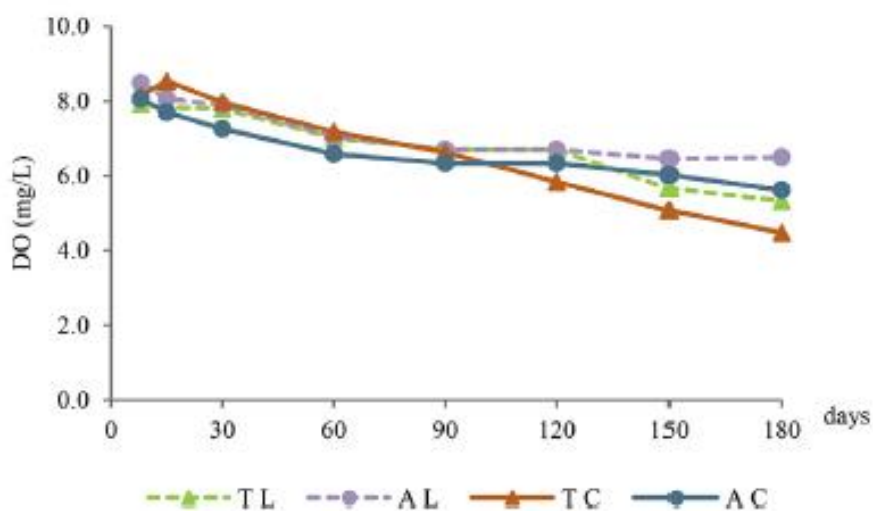
In fact, in traditional refinement, oxidation is promoted by the slow and continuous diffusion of oxygen through the wood, therefore through the space between the staves (Del Álamo-Sanza

& Nevares, 2014). With the passage of ageing time, compared to the initial phases, the wood becomes more humid, this therefore leads to a lower flow of oxygen, this because the diffusion coefficient of oxygen in the water is four orders of magnitude lower than the air (Nevares et al., 2016; Del Álamo-Sanza et al., 2017). With alternative ageing it is aimed to obtain the same results as the traditional one, but eliminating the latter problems also concerning the oxygenation of the product, thus adopting a faster and less expensive technology (Canas et al., 2019). If the product is stored in stainless steel tanks, very little oxygen can stay in contact with it, compared to storage in barrels for the passage through the pores of the wood, therefore through the MOX technique the quantities of oxygen that are bubbled, allowing to overcome this limitation. MOX is obtained by transferring a known volume of gas through a low pressure circuit inside the container where the distillate is present. Oxygen is dispersed in gaseous form, in the form of small bubbles with a diffuser that can be made of various materials, usually steel or ceramic. Among the influencing factors of this process there can be the added dose of oxygen, the size of the tank, in fact the height must be sufficient to allow the dissolution of the bubbles, the temperature of the distillate inside and the size of the oxygen bubbles. As can be seen from the image below, the oxygen cylinder is connected to a dosing system, with which, based on the characteristics of the volume present in the tank, the times and the quantity of oxygen to be introduced, this will develop this MOX. Diffusion occurs through diffusers inside the tank. In the Figure 6 are represented the various instruments used to carry out the MOX and the various connections between the cylinder that delivers alimentary oxygen with the dosing system and finally with the diffusers inside the tank (in this case their material is the ceramic).



**Fig 6. Image depicting the micro-oxygenation mechanism.** Sofia Catarino slides from the Stabilization and Ageing Wine course.

According to the study by Canas et al. (2019), the two ageing technologies were compared, the traditional one with the alternative one, the first using 250 L wooden barrels and the second with 1000 L stainless steel tanks (with MOX and use of wooden staves), using both wood of Limousin oak and chestnut for each test. This was done in order to compare the effects of these two ageing technologies, in the first 6 months, from the point of view of the chromatic characteristics of the aged WS and its phenolic composition. In these conditions, studying the evolution of oxygen dissolved (DO) in WS in the period between 0 and 180 days, in a traditional technology (TL) with barrels and Limousin wood, with a traditional technology but with the use of chestnut wood (TC), with an alternative technology with Limousin wood (AL) and an alternative technology with chestnut wood (AC). A lower concentration was observed up to the third month of ageing, between 5.5 mg/L and 8.5 mg/L of oxygen (Figure 7). In the case of traditional technology, this decrease reflects the lower release of oxygen caused by the passage of liquid in the wood. However, this phenomenon also has a second cause given by the fact that oxygen is still consumed in the various extraction and oxidation reactions (Avakiants, 1992; Cernîsev, 2017), contributing to the qualitative evolution of WS, whether they are aged in tanks in stainless steel with slaves or traditionally in barrels. These results show how the MOX carried out with the alternative ageing technology allows to perfectly emulate the passage of oxygen, as in traditional technology, also allowing savings in ageing times.



**Fig 7. Evolution of dissolved oxygen of wine spirits in the first six months of maturation according to the ageing technology (A - micro-oxygenation + staves; T - barrels) and the type of wood (L - Limousin oak; C - Chestnut).** Source: Canas et al. (2019). "DO" - dissolved oxygen (mg/L); "TL" - traditional technology with barrels and Limousin wood; "TC" - always corresponds to the

traditional technology but with the use of chestnut wood; "AL" alternative technology with Limousin wood; "AC" - alternative technology with chestnut wood.

Furthermore, again through this study it was possible to learn how the use of MOX combined with the staves was decisive in ensuring a faster extraction of the wood-derived compounds in the wine spirit, allowing a greater accumulation of single phenolic compounds to low molecular weight and higher total phenolic content than wooden barrels. It was therefore possible to obtain in the WS matured with this alternative technology a better evolution of the chromatic characteristics, since they had a lower brightness, greater saturation and greater intensity of the shades of red, yellow and brown (Canas et al., 2019).

## **2.6 Composition of wine spirit**

### 2.6.1 Physico-chemical composition

The general physico-chemical analyses of WS include various parameters which therefore serve to characterize a wine distillate or an aged WS, namely alcoholic strength, pH, acidity indexes (total, fixed and volatile acidities) and the dry extract.

Table 1 shows the results of WS samples deriving from an industrial scale process, analyzed through FT-Raman spectroscopy (Anjos et al., 2020), and that can be understood as representative for this type of WS. The table shows the various samples of WS that derive from six different modalities of ageing, for each modality the recorded times are 8, 30, 180 and 360 days (Anjos et al., 2020). These include the use of: new 250 L (TL) Limousin oak barrels (*Quercus robur* L.); new barrels of Portuguese Chestnut (*Castanea sativa* Mill.) of 250 L (TC); new Limousin oak barrels and new 250 L Portuguese chestnut barrels (made with the same proportion of both types of wood) (TM); use of stainless steel containers, with the use of fragments of oak wood and MOX (AL); use of stainless steel containers, with the use of fragments of chestnut wood and MOX (AC); use of stainless steel containers with the use of both types of wood and MOX (AM).

**Table 1. Alcoholic strength, pH, dry extract and acidity indexes of the WS aged by applying different technologies.** Source: Anjos et al., 2020.

