Impact of alternative wood ageing systems in the odorant profile of wine spirits with GC-O analysis

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Plurimas Gratias Ago Tibi Magister

BRANDY, n. A cordial composed of one part thunder-and-lightning, one part remorse, two parts bloody murder, one part death-hell-and-the- grave and four parts clarified Satan. Dose, a headful all the time. Brandy is said by Dr. Johnson to be the drink of heroes. Only a hero will venture to drink it.

Ambrose Bierce, The Devil’s Dictionary, 1911

ABSTRACT

Traditionally, wooden barrels are used in the ageing/maturation of wine and many more alcoholic beverages, especially wine spirits. However, production costs, as well as the general trend towards more sustainable management of forests and trees to produce such casks, have led the researchers to develop alternative methods for ageing and storing such beverages.

In this study, it was examined the application of wooden staves on wine spirits in order to accelerate the ageing process. It was evaluated the sensory composition and the odourant compounds of wine spirit aged in the traditional system (barrels) compared to the alternative system (stave) using two type of wood: Limousin oak and Portuguese chestnut.

The results of sensory analysis pointed out that the alternative ageing system with chestnut wood, bring to the wine spirit an accelerated and more complex ageing with concerning the attribute deriving from wood, namely vanilla, spicy, caramel, toasted, dry fruits, smoky, coffee.

Also, the wine spirits from alternative ageing system with oak wood presented a good evolution, in comparison with those from t the chestnut barrels, particularly for toasted, spicy, vanilla, dry fruit and smoke notes.

The GC-O analysis, permitted to detected and identified 23 odourant compounds, especially odourant with characteristic of fresh distillate with fruity and floral note deriving from fermentation process. Only a few odourant from wood (vanillin, HMF, syringaldehyde) was identified on the different samples. Nevertheless, the CA analysis for classify and find the difference between the ageing systems shown a low % of variance that didn't permit to discriminate the samples. These results pointed out one of the limits of GC-O analysis: contrary to the sensorial profile where volatile compounds and no-volatile compounds interact each other, giving a general perception of the product, in the GC-O analysis the volatile compounds are evaluated separately. Anyway, this work is a first approach to this subject and further studies are required to study the differences between the ageing systems after two years or more.

Keywords: Wine Spirit, Alternative Ageing System, GC-O, Odourant compounds

RESUMO

Tradicionalmente, as vasilhas de madeira são usadas no envelhecimento/maturação do vinho e de várias bebidas alcoólicas, nomeadamente as aguardentes vínicas. No entanto, os custos de produção, bem como a tendência geral para uma gestão mais sustentável das florestas e árvores para produzir tais vasilhas, levaram os investigadores a desenvolver métodos alternativos para o envelhecimento e armazenamento de tais bebidas.

Neste estudo, foi avaliada a aplicação de aduelas de madeira em aguardentes vínicas a fim de acelerar o processo de envelhecimento. Foi avaliada a composição sensorial e os compostos odorantes da aguardente vínica envelhecida no sistema tradicional (vasilhas) em comparação com o sistema alternativo (aduelas) utilizando dois tipos de madeira: carvalho Limousin e madeira portuguesa de castanheiro.

Os resultados da análise sensorial mostraram que o sistema de envelhecimento alternativo com madeira de castanheiro, promoveu um envelhecimento acelerado e mais complexo da aguardente vínica, relativamente aos atributos que derivam da madeira, nomeadamente baunilha, especiarias, caramelo, torrado, frutos secos, fumo e café.

Além disso, as aguardentes produzidas no sistema de envelhecimento alternativo com madeira de carvalho apresentaram boa evolução, em comparação com as produzidas nas vasilhas de madeira de castanheiro, particularmente para notas tostadas, especiarias, baunilha, frutos secos e fumo.

A análise de GC-O permitiu detectar e identificar 23 compostos odorantes, com aromas associados especialmente ao odor do destilado sem envelhecimento, com notas frutadas e florais decorrentes do processo de fermentação. Apenas alguns odorantes da madeira (vanilina, HMF, siringaldeído) foram identificados nas diferentes amostras. No entanto, a análise da CA para classificar e encontrar a diferenças entre os sistemas de envelhecimento evidenciou uma baixa percentagem de variância que não permitiu discriminar as amostras. Estes resultados realçam uma das limitações da análise de GC-O: contrariamente ao perfil sensorial, onde os compostos voláteis e os compostos não voláteis interagem entre si, dando uma percepção geral do produto, na análise de GC-O os compostos são avaliados separadamente. De qualquer forma, este trabalho constitui uma primeira abordagem a este tema, sendo necessários estudos posteriores para estudar as diferenças entre sistemas de envelhecimento após dois anos ou mais.

Keywords: aguardentes vínicas, métodos alternativos envelhecimento, GC-O, compostos odorantes

RESUMO ALARGADO

Tradicionalmente as vasilhas de madeira são usadas no envelhecimento/maturação do vinho e de várias bebidas alcoólicas, nomeadamente as aguardentes vínicas. No entanto, os custos de produção, bem como a tendência geral para uma gestão mais sustentável das florestas e árvores para produzir tais vasilhas, levaram os investigadores a desenvolver métodos alternativos para o envelhecimento e armazenamento de tais bebidas.

Neste estudo, foi avaliada a aplicação de aduelas de madeira, a fim de acelerar o processo de envelhecimento da aguardente vínica produzida na Lourinhã, a única denominação geográfica portuguesa que é exclusivamente delimitada para a produção de aguardente vínica envelhecida. Foi avaliada a composição sensorial e os compostos odorantes da aguardente vínica envelhecida no sistema tradicional (vasilhas) em comparação com o sistema alternativo (aduelas) utilizando dois tipos de madeira: carvalho de Limousin e madeira de castanheiro.

A aguardente foi produzida pelo processo de destilação em coluna, envelhecida em vasilhas de 250 litros e em depósitos de aço inoxidável de 1000 litros com aduelas e adição de oxigénio, a fim de reproduzir a mesma superfície de contato que a aguardente teria no método de envelhecimento tradicional.

Nos últimos anos, o estudo da qualidade das bebidas alcoólicas, concentrou-se não apenas na análise sensorial e química, mas também na análise de GC-O (cromatografia gás liquido-olfactometria). Este tipo de cromatografia gás líquido associa o nariz humano como detector para identificar e descrever o aroma dos compostos voláteis eluídos do GC.

A análise de GC-O seguiu o procedimento do método de frequência de detecção que consiste em usar um painel de 6 a 12 participantes, não obrigatoriamente treinados, que têm que assinalar os compostos detectados durante as corridas de GC. Cada avaliador (sniffer) regista cada odor detectado em conjunto com uma descrição que pode ser também registada. Os dados coletados dos sniffers foram analisados para avaliar o cromatograma e identificar o composto odorante detectado.

Antes dos testes de GC-O, as amostras de aguardente vínica foram submetidas a análise sensorial, a fim de avaliar as diferenças entre as amostras.

Os resultados da análise sensorial foram submetidos a análise estatística (ANOVA) que evidenciaram que o sistema de envelhecimento alternativo com madeira de castanheiro, trazem à aguardente vínica um envelhecimento acelerado e mais complexo relativamente aos atributos derivados da madeira, nomeadamente baunilha, especiaria, caramelo, torrado, frutos secos, fumo, café.

O efeito dos diferentes fatores, sistema de envelhecimento e tipo de madeira é evidente em quase todos os atributos, mas é mais significativo em relação ao sistema de envelhecimento.

Além disso, as aguardentes do sistema de envelhecimento alternativo com madeira de carvalho apresentaram boa evolução, em comparação com as provenientes de madeira de castanheiro, particularmente para notas tostadas, de especiarias, de baunilha, de frutos secos e de fumo.

A análise de GC-O e GC-MS permitiu detectar e identificar 53 compostos voláteis nas amostras. No entanto, apenas 23 compostos odorantes apresentaram uma frequência mais elevada, tendo sido detectados pelos *sniffers* e identificados por GC-MS. Foram identificados o acetato de isobutilo, 2-metilbutirato de etilo e isovalerato de etilo com notas frutadas; linalol, álcool benzílico e 2-feniletanol com notas florais; ácido acético com a nota típica de vinagre, característica comum na aguardente vínica; ácidos isovalérico e dodecanoico respectivamente com aroma de queijo gordo e ranço. Todos estes compostos odorantes derivados das uvas ou do processo de fermentação são característicos do destilado fresco. Apenas alguns compostos odorantes da madeira (vanilina, HMF, siringaldeído) foram identificados nas diferentes amostras. Contrariamente aos resultados da análise sensorial, onde o envelhecimento e a evolução foram notados especialmente para o sistema alternativo em madeira de castanheiro, na análise de GC-O as amostras ainda têm características e atributos de um destilado fresco após um ano de envelhecimento. No entanto, a análise CA para classificar e encontrar a diferenças entre os sistema de envelhecimento mostrou uma baixa percentagem de variância que não permitiu discriminar as amostras com base nos compostos odorantes. Estes resultados realçam uma das limitações da análise de GC-O: contrariamente ao perfil sensorial onde compostos voláteis e compostos não voláteis interagem entre si, dando uma percepção geral do produto, no GC-O os compostos voláteis são avaliados separadamente. De qualquer forma, este trabalho constitui uma primeira abordagem a esta temática sendo necessários estudos posteriores para avaliar as diferenças entre sistemas de envelhecimento após dois anos ou mais.

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CA: Correspondence analysis

EC: European Community

FD: Factor of dilution

GC-FID: Gas Chromatography coupled with flame ion detector

GC-MS: Gas Chromatography Mass Spectrometry

GC-O: Gas Chromatography Olfactometry

HMF: Hydroxymethylfurfural

NIF: Nasal impact effect

PCA: Principal component analysis

SNIF: Surface of nasal impact frequency

TPB: (E)-1-(2,3,6-trimethylphenyl)buta-1,3-diène)

1. INTRODUCTION

1.1 THE WINE SPIRIT

Wine spirits are the most known alternative products from wine grapes in the world; bring the fifth largest category of spirit beverage with 20 billion litre in total (Tsakiris et al., 2013). As it's well explained in the EC regulation, wine spirit is a spirit drink produced exclusively by the distillation at less than 86 % vol. of wine or wine fortified for distillation or by the redistillation of a wine distillate at less than 86 % vol. Wine spirit must contain a quantity of volatile substances equal to or exceeding 125 grams per hectolitre of 100 % vol. alcohol and having a maximum of methanol content of 200 grams per hectolitre of 100 % vol. alcohol. The minimum alcoholic strength by volume shall be 37,5 % vol. Furthermore, the spirit drink shall not be added with alcohol or flavour except for sugar addition in order to round off the final taste. However, the final product may not contain more than 20 grams of sweetening products per litre, expressed as invert sugar. It may also contain added caramel as a means to adapt colour. Where wine spirit has been matured, it shall mature for at least one year in receptacles or for at least six months in casks with a capacity of less than 1000 litres (Reg EC 19/787). However, for wine spirits with geographical denomination there are usually more restricted rules. For example, for wine spirit of Lourinhã it's only allowed the addition of caramel until the limit of 2% (Decreto-Lei nº 323/94).

1.2 ODOURANT COMPOUND

The olfactory system is one of the primordial senses for animals and humans, one of the first to have developed into living creatures. Observing the evolutionary context, the sense of smell and the ability to capture odours has always been decisive and important for human development and behaviour, such as for primitive ancestors to detect situations of danger, presence of predatory or contributed to the search for food; so, an important connection between the environment, animals and human (Leffingwell, 2002; Ruijten et al., 2009). The volatile chemical compounds in the vapor phase, spread from the environment are detected by giving a perception when they enter through the nostrils (orthonasal), coming into contact with the neuroreceptors, located in the olfactory epithelium on the nasal fossae when they are above the threshold of detection (Deibler et al., 1999; Portmann, 1999).

Outstanding is the range and accuracy of the olfactory system, enabling organism to detect and discriminate between thousands of low molecular mass compounds, mostly organic compound normally called odours (Firestein, 2001).

Nowadays leaving out the appearance of the ancestor's survival, the use of smell can be associated to odour that remind past experience, converted in a like, dislike, indifferent sensation and personal emotion, so the response is subjective to everyone with every kind of odour (Brattoli et al., 2013).

Through the development of new technology and increased interest in the quality of food and beverages, it was possible to identify a large number of chemical compounds involved in the aroma and flavour. Focus on the volatile compounds, just some of them contribute effectively to the flavour and aroma of food and beverages (Guth and Grosch, 1999). These are called odourant compounds or key compounds or active compounds.

The odourant compounds could be evaluated based on several characteristic and proprieties.

First of all, the *odour threshold* (OT) that is the minimum concentration at which 50% of a human panel can detect the presence of an odour or odourant without characterizing the stimulus. This is different from the *recognition threshold* (RT) which is the concentration that 50% of a human panel can detect and describe qualitatively the odour of the compound.

In addition, exist some physical and chemical properties. These include the *appreciable volatility* of a substance at ordinary temperatures (less than 300–400 relative molecular mass), to permeate the air near the sensory area, as well as the *slight water-solubility* which allows an odour to pass through the mucous layer to the olfactory cells and the *lipid-solubility,* which is necessary since olfactory cilia are composed primarily of lipid material.

The *intensity* is the relative strength of the odour above the recognition threshold. It is logarithmically related to odourant concentration (Stevens' law or the power law) which can be calculated with the equation one (Both et al., 2004).

$$I = K \log C \tag{1}$$

where I is the intensity, C is the concentration and K is a constant.

The *hedonic tone* is a measure of the pleasantness or unpleasantness of an odour mixture.

The *quality* is the property that identifies an odour and differentiates it from another odour of equal intensity.

Finally, the *molecular structure* or molecular geometry is the composition and structure of the functional groups within a molecule that can deeply affect the quality and features of an odour (Czerny et al., 2011).

Despite huge advances in research, the detection and identification of aroma and flavour compounds in wine, wine spirit and other food and beverage, remain one of the most important challenges in the food and beverage research and industry.

The goal is to better understand the role of the volatile compounds that effectively take part in to the aroma. The approach on food and beverage aroma study, use some techniques like sensorial analysis, electronic nose, high performance liquid chromatography (HPLC) and gas chromatography (GC). In the case of volatile compounds, the use of GC-O, i.e. gas chromatography olfactometry, became interesting and a key study for the determination and characterization of aroma of several products. It should be noted that odours are complex mixtures of many volatile chemicals which are present in different concentrations. These chemicals can interact synergistically or additively in the mixtures (Botelho and Climaco, 2011).
The human nose is the most powerful tool for the detection of odour compounds, but the GC-O permit only to detect and recognise every single molecule; we have no information regarding the role and impact of these odorous molecules in the whole of the aroma of a product (Botelho and Climaco, 2011).

1.3 AROMA EVALUATION

1.3.1 SENSORY ANALYSIS

As defined by several authors (Stone and Sidel, 1993; Lawless and Heymann, 1999) and international organization (Institute of food Technologists, ASTM), sensory analysis is ”the discipline used to evoke, measure, analyse and interpret to those characteristics of food and materials as they are perceived by the sense of sight, smell, taste, touch and earing”. This experimental discipline emphasizes the perception and behaviour of the sense. There are several national and international regulations UNI and ISO, that given guidelines about the type of sensory analysis room, the kind of glass or container to use, the training of the judges and the nature of tests for each type of product analysed.
Regarding wine and alcoholic beverage sensory analysis, it is very frequent the application of descriptive sensory analysis methodology.
The descriptive analysis is very common in sensory evaluation of wine and spirits around the world; it is very useful to evaluate every kind of wine, to find attributes and differences between them. The descriptive sensory analysis assumes the search for a minimum of descriptors to obtain the maximum information about the characteristics and sensory properties of a product, quantify the intensity perception by the judge for each of the chosen descriptors and finally obtain the profile of a product with such information (Caldeira et al., 1999).
The descriptive analysis for wine and spirits is carried out by a panel composed with trained judges that evaluate the flavour colour and taste of samples with a specific vocabulary, which

must be not influenced from the personal taste. In order to perform the task efficiently the judges must “calibrate themselves” before every test with standard solutions (Stone and Sidel, 2004). Normally the panel is selected and previously screened for their sensory acuity through tests such as threshold determination (ASTM, 1991) and odour identification. Under the guide of a panel leader they improve their self to use the appropriate vocabulary in order to have an analysis much objective and replicable. Hedonic terms as “delicious” “bad” or “high liking” are not used, instead of a proper qualitative and quantitative attribute for describe and quantify the level or intensity of a sensation detected (Meligaard et al., 2007).

1.3.2 GAS LIQUID CHROMATOGRAPHY OLFACTOMETRY

The principle of gas liquid chromatography is well known and permit to separate and quantify volatile compounds in a sample, exploiting the principle of differential migration in a biphasic system, consisting of a stationary phase (column) and a mobile phase (inert gas, that must not interact with the sample or stationary phase, generally is hydrogen or helium) that carries the sample along the column. The liquid samples vaporized on the injector, are carried out through the column by the carrier gas, and the compounds of the samples are separated based on their chemical affinity with the coating on the inside of the column. These compounds pass through the detector, located at the end of the column, such a FID, (flame ionization detector) or a MS, (mass spectrometer). Then with the chromatogram obtained the results can be analysed (Guiochon et Guillemin, 1990).

Gas liquid chromatography olfactometry (GC-O) is the technique that permit after the GC separation to detect, evaluate the duration, describe the quality and quantify the intensity of the odour perceived using human assessors and their olfactory system (Delahunty et al., 2006).

According to different properties of volatile compounds (absolute threshold, intensity and odour quality) several methods have been developed over the years to use the GC-O: detection frequency, dilution of threshold and direct intensity.

1.3.2.1 DETECTION FREQUENCY METHOD

It consists to use a panel test of 6-12 participants, not obligatory trained, that has to detect the presence or not of several compounds, with the minimum required number of GC runs. This method was further developed by Pollien et al., (1997). Each assessor records the duration for every odour detected together with a tape-recorded description. With this data is possible to develop an aromagram composed of two variables: NIF (nasal impact effect) and

SNIF. NIF consists in the number of assessors able to detect the odorous compound, corresponding to the peak in the aromagram. The SNIF instead, corresponds to the area of the peak and indicates the frequency% x s, the duration (Fig. 1.1). NIF and SNIF increase with concentration and, consequently, with odour intensity. Therefore, they can be used to compare peak intensities between two aromagrams (Pollien et al., 1997).
The main advantages of this method are the simplicity, it's repeatability and it´s applicability because doesn't require several and trained assessors (Pollien et al., 1997; Le Guen et al., 2000).
Although the only limitation of this method, the aromagram is not a direct measurement of the perceived odour intensity (Pollien et al.,1997).

1.3.2.2 DILUTION THRESHOLD METHOD

Is one of the most applied, based on a succession of dilution of the sample to analyse with the GC-O, until the volatile molecule is not anymore detected by the assessors. There are two techniques that applies this method: The Charm (combined hedonic aroma response method) analysis (Acree et al., 1984) and the AEDA, aroma extract dilution analysis (Ulrish and Grosch, 1987).
On the first one, the panellist records the start and end of each detected odour, presented with randomize and several dilution sample. The detection duration for each assessor is then compiled, and an aromagram is generated by plotting the duration of the odour sensation against the dilution Value (d'Acampora Zellner et al., 2008).
Charm values are calculated using an algorithm so that they are proportional to the amount of compound in the extract and inversely proportional to the odour detection threshold, as show in equation 2.

$$dv = F^{n-1}di \qquad (2)$$

Where dv is the dilution value, F is the dilution factor and n, the number of coincident odour responses detected at a single retention index, di.
The peak area is integrated from the duration of retention indices to yield the Charm value (Acree, 1993) as shown in the equation 3.

$$charm = \int_{peak} dv \qquad (3)$$

AEDA technique is based always on dilution rate of the sample but record just the maximum dilution at which an odour compound is detected. The aromagram of results is easily obtained by listing the FD values, factor of dilution, or the logarithm of FD (log FD) versus the retention index (Blank et al., 1989).

