REVIEW

THE HERBACEOUS CHARACTER OF WINES

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ABSTRACT

The herbaceous (vegetative) character of wine can be sensorially pleasant or not depending on different circumstances. This character can be specific to a particular grape cultivar (an endogenous varietal character) or produced during the winemaking procedure, from either an endogenous or exogenous cause. Several volatile wine components containing oxygen (aldehydes and alcohols with 6 carbon atoms), nitrogen (alkylmethoxypyrazines), and selected sulfur compounds (thiols, sulfides, disulfides) are reviewed and described. The effects of micro-oxygenation and storage in wood on the herbaceous post-fermentative character are discussed. Finally, the preventive tools and winemaking technologies used to reduce or to control this sensory character are described.

Keywords: green leaf volatiles, herbaceous, methoxypyrazines, thiols, vegetative, wine aroma

1. INTRODUCTION

The aroma complexity of wine is the result of several different factors (AMERINE and ROESSLER, 1976; RAPP, 1998). It consists of many hundred volatile compounds of various origin, which are present at concentrations ranging from fractions of ng/L up to mg/L and are able to interact with the olfactory epithelium to generate a sensory perception (ROBINSON *et al*., 2014a,b). Many of these odorous substances derive directly from the grapes (RAPP, 1998) and their formation is affected by both the grape metabolism and the viticultural ecosystem. Other impactful odorant compounds originate from the pre-fermentation biochemistry occurring during grape crushing and maceration. The 'fermentative aromas' (RAPP and MANDERY, 1986) originate from the metabolism of the microorganisms responsible for the alcoholic and malolactic fermentations. The chemical reactions occurring during the finishing and ageing of the wines also play a crucial role in the final bouquet of wine. Moreover, the effect of the addition of chemicals and adjuncts, the characteristics of the cooperage used for the processing and storage and the closure systems are also determining factors.

The fascination exercised by wine on the consumer is due in large part to the complexity of these orthonasal cues and to the retronasal perceptions evoked at the first sip. Due to the huge variability of odor notes, the volatiles are the components that often best define the parameters of quality and typicality of wine. Moreover, some aroma components are robust markers of identity, playing an important role in the protection of local products from commercial frauds (BOSELLI *et al*., 2008). A focus on 'varietal winemaking' is becoming increasingly important for countries characterized by an extremely variegated viticulture in terms of pedo-climatic conditions and cultivated genotypes which results in a large number of wines with specific geographical determination (e.g., over 400 in Italy).

Occasionally, wines may express excessive sensations of herbaceous ('vegetative') off-flavor, resulting in a reduction in fruit intensity and detrimentally impacting palate structure. Consumers rarely appreciate wines with pronounced herbaceous flavors; these products are described as possessing immature character, lacking refinement and elegance, and as such are not sought after by the modern consumer. In order to reduce or avoid these olfactory qualities in wines it is of critical importance to know the origins and causes of these characters.

This paper reviews the chemical compounds known to be responsible for the herbaceous character of wines and reports on the preventive tools and winemaking technologies used to minimize or control 'herbaceousness'. The herbaceous aroma in wines is described in reference to the genesis of the molecules responsible for the olfactory perception. These components can be ascribed to three different chemical classes:

- alkylmethoxypyrazines (endogenous varietal and exogenous herbaceous character)
- aldehydes and alcohols with 6 carbon atoms (pre-fermentative herbaceous character)
- selected low molecular weight sulphur compounds (herbaceous character in fermentation)

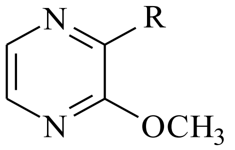
The contribution of any of these compounds to the final aroma depends on their concentration, their perception threshold and the extent to which they are modulated by interactions with both volatile and non-volatile components of wine.

2. HERBACEOUS CHARACTER DUE TO METHOXYPYRAZINES

2.1. Endogenous (varietal) herbaceous character

The varietal herbaceous character is usually described as 'green pepper', or 'tomato leaf' and is mainly derived from a group of nitrogen-containing compounds, the alkylmethoxypyrazines, which were first discovered in bell peppers (BUTTERY *et al.*, 1969) and are present in green plant tissues, including grapes (BAYONOVE *et al.*, 1975). Methoxypyrazines are used by insects and plants in chemical defense or, conversely, they may act in insects as pheromones.

The alkylmethoxypyrazines (MP) are chemical compounds based on the heterocyclic aromatic structure of pyrazine, originating from the metabolism of amino acids (Fig. 1).



R= CH_2CH_3, ETMP
R= $CHCH_3CH_2CH_3$, SBMP
R= $CH(CH_3)_2$, IPMP
R= $CH_2CH(CH_3)_2$, IBMP

Figure 1: Chemical structure of alkylmethoxypyrazines. ETMP: 3-ethyl-2-methoxypyrazine; SBMP: 3-sec-butyl-2-methoxypyrazine; IPMP: 3-isopropyl-2-methoxypyrazine; IBMP: 3-isobutyl-2-methoxypyrazine.

The main MP found in grapes, musts and wines are 3-ethyl-2-methoxypyrazine (ETMP); 3-sec-butyl-2-methoxypyrazine (SBMP); 3-isopropyl-2-methoxypyrazine (IPMP); and 3-isobutyl-2-methoxypyrazine (IBMP). The nature of the alkyl radical largely determines the olfactory perceptions of these compounds (SALA *et al.*, 2004) as summarized in Table 1.

Table 1: Odor descriptors and olfactory threshold in water (ng/L) of the main alkylmethoxypyrazines (MP) according to SALA *et al.* (2004).

Alkylmethoxypyrazines	Odor description	Threshold
ETMP	Raw potato, earthy, green, bell pepper	400-425
SBMP	Green, ivy leaves, bell pepper	1-2
IPMP	Earthy, cooked asparagus, green pepper	2
IBMP	Earthy, green, bell pepper, musty	0.5-2

Their aroma is generally described as 'bell pepper', 'leafy', 'ivy leaves', 'vegetable', 'peas', 'asparagus', 'ginseng', 'roasted', 'musty' and 'raw potatoes'.

MP represents a narrow, delineated group of extremely powerful odorants characterized by extremely low sensory perception thresholds (e.g., 1-2 ng/L in distilled water; KOTSERIDIS *et al.*, 1999). IBMP is typically the most predominant MP in grape and wine and, as such, is a major contributor to the vegetative character (SALA *et al.*, 2004; ALLEN *et al.*, 1991; LACEY *et al.*, 1991). This vegetative character is most commonly, although not exclusively, associated with Sauvignon blanc, Cabernet Sauvignon and other Bordeaux varietals (ALLEN *et al.*, 1991).

IPMP may also be present in certain grapes and thus found in the derived wine as a varietal character. However, it is the major exogenous contributor to the pool of methoxypyrazines because it is the most abundant and potent of the MP released by ladybugs, insects of the family Coccinellidae that can be accidentally incorporated with grapes during harvest operations (BOTEZATU *et al.*, 2012), as reported in the next section.

