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Chapter

Management of Wine Aroma Compounds: Principal Basis and Future Perspectives

Antia G. Pereira, Maria Fraga, Paula Garcia-Oliveira, Maria Carpena, Cecilia Jimenez-Lopez, Catarina Lourenço-Lopes, Lillian Barros, Isabel C.F.R. Ferreira, Miguel Angel Prieto and Jesus Simal-Gandara

Abstract

Wine's aroma is defined by volatile and non-volatile compounds that contribute to its make-up. The complex variety of volatile compounds, coming from grapes, interact with other non-volatile substances of the wine as precursors of wine's aroma, known as primary aromas, which give the aroma of the young wine. The volatile compounds present in the skin and in the grape juice change according to the grape variety. Most of wine volatile compounds responsible for aroma are linked to sugars and they initially form odorless glycosides. Through the process of hydrolysis, they are reverted into an aromatic form. Chemical reactions among these compounds occur during the fermentation and in the first months of a wine's existence, triggering fast and multiple modifications in wine's aroma at this point. As wine ages and matures, changes and development in aroma will continue to take place but at a slower and more gradual pace. The study of the compounds responsible for aroma and flavor, as well as their correlation with the wine quality, is ongoing. Improving the knowledge of wine aromatic compounds could increase the risk of its potential adulteration; however, consumers prefer wine for its natural origin, so this scenario is unlikely in the future.

Keywords: wine, aroma, compounds, grapes, volatile

1. Introduction

1.1 Wine, quality and aroma

Since ancient times, wine has had a fundamental role in diet for several reasons. It is a good method of liquid conservation because its composition prevents the development of microorganisms and rot, it presents properties against certain diseases and provides health benefits, as long as its consumption is moderate. Wine is rich in antioxidants, mainly phenolic compounds, which are natural bioactive compounds related to its diverse properties. Numerous epidemiological studies have

associated regular and moderate consumption of wine with a lower incidence of mortality and morbidity from cardiovascular diseases in European countries, mainly in the Mediterranean [1].

The general term “quality” is a complex idea, which is not simpler when applied to wine. Throughout history, different institutions and laws have tried to regulate this concept. The International Organization of Vine and Wine (OIV) considers wine quality as the set of characters that differentiate one wine from another, being one of these characters the consumer taste [2]. According to this definition, the quality of a wine is relative and may vary over time, depending on several factors, such as the country of consumption, its habits, or new advances in the wine industry, among others.

However, the Regulation (EEC) No 823/87 has established a more permanent concept of wine quality, through the definition of the following requirements:

- Delimitation of the production area.
- Distribution of varieties within the wine area.
- Cultivation systems and uses.
- Vinification methods.
- Minimum natural volumetric alcoholic strength.

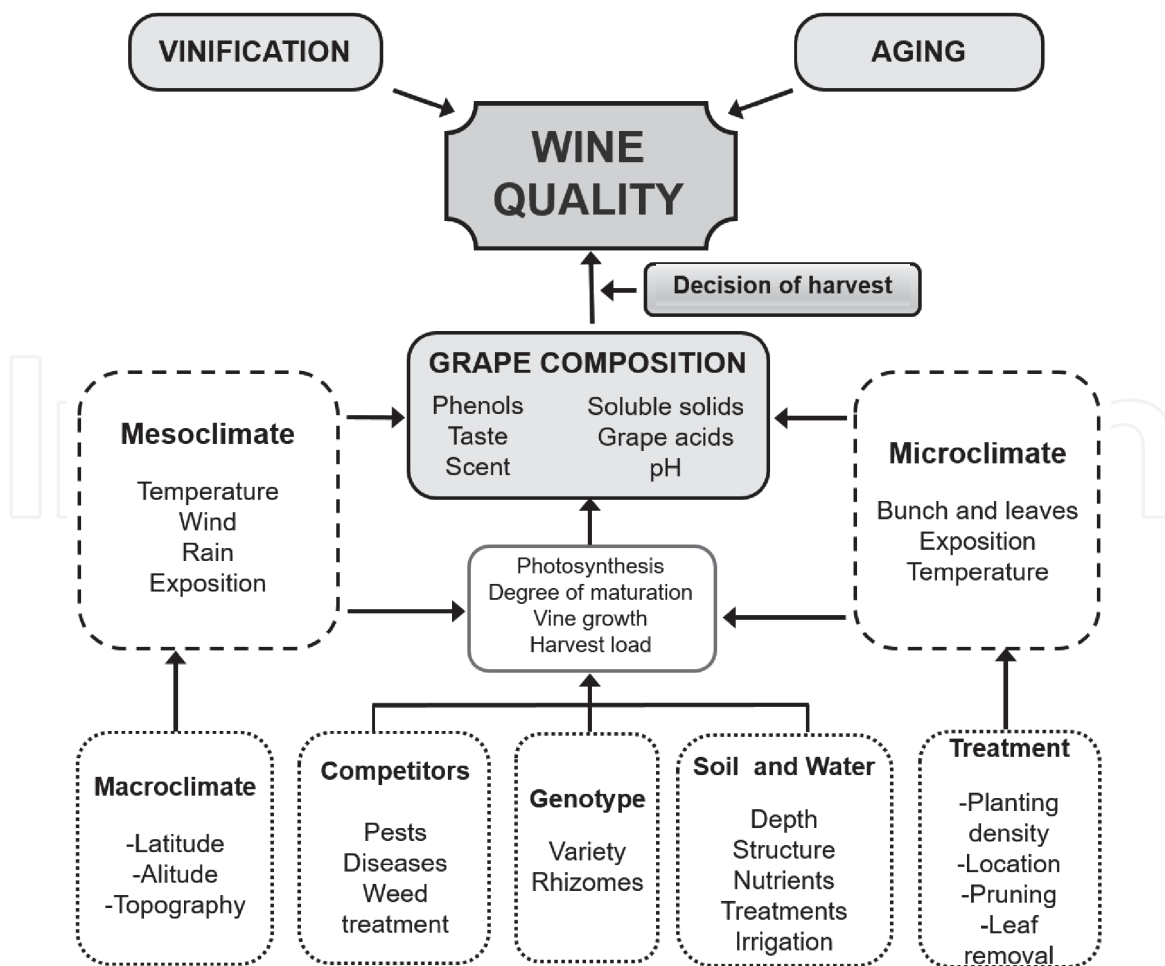


Figure 1. Environmental and viticultural factors that influence the composition of the wine. Adapted from [3].