Code	Ageing days	Alcohol Strength (% v/v)	Dry extract (g/L)	pH	Acidity (g of acetic acid/L)		
					Total	Fixed	Volatile
TL	8	77.02 ± 0.14	0.08 ± 0.01	5.05 ± 0.03	0.15 ± 0.00	0.02 ± 0.00	0.13 ± 0.00
	30	76.98 ± 0.20	0.14 ± 0.01	4.94 ± 0.02	0.16 ± 0.00	0.04 ± 0.00	0.12 ± 0.01
	180	76.64 ± 0.11	0.46 ± 0.02	4.65 ± 0.05	0.35 ± 0.02	0.07 ± 0.01	0.28 ± 0.03
	360	76.84 ± 0.08	0.80 ± 0.05	4.53 ± 0.04	0.51 ± 0.01	0.10 ± 0.00	0.42 ± 0.01
TC	8	76.77 ± 0.24	0.17 ± 0.03	4.96 ± 0.06	0.17 ± 0.01	0.04 ± 0.00	0.13 ± 0.01
	30	76.66 ± 0.23	0.31 ± 0.03	4.89 ± 0.16	0.18 ± 0.00	0.07 ± 0.00	0.12 ± 0.00
	180	76.25 ± 0.33	1.02 ± 0.08	4.37 ± 0.01	0.45 ± 0.03	0.15 ± 0.01	0.30 ± 0.02
	360	76.52 ± 0.33	1.72 ± 0.09	4.30 ± 0.02	0.65 ± 0.02	0.23 ± 0.01	0.41 ± 0.01
TM	8	76.96 ± 0.13	0.15 ± 0.02	4.93 ± 0.03	0.15 ± 0.00	0.03 ± 0.00	0.12 ± 0.00
	30	76.88 ± 0.09	0.21 ± 0.03	4.85 ± 0.02	0.19 ± 0.00	0.07 ± 0.00	0.12 ± 0.01
	180	76.42 ± 0.19	0.72 ± 0.04	4.45 ± 0.03	0.41 ± 0.01	0.11 ± 0.01	0.30 ± 0.01
	360	76.69 ± 0.11	1.22 ± 0.04	4.31 ± 0.02	0.59 ± 0.01	0.16 ± 0.00	0.43 ± 0.01
AL	8	77.01 ± 0.05	0.14 ± 0.02	4.97 ± 0.00	0.18 ± 0.00	0.04 ± 0.01	0.14 ± 0.01
	30	77.23 ± 0.13	0.20 ± 0.01	4.85 ± 0.01	0.18 ± 0.01	0.06 ± 0.00	0.12 ± 0.01
	180	77.00 ± 0.09	0.78 ± 0.01	4.59 ± 0.01	0.43 ± 0.02	0.08 ± 0.01	0.35 ± 0.02
	360	76.88 ± 0.11	1.54 ± 0.01	4.50 ± 0.01	0.52 ± 0.00	0.16 ± 0.00	0.36 ± 0.01
AC	8	77.03 ± 0.18	0.31 ± 0.02	4.66 ± 0.02	0.20 ± 0.01	0.06 ± 0.00	0.13 ± 0.01
	30	77.33 ± 0.08	0.59 ± 0.18	4.65 ± 0.08	0.25 ± 0.00	0.10 ± 0.00	0.15 ± 0.00
	180	76.97 ± 0.23	1.53 ± 0.05	4.22 ± 0.02	0.56 ± 0.02	0.20 ± 0.01	0.36 ± 0.03
	360	77.00 ± 0.05	2.70 ± 0.08	4.11 ± 0.05	0.70 ± 0.00	0.30 ± 0.00	0.40 ± 0.01
AM	8	76.74 ± 0.42	0.22 ± 0.03	4.82 ± 0.01	0.19 ± 0.01	0.06 ± 0.02	0.13 ± 0.03
	30	76.70 ± 0.50	0.33 ± 0.01	4.69 ± 0.07	0.23 ± 0.01	0.08 ± 0.01	0.14 ± 0.02
	180	76.58 ± 0.36	1.11 ± 0.02	4.41 ± 0.01	0.47 ± 0.00	0.13 ± 0.01	0.33 ± 0.01
	360	76.65 ± 0.32	2.08 ± 0.04	4.30 ± 0.01	0.58 ± 0.00	0.21 ± 0.00	0.37 ± 0.00
Wood (W)		4.7 (*)	9.0 (***)	10.8 (***)	3.6 (***)	14.9 (***)	0.3 (**)
Technology (T)		13.8 (**)	9.0 (***)	4.9 (***)	1.4 (***)	5.3 (***)	0.1 (**)
Ageing time (A)		28.0 (***)	66.6 (***)	74.4 (***)	90.5 (***)	63.3 (***)	94.2 (***)
WxS		11.7 (**)	0.5 (***)	0.5 (***)	0.5 (***)	0.7 (***)	n.s.
WxA		n.s.	5.9 (***)	2.7 (***)	2.2 (***)	11.6 (***)	n.s.
SxA		n.s.	8.5 (***)	n.s.	1.3 (***)	3.3 (***)	4.0 (***)
Error		41.8	0.4	6.9	0.6	0.8	1.5

n.s., \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001.

Results expressed as mean ± standard deviation; Means within the same row followed by different letters are significantly different ( $p < 0.05$ ); T – Traditional ageing; A – Alternative Ageing; L – Limousin oak; C – Chestnut; M – Both use of Limousin oak and chestnut.

As shown in the Table 1, the results show how the alcoholic strength decreases with the ageing time, in fact a total variation of 28% of the parameter is observed. This fact strictly depends on the fact that the ethanol evaporates during ageing, this happens more in traditional technologies with barrels than in tanks, because they have a greater porosity in the wood, precisely for this reason the WS aged with alternative technologies showed higher alcohol content (Anjos et al., 2020).

The acidity level was also influenced with the ageing time, in fact both the total, the fixed and the volatile increase with the ageing time, while the pH decreased. Volatile acidity is the index

that contributes the most to total acidity among all the samples, due to the greater release of acetic acid from the wood (Conner et al., 1999), a phenomenon mainly caused by the thermal degradation undergone by the hemicellulose of wood (Perez-Prieto et al., 2003) and also from the oxidation that acetaldehyde undergoes (Liu & Pilone, 2000). An increase in fixed acidity could also be noted, but less in the contribution to the total acidity index. As ageing progresses, another effect was the increase in dry extract, mainly due to the contact between the WS and the wood and therefore from the extraction of various wood compounds. This depends on the technology and the wood that can be used, although the increase in this parameter can be highlighted both with the use of oak and chestnut wood (Anjos et al., 2020).

### 2.6.2 Mineral composition

During the ageing process, there are various changes that affect the wood and the wine distillate. These derive from different physico-chemical processes, which can be of a dual nature, additive and subtractive, in particular involving extraction processes and oxidation reactions (Canas et al., 2020). Oxidation is a very important process that is conditioned by mineral elements that can be extracted from the wood, or are already present in the distillate (Cameàn et al., 2000). Of special importance are iron (Fe) and copper (Cu), which play the role of catalysts of oxidation reactions through the donation or acceptance of electrons (Canas et al., 2020). By this reason and also because of physico-chemical stability and safety it is of great importance to characterize the WS in terms of mineral composition.

The origin of the various elements that can be present in aged WS is mainly related to the contact with metal surfaces, e.g. during the distillation operation and during conservation and stabilization steps.. They can include potential mineral transference from the distillation device, and ageing process. Some of these elements were identified in the study reported by Szymczycha-Madeja et al. (2014), involving various distillates, including WS, analysed by atomic spectrometry. From research on the mineral elements of wine, it emerges that a large part of the metal content comes from the soil of the vineyard through the roots of the vine. This is because it is influenced by the geochemistry of the soil and the rootstock of the vine (Catarino et al., 2018). In addition, other potential sources of these elements include air pollution, soil amendments, fertilizers, pesticides, irrigation water, contact materials during transport, winemaking and ageing processes, oenological technological adjuvants and additives (Catarino et al., 2018).

Table 2 displays the concentrations of Fe and Cu in WS, reported by Canas et al. (2020) in a study carried out within the Oxyrebrand R&D project. The data was treated by ANOVA being

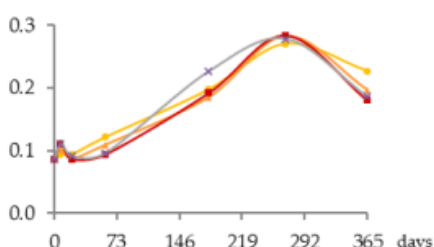
the corresponding results presented together with the corresponding graphs (Table 2). This exhibition aims to show the level of significance of the differences found and to clarify the behavior of these mineral elements according to the different ageing methods in the different ageing times (0, 8, 21, 60, 180, 270 and 365 days) (Canas et al., 2020). The different ageing modalities were as follows: O15 - oxygenation with 2 mL/L/month from 0 to 15 days and from 15 to 365 days with 0.6 mL/L/month with alimentary oxygen; O30 - oxygenation with 2 mL/L/month from 0 to 30 days and from 30 to 365 days with 0.6 mL/L/month with alimentary oxygen; O60 - oxygenation with 2 mL/L/month from 0 to 60 days and from 60 to 365 days with 0.6 mL/L/month with alimentary oxygen; N - use of 20 mL/L/month of nitrogen from 0 to 365 days. It shows the evolution of Fe and Cu concentrations in the various ageing processes, at different levels of MOX divided into times ranging from 0 to 365 days. The trend of the curves instead represents the trend of the Fe and of Cu in the table, described by the time span (from zero days to one year) of the various ageing processes on the abscissa, while on the ordinates there are the values of their concentrations.