1.3.2.3 DIRECT INTENSITY METHOD

Compared to the other methods explained so far, the direct intensity methods, are characterized by a psychophysical measurement by the assessors which must detect the intensity of the acquired signal from the sniffer (van Ruth, 2001).

Two techniques use this method: the posterior intensity method and the time-intensity method which is known as Osme (that means smell in Greek) method. The first one consists to rate the maximum intensity of the odour after the elution. As the principle of sensory analysis this method obtain the best results with a trained panellist (van Ruth and O'Connor, 2001). The Osme technique developed by McDaniel et al. (1990) develop an osmegram with the recorded by the assessors of the intensity of the odour compound detected, with a score from 0 (none) to 16 (extreme). Also, the description of the odour by the assessors is tape-recorded. Van Ruth (2001) found a positive correlation (R=0.822) of the posterior intensity method with the detection frequency method results.

According to the GC-O technique used, as anticipated, different aromagrams are obtained (Fig. 1.1).

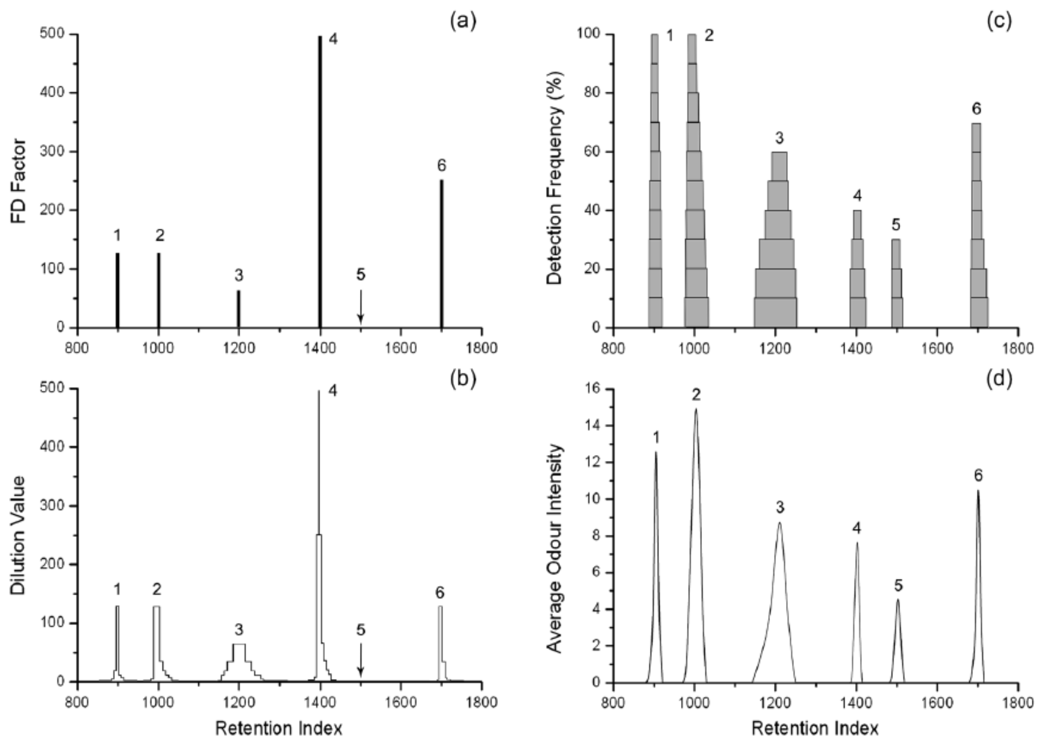

Figure 1.1: Comparison of results simulated for six compounds in the same extract using four different GC-O methods: (a) AEDA; (b) CharmAnalysis; (c) detection frequency; and (d) direct intensity (Osme). From Delahunty et al. 2006.

Regardless of the method used, attention must be paid to certain aspects. Taking into account that detector is always a human being, any environmental or external factor can influence the test. Therefore, the laboratory must be isolated from noises and distractions that could impair the normal performance of the test and modify the results of the analysis. The assessors must be in a comfortable position and the GC-O must be adequately calibrated, the sniffer as well, and the best results, as in the sensory analysis, are obtained using a suitably large and well-trained panel (Delahunty et al., 2006). Moreover, about the Charmanalysis, the randomization of the samples is very useful to avoid prejudices by the tester (d'Acampora Zellener et al., 2008).

1.4 ORIGIN OF THE AROMA IN WINE SPIRIT

From the grapes to the final wine spirit there are several steps that influence the aroma compounds and their amounts. All the different classes of aroma from the grapes, from ageing and evolution of compounds during fermentation, distillation and ageing are resumed here.

1.4.1. GRAPE AROMAS

Thanks to the transformation during the winemaking, a lot of aromas coming from the grapes are released (Cantagrel et al., 1998). Mostly giving floral, fruity and vegetal notes are present in new and aged wine spirits. The composition of precursors is conditioned by terroir, climate, vineyard and harvest management. Grape compounds in wine spirits include higher alcohols as hexanol and hexenol; terpenes, (the most important class of varietals aromas), typical and recognizable in the Muscat, Malvasia and Traminer grapes (also known as aromatic grapes) (Günata et al., 1985; Wilson et al., 1986). The most important are linalool and derivates and α -terpineol. Another family of varietal aromas are aldehydes and as last norisoprenoids, derivated from carotenoids degradation as vitispiranes, α -ionone, β -damascenone and (E)-1-(2,3,6-trimethylphenyl)buta-1,3-diène) (TPB) (Lurton et al., 1991).

1.4.2 PREFERMENTATIVE AROMA

From the harvest until the fermentation, several enzymatic phenomena happen to the grapes subject to storage, mashing, pressing and maceration (Coordonnier and Bayonove, 1981).
The main products of these reactions are hexanal, Z-3-hexenals, E-2-hexenals and their corresponding alcohols (Rapp et al., 1976; Schreier et al., 1976; Moio et al., 2004); they all have herbaceous and green connotation, with low perception threshold that can influenced the wine bouquet. In the past, precisely because of their olfactory characteristic, they were attributed to the leaves that were collected by mechanical harvesting (Joslin and Ough, 1978; Ramey et al., 1986).

1.4.3 FERMENTATIVE AROMAS

These class of aromas origin as secondary products of the metabolism of the yeast during the alcoholic fermentation and, a small part, during the malolactic fermentation. The synthesis of these compounds is influenced not only by the yeast strain, but also its growing conditions, as temperature, oxygen, acidity of the medium and sanitary condition of the grapes (Nedjma, 1997). Among the metabolites produced by the yeasts during the fermentation, there are higher alcohols that can also reach threshold of 100 mgL-1. It was found that exceeded the threshold of 400 mgL-1 these compounds may contribute negatively to the aromatic character of wines (Bell & Henscke, 2005).
The main alcohols are, active amyl alcohol, and 2-phenylethanol, 1-propanol, 1-hexanol (Czerny et al., 2008; Ferreira et al., 2000).
Esters are mostly formed through the esterification of alcohols with fatty acid during fermentation, distillation and ageing process (Zhao et al., 2009).
It is therefore possible to distinguish two categories of esters: acetate esters and ethyl esters. The most important and characteristic are the isoamyl acetate, with its tick smell of banana, phenylethyl acetate, with the smell of rose and honey; isobutyl acetate with fruity aroma, ethyl acetate characteristic for the solvent aroma. From medium chain fatty acid (MCFA), ethyl esters including ethyl hexanoate (aniseed, apple flavoured) and ethyl octanoate (sour apple flavouring) (Molina et al., 2009; Vilanova et al.,2012 Saerens et al.; 2008).
Volatile fatty acid, in particular acetic acid, can be easily found even within the distillate;
Some of them are propanoic acid, isobutyric acid, isovaleric acid, 3-methylbuty-acid and phenylacetic acid. (Vilanova and Oliveira, 2011), butanoic acid (butyric acid), hexanoic acid (caproic acid), octanoic acid (caprylic) and decanoic acid (capric) (Dubois, 1994), oleic and linoleic acids (Ribereau-Gayon et al., 2006 a,b; Vilanova et al., 2012).

1.4.4 AROMA DEVELOPING DURING DISTILLATION

The volatile compounds from the grapes (see 1.4.1), from the prefermentative process (see 1.4.2) and from fermentative process (see1.4.3) pass to the wine spirit during distillation (Léauté, 1990). Additionally, several chemical reactions during the distillation process that leads at the formation of new compounds and elimination of others, improving the quality of wine spirits (Cantagrel et al., 1990a).

Hydrolysis of esters and terpenes, as well as the formation of new compounds thanks to Maillard reactions as furans, pyridines and pyrazines with toasted nuts and cocoa notes.

Although by Streker degradation is possible to obtain aldehydes as furfural and acetals (Léauté, 1990)

1.4.5 WOOD AROMAS- VOLATILE COMPOUNDS FROM WOOD RECEPTACLES

The wine spirit must age for at least six months in wooden barrels (Reg. EC 19/787).

During this period there are several modifications that improve the quality and the characteristic of the wine spirit. From an aromatic point of view, several molecules are released into the wine spirit.

The quantity and type of aromas that the spirit acquires, depend first of all on the quantity of precursor content in the wood's matrix, then on botanical species (Canas et al., 2000), the toasting level of the barrels (Caldeira et al., 2006a) as well as their size (Canas et al., 2008) and the time that ageing the spirits (Caldeira et al., 2006b).

One of the most important and more potent compound extracts from wood is vanillin. This aldehyde derived from the lignins degradation and also from of coniferaldehyde oxidation (Puech et al., 1984), and its amount increase during the aging due to the extraction process and release from the wood (Puech et al., 1984; Canas, 2003).

Vanillin is normally present above the threshold and give pleasant vanilla aroma to the spirit.

The isomers *cis* and *trans* β-methyl-γ-octalactone are two important aromatic compounds of oak wood. These are present above the threshold, giving to the spirit a strong aromatic impact; the isotope *cis* is discriminatory for wine and spirits aged in American oak (*Quercus alba*) barrels as it releases the drink the typical coconut aroma. The *trans* isomer, on the other hand, has a more floral and sweeter feature and is discriminant for the French oak (*Quercus robur*) (Guichard et al., 1995).

Other two relevant compounds from wood are syringol and eugenol, which came also from the degradation of lignin (Fengel and Wegener, 1989). These two phenols, as other compounds, increase their amount as consequence of the increase of the toasting level during the making barrel in the cooperage. Although in a different way and probably with the

contribute of other molecules that interact with each other they both give a spicy and woody note to the spirit (Caldeira et al., 2008).
The smoky note, positively associated with the overall quality of wine spirits (Caldeira et al., 2006b), is due to the syringol, along with a large class of molecules with aromatic characteristics such as hexanoic acid, guaiacol, 4-propylguaiacol, syringol, 4-methylsyringol; 4- allylsiringol. Other unidentified molecules participate in this aroma note (Caldeira et al., 2008).
During the toasting process when the making barrel in the cooperage, the heating of the parietal polymers of the wood and in particular the cellulose and the hexoses that constitute it, lead to the formation of 5-hydroxymethyl furfural (HMF) and methyl-5-furfural. The degradation of pentoses from wood hemicellulose results in formation of furfural (Bierman et al.,1987; Chatonnet P and Boidron, 1989b).
These furanic aldheydes are associated with dry fruit attribute like almond, caramel and toasted attribute typical of spirits aged in wood, to which also contribute 4-propylguaiacol, syringol, 4-methylsyringol and 4-allylsyringol (Janácová et al., 2008; Caldeira et al., 2008).
Acetic acid is a secondary product due to the toasting process, which derives from hydrolysis of acetyl and xylanine groups (Biermann et al., 1987). Nevertheless, it remains at relatively low levels as it’s bonded on acetyl groups, being released progressively due to hydrolysis during the ageing of wines and spirits (Puech, 1987).
Acetovanillone and propiovanillone are ketons derived from the lignans of wood (Chattonet and Boidron, 1989b). The perception threshold of these molecules is 3 mg/l in water, 15 mg/l hydro-alcoholic solution model. During wine spirit ageing, it was noticed an increase of vanillin from the degradation of vanillone (Nishimura et al., 1983).

1.5 SPIRIT TECNOLOGY AND ITS INFLUENCE ON THE WINE SPIRIT AROMA

Some guidelines must be followed in order to obtain a good wine for distillation. Regarding the variety, the grapes should have a potential alcohol content that must not exceed 12% v/v preferably from 8 to 9% v/v. The grapes must have a good acidity, with a neutral aroma (Garreau, 2008). Talking about the neutral variety in the region of Armagnac and Cognac, the most important and most cultivated is the Ugni Blanc (Trebbiano Toscano), originary from Italy. (Garreau, 2008).
In the vine management, a particular care about the amount of certain phytosanitary product must be taken. The use of sulphurs fungicide to protect grapes from fungi disease as powdery mildew can develop on the wine spirit some nasty reduction odour (Estreguil et al., 2007).

On the other hand, and common problem in the vineyard is the presence of Botrytis cinerea on the grapes at harvest. The presence of this mould leads to an unpleasant consequence to the quality of the raw material and the wine spirit: less aroma, quality and complexity, nevertheless the presence of aroma of earth, fungus that is not always perceptible in the fresh distillate but after or during the ageing time. (Cantagrel et al., 1990b). In particular the 1-octen-3-ol is a compound with the typical odour of fungus on the grapes affected by mouldy (La Guerche et al., 2006) and is a specific marker of the presence of this mildew that pass easily during distillation into wine spirit (Urruty et al., 2007).

During the winemaking process, further precautions and strategies must be taken to obtain a good wine to be distilled.

At the beginning, after the grape harvesting it is very important do not exceed in the pressing in order to avoid an excesses of ethyl esters of long chain fatty acids that can give instability of the distillate forming an oil film and turbidity (Hervé et al., 2007). After a gentle pressing of grapes, the use of pectolytic enzymes is not recommended, as they could induce a higher production of methanol. However, the use of specific enzymes to produce base wines for distillation, limit the production of higher alcohols (quality markers in the distillate) and release an acceptable level of methanol in the wine (Bajard-Sparrow et al., 2007).

Contrary to what happens with modern technology for white wine making, it is possible to leave the lees after pressing to increase the complexity and aroma of the distillate, which has a higher level of ethyl laurate and ethyl decanoate, as found in the study of Jurado et al., (2007).

The grape juice must ferment without the addition of SO_2 that could be responsible for sensory defects in freshly distillate (Belchior et al; 2015) and can significantly decrease the complexity of aroma of the distillate in particular of some compounds such as ethyl acetate, 1-propan-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol and ethyl lactate. Moreover, after the fermentation should be a raking of the lees to avoid higher amount of higher alcohol (Jurado et al., 2007; Tsakiris et al., 2013).

The influence of malolactic fermentation seems to have significant effects with regard to fruity aromas and freshness of the distillate. According to the studies of du Plessis et al., (2002) in the basic wines subjected to malolactic fermentation the fermentation esters as isoamyl acetate, ethyl caproate, hexyl acetate and 2-phenethyl acetate diminish, influencing the quality and complexity of the distillates. On the other hand, it is possible to discriminate against the effects of malolactic fermentation based on the strains used: the use of *Lactobacillus* negatively influences the quality of the base wine compared to the use of *Oenococcus oeni* that seems to have a less pronounced effect (du Plessis et al., 2004).

The distillation can be carried out just after the alcoholic fermentation, to preserve as much as possible of esters aroma (such as isoamyl, diethyl succinate hexyl acetate, phenylethyl acetate) and to avoid the formation of undesirable flavour (like ethyl acetate and acetals) (Cantagrel, 2003).

During the distillation process, in addition to extraction and concentration of the aromas, several reactions happened during the production of the spirit.

Among the aromas that can be developed during the distillation there's 2-methylpropanal (isobutanal). This aldehyde above a certain threshold, 35 mg/L leads to evident and unpleasing hints of herbaceous and rancid that can affect the quality of the spirit (Galy et al., 1993). The main precursors are valine and glyoxal (Loizeau 2002).

The concentration of valine reaches 10 to 30 mg/L depending on the wine variety, cultivation and fermentation process (Vanderlinde, 1995).

The concentration of glyoxal, depends mainly on the malolactic fermentation (de Revel, 2000) and the presence of *Botritis cinerea* that with oxidation processes seems able to generate a greater accumulation of this precursor in the wine, especially if the grapes are not Sulphitate (Guillou, 1996).

Moreover, the oxidation of basic wines before distillation appears to have a positive relationship with the increase in concentrations of isobutanal precursors (Giraud, 2003).

The formation of the isobutanal occurs during the distillation where the high temperatures catalyse Maillrd's reactions.

The use of gas boilers rather than direct fire on the still, allow a better control of the temperatures and decrease the level of Isobutanal (Galy et al., 1993). Fortunately, even during ageing time on wooden barrels, there is a decrease in the levels of isobutanal due to selective evaporation (Giraud, 2003).

Another important factor to take care to obtain a fragrant and balanced spirit, regarding the aroma, is the control of the alcohol concentration in distilled making process. Study on Chinese wine spirit compared different product with different alcohol level: 51%, 65%, 77%, 80% (v/v). Volatiles in spirits, which were made from the same homogenized grapes, showed significant differences in some aromatic attribute. As the alcohol content increased, it was possible to notice an increase in the level of esters and a decrease in acids. At 80% v/v no terpenes were found. The fruity notes are the most marked in this spirit and at 77% v/v the product seems more balanced and expressive at the aromatic level (Wei et al., 2018).

1.6 DISTILLATION

The aim of the distillation is to increase the content of ethanol and the volatile constituents of the wine (Tsakiris et al., 2013). Distillation allows the separation of alcohol and volatile compounds contained in the wine (Belchior, 1987, Léauté, 1990, Cantagrel, 2008, Garreau, 2008). It consists of heating the wine to the boiling point of the volatile constituents and condensing the released steams. During the distillation, since the volatility of the compounds is related to the boiling points, the rate with which the volatile compounds are separated by the hydro-alcoholic steam varies according to the different chemical group and boiling point: alcohols, aldehydes, ketones, esters, nitrogen compounds, others.

The different distillation technologies lead to the production of distillates with different characteristics. French Cognac is obtained from a double distillation process in the typical "Charentais" alembic. Armagnac is produced from a single column distillation. In Portugal, the distillation take place in the alembic or column distillation (Belchior, 1987), depending on the region and the producer.

There are two types of distillation: continuous or in-batch. Normally, continuous distillation takes place in column still, while in-batch distillation takes place in alembic. The distillate, according to Reg EU 19/787, can reach a maximum alcohol content of 86% v/v, to retain some of the volatile compounds of the raw material.

An alembic consists of: boiler (or pot), head, swan's neck, low wine heater and a condenser as show in figure 1.2.

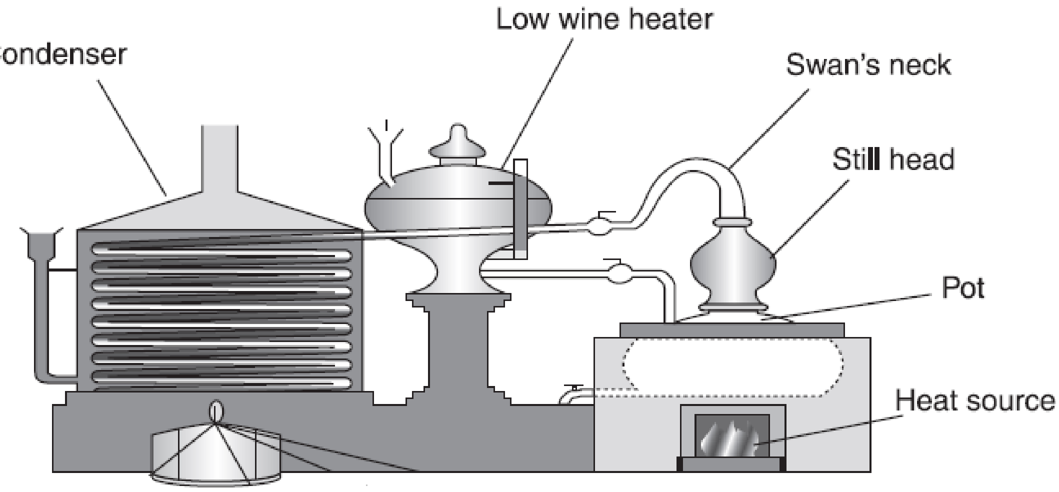

Figure 1.2: Alembic “Charentais” from “Alcoholic beverages”, © Woodhead Publishing Limited, 2012.