Different pathways leading to the MP responsible for the herbaceous character of wines have been proposed. One utilizes the amino acid leucine (for IBMP) or valine (for IPMP) and an unknown 1,2-dicarbonyl compound leading to the formation of 3-alkyl-2-hydroxypyrazine (HP). The final steps of these pathways involve the methylation of HP to form the associated MP, catalyzed by an O-methyltransferase enzyme (DUNLEVY *et al.*, 2010; DUNLEVY *et al.*, 2013). A very wide range of MP levels can be found in grapes due to changes in their concentration throughout the ripening process. Although the content of both IPMP and IBMP increases during early berry development, IPMP was found to decrease before veraison, whereas IBMP decreased rapidly after veraison in Cabernet Sauvignon grapes (HASHIZUME and SAMUTA, 1999). Similarly, MP levels were found to decrease progressively and rapidly with grape maturity both in Cabernet Sauvignon and Merlot (ROUJOU DE BOUBEE *et al.*, 2000). In Sauvignon blanc grapes, LACEY *et al.* (1991) found the same trend, although ALLEN *et al.* (1991) found an increase in IBMP during ripening.

A positive linear relationship was found between the content of IBMP in grapes at different ripening stages and the derived wines from the Bordeaux region (produced from Merlot, Cabernet Franc and Cabernet Sauvignon grapes) (KOTSERIDIS *et al.*, 1999). RYONA *et al.* (2010) hypothesized that the IBMP formation during ripening was due to the methylation of 3-isobutyl-2-hydroxypyrazine (IBHP) to IBMP. Therefore, IBHP is a key intermediate in both the formation and degradation of IBMP (HASHIZUME *et al.*, 2001; HARRIS *et al.*, 2012) although previous studies attributed this decrease to the photolability of IBMP (HASHIZUME and SAMUTA, 1999; RYONA *et al.*, 2008). Further research is necessary in order to better understand the formation of these compounds and control their concentrations in wines.

The excessive green bell pepper aroma found in red wines containing IBMP is generally considered unfavorable to wine quality. However, the presence of this compound at low levels is often noted to augment the quality of certain wines obtained from red varieties (e.g., Cabernet Franc, Cabernet Sauvignon, Carmenere, Merlot) or from white varieties (Sauvignon blanc, Semillon) by adding to the intrinsic flavor complexity of these varietals (ALLEN *et al.*, 1995; BELANCIC and AGOSIN, 2007). The presence of IBMP can be a positive quality factor when it is not dominant but is in balance and complemented by other herbaceous and fruity aromas (SALA *et al.*, 2004).

The content of MP in the wine depends primarily on grape composition (ROUJOU DE BOUBEE *et al.*, 2002). Viticultural factors may also influence the MP content in grapes and wines. Understanding the mechanisms that influence the formation of wine volatile compounds through grape growing, winemaking and storage is important in developing strategies for the production of wines with specific sensory attributes that appeal to target markets (ROBINSON *et al.*, 2014b).

IBMP concentrations were found to be significantly negatively correlated with the number of buds per vine (CHAPMAN *et al.*, 2004). The levels of MP can also be controlled in the vineyard by either extending the maturation or by defoliation (SALA *et al.*, 2004). High correlations between the decrease of malic acid and the decrease of IBMP as the grapes ripened, irrespective of other cultural and climatic variables, has been reported (ROUJOU

DE BOUBEE *et al*., 2000), indicating that IBMP levels could serve as markers indicative of poor ripening.

In general, light-exposed clusters are reported to have reduced IBMP levels at harvest, although there is some ambiguity in the literature (RYONA *et al*., 2008). Light exposure seems to have two opposing effects on the concentration of MP in grapes: (a) by promoting the biological formation of MP in immature grapes, and (b) by reducing the MP in ripening grapes (whether by photodegradation or initiating demethylation) (HASHIZUME and SAMUTA, 1999; HARRIS *et al*., 2012; HEYMANN *et al*., 1986). The actual content of MP in grapes would result from the total sum of these two processes.

Light exposure has previously been shown to affect other wine constituents, such as superior alcohols, phenols and esters (D'AURIA *et al*., 2003), dimethylsulfide (MARAIS and POOL, 1980), terpenes and norisoprenoids (CALO' *et al*., 1996). Cluster exposure to sunlight will inhibit IBMP accumulation pre-veraison (RYONA *et al*., 2008) and several studies have reported that pre-veraison cluster light exposure will result in decreased IBMP levels at harvest (DUNLEVY *et al*., 2010; RYONA *et al*., 2008). MARAIS and POOL (1980) reported a decrease of the IBMP levels in Sauvignon blanc by up to 50% in light-exposed berries across several sites in South Africa studied over two years, and they showed a reduction in IBMP at both veraison and harvest. Thus, the incident light conditions can affect the IBMP levels in the final wine.

The effect of the light exposure has been linked to the number of leaf layers (SALA *et al*., 2004). SCHEINER *et al*. (2010) evaluated the effects of basal leaf removal timing and severity on IBMP concentration in grape berries. The complete leaf removal significantly reduced the IBMP concentrations and consequently the herbaceous character. Early leaf removal reduced the IBMP concentrations more than later removal.

Another factor that can affect IBMP concentrations is the vine water status. SALA *et al*. (2005) reported that irrigation increased the IBMP content significantly; similar results have been reported by other authors (MENDEZ-COSTABEL *et al*., 2014a). Rainfall during the growing season may result in higher IBMP concentrations in the grapes at harvest, due to increased and prolonged vegetative growth (ROUJOU DE BOUBEE *et al*., 2000). Lack of rainfall has been shown to decrease perceptual green characters in wine (MENDEZ-COSTABEL *et al*., 2014b). In summary, all of these studies demonstrate the complex relationship between viticultural practices and varietal aroma. It is difficult to predict the final wine aroma because of the multiple compounds and pathways involved.

2.2. Exogenous herbaceous character

Lady beetles (*Coleoptera: Coccinellidae*) have been identified as a second source of high MP content in wine. This off-flavor has been termed 'ladybug taint' (LBT) (PICKERING *et al*., 2005). LBT is a wine defect caused by the inadvertent incorporation of ladybeetles into the wine during the winemaking process. *Harmonia axyridis*, also known as the multicolored Asian ladybeetle (MALB), and *Coccinella septempunctata* (seven spots) are the species considered responsible for causing the taint in many wine regions in Europe and in the United States (BOTEZATU *et al*., 2012; KOGEL *et al*., 2015; GALVAN *et al*., 2008).

Coccinellidae can emit a potent mix of odor-active compounds that may serve various behavioral functions, including defense, aggregation and mate-attraction (AL ABASSI *et al*., 1998; CUDJOE *et al*., 2005). The MP composition varies among species, although IPMP is the major exogenous contributor to LBT in wines. BOTEZATU *et al*. (2012) reported that *Coccinellidae* could release dimethyl-, isopropyl-, sec-butyl- and isobutyl-2-methoxypyrazine into wine at concentrations that negatively impact product quality when the ladybeetles are incorporated with grapes during the harvest operations. PICKERING *et al*. (2005) showed that the inclusion of *Harmonia axyridis* during fermentation of white and red musts significantly modified the wine aroma and affected the sensory properties. White wines acquired a peanut, bell pepper and asparagus flavor, while in red wines peanut, asparagus, bell pepper, earthy and herbaceous flavors predominated. The same authors observed a correlation between the decrease of the positive fruit, floral aroma and the increase of the number of beetles added both in white and red wines. Further research is needed to investigate treatments aimed at reducing the impact of these beetles and manipulating their concentration in wines for specific commercial targets.