**Table 2. Iron and copper content in WS aged according to ageing methods and ageing time.**

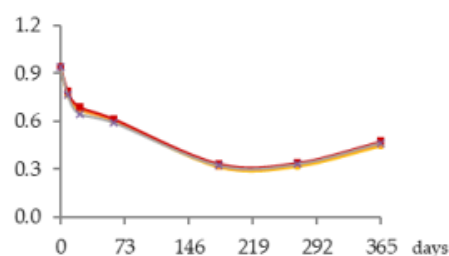
Adapted from the source: Canas et al., 2020.

Modality	Time (days)							
	0	8	21	60	180	270	365	
Fe (mg/L)	O15	0.086 ± 0.002 a	0.095 ± 0.003 ab	0.095 ± 0.004 ab	0.12 ± 0.01 b	0.20 ± 0.03 c	0.27 ± 0.01 d	0.23 ± 0.02 c
	O30	0.086 ± 0.002 a	0.10 ± 0.01 ab	0.087 ± 0.006 a	0.109 ± 0.001 b	0.185 ± 0.005 c	0.28 ± 0.02 d	0.20 ± 0.01 c
	O60	0.086 ± 0.002 a	0.111 ± 0.005 a	0.087 ± 0.004 a	0.094 ± 0.003 a	0.19 ± 0.03 b	0.28 ± 0.03 c	0.18 ± 0.02 b
	N	0.086 ± 0.002 a	0.111 ± 0.004 b	0.09 ± 0.004 ab	0.10 ± 0.01 ab	0.23 ± 0.02 d	0.279 ± 0.008 e	0.186 ± 0.006 c
Cu (mg/L)	O15	0.939 ± 0.009 f	0.767 ± 0.004 e	0.672 ± 0.006 d	0.606 ± 0.016 c	0.314 ± 0.020 a	0.318 ± 0.002 a	0.445 ± 0.003 b
	O30	0.939 ± 0.009 f	0.78 ± 0.03 e	0.68 ± 0.02 d	0.606 ± 0.004 c	0.321 ± 0.005 a	0.330 ± 0.009 a	0.457 ± 0.002 b
	O60	0.939 ± 0.009 f	0.79 ± 0.004 e	0.691 ± 0.016 d	0.616 ± 0.027 c	0.34 ± 0.01 a	0.34 ± 0.02 a	0.47 ± 0.03 b
	N	0.939 ± 0.009 f	0.77 ± 0.04 d	0.65 ± 0.03 c	0.59 ± 0.01 c	0.32 ± 0.02 a	0.33 ± 0.03 a	0.46 ± 0.05 b

Fe



Cu



Results expressed as mean ± standard deviation. For each element: ageing time (days)—means within the same row followed by different lowercase letters (a,b,c,d,e,f) are significantly different ( $p < 0.05$ ); ageing modality—means within the same column followed by different uppercase letters (A,B) are significantly different ( $p < 0.05$ ); 0 days—corresponds to the wine distillate used to fill the demijohns. Fe – iron; Cu – copper. O15, O30, O60 – MOX levels; N – Nitrogen (control).

As already mentioned, the contents of Fe and Cu are of great importance for the quality of the WS. The concentration of Fe in this product and in similar distillates is normally lower than 1 mg/L, an element that can be introduced during the ageing process due to the contact with metal surfaces and potential release from the wood (Canas et al., 2020). For Cu, concentrations often vary between 1 and 3 mg/L in wine distillates, often originated from the distillation devices in Cu (column still and alembic) (Tamasi et al., 2018). This metal is used in distillation devices for its valuable characteristics, its resistance to corrosion, its ability to conduct heat, and its malleability. It is precisely the Cu surfaces that release this element, which can emit it in significant quantities, especially as the contact time and alcoholic strength increase, due to the fact that the product is dragged through the condensation of the vapor along the compartments of the distillation apparatus (Canas et al., 2020).

The total concentrations of Fe found in wine distillates, in the safe range for the product, are usually low. Furthermore, it was noted for each sample that the Fe content did not significantly change depending on the ageing methodology (Canas et al., 2020). It was observed a significant effect of the ageing time on the Fe concentrations of the WS. In fact, there were increases of up to 270 days of ageing, due to the transfer of this element from the wood to the spirit of the wine. Kaya et al. (2017) conducted a study to evaluate the effect of ageing in wood on the mineral composition of wine. Although the wine being a different matrix from that of the distillate, this study is nevertheless important to analyze the effect that ageing in wood has on the mineral composition. It is well known that Fe forms soluble compounds with organic acids in wine (Ribereau-Gayon et al., 2006). When present in excessive quantities can cause turbidity phenomena, known as ferric casse. Undesirable sensory characteristics can manifest themselves in wine as in other alcoholic beverages with concentrations > 10 mg/L (empirical technical limit) (Kaya et al., 2017). In the case of minor elements, however, there was no influence on the part of ageing on the wood and the timing of ageing on the quantity. Among these, the Cu also had no variations. Instead, on other elements including Fe also had a greater effect (Kaya et al., 2017).

Like Fe, Cu also was present in low concentrations in WS, in the safe range for the human health. According to the data provided, no significant effect of the ageing modality was observed on this element, the concentration values were higher in WS that have undergone a higher concentration of oxygen (Canas et al., 2020). This is due to the different redox potential that the medium presents, which allows a regulation of the balance of the forms of Cu oxidation, which are  $\text{Cu}^{2+}$  and  $\text{Cu}^+$ , thus affecting the various phenomena of solubilization and precipitation (Canas et al., 2020).

Furthermore, under the conditions of the study by Canas et al. (2020), as regards the mineral composition, it was shown that the content of both mineral elements depended on the ageing time, showing variations between the different ageing times, even if the overall content is always low. As for Fe element, with the progress of the ageing time, up to 270 days, it showed a constant increase, up to a final decrease from 270 days to 360 days. Conversely, Cu element exhibited the opposite behavior, an initial decline and a slight increase at the end. This study also highlighted a strong correlation between the Fe element and some WS compounds at the end of the ageing period, as a consequence of the effect this element has on the various oxidation processes and in other reactions in which is present. This effect was not found with Cu, thus having a weak correlation with these compounds.

The evaluation of Cu is important, because it also has a great impact on the phenomena of irreversible turbidity and precipitation in WS, in the event that this has a pH greater than 4.5 and this can lead, for example, to the formation of copper tannate (Lafon et al., 1964; Canas et al., 2020). The presence of Cu leads to a low level of unsightly volatile compounds that contain sulfur, resulting in a better taste and aroma of distilled alcoholic beverages. The disadvantages are mainly related to the catalytic formation of acetaldehyde and other aldehydes and ethyl carbamate (Szymczycha-Madeja et al., 2014). Al, Cu, Fe and Zn, influence the color, aroma, clarity, stability and organoleptic properties, as for the bitter taste, in the distillate. As already mentioned above for Cu, Pb can also derive from the instruments used to carry out the distillation process, due to the surface corrosion of these materials, which causes their accumulation in the final product. The levels of these elements can increase during distillation due to the lowering of the pH resulting from the increase in the concentration of acids in the distillate (Szymczycha-Madeja et al., 2014). Like for Cu and Pb, this can be observed for As, the accumulation of which can derive from the raw materials used for the distillation processes, from the production characteristics and in the storage of finished products.

It is important to highlight their accumulation also in wine, because this is the basis for the production of the future aged WS, and above all their relationship with ageing. Na, K and Ca during the ageing period showed a decrease in their content, even if in this case they are due to the fact that during the ageing in wood of the wine precipitations, a physico-chemical stabilization, such as reactions, can occur of formation of potassium bitartrate and calcium tartrate. The loss may have been caused by the low contact time between the wood and the wine itself, which did not compensate for the loss for these phenomena. The Na underwent a greater increase over time due to the contact time, sufficient to have a correct concentration of the element. Mg was instead influenced by wood ageing, with an increased level (Kaya et al., 2017).