The boiler contains the wine and accumulating heat from the heat source (Belchior, 1987).

The head is the part of the alembic that is just above the boiler, its shape and volume determine concentration, selection and separation of the different volatile compounds of the wine (Cantagrel, 2008). This part helps to collects and send the vapours to the “Swan neck”, which has the purpose of leading the vapours from the hat to the coil (Cantagrel, 2008). The head also allowing a partial condensation of the same vapours that return into the boiler to be redistilled, improving the separation of the wine volatiles (Léauté, 1990).

The low wine heater of the alembic is an optional structure that permit, thanks to the vapor flow to pre-heating the wine destined for the next distillation (Cantagrel, 2008).

The condenser coil follows the swan's neck, it is formed by a cylindrical spiral tube, immersed in water, inside the condenser. The initial part of the coil has a wider diameter, to facilitate condensation and progressively goes decreasing until it reaches the alcohol meter (Léauté, 1990).

During condensation, the reaction of copper with sulphur compounds and fatty acids leads to the formation of insoluble compounds (Cantagrel et al., 1990a) which are removed through filtration.

The alcoholometer verifies the alcoholic strength and the temperature of the output distillate, allowing to check the progress of the distillation (Léauté, 1990; Cantagrel, 2008).

The distillation in alembic required a double distillation for the enrichment in alcohol which increase the yield, purity and the quality of wine spirit. The first distillation, which lasts 6-7 hours, depending on the volume of wine to distill and alcoholic degree of the wine, gives a heart fraction of about 28 to 32% v/v. Heads are re-distilled with the succeeding batch of wine. The heart is re-distilled for 15 hours, during the second distillation. The distillate became 70-86% v/v (Belchior et al., 2015).

The ethanol concentration increases in the first phase, then decreases (Leautè, 1990).

Batch distillation produces a complex, aromatic and more appreciated product.

The continuous distillation system (Fig 1.3), on the other hand, consists of a column containing several overlapping plates in which the different volatile compounds of the spirit are separated.

The wine is placed in the upper part of the heater, covering all the dishes until it reaches the base.

A steam flow passing through the wine permit the heating and the boiling. During the transition between the different plates, the steam separates the different volatile components and condensed (Lafon et al, 1973; Belchior, 1987; Garreau, 2008). The higher boiling components returns to the boiling flask.

Once the equilibrium (temperature, flow rate and alcohol content of the distillate) is reached, the distillate is collected in containers.

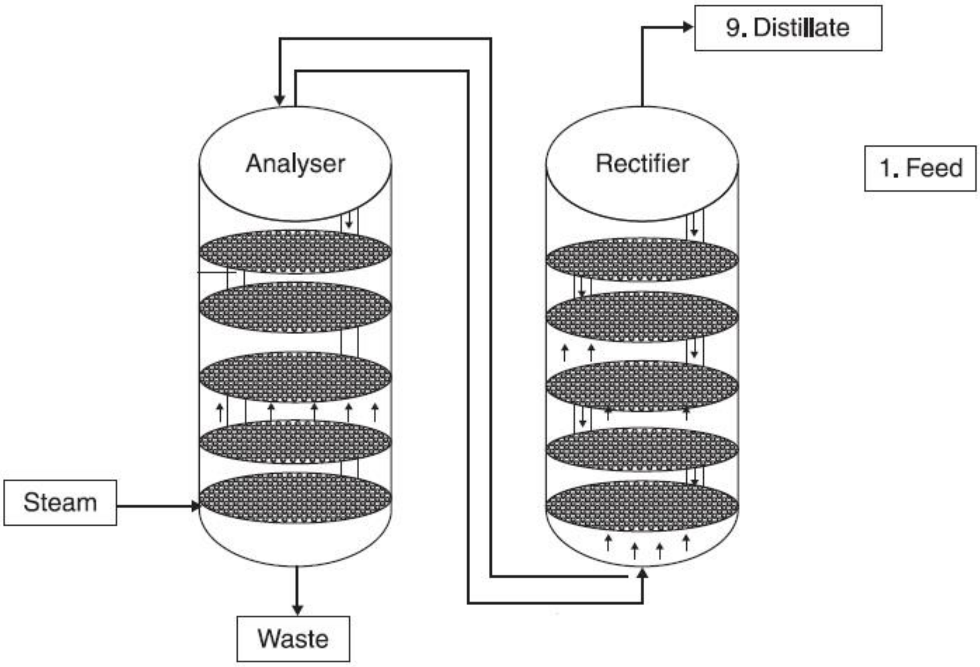

Figure 1.3: Distillation continuous in column. From “Alcoholic beverages”, © Woodhead Publishing Limited, 2012.

Continuous distillation, according to Lafon et al, (1973) and Garreau (2008) is considered economically advantageous, as it allows the distillation of large quantities of wine in a short time, although with less aromatic and richness than the "Charentais" system (Lafon et al, 1973).

The alembics and columns are made with copper which have several advantages: it is malleable, it is a good conductor of heat, it resists to corrosion from the fire and the wine, it is a catalyst for favourable reactions between wine components, it reacts with sulphur components and fatty acids forming insoluble salts that precipitate (Leauté, 1990; Belchior et al. 2015). On the other hand particularly attention on the cleaning of the alembic must be taken to avoid excessively copper residual inside the wine spirit. Nowadays, there is no precise regulation on the content of copper in spirits but it can be used as a guideline the limit placed for wines of 1mg/L (REG EC 19/606)

1.7 AGEING OF WINE SPIRITS

From the new distillate, qualitative and quantitative modifications occur on the flavour and aroma characteristics, due to the prolonged maturation period in wood (Leauté et al., 1998; Caldeira et al., 2008). These changes are mainly the result of the release of extractable wood compounds on wine spirits (Puech et al., 1984; Puech et al., 1985).

According to the European regulation. high-quality wine spirits must mature for at least 6 months in wooden receptacles with a capacity of less than 1 000 litres before sale and consumption (Reg EC 19/787). In the specific case of protected designations of origin, the wine spirit must stay in wooden barrels during one year for Armagnac (Déc. N ° 2009-1285) and two years for Cognac (Déc. N ° 2009-1146). For the Lourinhã DOC, (the unique Portuguese Geographical Denomination that is exclusively delimited for the production of aged wine spirit) (Fig 1.4), the spirit must age for at least two years in wooden barrels (Dec. Lei n º 39 of 1994).

Figure 1.4: Wine spirit's Protected Designation of Origin, Portugal. In IVV.pt

Nowadays the use of wooden barrels is not only used as simple containers for storage and transport but are necessary for an organoleptic evolution of wine or spirit aged in these barrels (Feuillat et al., 1998; Caldeira et al., 2006a).

In order to better understand how barrels can induce changes in wine spirits, it is necessary to mention the complexity of the wood's structure: it consists essentially of a series of glycosidic chains of cellulose, hemicellulose and lignin, bonded together with hydrogen and covalent bond, (in particular of the ester type), forming a complex and non-repeating three-dimensional ensemble, allowing the accessibility of the solvent (Ernis et al. 1976; Mosedale and Puech, 1998) (Fig.1.5).

These chemically and sensory changes are influenced by the wood botanical species (Miller et al., 1992; Viriot et al., 1993; Canas et al., 2000; Prida and Puech, 2006;), the cooperage production phase (Rabier and Moutounet, 1991; Canas et al., 2007), the dimension of the barrels (Guymon and Crowell, 1970; Singleton, 1995; Canas et al., 2008) and ageing cellar condition (Cantagrel et al., 1992).

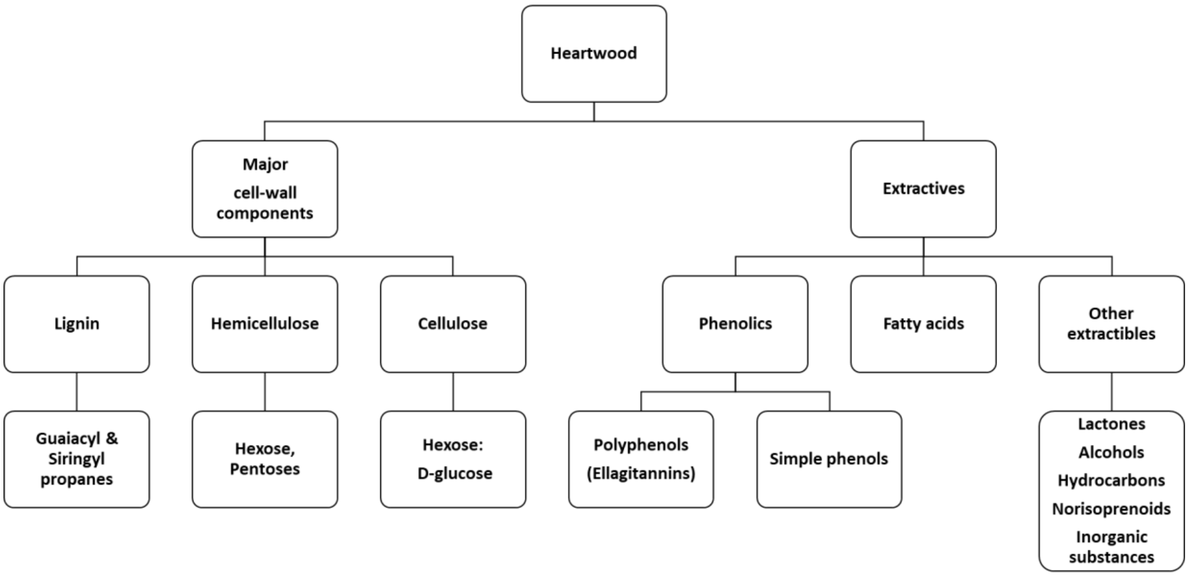

Figure 1.5: Component of oak heartwood (from Mosedale and Puech, 1998).

1.7.1 THE INFLUENCE OF THE BOTANICAL SPECIES OF THE WOOD

About the influence of using botanical species of wood for cooperage, several studies about the physic chemical proprieties and chemical composition were made, especially for the French and American oaks (Chatonnet and Dubourdieu, 1998; Zahri et al., 2007). Therefore, some authors support that only oak species enhance the quality of aged wine spirit (Singleton, 1995; Haluk and Irmouli, 1998).

Traditionally *Quercus robur L*. from Limousin Forest (France) was used for making barrels to aged wine brandy, especially Cognac and Armagnac.

Nevertheless, other botanical species are used for this purpose like the chestnut wood (*Castanea sativa* Mill.). On the 18th century chestnut barrels were very appreciated for storage and ageing wines (Taransaud, 1976) such as Porto wines (Filipe et al., 1998).

Study about the chemical composition of chestnut wood, shows the richest of gallic acid and hydrolysable tannin (Salagoity-Auguste et al.,1987) and other several research about chestnut wood take place, (Scalbert et al., 1989; Peng et al., 1991; Viriot et al., 1994), showing the importance of this species for cooperage and oenological use (like for oenological tannins). The botanical species and the geographical origin explain important differences between the different type of wood, regarding the total content of low molecular weight phenolic compounds: studies show that chestnut wood have the highest content (mainly phenolic acid with the 90-95% contribution, the aldehydes represent just the 1-5%) in contrary with the Portuguese, French and American oak. The gallic acids and the vanillin are also predominant in chestnut wood; and by the gallic acid present high amounts in the oak species (Canas et al., 2000).

Moreover, the origin of the wood influences the quantity of volatile compounds as *cis*-β-methyl-γ-octalactone, eugenol, acid, furfural, 4-hydroxy-2-butenoic acid lactone, hexanoic acid, *trans*-β-methyl-γ-octalactone, vanillin and guaiacol (Caldeira et al., 2006a). The same study confirm that is possible to discriminate the American white oak from the other species based on amount of eugenol and *cis*-β-methyl-γ-octalactone This last compound is was demonstrated that is absent or in a very low concentration in chestnut wood (Caldeira et al., 2010).

1.7.3 THE INFLUENCE OF THE COOPERAGE

Regarding the cooperage process there are several aspects that can modify the properties of wood, first of all, the period of drying outside that induces physico-chemical changes (leaching for the degradation of rainwater from peroxidase and microorganisms), and, most importantly, the barrel manufacturing process, in particular the heat treatment which consists of heating the inner surface of the wooden staves to allow the toasting and bending of them (Puech et al.,1984; Sarni et al.,1990).

Traditionally the heating and toasting process is handled differently depending on the manufacturer, however, according to the studies of Chatonnet and Boidron (1989a), the following parameters are used to obtain the different types of barrel's toasting level: for a light toasting level 5 minutes at 180°C; for a medium toasting 7,5 minutes at 200°C; for a strong and really strong toasting respectively 11,5 minutes at 220°C and 17,5 minutes at 230°C.

The first consequence of the heating process is the breakdown of the bonds between lignin polymers and the increase in the number of extractable compounds. The study carried out in 1990 by Sarni et al., clearly illustrates that with the heat treatment there is a decrease of ellagic tannins, a simultaneous increase of ellagic acid and an increase in aldehydes from the degradation of lignin. The temperature is a fundamental aspect: as the temperature and toasting time increase, the concentration of volatile compounds rises, such as furfural, 5-methylfurfural, 5-hydroxy-methylfurfural and acetic acid (Caldeira et al. 2006a). These compounds derive from the thermal degradation of cellulose and hemicellulose as explained by Fengel and Wegener (1989). Moreover, the amount of eugenol, syringol and 4-allyl-syringol and furan, is proportional and so discriminant for the toasting level of wood (Caldeira et al., 2006a). During the bending phase, low temperature (120-125 ° C) seems to be preferable for the mechanical properties, thermal uniformity along the stave and for a better extraction of low molecular weight extractable compounds (Canas et al., 2007);

1.7.4 ALTERNATIVE AGEING SYSTEM

The wine brandy as already anticipated, must age for a minimum of six months in wooden barrels, to reach the appropriate maturity before sale and consumption and to improve the sensory quality (Reg EC. 19/787). Nowadays, the interest towards sustainable economic and environmental production has stimulated researchers and industries to find alternative systems to achieve this goal in the wine and wine spirit.

The most interesting and recent are the physical methods that use various techniques to accelerate the aging process with excellent results on the stability and quality of wine, like ultrasonic waves, gamma radiation and application of electric field (Tao et al., 2014).

More traditional and well-known techniques with the use of wood fragments such as chips and stave, ageing on the lees and the use of micro-oxygenation. The production of chips and stave as alternative ageing techniques follows the same procedure for the cooperage, (drying out, heating and toasting and moistening at different levels) and on the market, we can find several options for different botanical species.

According to the study of Caldeira et al. (2010), the use of tablets improves the sensory aspect of the wine brandy, showing a more mature character than the ageing in barrels after only six months of refinement. About the colour, the spirits turn in a topaz hue; the aroma results more complex with of roasted and coffee notes. Even in these cases the botanical species is discriminatory: the use of the stave and the chestnut wood tablet seems to lead to a better evolution of the sensory characteristics of the brandy such as the colour of topaz, the toasted aroma and the coffee and the less bitter taste. More recent work (Caldeira et al.,2017), shows simile results: alternative system has a good impact on the sensory profile of a 24 months aged wine distillate, with a higher overall quality and strong notes of coffee, caramel and unctuous respect the same wine spirit in barrels. Is important to underline that this work on alternative ageing system is correlated with other parallels works with Canas et al., (2019) studying the impact on the phenolic composition and the antioxidant proprieties deriving from ageing. This work underline the faster extraction and evolution also of the phenolic compounds an the evolution of the colour of wine spirit, giving a good starter point to operationalize the ageing system in the wine spirit industries.

1.7.5 THE ROLE OF MICRO-OXYGENATION

It has always been known that barrels are an active container, influencing the aroma and, as regards the red wine, it allows the stabilization of the colour thanks to the formation of polymers, origin mainly phenolic, mediated by the oxygen (Timberlake and Bridle ,1976). Also, in this case the influence of the botanical species is relevant to the demand for oxygen in the various alternative ageing systems for red wines, as confirmed by the studies of De Alamo et al. (2010). The authors verified that the French-oak woods (*Q. petraea*) require more oxygen than the American oak (*Q. alba*). Moreover, about the wood fragments for the refinement, the larger the size of the latter and the greater the demand for oxygen in the wine (Del Alamo et al., 2010). About the aging of wine spirit, it has been found that the consumption of oxygen in the alternative refining systems (chips and stave) is greater than in wooden barrels (Canas et al., 2009)
The oxygenation of the wine spirit, during the ageing process in stainless steel with the addition of stave and micro-oxygenation, negatively influenced the release of extractives and lignin from wood to wine spirit (Anjos et al., 2013). Moreover with the studies of Canas et al. (2009), it seems that the wine spirits aged in alternative system by the addition of oxygen did not stabilize the colour after the first year, and during the sensory analysis, the spirits obtained a score lower than the spirit aged in barrels, then further studies must be carried out in order to better understand the role of oxygen in the alternative ageing systems.

1.8 GC-O AND SPIRITS QUALITY

The qualitative study of the volatile compounds and the search for key aromas in food and drink has been in use for many years now. The type, content and sensory threshold of the aroma compounds are the primary factors that affect the quality of spirits. These factors also influenced the flavour and sensory quality of wine spirits (Caldeira et al., 2002; Zhao et al., 2009) as well as for other alcoholic beverages such as rum and whiskeys. Advances and continuous researches in this field have led over time to discovery more and more key aromas within beverages, among the hundreds of volatile compounds that make up these products.
In rum, for example, the first studies on the volatile component were carried out by Maarse et al. in 1966, where more than a hundred volatile compounds were identified. More recently an interesting study with GC-O by De Souza et al. (2006) compared the Brazilian cachaça (spirit obtain from the sugar cane) with rum, identifying some of the most powerful odour components of the rum such as β-damascenone, 1,1-diethoxyethane, ethyl 2-

methylbutanoate, ethyl 2-methylpropanoate, ethyl butanoate, oak lactone, vanillin and two unknown compounds.

One of the aromatic characteristics of rums is the strong scent of first-impact alcohol, due to the presence of ethanol and other alcohols participating in this aroma as 3-methylbutan-1-ol and 2-methylpropan-1-ol, making it the largest class of odorous compounds within the distillate (Pino et al., 2012).

Regarding β-damascenone, several studies found it as an important key aroma for wine and several spirit like Cognac (Uselmann and Schieberle, 2015), pear brandy (Willner et al., 2013) and whiskey (Vocke, 2008), giving the fruity typical aroma that remind peach and jam (Pino et al.,2012). Its origin could be varietal in grapes, but it can also develop with acid-catalysed reaction during the fermentation and distillation (Daniel et al., 2008).

From the same studies of several spirits, also the 1,1-diethoxyethane was found as potent key aroma with a fruity connotation (Franitza et al., 2016).

Another world-known distillate that is the subject of studies in the field of the research of key compounds aroma is whiskey. Produced by fermentation and subsequent distillation of malt and cereals, this spirit refines in wood for several years until it reaches unique characteristics that make it one of the most appreciated alcoholic beverages (Poisson and Schieberle, 2008). On the research of odorant compounds in bourbon whiskey made by Poisson and Schieberle, (2008), was found again as determinant β-damascenone with fruity note with α-damascone, exhibiting a cooked apple-like smell, and β-ionone exhibiting a violet-like odour. But as mentioned before a large contribute of the aroma of whiskey is given by the wood's compounds like *cis* and *trans* -β-methyl-γ-octalactone (coconut like) and eugenol (clove like) that are between the most important odour compound in whiskey.