2.3. Techniques mitigating the herbaceous character due to methoxypyrazines

The increase of the MP content happens primarily on the first day after destemming and crushing of the grapes, before the start of the alcoholic fermentation. It is possible that the duration of maceration may influence the levels of MP in the final wines (SALA *et al.*, 2005). Various strategies have been investigated to reduce the levels of MP in the finished product.

The Spinning Cone Column is a modern, fast and efficient technology for extracting volatile components, such as ethanol to obtain low-alcohol wines, at high speed and low temperature (PICKERING, 2000). It was first applied by dairies in New Zealand to remove grassy pyrazine flavors from whey and it has been suggested that this method could be adapted to remove MP from wine (SCHMITT, 2014). Other strategies have focused on methods to reduce MP in finished white and red wines without altering the concentrations of other volatiles. The levels of IPMP (as well as other volatiles) were lowered by activated charcoal addition to white wine. Treatment with oak chips reduced the intensity of ladybug taint in both white and red wines, while other applications generally had no effect on white wine and limited effect on red wines (PICKERING *et al.*, 2006). The treatment of grape juice and must with silicone (a non-polar sorbent) prior to fermentation was found to reduce MP without altering the concentrations of the main fermentation-derived compounds. Therefore, it was used as a strategy for reducing all the MP without affecting the other positive volatile compounds (RYONA *et al.*, 2012).

The exposure of wine to oxygen during and after bottling is a crucial interval affecting the wine aroma compounds. The closure and the packaging can effect post-bottling modifications with resultant changes in MP concentrations during aging. All types of closure reduced significantly MP concentrations in wine (SILVA *et al.*, 2011). The greatest decrease in the MP content was evident in multilayer aseptic packages, due to the higher sorption capacity and gas permeability of this material (BLAKE *et al.*, 2009). Light and storage temperature did not influence significantly the MP decomposition in wines during a 12-month ageing period (BLAKE *et al.*, 2010).

Thermovinification (or hot maceration) is a process used to increase productivity, modify the aroma profile and reduce the risk of microbial contamination. It has the potential to reduce IPMP in MALB infested grapes (PICKERING *et al.*, 2008). Heating of *Coccinellidae*-affected grape must (Riesling and Pinot noir) prior to fermentation as a possible remedial intervention resulted in a moderate decrease of all MP (KOGEL *et al.*, 2015).

An alternative approach is the flash-détente technique, also reported in the literature as flash vacuum expansion. In this technique, plant materials are heated quickly (60-90°C) and then exposed to high vacuum (20-50 hPa) (BRAT *et al.*, 2000). Flash-détente has been also applied to crushed and destemmed grapes which were heated at 80-85°C, submitted at 20-50 hPa and then cooled down to 30°C (AGERON *et al.*, 1995; MOUTOUNET and ESCUDIER, 2000). The heating of the must followed by sudden expansion and instantaneous evaporation of water explosively ruptures the cellular tissue structure resulting in the extraction of pigments, phenols, aroma compounds and other dry matter present in the cells. Initially, this technique was used to promote the release of anthocyanins and tannin (HE *et al*., 2012; ESCUDIER *et al*., 1998); however, its ability to remove deleterious volatiles was soon recognized. According to VINSONNEAU *et al.* (2006) the flash-détente technique can improve the quality of red wines aged for 9 months; the most significant decrease of the herbaceous character was observed for Cabernet Sauvignon and Cabernet Franc. It must be noted that the sequential application of a heating treatment and of high vacuum is not a selective approach and contributes to the evaporation of both positive and undesired components, including MP (JONES and GNEKOW, 2011). For this reason, it is also possible to remove aldehydes and alcohols with 6 carbon atoms, which are pre-fermentative herbaceous aroma compounds formed during destemming (ESCUDIER, 2001; RAZUNGLES, 2010; FAVAREL, 2013) as discussed in the next section.

3. PRE-FERMENTATIVE HERBACEOUS CHARACTER

Aldehydes and alcohols with 6 carbon atoms (C6) are volatile, odorous molecules which can contribute to the herbaceous aroma in wine. They are mainly generated through the enzymatic breakdown of C18 polyunsaturated fatty acids contained in plant membranes. Their concentrations in must can be in the order of several hundreds of μg/L (MORENO and PEINADO, 2012) or even more than 13000 μg/L (FERREIRA *et al*., 2000), with very variable odour thresholds (400-8000 μg/L) (GUTH, 1997). Their levels depend on several factors, including the grape variety and ripeness, treatments prior to fermentation, and temperature/duration of contact with the skins. Therefore, they can be considered pre-fermentative volatile compounds. Their cut-grass-like aroma is the characteristic odor of freshly damaged green leaves; therefore, these compounds are often referred as Green Leaf Volatiles (GLV) (HARSCH *et al*., 2013). The C6 alcohols frequently found in grapes include hexanol, (Z)-3-hexenol and (E)-2-hexenol. As well as (E)-2-hexenol, (E)-3-hexenol may also be found in wine at levels of μg/L (GIGOT *et al*., 2010).

The C6 aldehydes commonly identified in grapes are hexanal and (E)-2-hexenal; also C7 compounds such as heptanal, and (E)-2-heptenal (KALUA and BOSS, 2008) have been found, but at lower concentration with respect to C6 aldehydes. At low concentrations (generally less than 0.5 mg/L threshold), these C6 volatiles compounds contribute positively to the overall aroma of the wine. However, at higher levels they lead to unwanted organoleptic notes such as herbaceous, green fruit, and crushed leaf and may also impart a bitter flavor (D'ONOFRIO, 2011; FERRANDINO *et al*., 2012).

These C6 compounds may be present in free volatile form or in bound form, as glycosides (CABRITA *et al*., 2006; DI STEFANO *et al*., 1998; LANATI *et al*., 2000; SEFTON, 1998). The C6 alcohols were identified only as free volatile compounds in Casorzo Malvasia and Schierano Malvasia, while in the aromatic Candia Malvasia they were present both as free and glycosylated forms, although the former predominated (BORSA *et al*., 2008).

Several studies were conducted in order to distinguish grape varieties on the basis of their C6 compounds composition. RAPP *et al.* (1993) have shown that the content of (E)-3-hexenol and its isomer (Z)-3-hexenol are the most important analytical parameters to discriminate monovarietal wines of Riesling, Müller-Thurgau, Kerner, Scheurebe, Ehrenfelser and Bacchus. Also, MORET *et al.* (1984), references these two compounds among the significant parameters discriminating Venetian white wines. More recently, OLIVEIRA *et al.* (2006) showed that (E)-3-hexenol/(Z)-3-hexenol ratio clearly discriminates Loureiro wines from those of Alvarinho, Avesso and Trajadura from Portugal. Moreover, 1-hexanol/(E)-3-hexenol and 1-hexanol/(Z)-3-hexenol ratios may also be able to discriminate Vinhos Verdes monovarietal wines (north of Portugal).

Aldehydes are the major C6 compounds in ripe grapes of Riesling, while in Cabernet Sauvignon C6 alcohols prevail (KALUA and BOSS, 2010). Analyzing the evolution of C6 volatile compounds during berry development of Cabernet Sauvignon, it was found that aldehydes increased significantly during the middle development stage but then decreased throughout the late development stage. The alcohols were present throughout the growth of berry, but reached significant levels only towards the end of ripening (KALUA and BOSS, 2010). The greater increase of the alcohols over the aldehydes across the course of ripening is a positive aspect because alcohols have a higher perception threshold than the aldehydes, so the resulting herbaceous character is less pronounced (D'ONOFRIO, 2011).