The mineral elements potassium (K), sodium (Na) and calcium (Ca) were found in the highest concentrations in almost all the distillates analyzed (Szymczycha-Madeja et al., 2014). According to these authors, the high content of these elements, together with lithium (Li) and magnesium (Mg), is linked to the water used for dilution of the distillates and for sanitizing the barrels used. (Szymczycha-Madeja et al., 2014). The presence of sulfur (S) derives mainly from sulfur dioxide (SO<sub>2</sub>) used in the initial preparation of the barrels, in order to sanitize them, before the ageing process takes place. The precipitation of heavy metals in wine, in the form of insoluble salts, such as sulphides, is facilitated by the addition of sulfur dioxide during winemaking (Catarino et al., 2018).

It is important to evaluate the presence of certain elements, including aluminium (Al), arsenic (As), cadmium (Cd), Cu, chrome (Cr), mercury (Hg), nickel (Ni), lead (Pb), antimony (Sb) and zinc (Zn), as they contribute to the overall quality of alcoholic beverages and determine their suitability and safety for consumption. In fact, certain elements, according to the OIV, may present toxicity if present above the tolerable and/or admissible limits (Catarino et al., 2006; Szymczycha-Madeja et al., 2014).

The distillation process itself does not allow the passage of these metals from the wine to the distillate. In spite of that, it is important to analyse distilled spirits to evaluate the concentration of some elements, for quality control reasons (Catarino et al., 2006; Szymczycha-Madeja et al., 2014). It should be highlighted that usually, the content of potentially toxic elements is low or even undetectable in WS (Szymczycha-Madeja et al., 2014).

The water that is used to dilute the distillates can be a source of trace elements, for this reason it is essential to periodically check it (Szymczycha-Madeja et al., 2014). If the water used is of low quality or if it is polluted it could contaminate distilled spirits with unwanted elements. Among these are barium (Ba), Hg, manganese (Mn), molybdenum (Mo), thorium (Th) or uranium (U) (Szymczycha-Madeja et al., 2014). All the elements mentioned above may also have implications from a sensory point of view.

### **3. Material and methods**

The WS analysed in this study resulted from an ageing experiment carried out within the project Oxyrebrand (POCI-01-0145-FEDER-027819), involving different ageing technologies (barrel ageing vs wood staves and MOX), including two types of wood (Limousin oak and chestnut) and different levels of MOX.

As previously mentioned, this specific study aims to evaluate the effects of the two ageing technologies (barrel ageing vs wood staves + MOX), using Portuguese chestnut wood (*Castanea sativa Mill.*), on the mineral composition (after one year of ageing) and physico-chemical (after 6 months of bottling) of the corresponding WS. The tests were carried out in the laboratory of Instituto Superior de Agronomia as regards the physico-chemical analyzes, while the mineral elements analysis was carried out in the Laboratory of Mineral Analysis of Instituto Nacional de Investigação Agrária e Veterinária - Dois Portos.

#### **3.1 Ageing experiment**

##### 3.1.1 Wine distillate and wood material

A wine distillate produced by the Adega Cooperativa da Lourinhã (Lourinhã, Portugal) with the following physico-chemical characteristics, alcoholic strength of 78.3% v/v, total acidity of 0.12 g of acetic acid/L of absolute ethanol, volatile acidity of 0.09 g of acetic acid/L of absolute ethanol and a pH of 5.33, was used in the ageing essay.

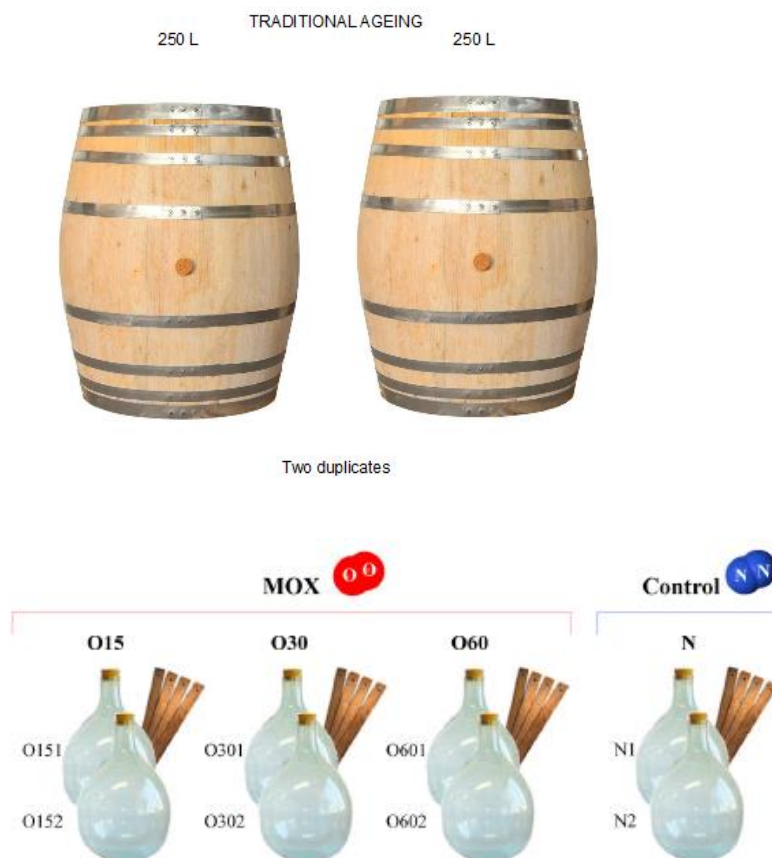
This wine distillate was aged in paralel, in barrels (traditional technology) and applying wood staves combined with MOX (alternative technology). Barrels and staves were produced by the J.M. Gonçalves cooperage (Palaçoulo, Portugal) using Portuguese chesnut wood. The staves (50 cm length x 5 cm width x 1.8 cm thickness), as well the barrels, were submitted to medium plus toasting (QM<sup>+</sup>) (Canas et al., 2020).

##### 3.1.2 Experimental design

As previously mentioned the wine distillate was aged in paralel through the traditional technology in 250 L barrels, and on a pilot scale in 50 L demijohns with wood staves combined with MOX. The number of staves were inserted based on the surface/volume ratio present in the corresponding 250 L barrel, this ratio corresponds to 85 cm<sup>2</sup>/L (Canas et al., 2020). Different conditions of MOX were tested: flow rate of 2 mL/L/month during the first 15 days

followed by 0.6 mL/L/month until 365 days (O15); flow rate of 2 mL/L/month during the first 30 days followed by 0.6 mL/L/month until 365 days (O30); flow rate of 2 mL/L/month during the first 60 days followed by 0.6 mL/L/month until 365 days (O60). It was made also a control of this trial with the use of nitrogen with a flow rate of 20 mL/L/month of nitrogen from 0 to 365 days (N). All the experimental modalities were carried out in duplicate.

For MOX pure oxygen (X50S Food, Gasin, Portugal) was added through a volumetric micro-oxygenator with multiple ceramic diffusers (VISIO 6, Vivelys, France), at different flow rates that change according to the modality of ageing, avoiding excessive oxidation thanks to an adaptation to provide low flow rates. Specific instrumentation was used for the modality with 100% pure nitrogen (X50S Food, Gasin, Portugal). This test was carried out as a control, in order to minimize oxygen as much as possible and allow the comparison with the other modalities (Canas et al., 2022). The demijohns were closed and then isolated with a silicone stopper (Canas et al., 2022). The demijohns as well as the barrels were placed in the same environmental conditions in the cellar. Figure 8 represents the traditional ageing that has been carried out, with 250 L barrels, both for ageing with oak and chestnut. Both types of wood were treated with a "medium plus QM +" toast, each with a replica. In the Figure is represents ageing that has been carried out with alternative technology, in which there are different MOX methods for the quantity of oxygen used in relation to the ageing time and that with the exclusive use of nitrogen.