In the same work a malty smell was identified as 3-methylbutanol, 2-phenilethanol (flower like) and various ester exhibiting fruity or flowery aroma characteristic as ethyl 2-methylbutanoate (considered by the authors the most important fruity odour in whiskey), 3-methylbutyl acetate, ethyl hexanoate, ethyl methylpropanoate, ethyl butanoate, 2-phenylethyl acetate, 2-phenylethyl propanoate, trans-ethyl cinnamate, and ethyl 2-phenylacetate.

Other interesting studies, about aroma compounds, were made by Cacho et al., on 2012 and 2013 on Peruvian Pisco, a wine spirit produced by aromatic and no-aromatic grapes. The study underlines the characteristic of fruity and floral aroma of this distillate, indeed, the most important odorants were 2,3-butanedione, isoamyl alcohol, ethyl butyrate and ethyl 3-methylbutyrate, by-product from yeast fermentation; guaiacol, β-damascenone, geraniol and linalool varietal aroma from grapes; sotolon and phenylacetaldehyde resulting from oxidation of the corresponding amino acid precursors during distillation (Ferreira et al.,2003). β-phenylethanol and its acetate derivative β-phenylethyl acetate, both with rosy attribute, were found as important key aroma for the aromatic piscos.

Probably not the most known spirit in France, Calvados is a spirit derivate from the fermentation of apple cider, then before the sells must age for at least two years in oak barrels. Considering already investigated the role and the aroma giving by the aging period in wood the team of Ledauphin et al., characterize the freshly distillate aromas on 2003.

Esters represent the largest group due to the apple constituent and fermentation process mainly methyl, butyl, 3-methylbutyl (isoamyl), and phenylethyl esters respectively characterise by fermented apple and flower note. Isopentanol was the main alcohol detected in the spirit with plastic aroma. This molecule is normally present in a large amount on wine spirit (Williams and Tucknott, 1971). The 3-methylbut-2-en-1-ol and 6-methylhept-5-en-2-ol were the most important alcohol active compounds. About the phenolic derivatives, 2-phenylethanol (rose, mushrooms) and its acetate 2-phenylethyl acetate (floral, underwood) and 4-vinylanisole (sweet, delicatessen) seems to be characteristic compounds in Calvados.

One of the most known wine spirits is Cognac, produced in the geographic area including the homonymous city and all Charente maritime, a large part of Charente and some neighbouring communities.

Several studies and research were made using GC-O, resulting on the identification and classification of key aromas compounds of these spirits.

One of the most interesting, were made by Ferrari et al. in 2004 that studied the fresh distillate, as goal to give some indicators of quality to the buyer of fresh distillate before the required ageing period.

The most important key aroma found in the fresh distillate was diacetyl with a strong impact of butter; nerodiol (Hay), *Z*-3-hexen-1-ol with the grass notes; pear and banana notes were identified as 2- and 3-methylbutyl acetates; the rose descriptor by 2-phenylethyl acetate; the higher alcohols methylbuyhyl acetates and methylbutanols are responsible for sweet and cacao attribute and the lime tree descriptor as linalool. These compounds with other with a minor impact are already present in the fresh distillate thereby giving to wine spirits a characteristic and specific aroma (Ferrari et al., 2004).

Another two work about comparing the aroma compounds and characteristic of wine spirit was made by Zhao et al., in 2009 and 2011, comparing respectively four and two Chinese wine spirit.

The results were almost according on other brandy and Cognac studies, underline that esters could be the most important aromas with the fruity impact and in particular ethyl esters as ethyl hexanoate, ethyl heptanoate ethyl octanoate. β-damascenone, *trans*-β-methyl-γ-octalactone and 1,1-diethoxyethane with the fruity, coconut and cream attribute; 2-methylpropanol and 3-methylbutanol with the fused note. The main key aroma was found in all the samples with difference in some amount of molecules that divided and describe the main difference in sensorial impact analysed and confirm with the description analysis (Zhao et al., 2009, 2011).

Regarding the principal odorant compounds on the aged wine spirits, several works have focused on the impact of wood extractive compounds, mainly phenols, vanillin, *trans*-β-methyl-γ-octalactone, furanic aldehydes and acid acetic. The correlation with overall quality between these compounds and the sensory impact that they have on the wine spirit is recognisable with attribute like toasted, smoke, vanilla, dried fruits, coconut and sweet (Caldeira et al., 2006b; Caldeira et al., 2008). Normally these compounds are present in the wine sprits with higher concentration that the corresponding odour threshold, that explain the sensory impact and importance of these compounds (Caldeira et al., 2016). Also, in the work of Janácová et al., (2008), on Slovakian wine spirit, was relevant the presence of active odour compound as HMF with Caramel and toasted notes or 5- hydroxymaltol giving roasted coffee and caramel notes.

2. AIM OF THE WORK

The objective of the thesis is to evaluate the impact and differences of alternative ageing systems such as staves, for the ageing of wine spirit produced in the delimited region of *Lourinhã,* Portugal, on the GC-O profile of aged wine spirits.

After a first sensory evaluation, the work will be focused on the impact at the level of the odorant profile, which is assessed by the use of the gas liquid chromatography-olfactometry.

3. MATERIAL AND METHOD

3.1 REAGENTS

For the preparation and extraction of the volatile compounds, the chemical reagents used were: dichloromethane, ethanol and sodium sulphate anhydrous, analytical grade, which were purchased from Merck (Darmstadt, Germany). Dichloromethane was redistilled in a Vigreux column. Ultrapure water was obtained through arium® comfort water Purification Systems by Sartorius, Göttingen, Germany.

3.2 STANDARDS

Acetic acid was purchased from Riedel-de-Haen (Seelze, Germany); ethyl isobutyrate, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl hexanoate, ethyl L-lactate, 1-hexanol, ethyl octanoate, linalool, butanoic acid, 3-methyl butanoic acid, hexanoic acid, guaiacol, 2-phenylethanol, eugenol, 4-ethylphenol, 3,4-dimethylphenol (IS), syringol, dodecanoic acid, 4-hydroxy-3-methoxy-benzaldehyde (vanillin), 5- methyl-2-hexanol (IS) were purchased from Fluka (Buchs, Switzerland); isoamyl acetate, *trans*-2-hexen-1-ol, *cis*, *trans*-β-methyl-γ-octalactone, 4-propylguaiacol, 4-methyl-syringol, 4-allyl-syringol were purchased from Aldrich (Steinheim, Germany); 4-ethylguaiacol, DL-malic acid diethyl ester were purchased from TCI, ethyl butyrate was purchased from Merck (Darmstadt, Germany). All the standards solutions were prepared at 20% vol/vol with ethanol/purified water.

These standards were used to do the quantification in the GC-FID, to screen the probable identification in GC-O and GC-FID and to confirm the identification in the GC-MS.

3.3 EXPERIMENTAL DESIGN AND SAMPLE SPIRITS

In order to evaluate the influence of the wood and ageing system, a two-way factorial experiment design was followed where factor one (type of ageing) had two levels and the factor two (kinds of wood) had also two levels (Fig. 3.1).

The wine distillate (alcohol strength, 77.4 % v/v; pH, 5.44; total acidity, as acetic acid, 0.13 g/hL of absolute ethanol; volatile acidity, as acetic acid, 0.11 g/hL of absolute ethanol), produced by the Adega Cooperativa da Lourinhã, Portugal, has used to fill the 250 L barrels and 1000 litre stainless steel tanks.

The wood used to produce the wooden barrels and the staves for the ageing in stainless steel came from Limousin French oak (*Quercus robur* L.), and from Portuguese chestnut (*Castanea sativa* Mill.). Both, barrels and staves were manufactured by J. M. Gonçalves cooperage (Palaçoulo, Portugal), with a medium toasting level (90 min at 240°C). The toasting of barrels followed the classic method over a fire of off cuts woods with control of temperature avoiding differences in toasting levels between the barrels. The staves (91 cm length x 5 cm width x 1.8 cm thickness), instead, were heated in industrial oven.

The staves were located in stainless steel tank in the appropriated quantity to reproduce the same contact surface between wood and wine spirit in 250 L wooden barrel (85 cm^2/L). After filling the tanks, micro-oxygenation was applied through multiple ceramic diffusers (VISIO 6, Vivelys, France) using pure oxygen (X50S Food, Gasin, Portugal) with a flow of 2 ml/L month.

The identification of the experimental units was as follow (Fig.3.1): chestnut barrels (CV1, CV2); oak Limousin barrels (LV1, LV2); stainless steel with chestnut stave and micro-oxygenation (CD1, CD2); stainless steel with oak Limousin stave and micro-oxygenation (LD1, LD2).

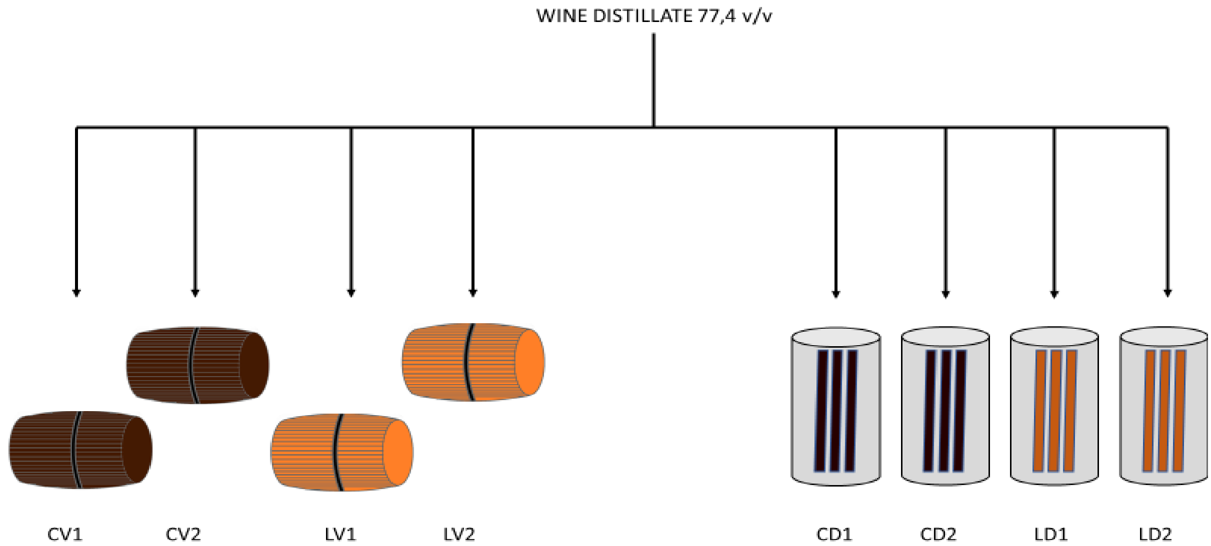

Figure 3.1: Experimental design

After one year of ageing, the samples were collected and submitted to a different chemical and sensory analysis.

The alcoholic strength of the wine spirits was evaluated by distillation followed by electronic densimetry according to the published methodologies (OIV, 2014).

3.4 ISOLATION OF VOLATILES FROM WINE SPIRITS

Volatiles from wine spirits were extracted using the procedure previously proposed by Caldeira *et al*. (2004). 100 cm^3 volume of wine spirit (diluted to 20% v/v) added with the internal standard (IS) 1.6 cm^3 of 5- methyl-2- hexanol (IS 81 mg. dm-3 of 50% ethanol solution) and 0.5 cm^3 of 3,4-dimethylphenol (IS 100 mg.dm-3 in ethanol) was extracted with dichloromethane. Extraction was carried out with the successive addition of 30, 10 and 10 cm^3 of dichloromethane by ultrasonification (P Selecta model 3000515) for 10 min, for each volume. Using separating funnel, the organic phases were collected, dried over anhydrous sodium sulphate and concentrated on a rotary evaporator (Büchi rotavapor R114 at 42,5± 0,5 ºC, without vacuum), to a final volume of 150- 300 mm^3. The extracts were stored at -20 ºC until analysis by GC-O, GC-FID and GC-MS.

3.5 GAS CHROMATOGRAPHY-OLFACTOMETRY ANALYSIS - (GCO)

GC-O analysis was carried out using an Agilent Technologies 6890 Series gas chromatograph (Wilmington, DE, USA) equipped with a fused silica capillary column of polyethylene glycol (HPINNO-Wax, Agilent technologies, Palo Alto, CA, USA), 30 m length, 0.32 mm i.d., 0.50 μm film thickness. The carrier gas was hydrogen with an average velocity of 62 cm.sec-1, flow 30 ml/min. The extract was injected (~ 8 μl) on the injector (250 ºC) in split mode (split ratio ½). The oven temperature program was 35 ºC (for 6 min), then increased at 3.5 ºC to 55 ºC (1 min), then increased at 10 ºC.min-1 to 85 ºC, then increased at 7.5 ºC.min-1 to 100 ºC, increased at 10 ºC.min-1 to 130 ºC (1min) then increased at 5 ºC.min-1 to 210 ºC and held for this temperature for a further 30 min. At the end of the capillary, the effluent was split into the flame ionization detector (FID) – 250 ºC and the olfactory detection port (ODP, Gerstel, Germany). The ODP was held at 220 ºC to prevent any condensation of volatile compounds. Humidified air (17 cm^3.min-1) was added at sniffing cone to reduce fatigue and drying of the judge's nasal passage.

Ten judges were selected for the identification of active odours. To each of them positioned comfortably in front of the detector door, in an appropriate laboratory and without elements that could disturb the analysis (as described by Delahunty et al., 2006), was asked to sniff and describe the odour perception of the volatile compounds coming out on the GC-O funnel. Through an "olfactometric button", which could be pushed by the sniffer, was recorded when an odour was detected (Gerstel, Germany). These signals and the FID signal were analysed simultaneously in HP Pascal workstation, and the judges interpreted the odour recognized with verbal descriptions, regularly recorded. Each wine spirit extract (CV1, CV2, LV1, LV2, CD1, CD2, LD1, LD2) was analysed by GC-O only once by each judge. The method used for the analysis of the graphs obtained during the analysis was the detection frequency method (Linssen et al., 1993; Pollien et al., 1997). Each peak recorded by the GC-O was interpreted as an active odour only when at least three or more judges were detecting the volatile compound in the same sample.

3.6 ODOURANT IDENTIFICATION - GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS - (GC–MS)

The active odourant compounds identified by the judges in the GC-O were then compared with the graphs obtained by the injection of pure standard compounds for a first screening of probable identification. For further confirmation, the retention indexes were approached with the retention Kovats indexes (KI) and MS fragmentation pattern with those of reference compounds or with mass spectra in the NIST and Wiley libraries, obtained by GC-MS.

The GC-MS analysis of dichloromethane wine spirit extracts were performed in a gas chromatograph– mass spectrometer (Magnum, Finnigan MAT, SanJose, CA, USA). The chromatograph was equipped with a fused silica capillary column of polyethylene glycol (HPINNOWax, Agilent technologies, Palo Alto, CA, USA), 30 m length, 0.25 mm i.d., 0.25 μm film thickness. Operating conditions were as follows: injector and interface temperature, 250 ºC; carrier gas helium (inlet pressure 12 psi and split ratio 1:60); oven temperature program: 3.5 ºC min-1 from 45 ºC (10 min isothermal) until 180 ºC and held at this temperature for 30 min, volume injected of 0.2–0.4 mm3. The MS was operated in the electron impact mode at 70 eV, scanning the range m/z 40–340.

Identification of volatile compounds was achieved by comparison of the GC retention times and mass spectra with those, when available, of the pure standard compounds. All mass spectra were also compared with those of the data system library (NIST and WILEY).

Kovats indexes of unknown compounds were determined in GC-O, GC-FID and GC-MS by injecting samples containing a series of alkanes (C9-C30), by linear interpolation (Philips, 1989).

3.7 ODOURANT QUANTIFICATION

The odorant compounds identified in aged wine spirits in previous work (Caldeira et al 2008), were quantified by GC-FID. The major volatiles compounds, which included two odorant compounds, namely 2-methyl-1-propanol and 2+3-methyl-1-butanol were analysed by direct injection of wine spirit distillate on the chromatograph Focus GC (Thermo Scientific, USA) using the methodology previously validated (Luís et al, 2011). The most odorant compounds of wine spirits are minor volatile, thus before the analysis by GC-FID these compounds should be extracted from the wine spirits and concentrated. It was followed the methodology proposed by Caldeira et al., (2004) and using the conditions described in Caldeira et al., (2010). As such, volatile compounds were extracted from 100 mL of wine spirit samples (previously diluted to 20% v/v) using discontinuous ultrasound liquid-liquid extraction with redistilled dichloromethane dried over sodium sulphate anhydrous and then concentrated to a final volume of about 200 μL.

The quantification was done through the analysis under the same conditions, of hydroalcoholic standard solutions (40% v/v, then diluition until 20% v/v) containing known amounts of the volatile compounds.

3.8 SENSORY ANALYSIS

The sensory panel was composed by judges previously selected and trained in the sensory descriptive analysis of wine spirits according to the methodology described by Caldeira et al., (1999). Moreover, the repeatability of the panel and the reliability of the judges was assessed as previously proposed by Caldeira et al., (2002), with the introduction of a sample replicate in each analysis session and through the recognition and quantification of the intensity of the odorous standards samples presented before each test.

The samples were previously diluted with water, thereby reducing the ethanol concentration to 40%. The samples were present to the judges, in wine tasting glasses (ISO 3591:1977) in balanced orders to eliminate first-order carry-over effects (MacFie et al., 1989). It was provided water for mouth rinsing between samples.

Each judge was asked to fill out a card with the following attributes: colour (citrus, straw, topaz, greenish); aroma (fruity, vanilla, woody, rancid, spicy, caramel, toasted, dried fruit, smoke, coffee, green, tails, glue, caoutchouc); flavour (sweet, soft, burning, tartness, harshness, bitter, body, unctuousness, evolution, complexity, aroma of mouth, persistence). Each descriptor was evaluated under a structured scale from 0 to 5 (0-not perceived, 5-maximum intensity). Additionally, the judges were asked for a general evaluation about the sample tasted with score from 1 to 20. Taking into account the aim of this work only the results of aroma attributes will be presented and discussed.

3.9 STATISTICAL ANALYSIS

A first analysis of the data of the descriptive sensory analysis was performed by the two-way ANOVA. When a significant effect was detected, a comparison mean test was done (LSD Test.). The calculations were done using Statgraphics statistical system, vs 7.0.

PCA (principal component analysis) and clustering analysis was applied to the average of sensory panel attributes, in order to evaluate the relationship between the different samples with the significant attribute find out with the ANOVA.

After the identification of the volatile compounds by GC-O, a correspondence analysis (CA) was applied to the matrix of the frequency of detection of aroma compounds for the wine spirits, in order to study the relationship between the different samples and the odourant compounds.

All the calculations (PCA, clustering and CA) were performed on NTSYS-pc package, version 2.1q (Rohlf, 2000).

4. RESULT AND DISCUSSION

4.1 SENSORIAL ANALYSIS

The sensory analysis was made with the panel to verify the difference between the samples. The analysis of variance was applied to the results of aroma attributes of the wine spirits, which is the focus of this work. Table 4.1 shows the ANOVA results for those sensory attributes, which are significantly influenced by the ageing system, wood type or both, namely vanilla, spicy, caramel, toasted, dry fruits, smoky, coffee.

The effect of the different factors, ageing system and type of wood is evident in almost all the attribute but is more significant regarding the ageing system. The ageing system influenced significantly the intensity of vanilla, spicy, caramel, toasted, dry fruits, smoke and coffee on the wine spirits. The wood type influenced the same attributes with exception for the spicy attribute that is not influenced by wood type. Only for vanilla attribute it was detected the interaction of the two factors.

The other aroma attributes namely alcohol, fruity, rancid, woody, sweet, green, tails and varnish were not influenced by the studied factors.

Toasted note presents a high significant influence based on the type of wood use, and also the smoke note for the type of system used. The spicy note is influenced only by the ageing system and, the vanilla attribute, is the only one that shows also a significant p value in the interaction of the ageing system and the wood used for the ageing.