- Yield per hectare.
- Analysis and evaluation of sensory characteristics.

Therefore, the quality of a wine will depend on many factors such as soil, type of grapes, presence of grape diseases, weather conditions, flavor complexity, alcoholic strength, balance, longevity, etc. (**Figure 1**) [4, 5]. These factors will also be important in the development and accumulation of the aroma compounds.

1.2 Accumulation of aroma compounds

The aroma of wine is complex, a particular wine contains more than 800 volatile compounds. When wine is ingested, these volatile compounds are dissolved in the palate and pharynx mucosa. Then, they reach the pituitary gland through the Eustachian tube. The perception of this odorous stimulus is the aroma of a wine and it cannot be evaluated without tasting it [6]. The concentration of these compounds may range from a few nanograms per liter to a few hundred micrograms or milligrams. They conform to a complex mixture of many different chemical compounds immersed in a hydroalcoholic solution [7].

Different factors determine the wine aroma such as the grape metabolism, the grape variety, the graft, the year, the weather conditions, the location of the vineyard, the region, the soil, cultural practices, the pre-fermentative biochemical phenomena (oxidations, hydrolysis) and the fermentative metabolism of microorganisms (conservation, aging) [8]. Since wine production is a long process that involves different stages, aromatic compounds from grapes can be transformed or revealed and many can be produced during alcoholic fermentation. In the case of aging wines in wood barrels, many reactions will occur in the wine forming new aroma compounds that will interact with the wood extracted aroma compounds.

The ripening of grapes also plays an important role since many compounds of interest are synthesized at the skin, such as terpenoids, linalool, alpha-terpineol, and geraniol, which will influence the final chemical composition and therefore, the accumulation of wine's aroma. The concentration of volatile compounds in grapes increases in advanced stages of ripening, when sugar accumulation in berries slows down. However, this cumulative process varies from red grapes to white grapes. In red ones, the maximum amount of varietal volatile compounds is reached at maturity and remains constant in the following weeks; whereas, in white grapes varietal volatiles content cannot be related to a maturity stage, because of its concentration changes during ripening [9].

1.3 Main factors affecting aroma quality

As mentioned before, different factors influence the wine quality: type of soil, grape variety, grape diseases, weather, balance, longevity, etc. (**Figure 1**) [10]. Thermal variation is an important factor since it affects grape ripening. Slight thermal variations along grape development will suppress grapes acidity, while stronger thermal fluctuations will yield grapes with better balance. Not just thermal variations but hours of sunshine exposition are another parameter to consider. Environmental conditions affect the grape taste and quality and they also influence the quantity and availability of nutrients in the soil. A deficiency of nutrients affects negatively to the development of vines and to the production and the quality of the wine. Regarding soil characteristics, granularity and texture are two important factors. Stony soils are considered favorable for wine quality, but unfavorable for

the plant production capacity due to its lower fertility. Clay soils are related to aromatic, well colored and, often thick, wines [11].

The variety of graft inserted in the vine is also important since the combinations of different varieties allow the obtaining of wines with greater complexity and creativity. Fruit physicochemical characteristics and, therefore wine properties define each variety [12].

Grape production, transformation and certification techniques may be considered, too. A good production is defined by several parameters. Grapes might be whole, healthy and mature. They should be grown following respectful cultivation and be manufactured using proper mechanization procedures. In addition, using appropriate technologies, oenological assessments should be performed for keeping high levels of quality along the control systems. As a result of all this chain of processes, a specific wine will be produced and therefore all these parameters will define its brand, image, authenticity and origin, among other aspects. All these factors lead to a new term “terroir”, which is used to express the distinctive characteristics that encompass the physical and biological environment and the applied wine practices that allow producing this wine in this situation [2].

1.4 Management of aroma in vineyards

One of the main factors that influence the evolution of the aroma is management in vineyards. Viticulture practices, which include management of vineyard cultivation, are controlled by human beings and, may be used to change the quality of the wine. These practices involve very varied factors as for example those based on decisions affecting the orientation vineyards' rows, their training system, density, the calendar for pruning, trimming, fungicide treatments, or the way in which soil surface is managed, which includes its tillage, the manipulation of the canopy structure, the nitrogen fertilization [13]. Additionally, vineyard cultivation may be affected by sunlight and water, among others, which will determine the final quality of berries since they are very sensitive to the microclimate. All the factors could change year by year, so the potential aroma of the wine depends on the year.

The amount of light absorbed by the vine leaf area determines the rate of photosynthesis, hence its metabolism. Therefore, light influences the development of grape aroma compounds. This relation is complex, as direct sunlight induces beneficial changes in photosynthetic pigment levels, but can cause stress by dehydration or temperature increase. Excessive sunlight exposure may burn the grapes' skin, resulting in a bitter flavor and affecting the quality of the wine [9]. Several studies reflect that sun exposure plays an important role in the variations of the volatile composition of grapes [14–16]. Some authors [17, 18] reported that high levels of terpene precursors are produced in over-ripe grape. The concentration of free and bound terpenes can also be increased by exposure of grapes to the sun [19, 20].

Canopy management is also important. Limiting canopy growth by removing leaves and vine shoots allows the increment of glucose production in the fruit, improving fruit's sunlight exposure. Nowadays, different ways for canopy structure management have been developed. The most common one is the plucking of leaves. It improves the microclimate of the clusters, reduces grape rot (caused by different pathogenic bacteria, yeast and, fungi, such as *Botrytis cinerea*) and provides better fruit maturation since less shadow is generated and enhances the pass of the sunlight. Another technique is head trimming which consists of cutting growing shoot tips in order to remove non-photosynthetic leaves. This technique reduces transpiration and induces the lignification of the plant, balances the development of

branches and increases airflow and insulation within the foliage [13]. Additionally, the removal of leaves may increase the content of total and phenol-free glycosides (one group of compounds that influence the aroma) [21]. In fact, wines obtained from defoliated grapes usually present higher fruity notes [22, 23]. Leaf-plucked grapes show the highest values of terpene and norisoprenoids compounds in Tempranillo, Merlot, and Gewürztraminer varieties where the synthesis of precursors is higher in warmest years and in grapes with more exposure to the sun [13].