**Fig 8. Ageing trial scheme for traditional and alternative ageing.** Adapted from: Canas et al. (2022).

The aged WS were analyzed at one year of ageing for the mineral composition and at time 0 and 6 months (bottling) for the physico-chemical composition. During ageing was limited the collection to avoid excessively the filling of the demijohns and to avoid the reducing the surface/volume ratio in the container.

### **3.2 Mineral analysis**

#### 3.2.1 Multielemental analysis by ICP-MS

The elemental composition of the WS after 12 months of ageing was analysed by means of inductively coupled plasma mass spectrometry (Q-ICP-MS) technique. Figure 9 displays the ICP-MS equipment used at the Laboratory of INIAV-Dois Portos.



**Fig 9. ICP-MS equipment at the Laboratory of Mineral Analysis, INIAV-Dois Portos.**

Previous to the analysis by ICP-MS, the wine spirits were centrifuged at 5000 g for 10 min (Heraeus Biofuge Stratos Centrifuge, Thermo Fisher Scientific, EUA), and concentrated (from 35 to 15 mL) using a SpeedVac concentrator (Labconco, model 7810033, Kansas City, MO, EUA) at  $65 \pm 1$  °C for 120 min, in order to reduce the high ethanol content. After, the volume was made up to the initial volume (35 mL) with ultrapure water.

Multi-elemental analysis was carried out with an Elan 9000 ICP-MS (Perkin-Elmer SCIEX, Norwalk, CT, USA) equipped with a cross-flow nebulizer, a Rytan Scott-type spray chamber, and nickel cones. A four-channel peristaltic sample delivery pump (Gilson model) and a Perkin-Elmer AS-93 Plus autosampler (Perkin-Elmer SCIEX, Norwalk, CT, USA) protected by a laminar-flow-chamber clean room class 100 (Max Petek Reinraumtechnik, Radolfzell am Bodensee, Germany) were used. The ICP-MS instrument was controlled by Elan 6100 Windows NT software (Version 2.4, Perkin-Elmer SCIEX, Norwalk, CT, USA). The operating conditions of the ICP-MS equipment were as follows: radio-frequency (RF) power of 1200 W; Ar gas flow rates of 15 L/min for cooling, between 0.94 and 0.98 L/min for nebulizer and 1.5 L/min for auxiliary; and solution uptake rate of 1.0 mL/min.

Element concentrations were determined in WS samples (after treatment as previously described and 10-fold dilution), in duplicate, by adapting the ICP-MS semi-quantitative method previously described by Catarino et al. (2006). A full mass spectrum ( $m/z = 6-240$ , omitting the mass ranges 16–18; 40, 41, 211–229) was obtained by full mass range scanning. Rh and Re (10 µg/L) were used as internal standards.

The reference response table (Perkin-Elmer TotalQuant III, Perkin-Elmer SCIEX, Norwalk, CT, USA) was updated with different multi-elemental standard solutions with appropriate

concentrations for WS samples. Between determinations, the equipment sampling system was rinsed with a 2% HNO<sub>3</sub> (v/v) for 75 s.

To avoid contamination, all polyethylene material (volumetric flasks, micropipette tips, and autosampler vessels) was immersed at least for 24 h in 20% (v/v) HNO<sub>3</sub>, and rinsed thoroughly with purified water before use. For decontamination solution preparation, reagent grade HNO<sub>3</sub> was double-distilled using an infra-red subboiling distillatory system (model BSB-939-IR, Berghof, Germany). Purified water (conductivity < 0.1 μS cm<sup>-1</sup>) was produced using a Seralpur Pro 90CN apparatus (Seral, Ransbach-Baumbach, Germany).

### **3.3 Physico-chemical analysis**

#### **3.3.1 Alcoholic strength**

The actual alcoholic strength by volume, or alcohol by volume (ABV), of spirit drinks is equal to the number of liters of ethyl alcohol contained in 100 L of a water-alcohol mixture having the same density as the alcohol or spirit after distillation. The determination of the alcoholic strength of the WS was carried out using the analytical method described by the OIV (OIV-MA-BS-01) (OIV, 2019). This method is based on the measurement of the alcoholic strength of WS by evaluation of the distillate density. The critical point of this method is the reference temperature of 20° C to be maintained during the analysis for the determination of the alcoholic strength of WS and spirit drinks. Figure 10 displays the automatic distiller used to produce the WS distillates.



**Fig 10. Automatic Distiller at the Laboratory of Enology, Instituto Superior de Agronomia.**

### 3.3.2 pH

The analytical determination of pH was carried out using the instrument of the pH-meter and the principle of the method described by the OIV (OIV-MA-BS-13) (OIV, 2019).

### 3.3.3 Total, fixed and volatile acidity

The analytical determination of total, fixed and volatile acidity of the WS was carried out by applying the method described by OIV (OIV-MA-BS-12), (OIV, 2019). Total acidity is the sum of the titratable acidities, the fixed acidity corresponds to the acidity of the residue left after the dry evaporation of the spirit drink, while the volatile acidity consists of acetic and aliphatic acids which are more volatile than are present in spirits. Total acidity is determined by the direct titration of the spirit drink. The determination of the fixed acidity takes place by titration of the aqueous solution obtained after having dissolved the evaporation residue of the spirit drink. Volatile acidity, on the other hand, is calculated by subtracting the fixed acidity from the total acidity.

Figure 11 shows the instruments used for the determination of acidity according to the OIV protocol. On the left the instruments used to carry out the titration, while on the right the pH meter.



**Fig 11. Laboratorial assembly for acid-base titration and pHmeter.**

#### 3.3.4 Dry extract analysis

The dry extract of WS was determined following the analytical procedure described by OIV (OIV-MA-BS-09) (OIV, 2019). This method is suited to the determination of the total dry extract in spirit drinks of viti-vinicultural origin which contain less than 15 g/L of dry matter. The total dry extract or total dry matter includes all matter that is non-volatile under specified physical conditions. The principle of the analysis is based on weighing the residue left by the evaporation of alcohol in a boiling water bath and drying in an oven. Figure 12 shows the ceramic capsules with the WS aged during the evaporation process to obtain the dry extract.



**Fig 12. Evaporation of WS in the water bath at 100°C.**

### ***3.4 Statistical analysis***

The statistical treatment of the data was carried out through the "R" program (R Development Core Team) for the analysis of variance (ANOVA) in order to evaluate the effect of ageing modalities and ageing time on the mineral and physico-chemical composition of the analyzed WS. A one-way ANOVA was performed to examine the effect of the ageing modality (B, O15, O30, O60 and N), as a fixed factor, on the mineral elements contents of the aged WSs at 365 days. For each ageing modality, another one-way ANOVA was carried out to assess the significance of the ageing time (0 and 365 days). Also, for physico-chemical composition, a one-way analysis of variance (ANOVA) was performed to examine the effect of the ageing modality (O15, O30, O60 and N) in each sampling time. Another one-way ANOVA was carried out to evaluate the time effect on the physico-chemical composition of the WSs (0 and 6 months). The test to calculate the minimum significant difference was carried out through the Shapiro Test, this therefore allowed a comparison between the means to detect a significant difference ( $p < 0.05$ ).

## 4. Results and discussion

### 4.1 Mineral composition

The content of the mineral elements Al, As, Co, K, Li, Mg, Mn, Mo, Na, Ni, Rb, Sr and Zn, quantified in the distillate and in the WS after 365 days of ageing, and corresponding one-way ANOVA results, are shown in Table 3.