The C6 aldehydes and alcohols derive from the oxidation of grape polyunsaturated fatty acids such as oleic acid (C18:1ω9), linoleic acid (C18:2ω6) and linolenic acid (C18:3ω3) initiated by the lipoxygenase pathway when the berries are crushed. Four enzymatic activities are sequentially involved in this pathway. First, an acyl-hydrolase frees the fatty acids with 18 carbon atoms from membrane lipids. Next, a lipoxygenase catalyzes the fixation of oxygen. The peroxides obtained are then split into C6 aldehydes (DRAWERT *et al.*, 1966). Some of these may be reduced to their corresponding alcohols by an alcohol dehydrogenase in the grape (CROUZET, 1986).

The C6 compounds increase from veraison to harvest, with the greatest concentration expressed at harvest (KALUA and BOSS, 2010; YANG *et al.*, 2009). For the neutral cultivar Nebbiolo grown in Piedmont (Italy), the pre-fermentative volatile compounds present in highest concentration were hexanal and E-2-hexenal (FERRANDINO *et al.*, 2012). The greater concentration of E-2-hexenal compared to Z-3-hexenal could be explained by an increased activity of 3Z,2E-enal-isomerase, which catalyzes the reaction from Z-3-hexenal to E-2-hexenal (KALUA and BOSS, 2010). In addition, the low concentration of these two aldehydes in the pre-veraison berries could be explained by an increased activity of the alcohol dehydrogenase that converts these aldehydes to the corresponding alcohols. During ripening, the concentration of aldehydes increases. Therefore, some C6 compounds that contribute to the grape aroma may be produced pre-veraison, rather than post-veraison.

The evolution of (E)-2-hexen-1-ol and (E)-2-hexenal to form thiols with tropical scents, such as 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA), has been recently confirmed during fermentation (HARSCH *et al.*, 2013). This pathway will further reduce the perceptual herbaceousness, both by reducing the C6 pool as well as by formation of the 'fruity' thiols which will serve to mask herbaceousness.

3.1. Avoiding the pre-fermentative herbaceous character: from the harvest to the winemaking process

The overall perceptual herbaceous character is related to the ripening stage of the grapes, but can arise in the must due to forceful mechanical action on the clusters. Therefore, it is important to consider the level of ripeness, the type of harvest, the transport, the crushing, the time and temperature of maceration, the type and the degree of pressing, the type of pumps, the degree of oxygenation and the use of enological adjuvants with regards to herbaceous expression. High temperature pretreatment of must immediately following crushing ("hot break") was shown to result in five- to six-fold higher concentrations of (E)-2-hexenal in Concord grape juice as compared to conventional hot press, especially with immature fruit, resulting in persistence of green aromas in the juice. In contrast, de-pectinization, pressing, and pasteurization decreased the content of (E)-2-hexenal of the juice of Concord grapes due to the reduction to (E)-2-hexenol (IYER *et al.*, 2010).

3.2.1. The harvest

Harvest timing is an important factor in minimizing/preventing the formation of herbaceous compounds. Additionally, accumulation of the C6 alcohols and aldehydes derived from the enzymatic oxidation of linoleic and linolenic acid can be reduced by limiting mechanical operations starting with the harvest. Mechanical harvesting increases herbaceous character due to uncontrolled enzymatic oxidation (NARDIN *et al.*, 2006); consequently, those grapes must be pressed quickly. In contrast, manual harvesting reduces the mechanical stress on the clusters with concomitant lower losses plus lower contamination with leaves, earth, water and other foreign substances. While mechanical harvesting certainly speeds up the overall process of winemaking, it comes at the expense of the mechanical stresses on the cluster and the acceleration of the oxidation reactions.

3.2.2. Destemming and crushing

Destemming (separating and removing the stalks from the grape berries) and crushing (breaking the grape in order to release the juice and some pulp material) are the next mechanical processes involved in winemaking. The influence of crushing on the herbaceous aroma in must and in the corresponding wine is well established (RIBEREAU-GAYON *et al.*, 2005; HASHIZUME and SAMUTA, 1997). Usually, destemming and crushing are performed by a single machine, a crusher-destemmer or destemmer-crusher, so-named in relation to the succession of the two processing stages. The destemmer-crusher first removes the stalks and then breaks open the grapes, thus avoiding the contact of the expressed juice with the stems. In contrast, in the crusher-destemmer undesired flavors can potentially be extracted from the stalks, leading to an increase of herbaceous aromas and bitter tastes. Furthermore, the presence of stalks reduces the intensity of the red wine color due to the absorption of anthocyanins (NARDIN *et al.*, 2006).

It is important to use equipment that reduces mechanical stresses, such as excessive pressure, abrasions and lacerations, in order to reduce the enzymatic oxidation activity on fatty acids. In this way, the polyphenol and potassium extraction from the stalks is also minimized. The use of inert gases or vacuum systems in concert with the crushing and pressing of the clusters is a very popular approach to remove oxygen and slow down the oxidation of the must and increase the formation of volatile 'fruity' esters and glutathione (BOSELLI *et al.*, 2010; DI LECCE *et al.*, 2013).

In the case of the wines produced by carbonic maceration, crushing is completely skipped and the tank is filled with whole clusters by means of a conveyor belt that minimizes the damage to the plant tissues. The herbaceous and bitter flavors may arise in some varieties as the stalks are not separated and removed in this process. Although wines produced by using carbonic maceration can be obtained by grapes harvested prematurely, the aromatic profiles of such wines do not exhibit herbaceous characters but instead show pleasant fruity notes (ETAIO *et al.*, 2011). This phenomenon is explained by the limited production of C6 aldehydes and alcohols due to both the lack of oxygen in the tank and the lack of crushing of the grapes.

3.2.3 Pre-fermentative maceration

The enzyme that catalyzes the peroxides scission to aldehydes is associated with the membranes, consequently the aldehyde levels are proportional to the intensity of maceration of these solid parts. To limit their concentration in red wines, it is important to control the maceration temperature, not to employ long pre-fermentative maceration and to continually monitor the maceration stage.

4. HERBACEOUS CHARACTER IN FERMENTATION

Sulfur is present in many organic compounds within the grape such as amino acids, peptides, proteins, vitamins and co-enzymes and may also result from sulfur spraying (KWASNIEWSKI *et al.*, 2014). Volatile sulfur compounds (VSC) may then originate from yeast metabolism during the alcoholic fermentation. These molecules are characterized by a strong aromatic impact at low concentrations due to very low odor thresholds (MESTRES *et al.*, 2000).

VSC can be distinguished based on their functional groups (thiols, sulfides, disulfides etc.). However, there is another classification based upon the boiling point (b.p.) of 3-methylthiopropanol (b.p. 90°C): light sulfur compounds show a b.p. lower than 90°C, whereas heavy sulfur compounds have a b.p. higher than 90°C (MESTRES *et al.*, 2002). The light sulfur compounds such as hydrogen sulfide, ethanethiol and methanethiol arise from the degradation of the sulfur-carbon bond of methionine or cysteine derivatives (LANDAUD *et al.*, 2008). They usually have an unpleasant effect on the quality of the wine, are characterized by off-flavors of 'rotten egg', 'sewer', 'cooked vegetable' or 'cooked cabbage', 'onion', 'garlic' or 'rubber' and show high volatility and low perception thresholds (μg/L) (MESTRES *et al.*, 2000; GONIAK and NOBLE, 1987). Dimethyl sulfide at low concentration is considered less undesirable since it shows aromas of 'cooked corn' and 'asparagus' (BELL and HENSCHKE, 2005).