As it has been previously described, soil and weather have an important effect on wine aroma, and this is mostly mediated by water. In general terms, water deficit has been proved to improve wine quality, enhance color, flavor and/or aroma [24]. However, no definitive scientific results can prove that the lack of water has such direct and positive impact on the quality since the effect of water depends on the species. For instance, during the maturation of Riesling grapes, the accumulation of terpene precursors is lower under irrigation [25]. In Tempranillo grapes, a positive correlation between irrigation and aroma potential at harvest has been detected [26, 27]. In Agiorgitiko grapes, precursors' synthesis is promoted by the limited availability of water [28]. There are several studies about the impacts of irrigation on fruit composition and wine sensory properties. However, the majority of these studies have been conducted in arid regions where irrigation is considered necessary and only a few have been carried out in humid regions where irrigation is mostly not necessary [29].

Among the nutritive compounds of must, the amino acids are of great importance. They can serve as precursors of esters and also may play an indirect role in supporting cellular metabolic activities [30]. Consequently, wine growers can also modify soil fertilization to change the aromatic profile of the resulting wine. The nitrogen soil composition can lead to excessive vine vigor and susceptibility to gray rot, but can also enhance aroma expression since yeasts use that same nitrogen [31]. Nevertheless, when juice presents a sufficient amount of nitrogen, the addition of amino acids does not improve the volatile composition of wine, therefore the excess of the amino acids will probably be consumed by other cellular processes [30].

As wine quality strongly depends on the grape quality, it is necessary to process healthy grapes at the correct ripeness stage in order to obtain high-quality wines. For this reason, winegrowers have to be especially careful in the prevention of parasite attacks on the grapevine. Fungicide treatments are applied to prevent diseases such as botrytis, powdery mildew or downy mildew, which cause serious problems in the production. However, these fungicides can pass to wines at trace levels and can affect the aroma, and also the safety of the wine [9]. In addition, pesticide residues on grapes can be transferred to the must and influence the selection and development of yeast strains. On the other hand, yeast can reduce the levels of the pesticides in the wine or adsorb them on the dregs of the wine. The persistence of pesticides depends on several factors such as the chemical characteristics of the active ingredients, photodegradation, thermodegradation, co-distillation and enzymatic degradation [32]. However, more studies are needed to orient the preferences of winegrowers for one or another fungicide.

2. Determination of aroma compounds

The analysis of the food aroma can be performed through tastings or instrumental methods, being necessary to correlate the latter with the former (**Figure 2**). Traditionally, sensory analysis was used to carry out the aroma determination. However, this method has the disadvantage (**Table 1**) of being subjective, and so,

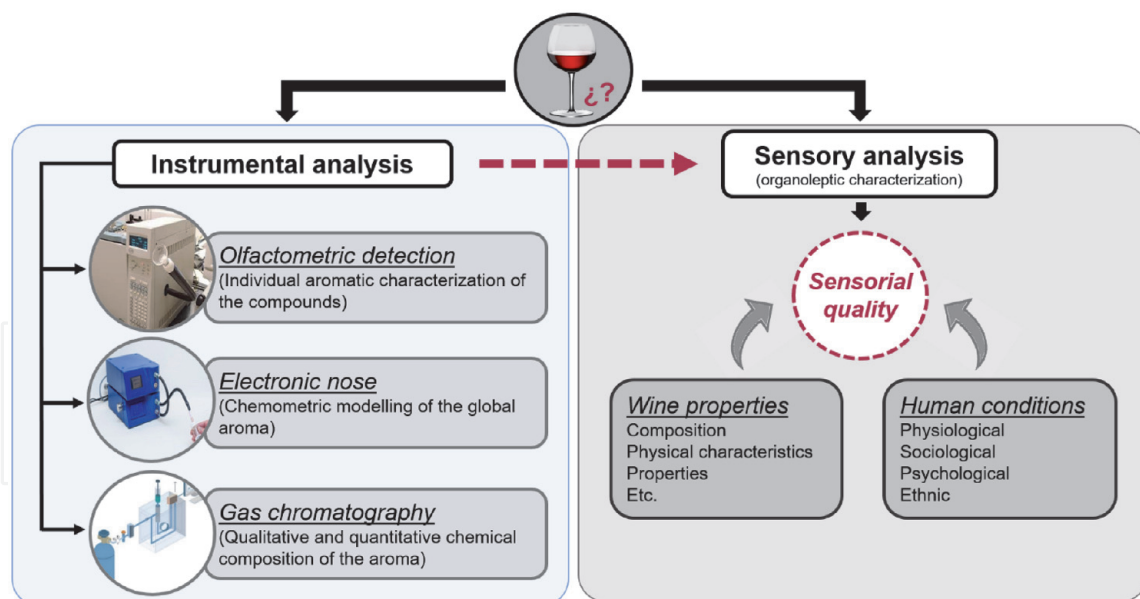


Figure 2.
Different techniques that can be applied to wine aroma analyses.

Advantages		Disadvantages	
Sensory	Instrumental	Sensory	Instrumental
Direct	Objective	Subjective	Indirect
Few material	Low quantity of sample	High quantity of sample	Expensive equipment
More realistic	One person	Several persons	Need of sensory analysis for realistic results
	Repeatable	Non repeatable	
	Can be toxic or no innocuous	Innocuous	

Table 1.
Advantages and disadvantages of each type of analysis methodology.

numerous regulations and protocols were elaborated to standardize the methodology and aromatic descriptors. The obtained results were significant but never distinguishable by the subjectivity of the taster. In this regard, technological advances that allow the investigation of the compounds responsible for the aroma and aromatic profile of a wine have been carried out. Nonetheless, there is no analytical method that allows interpreting the perceptions received during the tasting [33].

As mentioned before, the aroma of wines is complex and more than 800 volatile compounds have been identified, some of them at the ng/L level. Because of that complexity, the determination of aromatic compounds is not simple and it is difficult to isolate a specific aroma character. However, the development of techniques such as gas chromatography coupled to mass spectrophotometry or electric nose has allowed to identify hundreds of them [34].

Sensory methods and gas chromatography are analytical methodologies often slow and expensive. Developing new analysis techniques to solve these limitations is of great interest. This could be the case of the electronic nose (e-nose), characterized by its high sensitivity, speed, low cost and minimal, or no sample preparation [35]. Therefore, there are complementary tools that in combination will provide a lot of information regarding the evaluation of aroma [33].