**Table 3. Mean values of the mineral elements concentrations (ng/L) quantified in the wine distillate (0 days) and aged WS (365 days) according to the ageing modalities.**

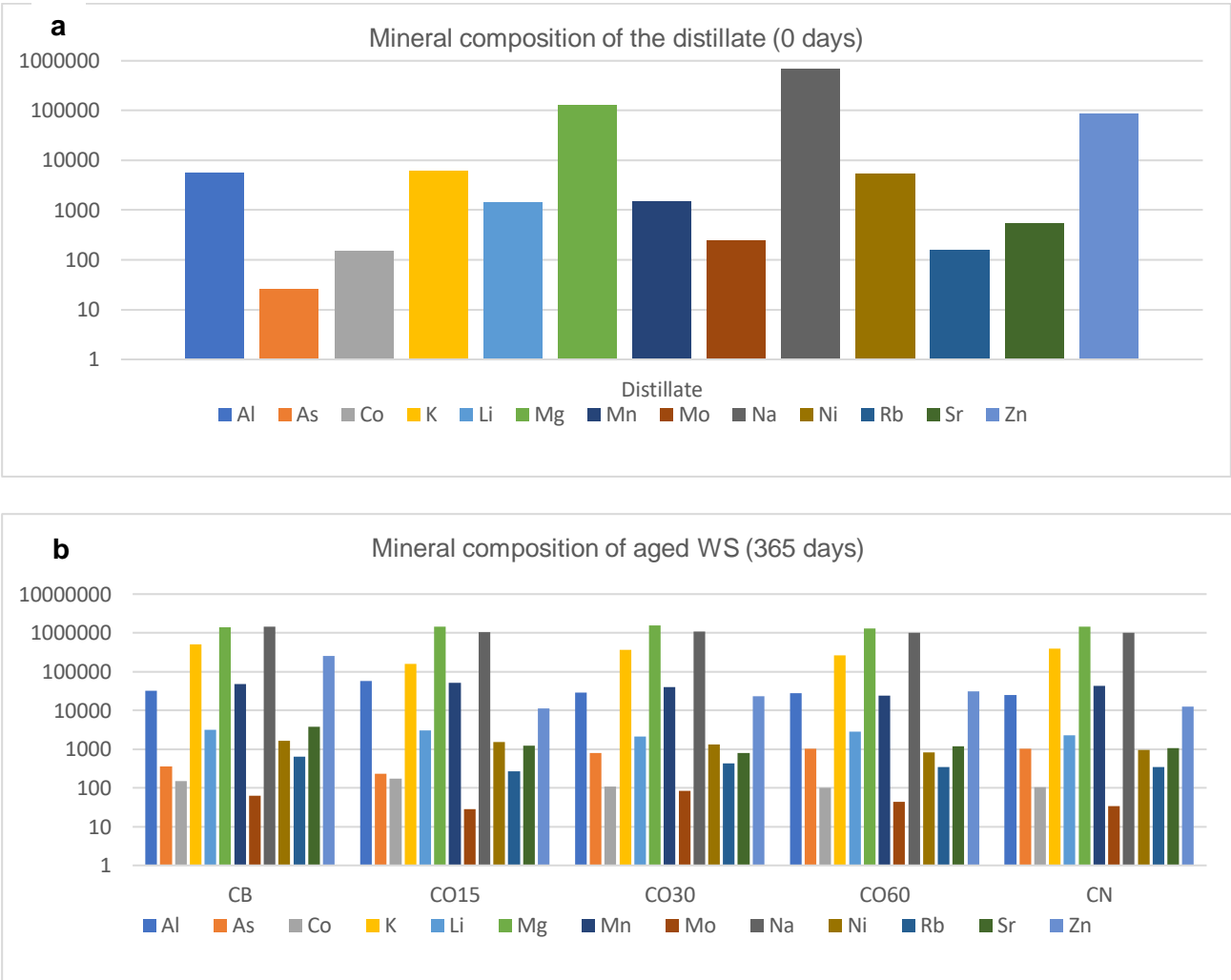
	Al		As		Co		K		Li	
	0	365	0	365	0	365	0	365	0	365
<b>CB</b>	5498 a	32386 b	25.9 a	353.1 b	149	149	6246 a	506474 b	1405	3150
<b>CO15</b>	5498	57304	25.9 a	234.4 b	149	174	6246 a	159137 b	1405 a	3011 b
<b>CO30</b>	5498 a	28459 b	25.9 a	785.8 b	149	107	6246 a	366913 b	1405	2113
<b>CO60</b>	5498 a	27425 b	25.9 a	1019.6 b	149	102	6246	264242	1405	2852
<b>CN</b>	5498	24605	25.9	1041.1 b	149	106	6246	385667	1405	2269

	Mg		Mn		Mo		Na	
	0	365	0	365	0	365	0	365
<b>CB</b>	127813 a	1412581 b	1452 a	47282 b	246.3 b	62.2 a	698436	1459089
<b>CO15</b>	127813 a	1440223 b	1452 a	52280 b	246.3 b	28.2 a	698436	1061357
<b>CO30</b>	127813 a	1544115 b	1452	39262	246.3 b	83.4 a	698436 a	1075124 b
<b>CO60</b>	127813 a	1310190 b	1452 a	23919 b	246.3 b	43.5 a	698436 a	1013089 b
<b>CN</b>	127813 a	1464070 b	1452 a	43335 b	246.3 b	33.6 a	698436 a	1011545 b

	Ni		Rb		Sr		Zn	
	0	365	0	365	0	365	0	365
<b>CB</b>	5393 b	1620 a	151 a	643 b	544 a	3726 bB	85507	255701
<b>CO15</b>	5393 b	1502 a	151	270	544	1238 AB	85507 b	11033 a
<b>CO30</b>	5393	1332	151	424	544	804 A	85507 b	23133 a
<b>CO60</b>	5393 b	819 a	151	348	544	1169 AB	85507 b	31484 a
<b>CN</b>	5393 b	939 a	151	341	544	1048 A	85507 b	12498 a

Results are expressed as mean. For each element: ageing time (days) - means within the same row followed by different lowercase letters (a,b) are significantly different ( $p < 0.05$ ) according to Shapiro test; ageing modality - means within the same column followed by different uppercase letters (A,B) are significantly different ( $p < 0.05$ ); 0 days - corresponds to the wine distillate used to fill the demijohn O15, O30, O60 – MOX levels; N – Nitrogen (control).

Figure 14 displays graphs (logarithmic scale) of the mineral composition of the distillate (a – 0 days) and aged WS according to the ageing modalities (b – 365 days).



**Fig 13. Graph of the mean values of mineral composition of the distillate and aged WS according to the ageing modality: a - distillate (0 days); b - aged WS (365 days)**

Monitoring the content of mineral elements is important for obtaining a high quality and safe product. In particular, the assessment of metal elements such as Al, As, Ni, and Zn is important for safe consumption of WS because of their potential toxicity. In the characterization of WS that was performed, the trends differed among the mentioned elements, while there was an increase in Al and As elements, for Ni and Zn there was a decrease during ageing, with concentrations remaining low. Currently, the OIV does not establish maximum acceptable limits for mineral elements in WS.

Al is known as an element that if taken in modest quantities leads over time to neurodegenerative and neurodevelopmental diseases, in particular in Alzheimer's disease

(Swegert et al., 1999). It has a bioavailability skill, the ability to combine with organic acids (Catarino et al., 2008). This element can derive from various products used during winemaking, such as tannins, bentonites and adjuvants for filtration, or from the contact with steel surfaces, in different steps of the production e.g. vinification, distillation and maturation (Catarino et al., 2008). In addition to the toxicity problems for humans described, it can also lead to physicochemical instability or unpleasant odors to the product (Catarino et al., 2008). The levels of Al found in the aged WS ranged from approximately 24 µg/L (CN) to 57 µg/L (CO15). These concentrations can be considered admissible contents and food safety, although there is no limit set by the OIV, but only by Germany, for wine, of 8.00 mg/L. Like the elements Fe and Cu that catalyze the oxidation phenomena on WS and in others alcoholic beverages, also Al and Zn can affect the stability, the clarity, the color, the aroma and some other organoleptic properties, as bitterness of the WS (Szymczycha-Madeja et al., 2014).

As is a potentially dangerous element for human health. It is therefore important a periodic control of the water used for processing, in fact if it should contain and exceed, according to EU legislation, 10 µg/L, it can become dangerous. Talking about its chemistry, it presents inorganic forms, with the most toxic form being monomethylarsenate together with dimethylarsenate, respectively As (III) and As (V). These can cause intoxication related to the formation of various cancers, for example if ingested in modest amounts it can lead to skin cancers. However, it is generally present in the less dangerous organic forms, thanks to the action that yeasts operate during biomethyl fermentation, and especially in minimal amounts (Catarino et al., 2008). In wine we can find it due to treatments carried out in viticulture, if pesticides are used in the field, although its origin can also come from the use of bentonites in winemaking. In this case, As is present within the limits of 0.2 mg/L in wines, given by OIV. In the case of the results presented by this study, As values were quite low, even if a slight increase was observed overtime.