Hydrogen sulfide, ethanethiol and methanethiol are present in reduced wines and, due to their low boiling points, can be volatilized by racking and aeration (MOREIRA *et al.*, 2002). In Table 2, the sensory properties of light sulfur compounds are listed according to different authors and their boiling point is reported (RIBEREAU-GAYON *et al.*, 2005; LANDAUD *et al.*, 2008; BENKWITZ *et al.*, 2012). For a review of the lower molecular weight sulfur compounds see MESTRES *et al.* (2000).

Table 2: Sensory properties and boiling point of light sulfur compounds (C_0-C_2) according to RIBEREAU-GAYON *et al.*, 2005; LANDAUD *et al.*, 2008; BENKWITZ *et al.*, 2012.

Light sulfur compounds	Chemical Formula	Flavor note	Perception threshold (μg/kg)	Boiling point (°C)	Probable precursor
Hydrogen sulfide	H_2S	rotten egg	10-80	-61	Cysteine, sulfate, sulfite
Methanethiol	CH_3SH	cooked cabbage, onion	0.3	6	Methionine
Ethanethiol	CH_3CH_2SH	onion, rubber	1.1	35	Unknown
Dimethyl sulfide	CH_3SCH_3	cooked corn, asparagus	25	35	Cysteine

Other volatile sulfur compounds include the higher molecular weight grape-derived thiols, such as 3MH, 3MHA, 4-mercapto-4-methyl-pentan-2-one (4MMP), and 4-mercapto-4-methyl-pentan-2-ol (4MMPOH), all of which contribute positively to the varietal aroma of wine and are thus referred to as varietal sulfur compounds (BELL and HENSCHKE, 2005).

The interaction of methoxypyrazines with varietal thiols is still unclear (WYNGAARD *et al.*, 2014). CAMPO *et al.* (2005) showed that the fruity character is related to the presence of varietal thiols (3MHA) and is negatively affected by the presence of methoxypyrazine. The vegetative aroma due to IBMP, such as 'bell pepper' has been shown to be slightly masked by the fruity aroma, as berry flavorings were added to a neutral red wine containing added bell pepper (HEIN *et al.*, 2009).

As mentioned earlier, the volatile thiols also have an important sensory role due to their biosynthesisis metabolizing C6 compounds (C6-thiol precursors); ROLAND *et al.* (2010) demonstrated that (E)-2-hexen-1-ol played a key role in the formation of the glutathionylated conjugate 3-*S*-glutathionylhexan-1-ol (G3MH). This conversion diminishes the perceptual herbaceousness by reducing the C6 pool while concomitantly increasing the masking pool. HARSCH *et al.* (2013) found that the addition of sodium hydrosulfide (NaSH) to grape juice greatly increased the formation of 3MH and 3MHA from (E)-2-hexen-1-ol and (E)-2-hexenal precursors during the fermentation of Sauvignon blanc musts under commercial winemaking conditions. While these two precursors exhibit a thiol-forming potential during fermentation, (E)-2-hexenal and (E)-2-hexen-1-ol are quickly catabolized in the very early stages following yeast addition, whereas the synthesis of H_2S (the thiol source) by yeasts begins after the conversion of sugars. This timing problem may be responsible for the low content of 3MH and 3MHA in finished Sauvignon blanc wines in spite of the high potential for their formation.

4.1. Oxidation of volatile thiols

Oxygen plays an important role in controlling the sensory defects associated with sulfur compounds. Micro-oxygenation can reduce undesired sulfur compounds, such as methanethiol and ethanethiol, to levels below their odor threshold, with no significant increase in dimethyl disulfide (GOMEZ-PLAZA *et al.*, 2011). This may be explained by the role of quinones as oxygen oxidizes *trans*-caftaric acid and other phenolic compounds and leads to the formation of the corresponding *o*-quinones (VIDAL and AAGAARD, 2008; WATERHOUSE and LAURIE, 2006). Furthermore, glutathione plays an important role during the oxidation of white must, forming the 2-S-glutathionyl-caftaric acid or Grape Reaction Product (GRP) which is very common in wines (BOSELLI *et al.*, 2006). This derivative is not oxidized by polyphenol oxidase and does not modify the color of the must (DU TOIT *et al.*, 2007; ROLAND *et al.*, 2010).

Pre-fermentative operations could provide a positive contribution to the aroma profile of white wine, such as Sauvignon blanc, by increasing the G3MH precursor. At this stage of winemaking, the cysteinylated and glutathionylated precursors of varietal thiols are not oxidizable because of the chemical stability of the thioether bond under oxidative conditions (ROLAND *et al.*, 2011).

Varietal thiols, however, are highly reactive compounds and their concentrations can decrease during storage (HERBST-JOHNSTONE *et al.*, 2011). Oxygen and iron can oxidize these compounds into their disulfide forms. In addition, they can participate in chemical reactions with the products of phenolic oxidation (*o*-quinones) catalyzed by transition metals (e.g., iron and copper) (HERBST-JOHNSTONE *et al.*, 2011; NIKOLANTONAKI *et al.*, 2010; NIKOLANTONAKI and WATERHOUSE, 2012).

5. CONTROL OF THE HERBACEOUS CHARACTER AFTER THE ALCOHOLIC FERMENTATION

The aging stage of a wine is a delicate technological phase linked to a variety of chemical-physical modifications that help define the final organoleptic characteristics. Micro-oxygenation is a technique that may be used during this stage, employing a controlled flow of oxygen in tanks to simulate the complex reactions that occur during wine maturation in barrel (CELOTTI and ZUCCHETTO, 2003). This treatment results in many changes to the organoleptic qualities of the wine; these changes have been empirically separated into two main phases (MOUTOUNET *et al.*, 2001):

Structuring: this takes place from the end of the alcoholic fermentation to the beginning of malolactic fermentation (MLF). In this phase, oxygen is consumed by polymerization reactions, which increase the aggressiveness and intensity of tannins on the palate. At the same time a decline in the aromatic intensity and complexity of the wine is observed. The changes in the chemical composition of wine caused by MLF are complex and often involve an enhancement of the fruity aroma and 'dairy' note and a decrease of the herbaceous character (LIU, 2002).

Harmonization: in this phase the intensity and the aromatic complexity increases, whereas the herbaceous character decreases. Furthermore, there is an increase in the color intensity, especially the purple hue. It is important to end the micro-oxygenation at the second stage in order to prevent excessive oxidation of the wine components leading to off-flavors. It is critical to prevent violent oxygenation during either stage to avoid generalized, uncontrolled oxidation.