Table 4.1: ANOVA output of significant attribute of sensorial analysis.

Attribute	Effect	SS	Degr. of freedom	MS	F	p	Effect
Vanilla	system	0.39506	1	0.39506	66.065	0.001	**
	wood	0.11150	1	0.11150	18.645	0.012	*
	system X wood	0.12500	1	0.12500	20.903	0.010	*
	Error	0.02392	4	0.00598			
Spicy	system	1.00347	1	1.00347	17.3980	0.014	*
	wood	0.34722	1	0.34722	6.0201	0.070	n.s.
	system X wood	0.00039	1	0.00039	0.0067	0.939	n.s.
	Error	0.23071	4	0.05768			
Caramel	system	2.46914	1	2.46914	66.3212	0.001	**
	wood	0.64853	1	0.64853	17.4197	0.014	*
	system X wood	0.15432	1	0.15432	4.1451	0.111	n.s.
	Error	0.14892	4	0.03723			
Toasted	system	0.43297	1	0.43297	16.4432	0.015	*
	wood	0.57186	1	0.57186	21.7179	0.010	***
	system X wood	0.08111	1	0.08111	3.0806	0.154	n.s.
	Error	0.10532	4	0.02633			
Dry fruits	system	0.47261	1	0.47261	22.897	0.009	**
	wood	0.55710	1	0.55710	26.991	0.007	**
	system X wood	0.06520	1	0.06520	3.159	0.150	n.s.
	Error	0.08256	4	0.02064			
Smoke	system	0.420139	1	0.420139	17.2857	0.014	*
	wood	0.781250	1	0.781250	32.1429	0.005	**
	system X wood	0.046682	1	0.046682	1.9206	0.238	n.s.
	Error	0.097222	4	0.024306			
coffee	system	1.023245	1	1.023245	130.9753	0.000	***
	wood	0.213059	1	0.213059	27.2716	0.006	**
	system X wood	0.027874	1	0.027874	3.5679	0.132	n.s.
	Error	0.031250	4	0.007812			

*significant ($p<0.05$); ** very significant ($p<0.01$); *** highly significant ($p<0.001$), SS - sum of squares; DF – degree of freedom; MS – medium of squares; F – Fisher' F; p - significance.

When a significant effect was detected, it was applied least significant different (LSD) post hoc-test, as shown in the tables 4.2 and 4.3.

Table 4.2: LSD post-hoc test: influence of the ageing system

		vanilla	spicy	caramel	toasted	dry fruits	smoke	coffee
Mean	Barrels	1.62 a	1.48 a	0.83 a	1.06 a	1.42 a	0.69 a	0.28 a
	Alternative	2.06 b	2.19 b	1.94 b	1.52 b	1.90 b	1.15 b	1.00 b

Table 4.3: LSD post-hoc test: influence of the type of wood

		vanilla	spicy	caramel	toasted	dry fruits	smoke	coffee
Mean	Limousin	1.72 a	n.s.	1.72 a	1.02 a	1.40 a	0.61 a	0.48 a
	Chestnut	1.96 b	n.s.	1.96 b	1.55 b	1.93 b	1.24 b	0.80 b

It was observed that both, alternative ageing system and the use of chestnuts (except for the spicy note), can increase the intensity of these significant attributes (Table 4.2 and 4.3).; In table 4.2 is confirmed that the wine spirits aged in alternative systems presented higher intensity, in particular the vanilla, spicy and caramel attribute that present more than double of the intensity of the corresponding wine spirits aged in wooden barrels. Only for the vanilla there was a significant interaction between wood and ageing system (Tab. 4.4), that shows that the use of limousine barrels for the ageing giving the lowest aroma of vanilla and a higher score for the other system: chestnut barrel, and alternative system, both for chestnut and oak staves. That's result is in accord of the study of Canas et al. (2000) that underline the fact that chestnuts wood has the higher content of vanillin that is associated to the vanilla attribute. Thus, considering the evolution of sensory attributes over the time, according to the studies of Caldeira et al., (2006b) the wine spirits aged in the presence of chestnut seem to be more matured, and this could explain the highest overall quality of that spirit.

Table 4.4: LSD post-hoc test: influence of the interaction ageing system x type of wood on vanilla attribute

			vanilla
Mean	Barrels	Limousin	1.37 b
	Barrels	Chestnut	1.86 a
	Alternative	Chestnut	2.05 a
	Alternative	Limousin	2.07 a

A general and clearer overview about the results of sensorial analysis is show in Figure 4.1.

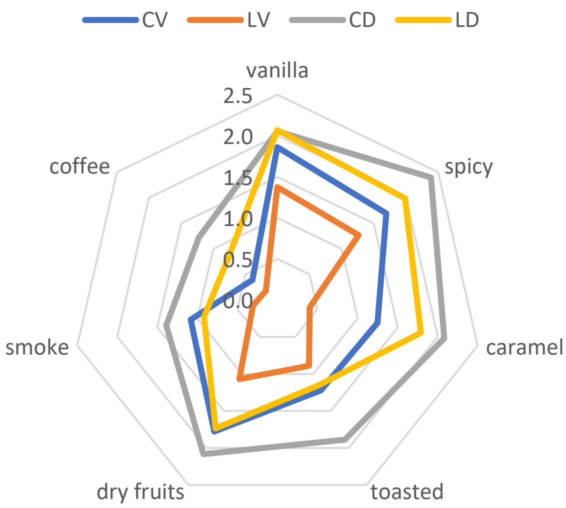

Figure 4.1: Radar plot of the significant value of aroma attribute in the sensory analysis

The radar plot was made with the average values of the score obtained in the sensory analysis session. The radar, underline that the wine spirit aged in stainless steel with chestnut wood staves (CD), after one-year present highest values for every significant attribute, so this alternative system is the best for a faster and completed evolution of the product after one year of ageing.

The alternative ageing system on stainless steel with oak staves (LD), permit also a faster and matured evolution of the spirit, showing the same result for the attribute vanilla, and presenting

almost the same score of the traditional ageing system in chestnut barrels (CV) for the attribute toasted, dry fruits and smoky.

The traditional method with oak barrels (LV), presented the lowest score for every attribute after one year of ageing, except for the coffee note that is almost the same of wine spirit aged in chestnut barrel (CV). Nevertheless, other studies are required next years to see the evolution of every spirit aging in different system.

The aroma attributes (vanilla, caramel, dry fruits, coffee, smoky, spicy, toasted), influenced by the studied factors, according to the previous ANOVA output were submitted to a PCA and clustering analysis.

The first two principal components accounted for 93% of the total variance (.87% for the first component and 6% for the second component). The plot of the wine spirits samples in the plane defined by the two components is shown in the Fig. 4.2.

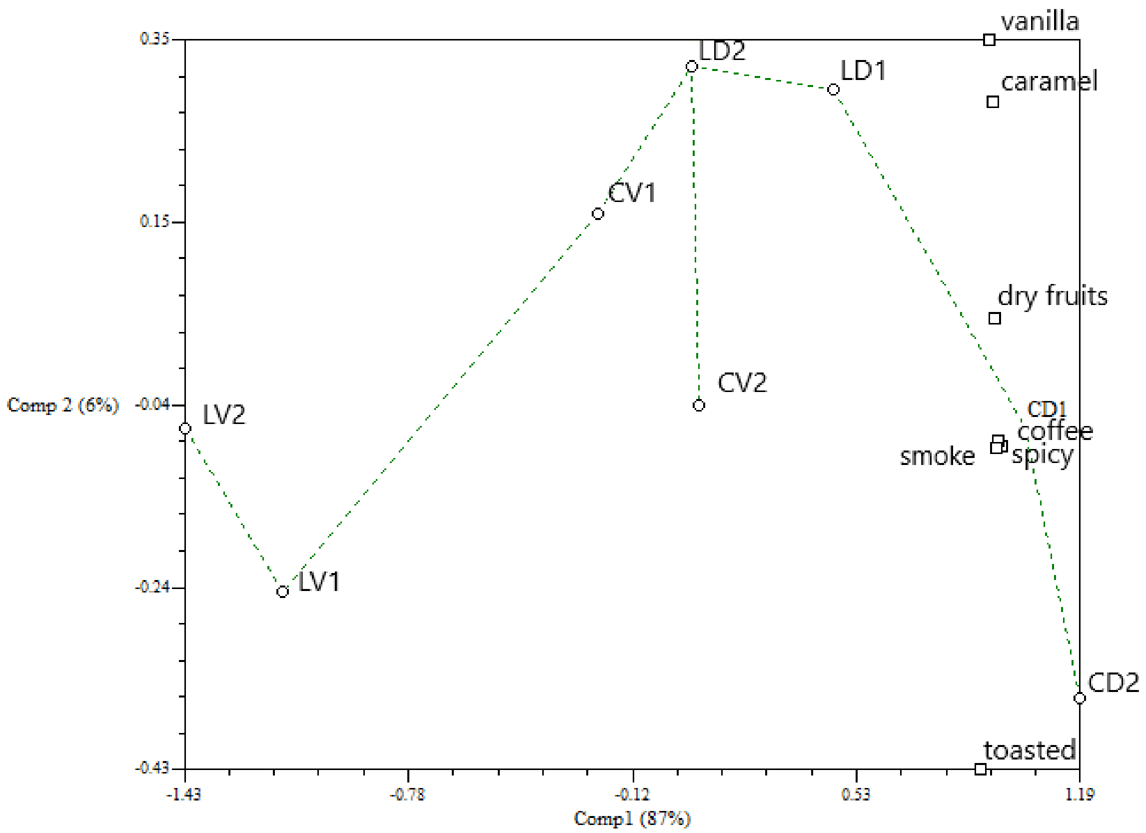

Figure 4.2: Scatter plot of PCA about the significant aroma attribute from sensorial analysis of wine spirit aged in different system

The first component of the PCA, seems to separate the samples based on the attributes related with ageing time and evolution. In fact, CD1 and CD2 are located in positive side of the component 1, related with high values of vanilla, caramel, dry fruits, coffee, spicy and toasted, which are attributes that increase with ageing time (Caldeira et al., 2006b) and they are also correlated with compounds released from the wood (Caldeira et al., 2008). LV1 and LV2 seems to be the less evolved, since they are located at the opposite side in the negative side the first component. LD1 and LD2 were also located in positive side of component 1, confirming that the alternative system regardless of the type of wood used, accelerates the aging of spirits and the evolution of the aroma (Caldeira et al., 2017). CV1 and CV2, as the position of the alternative system based on chestnuts wood (CD1, CD2), confirm precedent studies that wine spirits aged in presence of chestnut wood seem to be more matured considering the evolution of the sensory attribute over the time and present a highest overall quality (Caldeira et al., 2006b; 2010).

On the other hand, the second component of the PCA show an eigenvalue of 6%, so is not possible to evidence clearly the distribution of the aroma between the different samples but as the plot shows, are all in the area of the alternative system, near CD1 and CD2, confirming that this samples after one year present the best aroma evolution and characteristic.

The presentation of the phenogram below (Fig. 4.3) point out all this conclusion, a first separation between the oak barrels (LV1, LV2) and the rest of the aging system, confirm on first place that the chestnut wood has the best characteristic for this purpose and the alternative system permit a faster evolution of the aroma, concerning the attribute with higher significance.

Another two cluster is formed one by Chestnut alternative system (CD2) and one with chestnut barrels (CV1). The other two cluster related the two type of wood, one with CD1 and LD1 and the other with CV2 and LD2. This correlation could be explained that after one year there's no an evident separation between the samples.

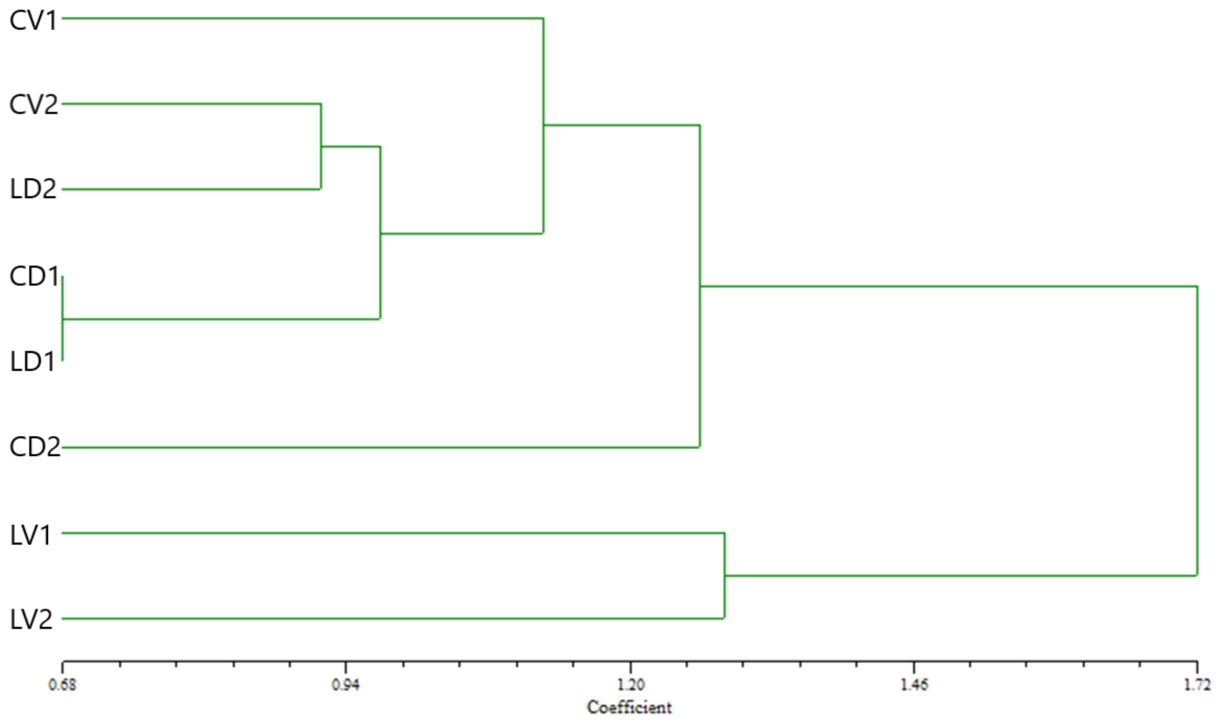

Figure 4.3: Phenogram of distribution and role of the variable on the principal component analysis

4.2 AROMA COMPOUNDS IN WINE SPIRITS-GCO RESULTS

The register of FID (in the top) and the register of sniffer (in the bottom) of the analysis by GC-O of the extract of wine spirit, aged in chestnut wood is show in figure 4.4.

In this work 57 compounds were detected from different categories of volatiles and aroma, but only 53 compounds were identified. The list of all the volatile compounds identified is reported in annex 1.

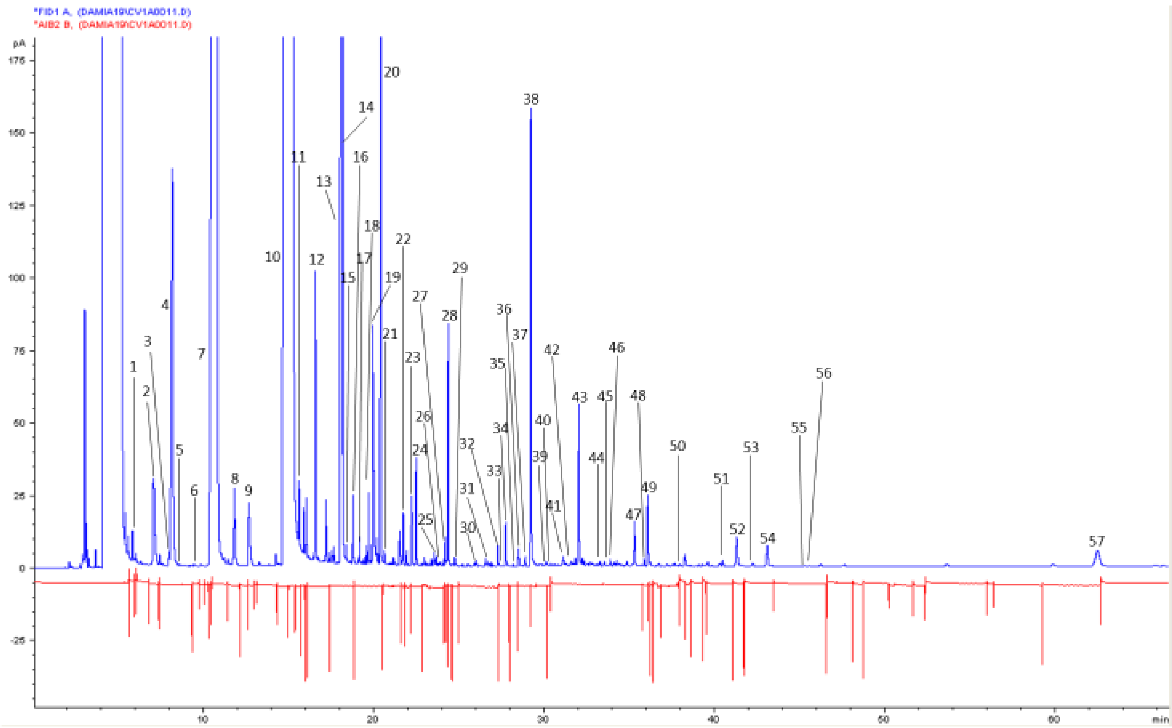

Fig. 4.4: Chromatogram of sample CV1: FID response (blue) and sniffer detection response (red).

Six volatile compounds could not be identified: some of them were present in trace level; others the relative peaks weren't confirmed by GC-MS analysis since contained more than one compound.

The first part of the chromatogram contains the esters and alcohol, the second part is characterising for the presence of wood derivate compounds, eluted lately due to the molecular structure and the major affinity with the stationary phase of the column (Guiochon et Guillemin, 1990).

Even if the chromatogram represents the sample of wine spirit aged in chestnuts wood barrel (CV1), intentionally, the position of the lactones (*cis* and *trans* β-metyl-γ-octalactone) that are normally not present or in a very low concentration in chestnut wood (Caldeira et al., 2010), has been reported (pick 37 and 40). The position was obtained by the overlap of chromatograms and control of retention times, besides identification with GC-MS.

More than ones, the judges notice the presence of “woody” and “toasted” on the last part of the chromatogram but wasn’t enough to determinate some odorant compounds.

The table 4.5 shows the results of GC-O analysis with the 23 compounds and respectively odour description determinate on the eight samples analysed.

The odorant compounds identified were eight esters, four acids, six alcohols, one terpene, one phenol, one furan and one aldehyde.

In contrast with the results of the sensory analysis, most of the odorants are esters and alcohols, typical of fresh distillate and just a few aldehydes and phenols which came from wood extraction. Linalol is the only mono-terpene present as odourant. Terpenes comes directly from the grapes; they are responsible for the fruity and flower aromas of aromatic grapes as Gewürztraminer (Günata et al., 1985; Wilson et al., 1986). Normally their concentration rises gradually during the ripening, until maturity after which concentration fall off (Günata et al., 1985). Linalol as key aroma compound on wine spirit seems to be a fundamental, as it was found in fresh wine distillate (Ferrari et al., 2004) and even in age wine spirit (Caldeira et al., 2008;2016).

The esters identified as odourant compounds (ethyl 2-isobutyrate, isobutyl acetate, ethyl butyrate, 2-methyl ethyl butyrate, ethyl isovalerate, isoamyl acetate, ethyl hexanoate) seems have a good impact on the odour profile of one-year aged wine spirit. Esters are synthetized by yeast during the fermentation of wine and give fruity fresh and floral aroma to the product; their amount depend by several factor as pH, temperature of fermentation and the quantity of nutrient and precursor contained in the must (Bertand, 2003). With the distillation some of them are released in the freshly spirit. These results are in accordance with the study of Ferrari et al., (2004) on the fresh distillate, where esters have a fruity impact characteristic. On the other hand, those results are in contrast with the sensory analysis where fruity and floral notes were not between the significant attributes. This could be explained with one of the criticism a and limitation of the GC-O method: instead of volatile compounds that give a certain aroma to the spirit, detected with the sensory analysis, in the GC-O study the molecules are eluted one by one to the sniffing port so even if the human nose is the best detector, still now is difficult to determinate and to understand the role of single molecules interacting together in the aroma formation (Botelho et al., 2011).