2.1 Sensory analysis

The sensory analysis includes wine tasting, its sensory estimation and appreciation, and its description. In this process, the subjectivity of the individual towards the sensations, emotions and memories that can awaken on him a certain smell (affective aspect) is very involved. Subjectivity is present because two stages coexist in the process: chemistry (biochemical reactions between the aroma/taste molecules and our papillae) and psychology (electrical signals processed in the brain that assigns a description) [36].

To avoid subjectivity in the obtained results, it is necessary to standardize the process and establish a series of standards. Various levels such as CEE, OIV, ISO, etc., have described regulations to carry out the tasting [37].

Even though sensory analysis does not provide information on the chemical composition of the aroma, it is useful for the search of certain sensory alterations, detection of adulterations or the typification of the product according to its origin and variety. However, the incorporation of instrumental techniques (olfactometric detection, electronic nose, etc.) requires sensory analysis for calibration and interpretation [37].

2.2 Instrumental analysis

Despite the complexity of the human smell and the study of wine's aroma, various techniques have provided valuable and complementary information on different aspects of aromas. Those include gas chromatography (qualitative and quantitative chemical composition of the aroma), olfactometric detection (individual aromatic characterization of compounds) and electronic nose (chemometric modeling of the global aroma). This analysis allows the quantitative determination and the qualitative evaluation of the aromatic compounds, as well as an instrumental interpretation of the overall aroma. It is necessary for the winemaker to know these techniques when seeking answers to their needs [35].

One of these techniques is gas chromatography (GC), which allows the identification and quantification of the volatile compounds of the wine. This system has been improved through stages of extraction and concentration of the volatile compounds, chromatographic separation and detection systems, such as mass spectrometry (MS) or flame ionization (FID) [38].

There is another technique coupled to the chromatographic analysis called olfactometric detection or sniffing (gas chromatography-olfactometry, GCO), which allows to determine the aromatic profile of a wine. In this assay, a person smells the compounds after they have been separated and eluted from the column. Thus, each peak is associated with an olfactory descriptor. Aromas are classified as active or inactive based on the value of aromatic activity (OAV) (This value allows measuring the aromatic activity of a compound (C) in a specific matrix as far as its activity is above the detection threshold (DT)). Thus, active aromas possess numerical OAV greater than one [39].

$$OAV = \frac{C}{DT} \quad (1)$$

This assay presents some limitations, for example, the evaluation of the compound β -damascenone, which is overestimating due to its very low detection threshold with GCO, its wide range and its dependence on the composition of the medium [34]. Nevertheless, this technique has been one of the most used in the last decade for overall identification of odor-active compounds in all types of wine

(white and red musts, young and aged wines, sweet wines) made from different varieties of grapes such as Chardonnay, Riesling, Gewürztraminer, Merlot, Cabernet Sauvignon, Grenache, Tempranillo, Zalema, Palomino Fino, Touriga Nacional, Aragonez or Trincadeira. It can also be applied to study the sensory profiles of wines produced with sound and sour rot affected grapes and compare them to understand the role of sour rot in the odor nuances of wines [40].

Recently, the electronic nose has been introduced in the wine industry. It consists of an instrument equipped with chemical sensors and a chemometric model recognition program, capable of identifying and comparing individual or complex odors. As its main objective is to obtain results comparable to those from the human olfactory system, the aim of this method is to relate the perceived aroma with a response that, after being stored in memory, will serve as a model in further analysis. It has been displayed as a useful tool due to the advantages it offers: short analysis time in chromatography position (5–10 min), continuous control, it is a non-destructive method and it does not require qualified personnel. However, it is limited by the effectiveness of the detectors [41]. Most of its applications are related to the discrimination of wines to prevent their adulteration or detection of disagreeable odors, but only a few of them consider the identification of the quality of wine aromas. Despite all, this system allows a good classification of typical red and white wine aromas [42].

Finally, along the scientific literature, it has been described as an innovative technique of aroma determination. It consists of an array of conducting polymer sensors coupled to a selective solid-phase micro-extraction (SPME) fiber. This assay allows carrying an analysis of the principal components, differentiating the aromas of the sample, even for wines with very similar sensory characteristics. Moreover, the response is fast and consistent. The selective adsorption of the fiber provides a better distinction, increasing the concentration of the minor compounds of an aroma [43, 44].

3. Types of aroma

Despite the massive amount of aromatic compounds present in wine, not all of them contribute to the perceived aroma, since some of them are found in concentrations below the perception threshold. Compounds that exceed this concentration are called active compounds. Aromas can be classified in different ways according to the parameter considered. In this way, a classification can be made based on its presence (basic, subtle and special) or according to its origin or sequence of wine production as described in **Figure 3**. In the last case, the classification based in the sequence of wine production allows to differentiate the aromas accordingly to its process as primary, secondary and post-fermentative aroma [9, 45], facilitating the inference of wine makers. The following paragraphs will describe the different stages of wine production and the aromas originated during the process.

3.1 Primary aroma

The primary aroma is formed by the varietal aromatic constituents. Three large groups of compounds can be distinguished: the free varietal aroma, the precursors of varietal origin (non-volatile or non-odorous precursors and odorous volatile compounds) and the substances that are formed from the precursors [46, 47].

3.1.1 Free varietal aroma

This type of aroma distinguishes the different varieties of grapes. Although relatively few studies have been able to identify a compound responsible for the

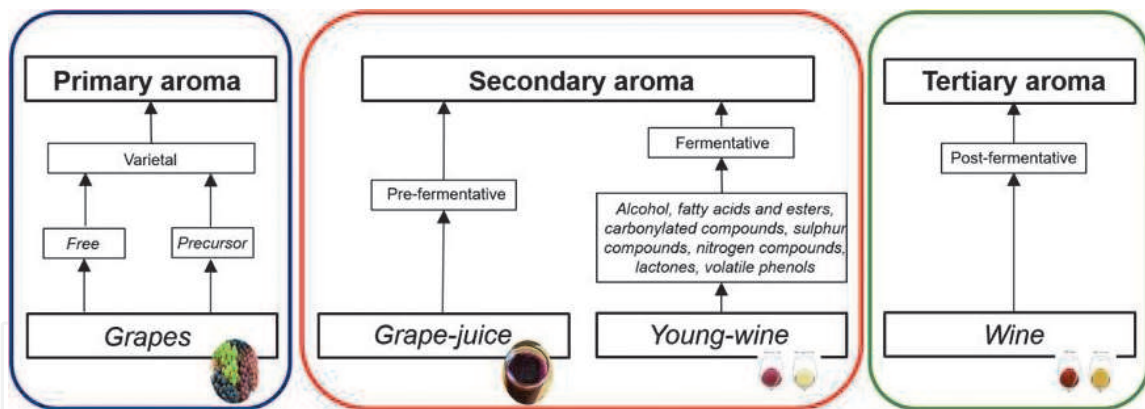


Figure 3.
 Classification of wine aroma according to its origin along wine production.