The sensory changes occurring in red wines exposed to different micro-oxygenation regimens and with different woods have been described (CELOTTI and ZUCCHETTO, 2003; MOUTOUNET *et al.*, 2001). It was emphasized how the controlled application of oxygen, as well as the wood contact in barrels, may decrease the astringency and the herbaceous characters of wine. Barrel-ageing of wines is costly and laborious and is thus not suitable for all wines and wine styles. Micro-oxygenation is an affordable alternative for accelerating the stabilization of color and phenolic compounds and for improving the sensory quality of red wines (LLAUDY *et al.*, 2006; PARISH *et al.*, 2000). Oxygen addition led to an increase of the fruity and floral notes, whereas the herbaceous character significantly decreased also in Cencibel red wines (CEJUDO-BASTANTE *et al.*, 2011). However, HERNANDEZ-ORTE *et al.* (2009) reported a strong dependence of the effects of microxygenation on the grape variety (Cabernet Sauvignon or Tempranillo), the use of barrels or steels vats and the period of maturation of the wines.

Sensory analysis was used to follow important perceptual sensations, such as astringency and herbaceousness, which are modified by controlled oxygenation. The herbaceous character showed a fairly rapid decrease during initial micro-oxygenation; however, for longer treatment times there was a general increase in the herbaceous note. The best results were obtained with up to 2 months of treatment of micro-oxygenation without the addition of tannin. The same studies (CELOTTI and ZUCCHETTO, 2003; MOUTOUNET *et al.*, 2001) highlighted the difficulty in accurately determining the oxygen dosage and the appropriate treatment times. However, it was demonstrated that the application of sensory analysis in concert with careful analytical control of the process allowed the optimal time for terminating the treatment to be decided.

6. CONCLUSIONS

The herbaceous character of wines is of important concern to the wine scientist, to the winemaker and finally to the wine consumer. Different classes of compounds with very different chemical structures can contribute to this particular sensory feature of wines. Alkylmethoxypyrazines, for instance, are key contributors to vegetativeness in Sauvignon blanc, as are some selected volatile thiols. The herbaceous character can be specific to a particular grape cultivar (an endogenous varietal character) or produced during the winemaking procedure, from either an endogenous or an exogenous cause. The positive or negative (off-flavor) attributes associated with the herbaceous character depend not only on the chemical composition (qualitatively and quantitatively) of the headspace of wine and/or the wine itself, but also on the odor activity values of the different volatile components as determined by sensory techniques.

For future perspectives, a deeper understanding of the origin of these herbaceous characters and, perhaps more importantly, their prediction in the finished wine (based upon chemical quality markers) remains a challenging subject, which deserves continued investigation.

This would be of benefit, not only to the wine scientist, but also to the global winemaking industry.

REFERENCES

Ageron D., Escudier J.L., Abbal P. and Moutounet M. 1995. Pré-traitements des raisins par flash détente sous vide poussé. Revue Française d'Œnologie 153: 50.

Al Abassi S., Birkett M.A., Pettersson J., Pickett J.A. and Woodcock C.M. 1998. Ladybird beetle odour identified and found to be responsible for attraction between adults. Cellular and Molecular Life Sciences 54: 876.

Allen M.S., Lacey M.J. and Boyd S. Methoxypyrazines in red wines: occurrence of 2-methoxy-3-(1-methylethyl) pyrazine. 1995. Journal of Agricultural and Food Chemistry 43: 769.

Allen M.S., Lacey M.J., Harris R.L.N. and Brown W.V. 1991. Contribution of methoxypyrazines to Sauvignon Blanc wine aroma. American Journal of Enology and Viticulture 42: 109.

Amerine M.A. and Roessler E.B. 1976. In Wines - Their Sensory Evaluation, W.H. Freeman and Company: San Francisco (USA).

Bayonove C., Cordonnier R. and Dubois P. 1975. Study of an aromatic characteristic fraction of Cabernet Sauvignon grape variety, identification of 2-methoxy-3-isobutyl-pyrazine. Comptes Rendus Hebdomadaires Des Seances De L'Academie Des Sciences Serie D 281: 75.

Belancic A. and Agosin E. 2007. Methoxypyrazines in grapes and wines of *Vitis vinifera* cv. Carmenere. American Journal of Enology and Viticulture 58: 462.

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

Benkwitz F., Nicolau L., Lund C., Beresford M., Wohlers M. and Kilmartin P.A. 2012. Evaluation of Key Odorants in Sauvignon Blanc Wines Using Three Different Methodologies. Journal of Agricultural and Food Chemistry 60: 6293.

Blake A., Kotseridis Y., Brindle I. D., Inglis D. and Pickering, G. J. 2010. Effect of light and temperature on 3-alkyl-2-methoxypyrazine concentration and other impact odourants of Riesling and Cabernet Franc wines during bottle ageing. Food Chemistry 119: 935.

Blake A., Kotseridis Y., Brindle, I.D., Inglis D., Sears M. and Pickering G.J. 2009. Effect of closure and packaging type on 3-alkyl-2-methoxypyrazines and other impact odorants of Riesling and Cabernet Franc wines. Journal of Agricultural and Food Chemistry 57: 4680.

Borsa D., Asproudi A., Nardi S., Costacurta A., Di Stefano R. and Calò A. 2008. Metaboliti secondari delle cultivar Malvasia. Confronto di due annate. Rivista di Viticultura ed Enologia 2-3-4: 99.

Boselli E., Di Lecce G., Alberti F. and Frega N.G. 2010. Nitrogen Gas Affects The Quality And The Phenolic Profile Of Must Obtained From Vacuum-Pressed White Grapes. LWT-Food Science and Technology 43 (10): 1494.

Boselli E., Giomo A., Minardi M. and Frega N.G. 2008. Characterization of phenolics in Lacrima di Morro d'Alba wine and role on its sensory attributes. European Food Research and Technology 227 (3): 709.

Boselli E., Minardi M., Giomo A. and Frega N.G. 2006. Phenolic composition and quality of white d.o.c. wines from Marche (Italy). Analytica Chimica Acta 563: 93.

Botezatu A.I., Kotseridis Y., Inglis D. and Pickering G.J. 2012. Occurrence and contribution of alkyl methoxypyrazines in wine tainted by *Harmonia axyridis* and *Coccinella septempunctata*. Journal of the Science of Food and Agriculture 93 (4): 803.

Brat P., Olle D., Reynes M., Alter P. and Cogat P.O. 2000. Free volatile components of Passion Fruit puree obtained by flash vacuum-expansion. Journal of Agricultural and Food Chemistry 48 (12): 6210.

Buttery R.G., Seifert R.M., Guadagni D.G. and Ling L.C. 1969. Characterization of some volatile constituents of bell peppers. Journal of Agricultural and Food Chemistry 17 (6): 1322.

Cabrita J.M., Freitas C.A.M., Laueano O. and Di Stefano R. 2006. Glycosidic aroma compounds of some Portuguese grape cultivar. Journal of the Science of Food and Agriculture 86: 922.

Calò A., Tomasi D., Crespan M. and Costacurta A. 1996. Relationship between environmental factors and the dynamics of growth and composition of the grapevine. Acta Horticulturae 427: 217.

Campo E., Ferreira V., Escudero A. and Cacho J. 2005. Prediction of the wine sensory properties related to grape variety from dynamic-headspace gas chromatography-olfactometry data. Journal of Agricultural and Food Chemistry 53: 5682.

Cejudo-Bastante M.J., Pérez-Coello M.S. and Hermosín-Gutiérrez I. 2011. Effect of wine micro-oxygenation treatment and storage period on colour-related phenolics, volatile composition and sensory characteristics. LWT-Food Science and Technol. 44: 866.