Also, higher alcohols are formed during the fermentation, some of them have an odour impact relevant as it can be the 2+3-methyl-1-butanol (isoamyl alcohol) with the characteristic banana note that was recognize in all the sample by almost all the judges. Other alcohols as 2-methylpropan-1-ol and 1-butanol give a sweet, fruity and fusel odorant note and seems to be the highest compounds present in spirits. Despite the high sensory threshold alcohols were often detected by the judge.

1-propanol, 2-methyl-1-propanol, 1-butanol, with sweet, alcoholic, balsamic and fruity notes, were detected several times by the judges, giving an odourant impact to the spirits samples. These results are in accordance to the results of Janácová et al., (2008) and these compounds seems really important in wine spirits aged in wooden barrels for a short period of time.

2-phenyl ethanol related to the rose and floral note (Table 4.5), is characteristic of wine spirits (Ferrari et al., 2004).

The green grass note was given by trans 2- hexenol. The green grass attribute was present also in 5 years old wine spirit analysed by Caldeira et al., (2008), but the intensity of the aroma was not correlated with the concentration inside the sample.

Acid acetic was found as odourant compound especially in wine spirit aged in barrels. With its typical vinegar aroma (Caldeira et al., 2008). This acid come from the distillate, but its amount increases during the ageing due to the ethanol oxidation and also from wood extraction (Puech et al., 1984), given the degradation of the acetyl groups present in the wood xylans, components of wood hemicelluloses (Fengel and Wengener, 1989).

The rancid and cheese attribute were giving by isovaleric acid. An important consideration is given observing the peak position of the chromatogram: isovaleric acid (peak 27) is just near to the ethyl succinate (pic 28). The high concentration, as show the pic of ethyl succinate was not detected by the judge, but the small concentration of the isovaleric acid was almost detected every time by the judge. This is related of course to the threshold limit but also is an important aspect of the GC-O analysis; several time new odour compounds were discovered with difficult in zone of the chromatogram were there wasn't peak or peak with a really small area, and relative concentration.

The presence of benzyl alcohol as odourant, especially in samples of wine spirit aged in chestnut wood, give a balsamic and floral note to the spirit. Its formation is due to the deamination and consequent oxidation of 2-phenylalanine inside the grapes and its level decrease with the maturity (Dunlevy et al., 2009), so it's possible that an early harvest to obtain a base wine for the distillation preserve a certain amount of this molecule.

The odourant 4-methylguaiacol was detected by the judges in wine spirit aged in chestnuts barrels and in wine spirit aged in alternative system with chestnut stave. Its attribute of sweet and toasted is really appreciated and in accordance with the work of Caldeira et al., (2010), it was found a positive correlation between this volatile compound and the smoke and toasted notes.

Vanilla is associated with the presence and detection of the vanillin as odorant compound (Table 4.,5) that seems to be a potent odorant especially in spirits aged in chestnut, in both ageing systems, barrels and staves, as show in tab 4.5. This could be related with the low sensory threshold between 0.1 and 4 mg dm^{-3} (Maga, 1985) in water and alcoholic solution. Vanillin is extract from the wood and its concentration is dependent to the toasting level

(Cadeira et al.,2006a). Also, the time of ageing is determinant for the concentration in spirits: the level increase owning to the extraction and the oxidation of coniferaldehyde (Puech et al., 1984).; As already present as strong odorant, the impact of vanillin aroma on the wine spirit could just increase the next years.

Syringaldehyde derived from the hydroalcoholysis of lignins (Puech et al., 1984) and was associated with the woody and floral attribute, the concentration of this benzoic aldehyde increases during aging and is present in higher concentrations in spirits aged in oak French (Madrera et al., 2003).

Table 4.5: Odourant profile of the wine spirits obtained with the GC-O analysis.

Compound	IR	Detection frequency								Odour Descriptor
		CV1	CV2	LV1	LV2	CD1	CD2	LD1	LD2	
Ethyl 2-isobutyrate	996	0	1	2	1	3	1	4	4	fruity, caramel
isobutyl acetate	1013	1	3	1	3	4	0	2	3	fruity, banana
ethyl butyrate	1035	4	1	0	0	1	1	2	0	Fruity, floral, kiwi
1-propanol	1040	2	3	5	3	1	3	5	3	fruity, yeast, apple
2-methyl ethyl butyrate	1050	5	3	3	5	5	4	1	1	fruity, tropical
ethyl isovalerate	1067	6	1	5	0	1	1	2	1	sweet, fruity
2-methylpropan-1-ol	1102	6	6	4	5	7	4	6	3	alcoholic, sweet, balsamic
Isoamyl acetate	1129	5	2	4	3	2	2	3	3	fruity, banana
1-butanol	1152	3	0	2	2	2	4	3	1	fusel oily sweet balsamic
2+3-methyl-1-butanol (isoamyl alcohol)	1233	10	10	10	8	9	9	10	9	fusel, alcoholic, fruity, banana
ethyl hexanoate	1245	2	1	4	2	0	1	2	1	fruity, tropical fruity pineapple
trans 2- hexenol	1387	5	7	4	3	5	3	2	2	grass
acetic acid	1449	4	4	5	1	1	1	2	1	vinegar, spirit
linalol	1545	3	3	1	4	1	0	2	1	fruity, citrus, floral
isovaleric acid	1682	6	6	7	6	4	2	4	1	cheese rancid
hexanoic acid	1849	4	3	1	0	3	2	2	2	fruity, floral
benzyl alcohol	1887	2	2	3	1	4	2	0	1	floral balsamic
2-phenyl ethanol	1923	3	3	2	0	3	1	3	4	flowers, rose
4-methyl guaiacol	1967	5	3	0	0	1	0	0	0	Sweet, candy, spice, toasted
dodecanoic acid	2487	3	5	2	1	2	2	2	0	fatty
HMF	2518	2	5	6	1	3	2	3	5	dry fruits
vanillin	2578	4	4	1	1	6	4	0	4	vanilla
syringaldehyde	2935	3	2	2	3	2	7	2	4	floral, woody, vanilla

Ir: retention index; CV1-CV2: Spirits aged in chestnut barrels; LV1-LV2: spirits aged in oak barrels; CD1-CD2: spirit aged with chestnut stave; LD1-LD2: spirts aged with oak stave. N° 0-10: number of detections by the judges of the correspondent odourant compounds.

The attribute of dry fruit, determinant in sensorial analysis could be correlated with the presence of HMF as potent odorant in the GC-O profile. As mentioned in previous work, this furanic compound can contribute to the aroma of wine spirit (Caldeira et al., 2008; Janácová et al., 2008).

Dodecanoic acid, detected by five judges just in one sample (CV2), gives a fatty unpleasant odour. On previous work on aged wine spirit, a correlation between certain acid as dodecanoic and the presence of tails on the distillate was searched without establishment of any result (Caldeira et al., 2008).

4.3 CORRESPONDENCE ANALYSYS

The matrix composed by the frequency of detection of the odourant compounds for the eight analysed samples were submitted to the correspondence analysis.

The plot in the Fig 4.5 and Fig. 4.6 shows the results of correspondence analysis (CA) between the different samples and odourant found

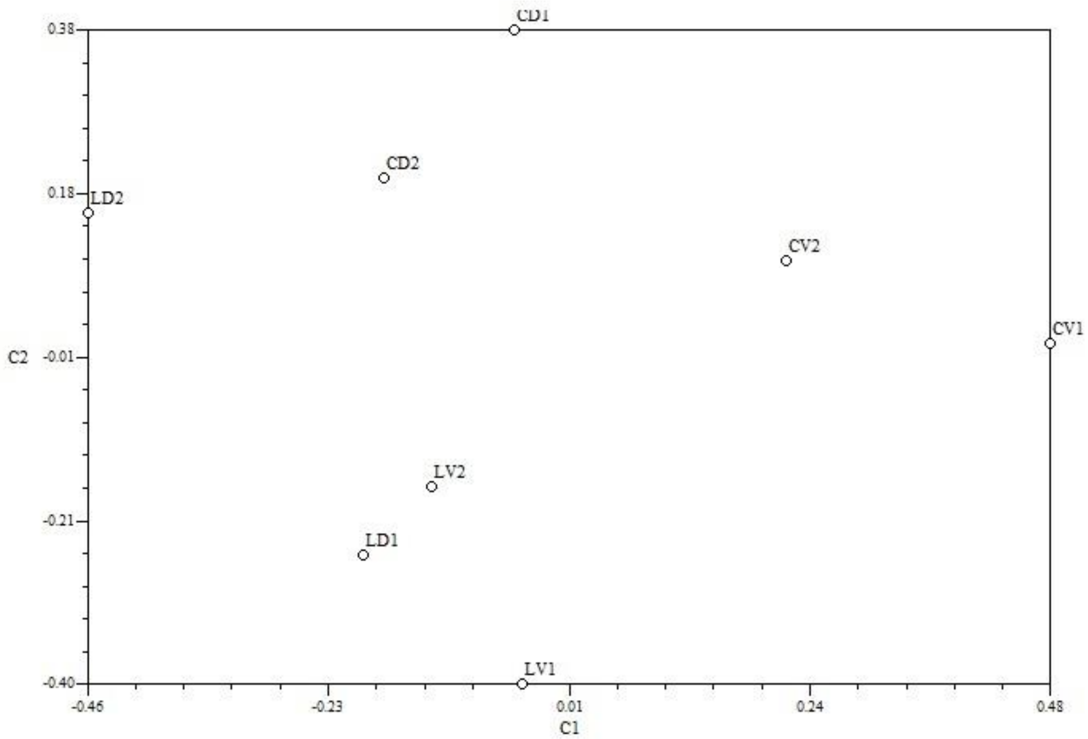

Figure 4.5: row factor plot of CA showing the distribution of the different samples

As shows the wine spirits aged in chestnut barrel (CV1, CV2) show a good association as the alternative ageing system (CD1, CD2, LD2), and the oak barrels (LV1, LV2). Only LD1 is well correlated with the oak barrel and that could be related at the short time of ageing that bring the same result of oak barrels for that sample. Even if the plot shows a first distribution well related, unfortunately, the eigen value of the first component is 27.87 % and don't explain really well the variance between the samples, also the second component present and explain 21,81 % of the variance. The cumulative is less of the 50% so was not possible to discriminate the difference between the samples.

As was mentioned before, after one year of ageing is not possible to discriminate the samples based on the odorant compound identified.

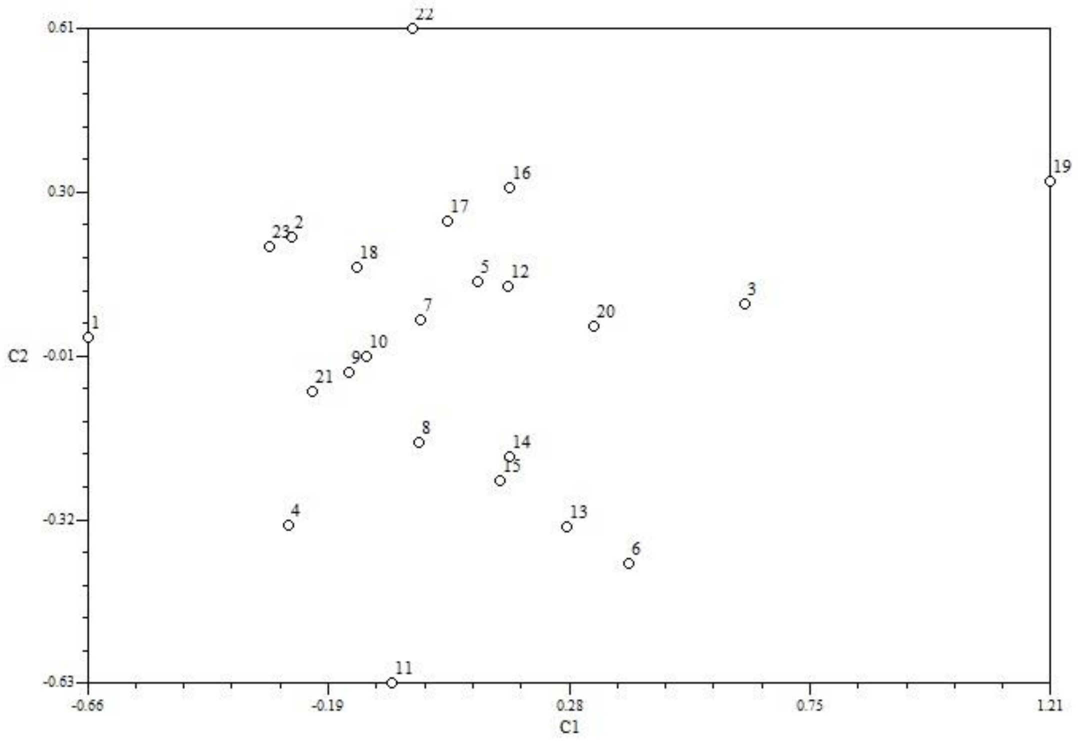

Figure 4.6: Column factor in the CA of the 23 odourant identified . 1. Ethyl isobutyrate, 2. isobutyl acetate, 3. ethyl butyrate, 4. 1-propanol, 5. 2-methyl ethyl butyrate, 6. ethyl isovalerate, 7. 2-methylpropan-1-ol, 8. Isoamyl acetate, 9. 1-butanol, 10. isoamyl alcohol, 11. ethyl hexanoate, 12. trans 2- hexenol, 13. acetic acid, 14 linalol, 15. isovaleric acid, 16. hexanoic acid, 17. benzil alcohol, 18. 2-phenylethanol, 19. 4-methyl guaiacol, 20. dodecanoic acid, 21. HMF, 22. Vanillin, 23. Syringaldehyde.

The second plot (Fig. 4.6) presented the distribution of the odourant, is possible to relate the position of 4-methylguaiacol (19) as strong odourant of chestnut barrels (CV1, CV2) and vanillin (22), highly related with alternative system with chestnut stave (CD1, CD2). HMF (21), another component related to the wood ageing was related at least to the samples aged in oak barrels (LV1, LV2). As mentioned before, the distribution of the odourant and the % of variance, doesn't permit a well separation and distribution of the samples.

4.4 ODOURANT POWER AND CONCENTRATION

The samples were submitted to GC-FID analysis, in order to obtain the concentration of the volatile compounds present inside the spirits. The concentration of the odourant compounds inside the samples is show in Table 4.6.

Table 4.6: Volatile compounds quantified in the wine spirits with GC analysis, related with sensory threshold.

Compound	Perception threshold mg dm3	source	CV1	CV2	LV1	LV2	CD1	CD2	LD1	LD2
Ethyl isobutyrate	0.1ppb	b								
isobutyl acetate	66 ppb	c, d	2,02	1,71	1,50	1,28	2,05	2,56	2,28	4,22
ethyl butyrate	1 ppb	b, c,d	0,79	0,76	0,73	0,70	0,77	0,79	0,77	0,66
1-propanol	>47 (H_2O)	6	156,77	156,15	157,64	156,22	168,12	162,93	163,61	168,28
2-methyl ethyl butyrate	1.6 µ/L	x	0,06	0,06	0,06	0,06	0,06	0,06	0,06	0,06
ethyl isovalerate	1.3 (beer)	5	0,11	0,11	0,108	0,171	0,108	0,108	0,108	0,108
2-methylpropan-1-ol	75 (H_2O); 75(EtOH-9.4%)	6, 1	778,53	791,19	788,21	777,28	800,09	795,59	796,11	808,27
Isoamyl acetate	0.05 ppm	7	3,54	3,33	3,46	3,11	3,49	3,46	3,61	3,34
1-butanol	>50 (H_2O); 150 (wine)	6, 5	5,67	6,00	5,97	5,52	6,40	5,92	5,73	6,01
isoamyl alcohol	60, 180 (wine)	5	1875,19	1883,34	1862,84	1870,27	1943,69	1908,62	1914,07	1946,56
ethyl hexanoate	0.076 (H_2O); 0.08 (EtOH-9.4%)	6, 1	1,29	1,19	1,18	1,16	1,29	1,25	1,19	1,16
trans 2- hexenol	nd	nd	0,62	0,58	0,58	0,59	0,57	0,58	0,58	0,58
acetic acid	26 (H_2O); 26 (EtOH-9.4)	6, 1	353,85	293,06	301,46	295,14	317,21	335,55	291,81	279,04
linalol	1.5 ppb; (H_2O)	31	0,37	0,35	0,37	0,38	0,33	0,33	0,35	0,34
isovaleric acid	2.5 ppb (H2O); 12 ppb (H2O); 0.75;0.7 ppm (H2O); 0.75 (EtOH-9.4%);	9, 31, 6, 7, 1,	1,57	1,48	1,28	1,40	1,42	1,40	1,37	1,38
hexanoic acid	8.6 (H_2O); 8.8 ppm	6, 7	2,08	2,12	1,98	2,13	2,05	1,89	1,98	2,01
benzil alcohol	1000 ppb	a								
2-feniletanol	7.5 (H2O); 2000 ppb (H_2O)	6, 31	16,91	17,44	16,15	17,04	16,65	16,06	15,78	15,95
4-methyl guaiacol	0.065 (H_2O); 0.01 (H2O), 0.03 (wine)	5, 42	0,00	0,00	0,00	0,00	0,24	0,23	0,19	0,21
dodecanoic acid	7,2	6	0,43	0,36	0,42	0,38	0,34	0,35	0,33	0,36

HMF	nd		20,23	14,06	9,53	10,91	19,53	20,34	19,06	17,92
Vanillin	1200ppb (H_2O); 0.1/0.5-4 (H_2O/beer); 0.5 (EtOH- 10%), 0.1 (EtOH- 40%); 0.11 (H_2O), 0.065 (wine); 0.25 (H_2O)	31, 5, 46, 42, 45	3,84	3,56	2,91	2,82	9,11	9,17	6,68	6,85
Syringaldehyde	30 (H_2O), 15 (EtOH-10%), 15 (EtOH- 40%); >50 (H_2O)	46, 42								

Source of threshold limit in Annex 2: 1- Nykanen e Suomalein (1983); 2- Etiévant (1991); 3- Salo et al., (1972); 4- Salo (1970); 5- Ong e Acree (1999); 6- Boidron et al (1988); 7- Riboulet (1982); 8- Maga (1985); 9- Buttery et al., (1988); 10- Takeoka et al., (1990); 11- Flath et al., (1967); 12- Fazzalari et al., (1978); 13- Pino et al., (2012).

It was not possible to obtain the concentration of all the odourant, especially for ethyl 2-isobutyrate and benzyl alcohol and syringaldehyde.

Isoamyl acetate is always present in high concentration and was always detected by the judges in all the spirit, meaning the low perception threshold (0.05 ppm) (Salo, 1970), and the importance of this odourant with fruity and banana note.

The odourant trans 2-hexenol, was always detected and present with high concentration in the samples, comparing to the threshold limit. With its grass, green note, *trans*2-hexenol derive from unripe grapes and continuous presses that may induce herbaceous taste, decreasing the overall quality of the wine spirit (Tsakiris et al., 2013).

2-Phenylethanol concentration, was always over the threshold limit and well detected by the judges, in particular on the samples from alternative ageing system (CD, LD) and on samples aged in chestnut barrels. With its rose note, 2-phenylethanol is key aroma in spirit, especially for Cognac (Ferrari et al., 2004).

4-Methylguaiacol, despite the high concentration and the low perception threshold was detected just in CV and once in CD.

Vanillin, as important marker of spirit aged in wood, was present in high concentration, but determinant as odourant compound in spirit aged in chestnut, both, barrels and alternative system.