Compound	Chemical formula	Chemical structure	Olfactory descriptor
3-Isobutyl-2-methoxypyrazine	$\text{CH}_2\text{CH}(\text{CH}_3)_2$		Green peppers
2-Methoxy-3-sec-butyl-pyrazine	$\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$		Green peppers
2-Methoxy-3-(2-methylpropyl) pyrazine	$\text{CH}(\text{CH}_3)_2$		Green peppers

Table 2.
 Principal pyrazines found in wines.

varietal character, notable difference between varieties have been reported. This difference is therefore due to the overall aromatic profile [48]. Among the compounds that determine the free varietal aroma, determined by volatile substances linked to the aromatic typicality of the variety, two chemical families are distinguished: pyrazines and terpenes [46].

3.1.1.1 Pyrazines

These compounds (**Table 2**) were first identified in Cabernet Sauvignon grape variety. They are nitrogen compounds derived from the catabolism of some amino acids such as leucine, isoleucine, valine and glyoxal. They are usually found in concentrations below the perception threshold. Its concentration has a positive correlation with the herbaceous note of some wines such as Cabernet Sauvignon and Sauvignon Blanc [46, 49]. The concentration of pyrazines has been estimated in different grape varieties, such as Sauvignon Blanc (3 ng/L), Semillon (2 ng/L), Cabernet Sauvignon (2–24 ng/L) [50]. Regarding wine, pyrazines content has been estimated at about 1 ng/L in white wines, while in red wines, the concentration reached 10 ng/L [51]. Several factors have been described to influence the pyrazines

content in grapes, especially grape variety and maturation. The degree of grapes' ripeness influences their content, being pyrazines' content inversely proportional to this factor. Thus, its content decreases appreciably from summer and disappears practically under optimal conditions of maturation. Other factors have been described to influence pyrazine content, such as temperature and irradiation of vineyard [46, 50]. The soil also plays a significant role in pyrazine levels. A higher amount of pyrazines has been found in vineyards grown in limestone and clay soils than in sandy soils [49].

3.1.1.2 Terpenes

Within this group, very abundant in the plant kingdom, are monoterpenes (formed by 10 carbon atoms), sesquiterpenes (15 carbon atoms) and the corresponding alcohols and aldehydes [52].

They are the most studied odoriferous compounds found in *Vitis vinifera*, having identified around 70 compounds in both wines and grapes. They can be found as free forms or as odorless precursors, mainly glycosylates. The most odoriferous monoterpenes are monoterpene alcohols like linalool, α -terpineol, nerol, geraniol, citronellol and ho-trienol (**Figure 4**), which provides floral aromas (rose, lily, citronella, linden, etc.). These floral attributes are characteristic of white wines [49, 53]. In addition, the content in terpenoles is determined by the state of the grape since they are very sensitive to the attack of *Botrytis cinerea* [54].

Both monoterpenoles and sesquiterpenes are synthesized from isopentyl pyrophosphate (IPP) and dimethylalkyl pyrophosphate (DMAPP). IPP and DMAPP precursors are produced through the cytosolic mevalonic acid (MVA) pathway (from three molecules of acetyl-CoA) or through 2-C-methyl-D-erythritol-4-phosphate plastidial (MEP) pyruvate and glyceraldehyde-3-phosphate [48].

Regarding the location of these compounds in grapes, more than 50% are concentrated in the solid part (pulp and skin), reaching concentrations of 90% of geraniol and nerol in these parts. In contrast, half of the linalool is in the juice; thus,

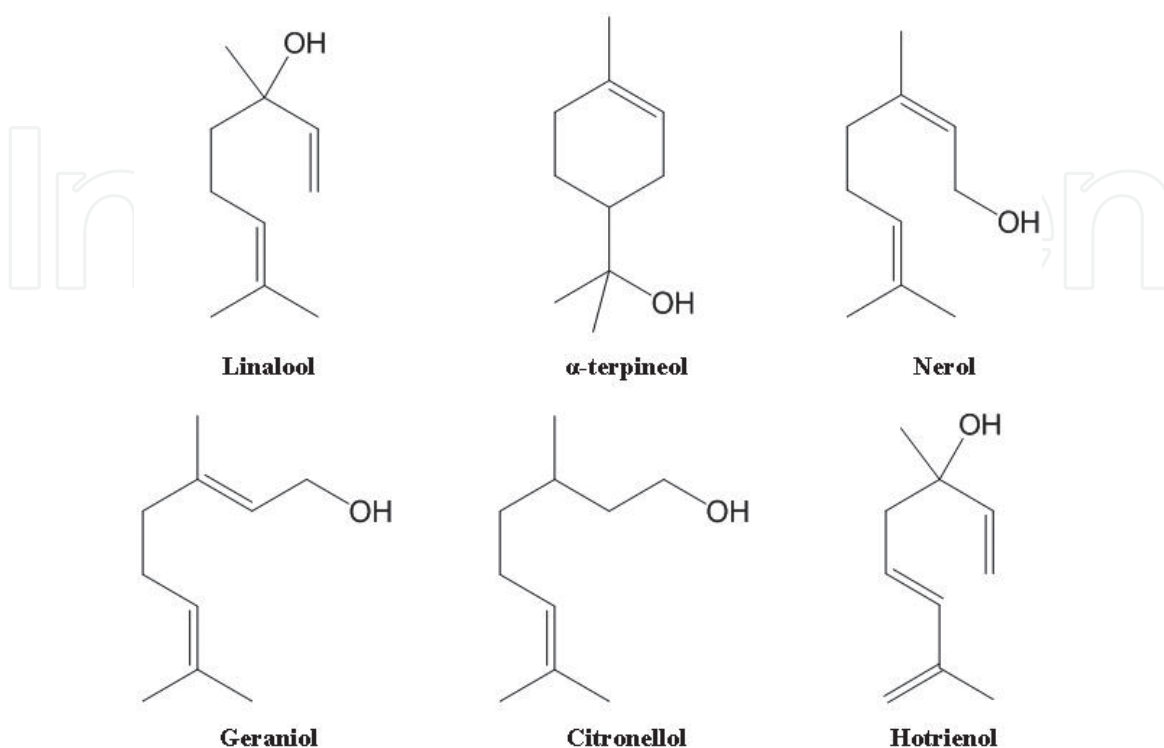


Figure 4.
Structure of the main monoterpene alcohols found in wine.

processes that involve an increase in the exchange of solid and liquid parts (e.g. maceration) have important implications on the final aromatic characteristics of the wine [46].