Celotti E. and Zucchetto M. 2003. Esempio di gestione ragionata della microssigenazione applicata ad un vino rosso. Vinidea.net-Rivista internet tecnica del vino. 11: 1.

Chapman D.M., Thorngate J.H., Matthews M.A., Guinard J.-X. and Ebeler S.E. 2004. Yield Effects on 2-Methoxy-3-Isobutylpyrazine Concentration in Cabernet Sauvignon Using a Solid Phase Microextraction Gas Chromatography/Mass Spectrometry Method. Journal of Agricultural and Food Chemistry 52: 5431.

Crouzet J. 1986. Les enzymes et l'arôme des vins. Revue Française d'Œnologie 102: 42.

Cudjoe E., Wiederkehr T.B. and Brindle I.D. 2005. Headspace gas chromatography-mass spectrometry: a fast approach to the identification and determination of 2-alkyl-3-methoxypyrazine pheromones in ladybugs. Analyst 130 (2): 152.

D'Auria M., Emanuele L., Mauriello G. and Racioppi R. 2003. On the origin of 'Gout de Lumiere' in champagne. Journal of Photochemistry and Photobiology A: Chemistry 158: 21.

Di Lecce G., Boselli E., D'Ignazi G. and Frega N.G. 2013. Evolution of phenolics and glutathione in Verdicchio wine obtained with maceration under reductive conditions, LWT - Food Science and Technology 53: 54.

Di Stefano R., Bottero S., Pigella R., Borsa D., Bezzo G. and Corino L. 1998. Precursori d'aroma glicosilati presenti nelle uve di alcune cultivar a frutto colorato. L'Enotecnico 63.

D'Onofrio C. 2011. Caratterizzazione funzionale della biosintesi degli aromi durante lo sviluppo dell'acino e controllo della qualità aromatica delle uve. Italus Hortus 18 (1): 39.

Drawert F., Heimann W., Emberger R. and Tressl R. 1966. EnzymatischeBildung von Hexen-(2)-al-(1), Hexanal und deren Vorstufen. Liebigs Annalen der Chemie 694: 200.

Du Toit W.J.,Lisjak K., Stander M. and Prevoo D. 2007. Using LC–MSMS to assess glutathione levels in South African white grape juices and wines made with different levels of oxygen. Journal of Agricultural and Food Chemistry. 55(8): 2765.

Dunlevy J.D., Dennis E.G., Soole K.L., Perkins M.V., Davies C. and Boss P.K. 2013. A methyltransferase essential for the methoxypyrazine-derived flavour of wine. The Plant Journal 75: 606.

Dunlevy J.D., Soole K.L., Perkins M.V., Dennis E.G., Keyzers R.A., Kalua C.M. and Boss P.K. 2010. Two O-methyltransferases involved in the biosynthesis of methoxypyrazines: grape-derived aroma compounds important to wine flavour. Plant Molecular Biology 74: 77.

Escudier J.L. 2001. Les nouvelles methods pour la vinificationet le traitement des vins rouges, in Guide de la vinification rhodanienne 5:31. http://www.institut-rhodanien.com/download/646

Escudier J-L., Mikolajczak M. and Moutonnet M. 1998. Pre-traitement des raisins par flash-detente sous vide etcaracteristiques des vins. Journal International des Sciences de la Vigneet du Vin 32: 105.

Etaio I., Elortondo F.J.P., Albisu M., Gaston E., Ojeda M. and Schlich P. 2011. Effect of winemaking process and addition of white grapes on the sensory and physicochemical characteristics of young red wines. Australian Journal of Grape and Wine Research 14: 211.

Favarel J.L. Flash-détente technology. Presentation at the 15th Australian Wine Industry Technical Conference (AWITC), Sidney July 16, 2013 (https://www.awri.com.au/wp-content/uploads/2013/08/pera-WS39-AWITC15.pdf) (April 13, 2015).

Ferrandino A., Schubert A. and Carlomagno A. 2012. Varietal and prefermentative volatiles during ripening of *Vitis vinifera* cv Nebbiolo berries from three growing areas. Food Chemistry 135 (4): 2340.

Ferreira V., López, R. and 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 (11): 1659.

Galvan T.L., Kells S. and Hutchison W.D. 2008. Determination of 3-alkyl-2-methoxypyrazines in lady beetle-infested wine by solid-phase microextraction headspace sampling. Journal of Agricultural and Food Chemistry 56 (3): 1065.

Gigot C., Ongena M., Fauconnier M.-L., Wathelet J.-P., Du Jardin P. and Thonart P. 2010. The lipoxygenase metabolic pathway in plants: potential for industrial production of natural green leaf volatiles. Biotechnologie, Agronomie, Société et Environnement 14 (3): 451.

Goniak O.J. and Noble A.C. 1987. Sensory Study of Selected Volatile Sulfur Compounds in White Wine. American Journal of Enology and Viticulture 38: 223.

Guth H. 1997. Quantitation and sensory studies of character impact odorants of different white wine varieties. Journal of Agricultural and Food Chemistry 45: 3027.

Harris S.A., Ryona I. and Sacks G.L.J. 2012. Behavior of 3-Isobutyl-2-hydroxypyrazine (IBHP), a key intermediate in 3-Isobutyl-2-methoxypyrazine (IBMP) metabolism, in ripening wine grapes. Journal of Agricultural and Food Chemistry 60: 11901.

Harsch M.J., Benkwitz F., Frost A., Colonna-Ceccaldi B., Garner R.C. and Salmon J.M. 2013. New precursor of 3-mercaptohexan-1-ol in grape juice: thiol-forming potential and kinetics during early stages of must fermentation. Journal of Agricultural and Food Chemistry 61: 3703.

Hashizume K. and Samuta T. 1997. Green odorants of grape cluster stem and their ability to cause a wine stemmy flavor. Journal of Agricultural and Food Chemistry 45: 1333.

Hashizume K. and Samuta T. 1999. Grape maturity and light exposure affect berry methoxypyrazine concentration. American Journal of Enology and Viticulture. 50(2): 194.

Hashizume K., Tozawa K., Endo M. and Aramaki I. 2001. S-Adenosyl-L-methionine-dependent O-methylation of 2-hydroxy-3-alkylpyrazine in wine grapes: a putative final step of methoxypyrazine biosynthesis. Biosciensce Biotechnology Biochemistry 65: 795.

He F., Liang N., Mu L., Pan Q., Wang J., Reeves M.J. and Duan C. 2012. Anthocyanins and their variation in red wines I. monomeric anthocyanins and their expression. Molecules 17: 1571.

Hein K., Ebeler S.E. and Heymann H. 2009. Perception of fruity and vegetative aromas in red wine. Journal of Sensory Studies 24: 441.

Herbst-Johnstone M., Nicolau L. and Kilmartin P.A. 2011. Stability of varietal thiols in commercial Sauvignon blanc wines. American Journal of Enology and Viticulture 62: 495.

Hernandez-Orte P., Lapena A.C., Escudero A., Astrain J., Baron C., Pardo I., Polo L., Ferrer S., Cacho J. and Ferreira V. 2009. Effect of micro-oxygenation on the evolution of aromatic compounds in wines: Malolactic fermentation and ageing in wood. LWT-Food Science Technology 42: 391.