Ni is also a potential toxic element to humans, and is classified as an allergen by the World Health Organization, as it can cause dermatitis and has a carcinogenic effect (Catarino et al., 2008). Very low enrichments can result from the contact with stainless steel tools during winemaking, distillation and ageing. Again, it should be stressed that the levels of Ni found in the WS were very low.

Zn is essential for the vegetative growth of plants, it is found in nature as a mineral associated to S, therefore it can be easily assimilated by the plant in the field, but it can also be found because of the use of products in viticulture, such as dithiocarbamate fungicides. There is a decrease of the content already during vinification due to fermentation, therefore a lower concentration in wines. For WS instead there can be an accumulation due to the contact with

metal surfaces, always of small quantities. Zn is not as toxic as the previous mentioned elements, however it is dangerous if inhaled and can lead to deficiencies of other trace minerals if included in the diet (Catarino et al., 2008). The OIV establishes a maximum acceptable level of 5 mg/L in wines, but again there is no limit for WS. These results highlight the importance of the distillation equipment, if adequately and certified, it does not release high amounts of harmful heavy elements, but rather low amounts as can be seen in the distillate, emphasizing how low or even undetectable the content of these elements is in this study and others.

Co mainly derives from the use of metallic materials used during the various production processes both for wine and aged WS. In the case of wine through the use of bentonites, however generally in wine the values do not exceed 10 µg/L (Catarino et al., 2008). It is important to keep the levels low because it is potentially toxic to humans, causing allergies. The aged WS of this study showed very low concentrations which remain stable or decrease during ageing.

K is an element that can lead to physico-chemical instability in the WS (Catarino et al., 2008). In this study, it was observed significant and strong increases during ageing, both in traditional and alternative technology.

Na and Mg, like K, are among the major alkali metals with acid-base buffering capacity in the medium in wines (Catarino et al., 2008). Both Na and Mg show an increase in their concentrations, however low levels compared to the previously mentioned ranges and without special practical meaning. Na has a maximum acceptable limit according to OIV of 60 mg/L (surplus Na) in wines, whereas for WS there are no legal limits established for this element neither for Mg. It should be stressed that the levels recorded in the aged WS are not high and not problematic for their consumption.

Likewise, Li is an alkaline metal that can originate from the tools used during production processes. In this study, the concentrations observed in the aged WS were very low.

Mn is a transition metal potentially involved in oxidation processes, just like Fe and Cu. It can mainly derive from plants as well as from soil, occurring in wines in mean concentrations of 3 mg/L. Moreover, in the soil it can derive from plant protection products which have Mn salts or in the winery from products such as pectolytic enzymes or from bentonite itself. In any case, the values remain low both before and after ageing, and balanced among the various ageing technologies (Catarino et al., 2008). Mn can be correlated with the ageing time and the type of wood used for barrels (Szymczycha-Madeja et al., 2014).

The levels of Mo in wines can increase with some products used in the winery during the winemaking process, such as bentonite. The aged WS presented very low levels, decreasing during the ageing (Catarino et al., 2008).

Rb and Sr were observed in the aged WS in very low concentrations, always lower than 1 µg/L, and far from the mean concentrations in wines Catarino et al., 2008).

#### 4.1.1 Effect of the ageing modality

With the exception of the Sr element, for the other elements no significant differences were observed in the concentrations of aged WS according to the different ageing technologies/modalities. Regarding Sr element, a higher concentration was verified in the WS obtained through the traditional ageing technology (CB), as shown in Table 3.

It should be highlighted that for most part of the elements, in general high differences were observed between replicates of the same ageing modality, which could explain the lack of statistical significance.

For As, even if the effect of the ageing modality is not significant, it seems that there is a trend of a slight increase of concentrations as the level of MOX increases. For Co, Mn, and Ni, the concentrations tend to decrease with MOX level.

For the Zn element, on the other hand, traditional ageing differs from the alternative ageing with MOX and from the nitrogen modality. It can be observed that Zn concentration tends to increase with MOX level. The highest concentration was verified in the CO60 modality followed by barrel ageing modality.

#### 4.1.2 Effect of the ageing time

The effect of the ageing time on mineral elements was significant for all the elements examined. The analysis of the variations of the mineral content between day 0 and day 365 of ageing showed a significant increase for the elements Al, As, K, Li, Mg, Mn, Na. The Rb and Sr elements, on the other hand, showed a significant increase only in the case of the traditional modality.

Zn significantly decreased in all the MOX modalities while higher concentrations were observed barrel-aged WS than in the distillate. This enrichment is most probably due to Zn extraction from wood both for traditional technology.

At last, the concentrations of these elements observed in the distillate and aged WS were very low and remain consistently very low. The decreases observed for Co, Mo, Ni, and Mo are probably due to insolubilization and precipitation phenomena.

#### 4.2 Physico-chemical composition

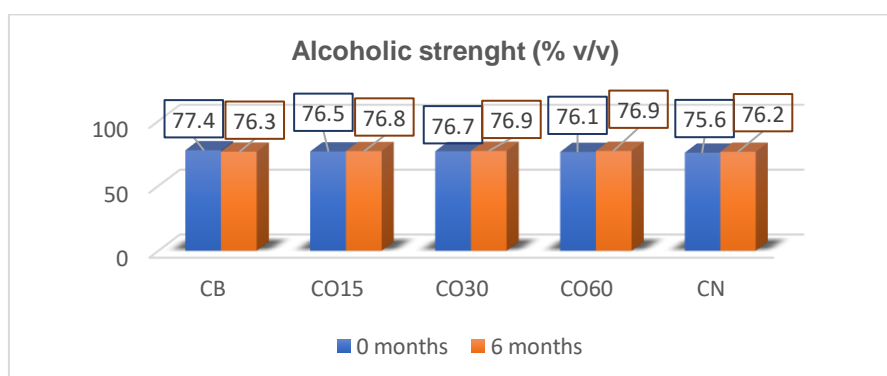
Table 4 shows the basic physico-chemical characteristics (alcoholic strength, fixed acidity, volatile acidity, total acidity, pH and dry extract) of the distillate and aged WS, according to the ageing modalities, after 12 months of the ageing essay (0 months of bottling ageing) and after 6 months of bottling ageing.

**Table 4. Mean values of the physico-chemical characteristics of the WSs aged according to the different ageing conditions at 0 and 6 months of bottle ageing.**