4.5 CONCLUSION

The study of alternative ageing systems, in particular the comparison between the use of traditional systems, i.e. barrels and the use of stave and micro-oxygenation on inox vats, after a year of ageing, presents the following results.

At the level of sensory analysis, the alternative system and in particular the use of chestnut wood staves, presents a more complete evolution and refinement, in particular to the aromatic connotations derived from wood such as vanilla, spicy, toasted, dry fruit and smoke.

Analysis through GC-O has led to the identification of 57 volatile compounds but only 23 odorant compounds. However, contrary to the sensory analysis, the key aroma compounds identified, reflect more characteristics of a young distilled with connotations resulting from terpenes (linalol), fermentation esters (fruity notes) and only a few odours from the ageing such as vanilla, dried fruits and woody notes derived from vanillin, HMF and syringaldehyde respectively. This discrepancy between the two analyses can be explained by one of the characteristics or shortcomings of GC-O analysis. Contrary to sensory analysis in which the interaction between the different volatile molecules and no-volatile compounds, leads to complexity and characteristic typical of a product; contrary GC-O separates the molecules one by one allowing a qualitative analysis of individual volatile molecules present in the wine spirit.

Although it wasn’t possible to discriminate the samples by the odourant with the CA analysis due to the low separation and low eigen value explaining the variance between the different spirits.

However, the results obtained from the respective analyses are the first step towards standardising and effectively using more sustainable alternative systems, both from an environmental and economic point of view.

Further studies, on the same wine spirits after 2- 5 years are necessary to evaluate the aromatic and sensory evolution of the product.

REFERENCES

Acree T. E., Barnard J., Cunningham D. 1984. A Procedure for the Sensory Analysis of Gas Chromatographic Effluents. Food Chemistry. 14:273-286.

Acree T. E. 1993. Flavor Science: Sensible Principles and Techniques, in Acree, T. E., Teranishi, R. (Ed.), pp. 1–22. American Chemical Society, Washington DC. USA.

Anjos O., Carmona C., Caldeira I., Canas S. 2013. Variation of Extractable Compounds and Lignin Contents in Wood Fragments Used in the Aging of Wine Brandies. BioResources. 8:4484-4496.

ASTM. 1991. Annual book of ASTM standards. Philadelphia, USA.

Bajard-Sparrow C., Grassin C., Fauveau C. and Pellerin P. 2007. Utilisation des pectinases pour la vinification charentaise des vins destinés à la production d'eaux-de-vie de Cognac. *In* Les Eaux-de-Vie Traditionnelles d'Origine Viticole. Bertrand A. Lavoisier (ed.), pp. 65–67. Tec & Doc, Paris, France.

Belchior A.P. 1987. Aguardentes Velhas III – Destilação. O Escanção. 7:21.

Belchior A.P., Canas S., Caldeira I., Carvalho E. 2015. Aguardentes vinícolas. Tecnologias de produção e envelhecimento. Controlo de Qualidade. 1-63.

Bell S.J., Henschke P.A. 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. Australian Journal of Grape and Wine Research. 11:242-295.

Bertand A. 2003. Volatiles from Grape Must Fermentation. In Flavour of Distillated Beverages, origin and development. Piggot J.R. (ed.), pp. 93-109. Ellis Horwood Limited, Chichester, England.

Biermann C.S., Me Ginnis G., Schultz T.P. 1987. Scanning electron microscopy of mixed harwoods subjected to various pretreatment process. Journal of Agricolture and Food Chemistry. 35:713-716.

Blank I., Fischer K. H., Grosch W. 1989. Intensive neutral odourants of linden honey differences from honeys of other botanical origin. Zeitschrift für Lebensmittel-Untersuchung und -Forschung. 189:426-433.

Boidron J.N., Chatonet P., Pons M. 1988. Influence du bois sur certaines substances odorantes des vins. Connaissance Vigne Vin. 22:275-294.

Botelho G., Climaco M.C. 2011. The human nose as detector: Importance to Wine Aroma Study. *In* The Biology of Odors. Weiss L.E., Atwood J.M. (ed), pp. 97-130. Nova Science Publishers, Inc, New York, USA.

Both R., Sucker K., Winneke G., Koch E. 2004. Odor intensity and hedonic tone - important parameters to describe odor annoyance to residents? Water Science Technology. 50:83-92.

Brattoli M., Cisternino E., Dambruoso P., De Gennaro G., Giungato P., Mazzone A., Tutino M. 2013. Gas chromatography analysis with olfactometric detection (GC-O) as a useful methodology for chemical characterization of odorous compounds. Sensors. 13:16759-16800.

Buttery B.G., Turnbaugh J.G., Ling L.C. 1988. Contribute of volatiles to rice aroma. Journal of Agricolture and Food Chemistry. 36:1006-1009.

Cacho J., Moncayo L., Palma J.C., Ferreira V., Culleré L. 2012. Characterization of the aromatic profile of the Italia variety of Peruvian pisco by gas chromatography-olfactometry and gas chromatography coupled with flame ionization and mass spectrometry detection systems. Food research international. 49:117-125.

Cacho J., Moncayo L., Palma J. C., Ferreira V., Culleré L. 2013. The impact of grape variety on the aromatic chemical composition of non-aromatic Peruvian pisco. Food Research International. 54:373-381.

Caldeira I., Canas S., Costa S., Carvalho E., Belchior A.P. 1999. Formação de uma câmara de prova organoléptica de aguardentes velhas e selecção de descritores sensoriais. Ciência e Técnica Vitivinícola. 14:21-30.

Caldeira I., Belchior A.P., Clímaco M.C., Bruno de Sousa R. 2002. Aroma profile of portuguese brandies aged in chestnut and oak woods. Analytica Chimica Acta. 458:55-62.

Caldeira I., Pereira R., Clímaco M.C., Belchior A.P., Bruno de Sousa R. 2004. Improved method for extraction of aroma compounds in aged brandies and aqueous alcoholic wood extracts using ultrasound. Analytica Chimica Acta. 513:125-134.

Caldeira I., Clímaco M. C., Bruno de Sousa R., Belchior A. P. 2006a. Volatile composition of oak and chestnut woods used in brandy ageing: Modification induced by heat treatment. Journal of Food Engineering. 76: 202–211.

Caldeira I., Mateus A.M., Belchior A.P. 2006b. Flavour and odour profile modifications during the first five years of Lourinhã brandy maturation on different wooden barrels. Analytica Chimica Acta. 563: 264-273.

Caldeira I., Bruno de Sousa R., Belchior A.P., Clìmaco M.C. 2008. A sensory and chemical approach to the aroma of wooden aged Lourinhã wine brandy. Ciência e Técnica Vitivinícola. 23:97-110.

Caldeira I., Anjos O., Portal V., Belchior A.P., Canas S. 2010. Sensory and chemical modifications of wine-brandy aged with chestnut and oak wood fragments in comparison to wooden barrels. Analytica Chimica Acta. 660:43-52.

Caldeira I., Santos R., Ricardo-da-Silva J.M., Anjos O., Mira H., Belchior A.P., Canas S., 2016. Kinetics of odorant compounds in wine brandies aged in different systems. Food Chemistry. 211:937-946.

Caldeira I., Anjos O., Belchior A.P., Canas, S. 2017. Sensory impact of alternative ageing technology for the production of wine brandies. Ciência e Técnica Vitivinícola. 32:12-22.

Canas S., Leandro M. C., Spranger M. I., Belchior A.P. 2000. Influence of Botanical Species and Geographical Origin on the Content of Low Molecular Weight Phenolic Compounds of Woods Used in Portuguese cooperage. Holzforschung. 54: 255-261.

Canas S. 2003. Estudos dos compostos extraìveis de madeira (Carvalho e Castanheiro) e dos processos de extração na perspectiva do envelhecimento em Enologia. 303p. Tese de dotouramento em Engenharia Agro-Industrial, UTS-ISA, Lisboa, Portugal.

Canas S., Belchior A. P., Falcão A., Gonçalves J. A., Spranger M. I., Bruno-de-Sousa R. 2007. Effect of heat treatment on the thermal and chemical modifications of oak and chestnut wood used in brandy ageing. Ciência e Técnica Vitivinícola. 22: 5-14.

Canas, S., Vaz M., Belchior A.P. 2008. Influence de la dimension du fût dans les cinétiques d'extraction/oxydation des composés phénoliques du bois pour les eaux-de-vie Lourinhã. In Les eauxde-vie traditionnelles d'origine viticole. Bertrand A. (ed.), pp.143-146. Lavoisier - Tec & Doc, Paris, France.

Canas S., Caldeira I., Belchior A. P. 2009. Comparison of alternative systems for the ageing of wine brandy. oxygenation and wood shape effect. Ciência e Técnica Vitivinícola. 24:33-40.

Canas S., Caldeira I., Anjos O., Belchior A. P. 2019. Phenolic profile and colour acquired by the wine spirit in the beginning of ageing: Alternative technology using micro-oxygenation vs traditional technology. LWT - Food Science and Technology. 111:260–269.

Cantagrel R., Lurton L., Vidal J.P., Galy B. 1990a. La distillation charentaise pour l'obtention des eaux-de-vie de Cognac. *In* Les eau de vie traditionelle d'origine viticole. Bertand A. (ed.), pp. 60-69. Tec & Doc, Paris, France.

Cantagrel R., Desache F., Lacouture J., Galy B., Lurton L., Roulland C., Vidal J.P., Mazzerolles G. 1990b. Bilan d'activité 1989 et principales orientations pour 1990. pp.168-171.

Cantagrel R., Mazerolles G., Vidal J.P., Galy B., Boulesteix J.M., Lablanquie O., Gaschet J., 1992. Evolution analytique et organoleptique des eaux-de-vie de Cognac au cours du vieillissement. 2ª partie: Incidence de la température et de l'hygrométrie des lieux de stockage. *In* Élaboration et connaissance des spiritueux. Cantagrel R. (ed.), pp.573-576. Lavoisier - Tec & Doc, Paris, France.

Cantagrel R., Galy B., Jouret C. 1998. Eaux-de-vie d'origine viticole. *In* Oenologie, Fondements Scientifi ques et Technologiques. Flanzy C. (ed.), pp. 1084–1107. Lavoisier, Tec & Doc. Paris, France.

Cantagrel R. 2003. Chemical composition and analysis of Cognac. *In* Encyclopedia of Food Science and Nutrition, 2nd edition. Caballero B., Trugo I.C. and Fingla P.M. (ed.), pp. 601-606. Academic Press, Oxford, UK.

Cantagrel R., 2008. La qualité et le renom du Cognac dans le monde, sa place dans L'histoire. *In* Les eaux-de-vie traditionnelles d' origine viticole. Bertrand A. (ed.), pp. 15-36. Lavoisier, Tec & Doc, Paris, France.

Chatonnet P., Boidron J.N. 1989a. Incidence du traitement thermique du bois de chêne sur sa composition chimique. 1ere partie: définition des paramètres thermiques de la chauffe des fûts en tonnellerie. Connaissance de la Vigne et du Vin, Journal International des Sciences de la Vigne et du Vin. 23:77-87.

Chatonnet P., Boidron J.N. 1989b. Incidence du traitement thermique du bois de chêne sur sa composition chimique. 2eme partie : Evolution de certains composés en fonction de l'intensité de brûlage. Connaissance de la Vigne et du Vin, Journal International des Sciences de la Vigne et du Vin. 4: 223-250.

Chatonnet P., Dubourdieu D., 1998. Comparative study of the characteristics of American white oak (Quercus alba) and European oak (Quercus petraea and Quercus robur) for production of barrel ageing wines. American Journal of Enology and Viticulture. 49:79-85.

Cordonnier R., Bayonove C. 1981. Etude de la phase préfermentaire de la vinification: extraction et formation de certains composés de l'arôme; cas des terpenols, des aldehydes et des alcools en C6. Journal international des sciences de la vigne et du vin. 15:269-286.

Czerny M., Christlbauer M., Christlbauer M., Fischer A., Granvogl M., Hammer M., Hartl C., Hernandez N.M., Schieberle P. 2008. Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. European Food Research and Technology. 228:265-273.

Czerny M., Brueckner R., Kirchhoff E., Schmitt R., Buettner A. 2011. The influence of molecular structure on odor qualities and odor detection thresholds of volatile alkylated phenols. Chemical Senses. 36:539-553.

D'Acampora Zellner B., Dugo P., Dugo G., Mondello L. 2008. Gas chromatography–olfactometry in food flavour analysis. Journal of Chromatography A. 1186 :123-143.

Daniel M.A., Puglisi C.J., Capone D.L., Elsey G.M., Sefton M.A. 2008. Rationalizing the formation of damascenone: Synthesis and hydrolysis of damascenone precursors and their analogues, in both aglycone and glycoconjugate forms. Journal of Agricultural and Food Chemistry. 56 :9183-9189.

De Revel G., Martin N., Pripis-Nicolau L., Lonvaud-Funel A., Bertrand, A. 1999. Contribution to the knowledge of malolactic fermentation influence on wine aroma. Journal of Agricultural and Food Chemistry. 47 :4003-4008.

De Revel G., Pripis-Nicoleau L., Barbe J.C., Bertrand A. 2000. The detection of α-dicarbolyl compounds in wine by formation of quinoxaline derivatives. Journal of Science Food and Agriculture.80

De Souza M.D.C.A., Vásquez P., Del Mastro N.L., Acree T.E., Lavin E.H. 2006. Characterization of cachaça and rum aroma. Journal of Agricultural and Food Chemistry. 54:485-488.

Décret nº 2009 -1146. Appellation d'origine contrôlée "Cognac" ou "Eau-de-vie de Cognac" ou "Eau-de-vie des Charentes". *In* Journal Officiel De La République Française, 24 septembre 2009, pp.12.

Décret nº 2009 -1285. Appellations d'origine contrôlée "Armagnac", "Blanche Armagnac", "Bas Armagnac", "Haut Armagnac" et "Armagnac-Ténarèze". *In* Journal Officiel De La République Française, 25 octobre 2009, pp.14.

Decreto-Lei nº 323/94. Estatutos da Região Demarcada das Aguardentes Vínicas da Lourinhã. *In* Diário da República – I Série – A, 29 de Dezembro 1994, pp.7486-7489.

Deibler K.D., Acree T.E., Lavin E.H. 1999. Gas Chromatography-Olfactometry (GC/O) of Vapor Phases. *In* Flavor Chemistry: 30 Years of Progress. Teranishi et al., (ed.), pp: 387-395. Kluwer Academic/Plenum Publishers, New York, USA.

Del Álamo M., Nevares I., Gallego L., Fernández de Simón B., Cadahía E. 2010. Micro-oxygenation strategy depends on origin and size of oak chips or staves during accelerated red wine aging. Analytica Chimica Acta. 660: 92–101.

Delahunty C.M., Eyres G., Dufour J.P. 2006. Gas chromatography-olfactometry, Review. Journal of Separation Science. 29:2107-2125.

Du Plessis H.W., Steger C.L.C., du Toit M., Lambrechts M.G. 2002. The occurrence of malolactic fermentation in brandy basewine and its influence on brandy quality. Journal of Applied Microbiology 92 :1005-1013.

Du Plessis H.W., Dicks L.M.T., Pretorius I.S., Lambrechts M.G., du Toit M. 2004. Identification of lactic acid bacteria isolated from South African brandy base wines. International Journal of Food Microbiology 91 :19-29.

Dubois P. 1994. Les arômes des vins et leurs défauts. Revue française d'oenologie. 146:39-50.

Dunlevy J.D., Kalua C.M., Keyzers R.A., Boss P.K. 2009. The production of flavour & aroma compounds in grape berries. *In* Grapevine Molecular Physiology & Biotechnology, 2nd edition. Roubelakis-Angelakis K.A. (ed.), pp :293-340. Springer, Dordrecht, The Netherlands.

Estregueil S., Galy B., Loiseau B., Lutron L. 2007. Maîtrise des risques liés à l'utilization de specialités phytosanitaires dans le vignoble de Cognac, I*n* Les Eaux-de-Vie Traditionnelles d'Origine Viticole. Bertrand A. (ed.), pp. 65-68. Lavoisier Tec & Doc, Paris, France.

Etiévant P. 1991. Wine. *In*: Volatile compounds in food and beverages. 483-545. Maarse H. (ed.), Marcell Dekker Inc., New York, USA.

Fazzalari F.A. 1978. Compilation of Odor and Taste Threshold Data. Fazzalari F.A. (ed.), DS 48A. American Society for Testing and Materials, USA.

Fengel D., Wegener G. 1989. Wood chemistry, ultrastructure, reactions. Walter de Gruyter & Co. (ed.), pp. 319-342. Berlin, Germany.

Ferrari G., Lablanquie O., Cantagrel R., Ledauphin J., Payot T., Fournier N., Guichard E. 2004. Determination of Key Odorant Compounds in Freshly Distilled Cognac Using GC-O, GC-MS, and Sensory Evaluation. Journal of Agriculture and Food Chemistry. 52:5670-5676.

Ferreira V., Lopez R., Cacho J.F. 2000. Quantitative determination of the odorants of young red wines from different grape varieties. Journal of the Science of Food and Agriculture. 80:1659-1667.

Ferreira A.C.S., Hogg T.,de Pinho P.G. 2003. Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. Journal of Agricultural and Food Chemistry. 51:1377-1381.

Feuillat F., Keller R., Masson G., Puech J.L. 1998. Bois de chêne. Oenologie, par Technologie. 1002-1052.

Filipe A.G.M.P., Mota I., G., Almeida J.N., Soares Franco J.M., Magalhães N., Magalhães V., 1998. O Vinho do Porto e os Vinhos do Douro. Enciclopédia dos Vinhos de Portugal. Chaves Ferreira Publicações, Lisboa, Portugal.

Firestein R. 2001. How the olfactory system makes sense of scents. Nature. 413:211-218.

Flath R.A., Black D.R., Guadagni D.G., McFadden W.H., Schultz T.H. 1967. Journal of Agricultural and Food Chemistry. 15:2935.

Franitza L., Granvogl M., Schieberle P. 2016. Characterisation of the Key Aroma Compounds in Two Commercial Rums by Means of the Sensomic Approach. Journal of Agricultural and Food Chemistry. 64:637-645.

Galy B., Roulland C., Lurton L., Cantagrel R. 1993. Connaissance des paramètres influant sur la conservation des vins destinés à l'élaboration des eaux-de-vie de Cognac. *In* Elaboration et connaissance des Spiritueux. Cantager R. (ed.), pp. 218-224. Tec & Doc, Lavoisier, Paris, France.

Garreau C. 2008. L'Armagnac. *In* Les Eaux-de-Vie Traditionnelles d'Origine Viticole. Bertrand A. Lavoisier (ed.), pp. 39-62. Tec & Doc, Paris, France.

Giraud N. 2003. L'isobutanal dans le Cognac, causes de formation et devenir au cours du vieillissement. *In* Mémoire de fin d'études Station Viticole du BNIC - École Supérieure d'Agriculture de Purpan, Toulouse, France

Guichard E., Fournier N., Masson G., Puech J.L. 1995. Stereoisomer of β-metyl-γ-octalactone. I-quantification in brandies as a function of wood origin and heat treatment of the barrels. American Journal of Enology and Viticulture. 46:419-423.

Guillou I. 1996. Étude de substances de faibles poids moléculaires combinant le dioxyde de soufre dans les vins blancs issus de vendanges botrytisées. Mise en évidence et importance du rôle de l'hydroxypropanedial. Thèse de doctorat. Université de Bordeaux 2, France.

Guiochon G., Guillemin C.L. 1990. Gas chromatography. Review of Scientific Instruments. 61:3317-3339.

Günata Y.Z., Bayonove C.L., Baumes R.L., Cordonnier R.E. 1985. The aroma of grapes. I. Extraction and determination of free and glycosidically bound fractions of some aroma components. Journal of Chromatography A. 331:83–90.

Guth H., Grosch W. 1999. Evaluation of important odorants in foods by diluation techniques. *In* Flavor Chemistry. Teranishi R., Wick E.L., Hornstein I., (ed.), pp: 377-386. Kluwer Academic, Plenum, New York, USA.