3.1.2 Precursors of varietal origin

Although some precursors do not possess odoriferous characteristics, they can give rise to odoriferous substances. These are monoterpenes, diols or terpene polyols, fatty acids, carotenoids, glycosylated precursors of aroma and volatile phenols [55].

3.1.2.1 Monoterpenes

This group of compounds is one of the most studied in wine and includes a wide variety of compounds. The formation of these molecules is mainly due to the oxidative metabolism of linalool in grapes [56]. Monoterpenols are sensitive to the hydration and oxidation reactions that occur during winemaking and cause the transformation of one into another [57, 58]. Some yeasts can increase the content of this type of compound. This is the case of *Issatchenkia* spp. [59].

3.1.2.2 Diols

These compounds are characterized by transforming at relatively acidic pH, such as those found in musts or wines. Some of these compounds thus obtained are fragrant, but may be the cause of transfers of strange aromas to the wines [46]. Red grapes are not very rich in these compounds, but their action on the aroma is not improbable if the olfactory threshold is taken into account, since it is very low in some of its derivatives, either isolated or well mixed [8].

3.1.2.3 Carotenoids

Carotenoid content decreases throughout ripening, with a higher content in the grapes exposed to the shade than those exposed to the sun. In grapes grown in high altitude, the content is also lower, due to the low temperatures and higher humidity [60–62]. Its content in grapes ranges between 15 and 2.000 µg/kg. Lutein and β-carotene stand out as the most abundant, as well as neoxanthin, flavoxanthin and others in smaller quantities [63].

Carotenoids are not found in grape juices and in wines made without maceration, as they are degraded during the breaking of the grape and the vinification. Light and oxidases are capable of degrading carotenoids into smaller fragments, more soluble and more fragrant. Among the compounds that are formed in the decomposition of carotenoids, norisoprenoids are worth mentioning because they have low perception thresholds that make them play an important role in the aroma of wine. This degradation can be direct or with an intermediate step that is the formation of glycoconjugates, which can then release their volatile aglycone during fermentation through enzymatic and acid hydrolysis processes [64, 65].

3.1.2.4 Glycosylated precursors

All grape varieties have the same glycosylated derivatives, being Moscatel varieties the most concentrated. They appear in greater content in the skin than in the pulp or juice. These compounds are four types of glycosides: one monoglycoside (β-D-glucopyranoside) and three diglycosides

(*O*- α -L arabinofuranosyl- β -D-glucopyranoside, 6-*O*- α -L rhamnosyl- β -D-glucopyranoside and 6-*O*- β -D apiosyl- β -D-glucopyranoside) [66].

Although grapes contain enzymes (β -glycosidases) capable of releasing some of these compounds during winemaking, under normal winemaking conditions, glycosylated precursors have a poor effect on the development of the aroma since its optimal activity is developed in other pH values [67].

3.1.2.5 Volatile phenols

These compounds can be responsible for the originality or wine aroma defects. Phenolic compounds are non-odorous compounds, which can be submitted to different enzymatic reactions, transforming into volatile phenols and contributing to unpleasant aromas of pharmacy, smoke, forest, leather or pepper. These compounds are characteristic of carbonic maceration wines, although they are present in other wines [68].

Among phenolic acids, caffeic, ferulic and *p*-coumaric are found in the pulp and skin in the form of tartaric esters (caftaric acid, feruloyl tartaric acid) and coumaric (*p*-coumaroyl tartaric). Its olfactory threshold is very low and they strongly decrease during the ripening of the grape, with large variations within the same variety [46].

During vinification, free acids and then volatile phenols are formed. The process of hydrolysis causes the appearance of other phenolic compounds such as vanillin, methyl vanilla or homovanillyl alcohol [69].

3.2 Secondary aroma

3.2.1 Pre-fermentative aroma

This type of aroma is developed during the winemaking process, more specifically, between the harvest of the grapes and the beginning of the fermentation. The mechanical processes performed during this stage, such as grape transport, crushing, de-stemming, pressing and even carbonic maceration have a great influence in the pre-fermentative aroma. All these processes involve grape cellular rupture, allowing enzymes to come into contact with the aroma precursors. Thus, the availability of aroma precursors is directly proportional to the degree of grape rupture. Moreover, the mechanical process also favors the incorporation of oxygen, leading to enzymatic oxidation reactions. Throughout the process of crushing grapes, relatively large amounts of aldehydes and alcohols of 6 carbon atoms are formed. In some cases, these compounds give the smell of cut leaves, bitter taste and low olfactory threshold. The formation of C₆ aroma compounds varies depending on the ripeness of the grape, although the greatest potential occurs before the ripening date, where grape lipids levels are higher and also depends on the type of variety [70].

3.2.2 Fermentative aroma

Fermentation is the main phase in the transformation of grape juices into wine, with two biological transformations occurring at this stage: alcoholic (yeast transform sugars into alcohol and other secondary products) and malolactic fermentation (lactic acid bacteria cause the degradation of malic acid in lactic acid) [71].

While malolactic fermentation subtly modifies the aroma of wine, alcoholic fermentation is of great importance in the aroma since it is responsible for the winery note that constitutes the common aromatic base for all wines. Moreover,

volatile constituents formed during fermentation (**Table 3**) will quantitatively represent most of the aroma constituents [72]. Malolactic fermentation is not interesting in all situations. For example, in white wines it is interesting to avoid it since this type of fermentation leads to a loss of freshness and varietal aromas, which are desirable characteristics in this type of wine.

3.2.2.1 Alcohols

Main alcohols synthesized in alcoholic fermentation are 2- and 3-methylbutanol, 1-propanol, 2-methylpropanol, 1-butanol, 1-pentanol, 2-phenylethanol, 3-methylthio-propanol, tyrosol and tryptophol, being its average total content between 400 and 500 mg/L. These compounds are produced at the metabolism of amino acids, so their concentration depends on the nitrogen content of the grape juice, the yeast species and the factors that influence their development [73].