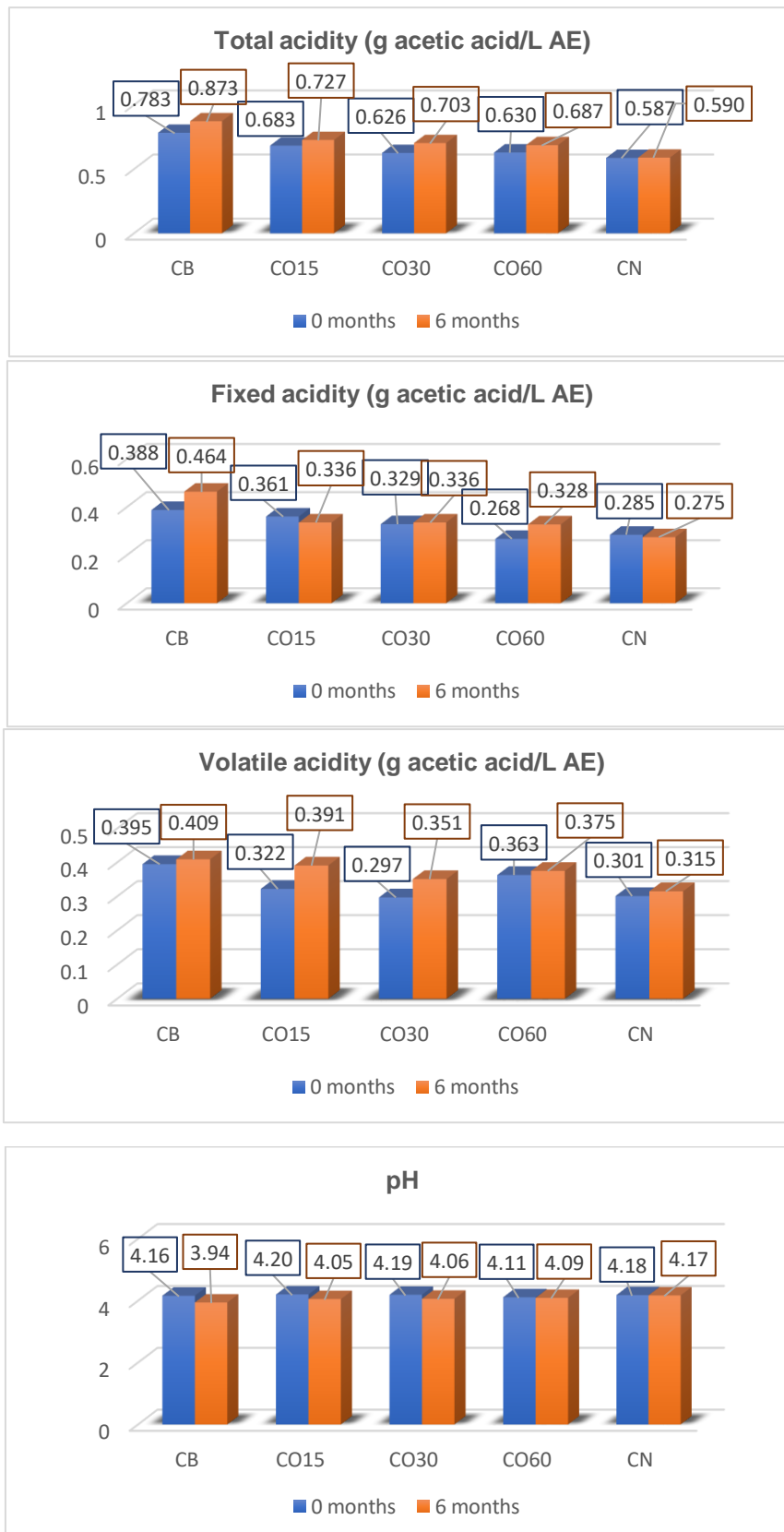
	AS (% v/v)		TA (g acetic acid/L AE)		FA (g acetic acid/L AE)		VA (g acetic acid/L AE)		pH		TDE (g/L)	
	0	6	0	6	0	6	0	6	0	6	0	6
<b>CB</b>	77.4	76.3	0.8	0.9	0.4	0.5	0.4	0.4	4.2	3.9	2.3	2.4
<b>CO15</b>	76.5	76.8	0.7	0.7	0.4	0.3	0.3	0.4	4.2	4.0	2.3	2.7
<b>CO30</b>	76.7	76.9	0.6	0.7	0.3	0.3	0.3	0.4	4.2	4.1	2.2	2.0
<b>CO60</b>	76.1	76.9	0.6	0.7	0.3	0.3	0.3	0.4	4.1	4.1	2.3	2.1
<b>CN</b>	75.6	76.3	0.6	0.6	0.3	0.3	0.3	0.3	4.2	4.2	2.1	1.9

Results are expressed as mean; Means within the same row followed by different letters are significantly different ( $p < 0.05$ ) according to Shapiro Test; MOX – micro-oxygenation; O15, O30, 60 – MOX levels; N – Nitrogen (control). AS - Alcoholic strength; TA - Total acidity; FA – Fixed acidity; VA - Volatile acidity; TDE - Total dry extract.

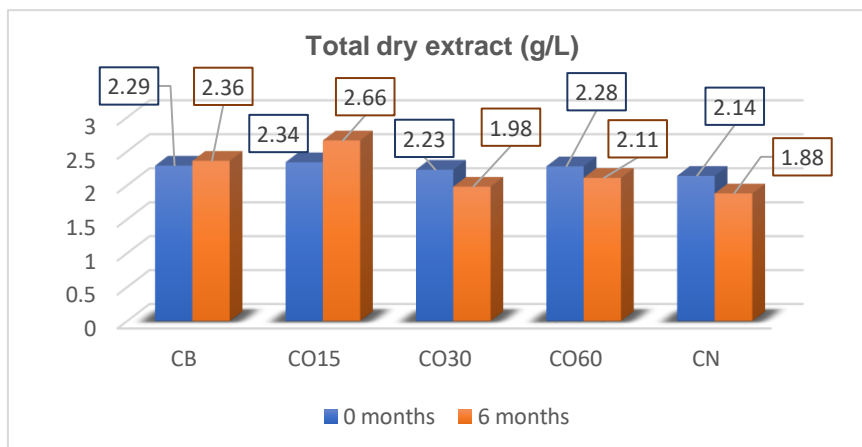
The figures 14, 15 and 16 show the physico-chemical composition of the aged WS.



**Fig 14. Graph on the analyzed alcoholic strenght parameter of the aged WS.**



**Fig 15. Graphs of the acidity-related parameters of the aged WS (total, fixed and volatile acidity; pH)**



**Fig 16. Graph on total dry extract of the aged WS**

#### 4.2.1 Effect of the ageing modality

Regarding the basic physico-chemical characteristics of the aged WS no significant effect of ageing modality was observed for any analytical parameter, with minimal differences between traditional and alternative technologies.

The decrease of the alcohol strength in barrel-aged WS overtime, due to ethanol evaporation, has been reported. This decrease is not evident in WS aged in stainless steel tanks.

Even if not significantly different, the volatile acidity tends to be higher in the WS aged in barrels, which is reflected in the total acidity and pH values. The same was reported in the study by Canas et al. (2022), in which slight increases of the fixed acidity and dry extract values were observed, especially in the ageing modality with lower oxygenation (CO15).

The pH however remains constant in the WS, as well as the dry extract does not show considerable variations, it's higher in the modality CO15 in the alternative technology.

#### 4.2.2 Effect of the ageing time

The effect of time on aged WS didn't have a significant impact among the different aging methods applied. In fact, if in the ageing of WS by the traditional technology the alcoholic strength decreases, due to the evaporation of ethanol from the pores of the barrels (Anjos et al., 2020), in alternative technology modalities this evaporation does not occur, but rather, the values remained constant (Anjos et al., 2020). However, even the effect of ageing time did not reflect the expected data, because of the phenomenon of ethanol evaporation, it was expected a lower value of alcohol by volume at the end of the ageing year. In contrast, this effect was shown in the study by Canas et al (2022), where alcohol strength decreased instead.

Increases in fixed and volatile acidity led to a consequent increase in total acidity. The major contribution to total acidity increase was made by volatile acidity. The increase in total acidity also resulted from the wood release of acetic acid to the WS.

The pH levels exhibited minimal variations given by the variation in the acidity content of the WS. The nitrogen control (CN) technology had a slightly lower pH, followed by the CO30 and CO60 modes of the alternative technology.

Regarding total dry extract there were no significant differences between month 0 and 6 months, with minimal variations in content.

## 5. Conclusions

This study aimed to investigate the effect of wood ageing with chestnut (*Castanea sativa* Mill.) on WS mineral composition and basic physico-chemical composition, comparing barrel aging and an alternative technology (wood fragments combined with MOX), and examining the effect of ageing time.

For that, WS resulting from a wider ageing experiment within a R&D project in course, were used in this work. Multi-elemental analysis of the 12 months aged WS was carried out by ICP-MS, while physico-chemical characteristics were determined by classical wet chemical methods as described by the OIV.

The concentrations of Al, As, Co, K, Li, Mg, Mn, Mo, Na, Ni, Rb, Sr, and Zn were assessed. With exception of Sr, no significant differences were observed between the different ageing modalities.

In respect to the time effect, for several elements significant increases, even if not relevant under the technological point of view, were observed between time zero and 365 days of ageing. For the physico-chemical composition, evaluated at the end of ageing (beginning of bottle ageing) and after 6 months from bottling, no changes were found between ageing technologies for alcoholic strength, acidity related parameters and dry extract.

It could be concluded that regarding the mineral and physico-chemical composition, the ageing technology did not resulted in significant differences between the aged WS.

In a more widen perspective, further studies for a better understanding of the role of oxygenation in WS ageing.

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