Guymon J.F., Crowell E.A. 1970. Brandy aging. Some comparisons of American and French oak cooperage. Wines & Vines. 1:23-25.

Haluk J.P., Irmouli M. 1998. The fixed polymer constituents in cooperage oak: cellulose, hemicelluloses and lignin. Journal des Sciences et Techniques de la Tonnellerie. 4:43-82.

Hervé É., Ségur M.C., Bertand A. 2007. Teneurs élévée en esters d'acides gras à lounge chaîne dans les eaux-de-vie d'Armagnac: une consequence indirecte de la trituration du rasain. *In* Les Eaux-de-Vie Traditionnelles d'Origine Viticole. Bertrand A. (ed.), pp. 65-68. Lavoisier TEC & DOC, Paris, France.

ISO-International Organization for Standardization. ISO standard 3591. 1977. Sensory analysis - Apparatus -Wine-tasting glass. Last reviewed in 2010.

Jánacová A., Sádecká J., Kohajdová Z., Spanik I. 2008. The identification of aroma-active compounds in Slovak brandies using GC-sniffing, GC-MS and sensory evaluation. Chomatographia. 67:113-121.

Joslin W. S., Ough C. S. 1978. Cause and fate of certain C6 compounds formed enzymatically in macerated grape leaves during harvest and wine fermentation. American Journal of Enology and Viticulture. 29:11-17.

Jurado M., Puercas B., Cantos E., Guillen D. 2007. Influence de l'anhydride sulfureux et de la lie sur la qualité du Brandy distillé à la Chaudiere. *In* Les Eaux-de-Vie Traditionnelles d'Origine Viticole. Bertrand A (ed.), pp. 79-87. Lavoisier Tec & Doc, Paris, France.

La Guerche S., Dauphin B., Pons M., Blancard D., Darriet P. 2006. Characterization of some mushroom and earthy off-odors microbially induced by the development of rot on grapes. Journal of Agricoltural and Food Chemistry. 54:9193-9200.

Lafon J., Couillud P., Gaybellile F. 1973. Le cognac. Baillière J.B. (ed.). Paris, France.

Lawless H., Heymann H. 1999. Sensory evaluation of food: principles and practices. Aspen Publisher. Mariland, USA.

Le Guen S., Prost C., Demaimay M. 2000. Critical comparison of three olfactometric method for the identification of the most potent odorants in cooked mussels (Mytilus edulis). Journal of Agricultural and Food Chemistry. 48:1307-1314.

Léauté R. 1990. Distillation in alembic. American Journal of Enology and Viticulture. 41: 90-102.

Léauté R., Mosedale J.R., Mourgues J., Puech J.L. 1998. Barrique et vieillissement des eaux-de-vie. *In* Oenologie fondements scientifiques et technologiques. Flanzy C. (ed.), pp. 1085-1142. Collection Science & Technology Agriculture. A, New York, USA.

Ledauphin J., Guichard H., Saint-Clair J.F., Picoche B., Barillier D. 2003. Chemical and sensorial aroma characterization of freshly distilled Calvados. 2. Identification of volatile compounds and key odorants. Journal of agricultural and food chemistry. 51:433-442.

Leffingwell J.C. 2002. Olfaction- Update n°5. Leffingwell Reports. 2 :1-34.

Linssen J.P.H., Janssens J.L.G.M., Roozen J.P., Posthumus M.A. 1993. Combined gas chromatography and sniffing port analysis of volatile compounds of mineral water packed in polyethylene laminated packages. Food Chemistry. 46:367-371.

Loizeau A. 2002. L'isobutanal dans les eaux-de-vie de Cognac, origine de ce composé dépréciatif - Mémoire de fin d'études Station Viticole du BNIC - École Supérieure d'Agriculture de Purpan, Toulouse, France

Lurton L., Mazerolles G., Galy B., Cantagrel R., Vidal J.P. 1991. Influence de la technologie de vinifi cation sur la qualité des eaux-de-vie de Cognac: exemple des norisoprénoïdes et des alcools supérieurs', *In* Les Eauxde- vie Traditionnelles D'Origine Viticole. Bertrand A. (ed.), pp. 127–136. Lavoisier Tec & Doc, Paris, France.

Maarse H., Ten Noever de Brauw M.C. 1966. The analysis of volatile components of Jamaica rum. Journal of Food Science. 31:951-955.

Macfie H.J.M., Bratchell N., Greenhoff H., Vallis L.V., 1989. Designs to balance the effects of order of presentation and first order carryover effects in hall tests. Journal of Sensory Studies. 4 :129–148.

Madrera R., Rodríguez D., Blanco G., Mangas J.J.A. 2003. Influence of distillation system, oak wood type, and aging time on composition of cider brandy in phenolic and furanic compounds. Journal of Agricolture and Food Chemistry. 51 : 7969-7973.

Maga J.A. 1985. Flavor contribution of wood in alcoholic beverages. *In* Progress in flavour research 1984. Adda J. (ed.), pp. 409-416. Elsevier, Amsterdam, The Netherlands.

McDaniel M.R., Miranda-Lopez R., Watson B.T., Micheals N.J., Libbey L.M. 1990. Flavors and Off-Flavors. Charalambous G. (ed.), pp. 23-36. Elsevier Science Publishers, Amsterdam, Netherlands.

Meligaard M.C., Civille G.V., Carr B.T. 2007. Sensory evaluation techniques. 4th Edition. CRC Press, Boca Raton, Florida, USA.

Miller D.P., Howell G.S., Michaelis C.S., Dickmann D.I., 1992. The content of phenolic acid and aldehyde flavor components of white oak as affected by site and species. American Journal of Enology and Viticulture. 43:333-338.

Moio L., Ugliano M., Genovese A., Gambuti A., Pessina R., Piombino P. 2004. Effect of antioxidant protection of must on volatile compounds and aroma shelf life of Falanghina (Vitis vinifera L.) wine. Journal of Agriculture and Food Chemistry. 52:891-897.

Molina A.M., Guadalupe V., Varela C., Swiegers J.H., Pretorius I.S., Agosin E. 2009. Differential synthesis of fermentative aroma compounds of two related commercial wine yeast strains. Food Chemistry. 117:189-195.

Mosedale J.R., Puech J.L. 1998. Wood maturation of distilled beverages. Review. Trends in Food Science & Technology. 9:95-101.

Nedjma M. 1997. Influence of Complex Media Composition, Cognac's brandy, or Cognac, on the Gas Chromatography Analysis of Volatile Sulfur Compounds – Preliminary Results of the Matrix Effect. American Journal of Enology and Viticulture. 48:333-338.

Nishimura K., Onishi M., Masuda M., Koga K., Matsuyama R. 1983. Reactions of wood components during maturation. *In* Flavour of distilled beverages: Origin and development. Piggott J. R. (ed.), pp : 241–255). Ellis Horwood Limited, UK.

Nykänen L., Suomalainen H. 1983. Evaluation of flavour. *In* Aroma of beer, wine and distilled alcoholic beverages. 1-3. Nykanen L., Suomalainen H. (eds.), D. Reidel Pulbishing Company, Dordrecht, Holland.

OIV, 2014. Compendium of international methods of analysis of spirituous beverages of vitivinicultural origin. OIV, Paris.

Peng, S., Scalbert A., Monties, B. 1991. Insoluble ellagitannins in Castanea sativa and Quercus petaea woods. Phytochemistry. 30:775-778.

Philips R.J. 1989. Qualitative and quantitative analysis. *In* High resolution gas chromatography (3rd edition). Hyver K.J., Sandra P. (eds), pp. 1-11. Hewlett-Packard, Ca, USA.

Piggiot J. 2012. Alcoholic beverages. Sensory evaluation and consumer research. Piggiot J (ed). Woodhead Publishing Limited, Cambrige, UK.

Pino J.A., Tolle S., Gök R., Winterhalter P. 2012. Characterisation of odour-active compounds in aged rum. Food Chemistry. 132:1436-1441.

Poisson L., Schieberle P. 2008. Characterization of the Most Odor-Active Compounds in an American Bourbon Whisky by Application of the Aroma Extract Dilution Analysis. Journal of Agricoltural and Food Chemistry. 56 :5813-5819.

Pollien P., Ott A., Montigon F., Baumgartner M., Muňoz-Box R., Chaintreau A. 1997. Hyphenated headspace-gas chromatography-sniffing technique: screening of Impact odorants and quantitative aromagram comparison. Journal of Agriculture and Food Chemistry. 45:2630-2637.

Portmann M. 1999. Anatomy of the organs of the senses. Journal international des Sciences de la Vigne et du Vin, Wine tasting, special issue. 7-13.

Prida A., Puech J.L. 2006. Influence of geographical origin and botanical species on the content of extractives in American, French, and East European oak woods. Journal of Agricultural and Food Chemistry. 54:8115-126.

Puech J.L., Léauté R., Clot G., Nomdedeu L., Mondiés H. 1984. Évolution de divers constituants volatils et phénoliques des eauxde-vie de cognac au cours de leur vieillissement. Sciences des Alimentes. 4:65-80.

Puech J.L., Jouret C., Goffinet B. 1985. Évolution des composés phénoliques du bois de chêne au cours du vieillissement de l'armagnac. Sciences des Alimentes. 5:379-392.

Puech J.L., 1987. Apport du bois de chêne au cours du vieillissement des eaux-de vie. *In* Le bois et la qualité des vins et des eaux-de-vie. Conn. Vig Vin, 151-162.

Rabier P., Moutounet M. 1991. Evolution d’extractibles de bois de chêne dans une eau-de-vie de vin. Incidence du thermotraitement des barriques. *In* Les eaux-de-vie traditionnelles d’origine viticole. Bertrand A. (ed.), pp. 220-230. Lavoisier - Tec & Doc, Paris, France.

Ramey D.D., Bertrand A., Ough C.S., Singleton V.L., Sanders E. 1986. Effects of skin contact temperature on Chardonnay must and wine composition. American Journal of Enology and Viticulture. 37 :99-106.

Rapp A., Hastrich H., Engel L. 1976. Gaschromatogaphiche untersuchungen über die aromastoffe von weinbeeren. I. Anreicherung und kapillarchromatographische auftrennung Vitis. 15 : 29-36.

Regulation (EC) No. 606/2019. laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions. Official Journal of the European Union 2019, L 193/1, 1-59

Regulation (EC) No. 787/2019. Definition, description, presentation and labelling of spirit drinks, the use of the names of spirit drinks in the presentation and labelling of other foodstuffs, the protection of geographical indications for spirit drinks, the use of ethyl alcohol and distillates of agricultural origin in alcoholic beverages. Official Journal of the European Union 2019, L130/1, 6–39.

Ribéreau-Gayon P., Glories Y., Maujean A., 2006a. Handbook of Enology. Volume 2. The Chemistry of wine, stabilization and treatment. II edition. John Wiley & Sons, LTD (ed), England.

Ribéreau-Gayon P., Dubourdieu D., Donèche B, Lonvaud A., 2006b. Handbook of enology. Volume 1. The microbiology of wine and vinifications. II edition. John Wiley & Sons Ltd, Chichester,UK.

Riboulet J.M. 1982. Contribution à l' étude chimique et microbiologique des "gôuts de bouchon" dans les vins. 191 p. Thèse 3me cycle, Université de Bordeaux II, Bordeaux, France.

Rohlf F.J., 2000. NTSYS-pc: Numerical taxonomy and multivariate analysis system. P. 130.Exeter Software, New York, USA.

Ruijten M.W.M.M., van Doorn R., van Harreveld A.P. 2009. Assessment of Odor Annoyance in Chemical Emergency Management. RIVM Report 609200001. RIVM, Bilthoven, The Netherlands. pp:11-12.

Saerens S.M.G., Delvaux F., Verstrepen K.J., Van Dijck P., Thevelein J.M., Delvaux F.R. 2008. Parameters affecting ethyl ester production by Saccharomyces cerevisiae during fermentation. Applied and Environmental Microbiology. 74:454-461.

Salagoity-Auguste M. H., Tricard C., Marsa F., Sudraud P. 1987. Preliminary investigation for the differentiation of enological tannins according to botanical origin: Determination of gallic acid and its derivatives. American Journal of Enology and Viticulture. 37:301-303.

Salo P. 1970. Determining the odor threshold for some compounds in alcoholic beverages. Journal of Food Science. 35:95-99.

Salo P., Nykänen L., Soumalainen H. 1972. Odour tresholds and relative intensities of volatile aroma components in an artifical beverage imitating whisky. Journal of Food Science. 37:394-398.

Sarni F., Moutounet M., Puech J.L., Rabier P. 1990. Effect of Heat Treatment of Oak Wood Extractable Compounds. Holzforschung. 44:461-466.

Scalbert A., Monties B., Janin G. 1989. Tannins in wood: Comparison of different estimation methods. Journal of Agricultural and Food Chemistry. 37:1324-1329.

Schreier P., Drawert F., Junker A. 1976. Identification of volatile constituents from grapes. Journal of Agricultural and Food Chemistry. 24: 331-336.

Singleton V.L., 1995. Maturation of wines and spirits: comparisons, facts and hypotheses. American Journal of Enology and Viticulture. 46:98-115.

Stone H., Sidel J.R. 1993. Sensory evaluation practices. Accademic press. San Diego, USA.

Stone H., Sidel J.R. 2004. Sensory evaluation practices. 3rd edition. Accademic press. San Diego, USA.

Takeoka G.R., Flath R.A., Mon T.R., Teranishi R., Guentert M. 1990. Volatile constituents of apricot (Prunus armeniaca). Journal of Agriculture and Food Chemistry. 38:471-477.

Tao Y., Garcìa J.F., Sun D.W. 2014. Advances in Wine Aging Technologies for Enhancing Wine Quality and Accelerating Wine Aging Process. Critical Reviews in Food Science and Nutrition. 54:817-835.

Taransaud J. 1976. Le livre de la tonnellerie. La Roue à Livres Diffusion (ed.), Paris, France.

Timberlake C.F., Bridle P., 1976. Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. American Journal of Enology and Viticulture. 27:97-105.

Tsakiris A., Kallithrakab S., Kourkoutasc Y. 2013. Review: Grape brandy production, composition and sensory evaluation. Journal of the Science of Food and Agriculture. 94:404-414.

Ullrich F., Grosh W. 1987. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. Zeitschrift für Lebensmittel-Untersuchung und -Forschung. 184:277-282.

Urruty L., Gaudin M., Snakkers G., Pineda C., Estreguil S., Ferrari G., Lurton L. 2007 Recherce de marqueurs analytiques de Botrytis cinereal dans les eaux-de-vie de Cognac. *In* Les Eaux-de-Vie Traditionnelles d'Origine Viticole. Bertrand A. Lavoisier (ed.), pp. 69-71. Tec & Doc, Paris, France.

Uselmann V., Schieberle P. 2015. Decoding the combinatorial aroma code of a commercial cognac by application of the Sensomics concept and first insights into differences from a German brandy. Journal of Agricoltural and Food Chemistry. 63:1948-1956.

Van Ruth S.M. 2001. Methods for gas chromatography-olfactometry: a review. Biomolecular Engineering 17:121-128.

Van Ruth S.M., O'Connor C.H. 2001. Influence of assessors' qualities and analytical conditions on gas chromatography-olfactometry analysis. European Food Research and Technology. 213:77-82.

Vanderlinde R. 1995. Étude de certains caractères des eaux-de-vie, rôle des composés carbonylés. Thèse de doctorat, université de Bordeaux 2, France.

Vilanova M., Oliveira J.M. 2011. Application of gas chromatography on the evaluation of grape and wine aroma in Atlantic viticulture (NW Iberian Peninsula). *In* Gas chromatography in plant science, wine technology, toxicology and some specific applications. B. Salih (ed.), pp:109-146. InTech, Rijeka, Croatia.

Vilanova M., Siebert T.E., Varela C., Pretorius I.S., Henschke P.A. 2012. Effect of ammonium nitrogen supplementation of grape juice on wine volatiles and non-volatiles composition of the aromatic grape variety Albarino. Food Chemistry. 133:124-131.

Viriot C., Scalbert A., Lapierre C., Moutounet M. 1993. Ellagitannins and lignins in ageing of spirits in oak barrels. Journal of Agriculture and Food Chemistry. 41:1872-1879.

Viriot C., Scalbert A., Hervé du Penhoat C.L.M., Moutounet M. 1994. Ellagitannins in woods of sessile oak and sweet chestnut dimerization and hydrolysis during wood ageing. Phytochemistry. 36:1253-1260.

Vocke M. 2008. The influence of the processing steps on the formation of important aroma compounds in American whiskey (in German). Ph.D. thesis, Technical University of Munich. Munich, Germany.

Wei X.F., MA X.L., Cao J.H., Sun X.Y., Fang Y.L. 2018. Aroma characteristics and volatile compounds of distilled Crystal grape spirits of different alcohol concentrations: wine sprits in the Shangri-La region of China. Food Science and Technology. 38:50-58.

Williams A.A., Tucknott O.G. 1971. Volatile constituents of fermented cider. I. Draught dry cider blend. Journal of the Science of Food and Agriculture.22:264-269.

Willner B., Granvogl M., Schieberle P. 2013. Characterization of the key aroma compounds in Bartlett pear brandies by means of the Sensomics concept. Journal of Agricoltural and Food Chemistry. 61:9583-9593.

Wilson B., Strauss C.R., Williams P.J. 1986. The distribution of free and glycosidically-bound monoterpenes among skin, juice and pulp fractions of some of white grape varieties. American Journal of Enology and Viticulture. 37:107-111.

Zahri S., Belloncle C., Charrier F., Pardon P., Quideau S., Charrier B. 2007. UV light impact on ellagitannins and wood surface colour of European oak (Quercus petraea and Quercus robur). Applied Surface Science. 253:4985-4989.

Zhao Y.P., Li J.M., Xu Y., Fan W.L., Jiang W.G., 2009. Characterization of aroma compounds of four brandies by aroma extract dilution analysis. American Journal of Enology and Viticulture. 60:269-277.

Zhao Y. P., Wang L., Li J.M., Pei G.R., Liu Q.S. 2011. Comparison of volatile compounds in two brandies using HS-SPME coupled with GC-O, GC-MS and sensory evaluation. South African Journal of Enology and Viticulture. 32:9-20.

WEB SITE REFERENCES

https://www.ivv.gov.pt/np4/Anu%C3%A1rio
https://webbook.nist.gov/chemistry/

ANNEX

Annex 1: Volatile compounds identified

peak n°	compounds	peak n°	compounds
1	ethyl 2-isobutyrate	30	unknown
2	isobutyl acetate	31	unknown
3	Ethyl butyrate	32	ethyl phenyl acetate
4	1-propanol	33	ethyl dodecanoate
5	2- methyl ethyl butyrate	34	hexanoic acid
6	ethyl isovalerate	35	guaiacol
7	isobutanol3	36	benzyl alcohol
8	Isoamyl acetate	37	*trans*-β-methyl-γ-octalactone
9	1-butanol	38	2-phenyll ethanol
10	2+3-methyl-1-butanol	39	4-methyl guaiacol
11	ethyl hexanoate	40	*cis*-β-methyl-γ-octalactone
12	5 methyl 2 hexanol (internal standard)	41	ethyl guaiacol
13	ethyl lactate	42	ethyl malate
14	1-hexanol	43	octanoic acid
15	*cis*-3-hexenol	44	unknown
16	*tran*s-2-hexenol	45	eugenol
17	ethyl octanoate	46	4- ethylphenol
18	unknown	47	3,4-dimethylphenol (internal standard)
19	acetic acid	48	syringol
20	furfural	49	decanoic acid
21	benzaldehyde	50	4-methyl syringol
22	linalol	51	dodecanoic acid
23	unknown	52	HMF
24	methyl furfural	53	4-allylsyringol
25	butanoic acid	54	vanillin
26	ethyl decanoate	55	ethyl vanillate
27	isovaleric acid	56	acetovanilone
28	ethyl succinate	57	syringaldehyde
29	terpeniol		