3.2.2.2 Fatty acids and esters

Together with alcohols, these compounds are the main markers of the fermentative aroma. Fatty acids are formed as a result of the interruption of the process of elongation of acetyl-CoA by hydrolysis. Regarding esters, most of them are formed enzymatically, by the initial activation of fatty acids combined with coenzyme A (CoA) [74]. These compounds are usually found in low concentrations, but above the threshold of perception. They are also pleasant aromas (fruit mainly) except ethyl acetate (over 100 mg/L) [75].

3.2.2.3 Carbonylated compounds

These compounds arise from the metabolism of yeasts by decarboxylation of the α -ketone acids formed in the biosynthesis of higher alcohols. They remain in the wine in small quantities, due to the strong reducing activity of yeast during fermentation. Sulfur dioxide and alcohols react to the pH of the wine with the carbonylated compounds, forming sulphydric combinations [69].

3.2.2.4 Sulfur compounds

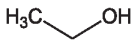
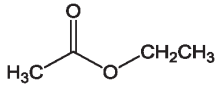
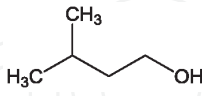
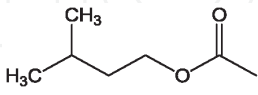
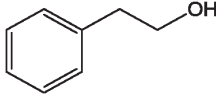
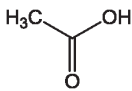
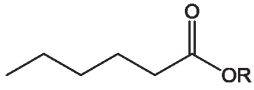
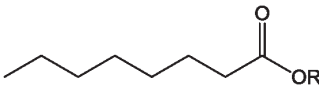
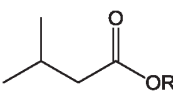
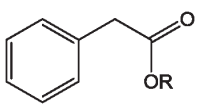
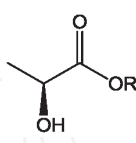
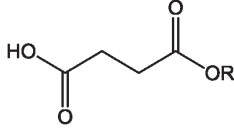
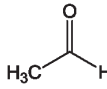
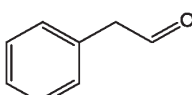
These are compounds that generally provide intense and unpleasant odors. Within this group are thiols, sulfides and thioesters. They can be classified according to their weight in compounds of low molecular weight (sulfur functional group predominantly on odor) or high molecular weight (complex participation in aroma due to its analogy with higher alcohols) [69].

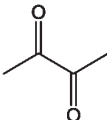
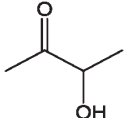

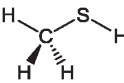
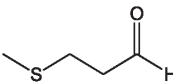
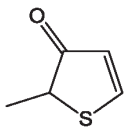
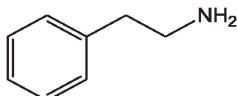
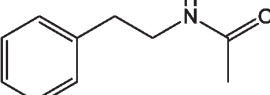

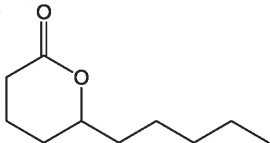
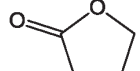
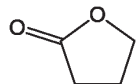
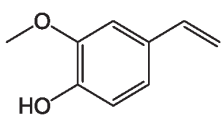
3.2.2.5 Nitrogen compounds

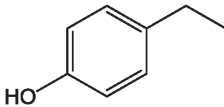
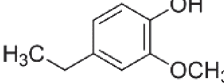
The most abundant nitrogen compounds are acetamides from primary amines and their corresponding amines. Acetamides have a smell similar to uric acid. They are produced by *Bacterium manitopeum* [76].

3.2.2.6 Lactones

They are compounds related to hydroxy acids because they are obtained by intramolecular esterification of these compounds: 4-hydroxy acids lead to γ -lactones and 5-hydroxy acids to δ -lactones; both are oxygenated heterocycles [69].

Compound	Molecular formula	Chemical structure	Olfactory descriptor	ODT (mg/l)
A. Alcohols, acids and esters				
Ethanol	C ₂ H ₆ O		Alcohol	0.150
Ethyl acetate	C ₄ H ₈ O ₂		Fruity	0.605
Isoamyl alcohol	C ₅ H ₁₂ O		Almond Cream, fusel oil	30
Isoamyl acetate	C ₇ H ₁₄ O		Banana	0.030
Chain R = H 2-phenylethanol Chain R = Acetate; 2-Phenylethyl acetate	C ₈ H ₁₀ O		Roses	14
Acetic acid	C ₂ H ₄ O ₂		Vinegar	2.3
Chain R = H; Hexanoic acid Chain R = Ethyl; Ethyl octanoate			Sweat, sour Pineapple	0.420 0.014
Chain R H; Octanoic acid Chain R = Ethyl; Ethyl octanoate			Butter Fruit	2.200 0.005
Chain R = H; 3-methylbutanoic acid Chain R = Ethyl; Ethyl 3-methylbutanoate			Cheese Fruit	0.250 0.003
Phenylacetic acid			Animal Rose	1 1.8
Chain R = H; lactic acid Chain R = Ethyl; Ethyl lactate			Sour milk Milk	0.065 150
Chain R = H; Succinic acid Chain R = R = Ethyl; Ethyl succinate			Cantaloupe	No data 1.2
B. Carbonylated compounds				
Acetaldehyde	C ₂ H ₄ O		Last apple	0.090
Phenylacetaldehyde	C ₈ H ₈ O		Floral	No data

Compound	Molecular formula	Chemical structure	Olfactory descriptor	ODT (mg/l)
Diacetyl	C ₄ H ₆ O ₂		Butter	0.100
Acetoin	C ₄ H ₈ O ₂		Butter	150
C. Sulfur compounds				
Hydrogen sulfide	H ₂ S		Rotten egg	0.016
Methanethiol	CH ₄ S		Light taste	1
Methional	C ₄ H ₈ OS		Cooked potatoes	0.200
Methylthiophene-3-one	C ₅ H ₆ OS		Breadcrumbs	No data
D. Nitrogen compounds				
2-phenylethylamine	C ₈ H ₁₁ N		Rot	No data
N-(2-phenylethyl)-acetamide	C ₁₀ H ₁₃ NO		Similar to uric acid	6
E. Lactones				
γ-nonalactone	C ₉ H ₁₆ O ₂		Coconut nut	0.025
Δ-decalactone	C ₁₀ H ₁₈ O ₂		Peach	0.110
γ-butyrolactone	C ₄ H ₆ O ₂		Coconut nut	0.400
F. Volatile phenols				
4-vinylphenol	C ₈ H ₈ O		Nail	0.375
4-vinylguaiaicol	C ₉ H ₁₀ O		Clove, roasted almonds	0.006

Compound	Molecular formula	Chemical structure	Olfactory descriptor	ODT (mg/l)
4-ethylphenol	C ₈ H ₁₀ O		Medicinal horse manure	0.620
4-ethylguaiaicol	C ₉ H ₁₂ O ₂		Wood	0.05

Olfactory detection threshold (ODT).

Table 3.
Relevant aroma compounds produced during wine fermentation [77, 78].

The most abundant lactones are γ -butyrolactone, 4-carbethoxy-8-butyrolactone and 4-ethoxy- γ -butyrolactone.

3.2.2.7 Volatile phenols

The main volatile phenols produced by *Saccharomyces cerevisiae* yeasts are 4-vinylphenol and 4-vinylguaiaicol. Within this group is also 4-ethylphenol, which is produced by yeasts belonging to the genus *Brettanomyces/Dekkera*. It is produced by enzymatic decarboxylation of *p*-coumaric and ferulic acids during alcoholic fermentation [69].

3.3 Post-fermentative aroma

This type of aroma includes all volatile compounds formed throughout the aging of the wine, giving rise to what is called “bouquet”. During the conservation of the wine, physicochemical and biological reactions transform the aromatic compounds produced in previous stages, so post-fermentative aroma suffers deep changes [79]. Depending on the type of aging, two types of bouquet can be distinguished:

- The bouquet of oxidation takes place in wooden barrels. It is produced by the synthesis of acetaldehydes and/or acetals, as well as by the extraction of a series of nuances from wood and the diffusion of oxygen through the wood pores [79].
- The bouquet of reduction is characteristic of aging in the bottle, determined by the interaction between the wine compounds synthesized throughout the fermentation [80].

During this stage, the fruity aroma of the wines disappears progressively, evolving towards a more complex aroma. These reactions are slow and limited, sensibly manifesting in the wines after several years.

In this type of wines, there are compounds given by wood such as furans (furfural, 5-methylfurfural, 5-hydroxymethylfurfural) which are responsible for the aromas of roasted almonds [81], phenolic aldehydes (vanilla), phenyl ketones (vanilla aroma), oxygenated heterocycles (caramel aroma), volatile phenols (toasted smell and burnt wood) [82], 4-vinylphenol (carnation aroma), 4-vinylguaiaicol (clove aroma), whiskey-lactones (coconut aroma) [83].

However, some compounds are transformed. During aging, the concentration of monoterpenic alcohols in wine from grapes (linalool, geraniol and citronellol) decreases. On the other hand, the concentration of isomeric oxides, whose olfactory thresholds are higher than the starting alcohols is increased. Hence, there is a loss of varietal aromatic load [84].

4. Future perspectives

The complexity of wine's aroma has been considered more an art than a scientific fact since little knowledge of the mechanisms involved in the process is available. However, in the last decades, this situation is changing as more and more wine growers turn to science to improve their production. In the nearly future, wine-related genomic, proteomic and metabolomic research will be applied to *Vitis vinifera* cultivars (the important ones from a commercial point of view), to other *Vitis* species, as well as to the yeast strains employed in fermentation [9]. Nowadays, scientific research helps, for example, to understand the impact of wine microorganisms and their derivatives on varietal aromas. This allows winemakers to generate different wines with different characteristics (including aromatic ones) from the same matrix (same grapes), using different species of microorganisms.

The aromatic quality of wines in the near future will depend on climate change and the effect of greenhouse gases. The increment of these gases will carry an average raise on global surface temperatures, evaporative demand, and the frequency and intensity of drought. Studies about changes in the volatile composition, phenolic content and antioxidant activity of wines produced at elevated CO₂ concentrations are very limited, so it will be necessary to optimize the quality of wine in a future scenario of climate change [9]. Climate change will also allow to cultivate vineyards in not cultivable areas until now. For instance, the viticulture sector of United Kingdom has undergone a rapid growth (148% during 2004–2013), in part attributed to a warmer temperature in the areas of England and Wales (13–15°C), deemed suitable for cool-climate viticulture [85].

There is a future concern about that wines in the future could being “manipulated” by using chemical additives to add enhance complexity and additional aromas to wine such as created a manufactured perfume. In fact, there have already been cases. In 2004, a South-African winery was found to have added illegal flavoring to their Sauvignon blanc to enhance the aroma [86]. Increasing the knowledge about the mechanisms and compounds responsible for wine aroma helps to develop new methods to allow the improvement of the quality of common wines to resemble high-quality wines. However, any artificial manipulation of wine aroma is currently forbidden, and there is a significant number of winemakers and consumers that prefer more natural wines, without or with minimum chemical manipulation. The wine is widely consumed around the world by its nice sensory taste and its natural origin from fermented grape juice. Change this view could be dangerous to the wine industry and can lead to a significant noteworthy wine devaluation towards by the wine consumers and connoisseurs.

5. Conclusions

Despite the plenty of advances for understanding how the grape berry is developed and which chemical components are important for wine aroma and flavor, there is still too much to do. The advances that are being carried out in different fields such as the determination of aromas and their evolution will allow to continue

expanding this knowledge. Nowadays, the quality of wines has improved due to many of the mechanisms involved in the quality of a wine are known. Thus, manipulation of grapes in vineyards through different production practices in order to improve wine's quality is possible.

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Author details


Antia G. Pereira^{1,2}, Maria Fraga^{1,2}, Paula Garcia-Oliveira^{1,2}, Maria Carpena¹, Cecilia Jimenez-Lopez^{1,2}, Catarina Lourenço-Lopes¹, Lillian Barros², Isabel C.F.R. Ferreira², Miguel Angel Prieto^{1*} and Jesus Simal-Gandara^{1*}

1 Nutrition and Bromatology Group, Analytical and Food Chemistry Department, Faculty of Food Science and Technology, University of Vigo, Ourense, Spain

2 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal

*Address all correspondence to: mprieto@uvigo.es and jsimal@uvigo.es

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