Heymann H., Noble A.C. and Boulton R.B. 1986. Analysis of methoxypyrazines in wines. 1. Development of a quantitative procedure. Journal of Agricultural and Food Chemistry 34: 268.

Iyer M.M., Sacks G.L. and Padilla-Zakour O.I. 2010. Impact of harvesting and processing conditions on green leaf volatile development and phenolics in Concord grape juice. Journal Food Science 75: C297.

Jones R. and Gnekow B. Flash Détente: implications for luxury winemaking. Presentation at the Napa Valley Wine Technical Group. 23 February 2011 (http://luxury1.org/flash-dtente-implications-for-luxury-winemaking-e1.html) (April 13, 2015).

Kalua C.M. and Boss P.K. 2008. Sample preparation optimization in wine and grapes: dilution and sample/headspace volume equilibrium theory for headspace solid-phase microextraction. Journal of Chromatography A 1192 (1): 25.

Kalua C.M. and Boss P.K. 2010. Comparison of major volatile compounds from Riesling and Cabernet Sauvignon grapes (*Vitis Vinifera* L.) from fruit set to harvest. Australian Journal of Grape and Wine Research 16: 337.

Kögel S., Botezatu A., Hoffmann C. and Pickering G. 2015. Methoxypyrazine composition of Coccinellidae-tainted Riesling and Pinot noir wine from Germany. Journal of the Science of Food and Agriculture 95 (3): 509.

Kotseridis Y., Baumes R.L., Bertrand A. and Skouroumounis G.K. 1999. Quantitative determination of 2-methoxy-3-isobutylpyrazine in red wines and grapes of Bordeaux using a stable isotope dilution assay. Journal of Chromatography A 841: 229.

Kwasniewski M.T., Sacks G.L. and Wilcox W.F. 2014. Persistence of elemental sulfur spray residue on grapes during ripening and vinification. American Journal of Enology and Viticulture 65: 453.

Lacey M.J., Allen M.S., Harris R.L.N. and Brown W.V. 1991. Methoxypyrazines in Sauvignon blanc Grapes and Wines. American Journal of Enology and Viticulture 42: 103.

Lanati D., Bonino M. and Mazza G. 2000. Precursori d'aroma e profilo varietale della cultivar ‘Sangiovese’. Atti del Simposio Internazionale, V Sessione: Aspetti enologici 447-452.

Landaud A., Helinck S. and Bonnarme P. 2008. Formation of volatile sulfur compounds and methabolism of methionine and other sulfur compounds in fermented food. Applied Microbiology and Biotechnology 77: 1191.

Liu S.Q. 2002. Malolactic fermentation in wine – beyond deacidification. Journal of Applied Microbiology 92: 589.

Llaudy M.C., Canals R., Gonzales-Manzano S., Canals J. M., Santos-Buelga C. and Zamora F. 2006. Influence of Micro-Oxygenation Treatment before Oak Aging on Phenolic Compounds Composition, Astringency, and Color of Red Wine. Journal of Agricultural and Food Chemistry 54: 4246.

Marais J. and Pool H. 1980. Effect of storage time and temperature on the volatile composition and quality of dry white table wines. Vitis 19: 151.

Mendez-Costabel M.P., Wilkinson K.L., Bastian S.E.P., Jordans C., McCarthy M., Ford C. M. and Dokoozlian N.K. 2014. Effect of increased irrigation and additional nitrogen fertilisation on the concentration of green aroma compounds in *Vitis vinifera* L. Merlot fruit and wine. Australian Journal of Grape and Wine Research 20 (1): 80, a.

Mendez-Costabel M.P., Wilkinson K.L., Bastian S.E.P., Jordans C., McCarthy M., Ford C.M. and Dokoozlian, N. 2014. Effect of winter rainfall on yield components and fruit green aromas of *Vitis vinifera* L. cv. Merlot in California. Australian Journal of Grape and Wine Research. 20, 1: 100, b.

Mestres M., Busto O. and Guasch J. 2000. Analysis of organic sulfur compounds in wine aroma. Journal of Chromatography A 881: 569.

Mestres M., Busto O. and Guasch J. 2002. Application of headspace solid-phase microextraction to the determination of sulphur compounds with low volatility in wines. Journal of Chromatography A 945: 211.

Moreira N., Mendes F., Pereira O., Guedes de Pinho P., Hogg T. and Vasconcelos I. 2002. Volatile sulphur compounds in wines related to yeast metabolism and nitrogen composition of grape musts. Analytica Chimica Acta 458(1): 157.

Moreno J. and Peinado R. 2012. "Enological Chemistry" First ed. Academic Press, USA.

Moret I., Scarponi G. and Gescon P. 1984. Aroma components as discriminating parameters in the chemometric classification of venetian white wines. Journal of the Science of Food and Agriculture 35: 1004.

Moutounet M. and Escudier J.-L. 2000. Prétraitements des raisins par flash-détente sous vide, influence sur la qualité des vins. Bulletin de l'O.I.V. 73 (827-828): 5.

Moutounet M., Mazauric J.-P., Ducournau P. and Lemaire T. 2001. Wine micro-oxygenation. Principles and technological applications. Industria delle Bevande 30: 253.

Nardin G., Gaudio A., Antonel G. and Simeoni P. 2006. Impiantistica Enologica. Hoepli, Milan (Italy).

Nikolantonaki M., Chichuc I., Teissedre P. L. and Darriet P. 2010. Reactivity of volatile thiols with polyphenols in a wine-model medium: Impact of oxygen, iron, and sulfur dioxide. Analytica Chimica Acta 660: 102.

Nikolantonaki M. and Waterhouse A.L. 2012. A method to quantify quinine reaction rates with wine relevant nucleophiles: a key to the understanding of oxidative loss of varietal thiols. Journal of Agricultural and Food Chemistry 60: 8484.

Oliveira J.M., Faria M., Sà F., Barros F. and Araùjo I. M. 2006. C_6-alcohols as varietal markers for assessment of wine origin. Analytica Chimica Acta 563: 300-309.

Parish M., Wollan D. and Paul R. 2000. Micro-oxygenation, a review. Australian Grapegrowing Winemaking, 47.

Pickering G.J., Lin J., Reynolds A., Soleas G., Riesen R. and Brindle I. 2005. The influence of *Harmonia axyridis* on wine composition and aging. International Journal of Food Science and Technology 70: 128.

Pickering G., Lin J., Reynolds A., Soleas G. and Riesen R. 2006. The evaluation of remedial treatments for wine affected by *Harmonia axyridis*. Journal of Food Science and Technology 41: 77.

Pickering G.J. 2000. Low- and reduced-alcohol wine: a review. Journal of Wine Research 11(2): 129.

Pickering G.J., Spink M., Kotseridis Y., Brindle I.D., Sears M. and Inglis D. 2008. Morbidity of *Harmonia axyridis* mediates ladybug taint in red wine. Journal of Food Agriculture Environment 6: 133.

Rapp A. 1998. Volatile flavour of wine: correlation between instrumental analysis and sensory perception. Nahrung.42: 351.

Rapp A. and Mandery H. 1986. Wine aroma. Experientia. 42: 873.

Rapp A., Volkmann C., Niebergall H. 1993. Untersuchung flüchtiger Inhaltsstoffe des Weinaromas: Beitrag zur Sortencharakterisierung von Riesling und Neuzüchtungen mit Riesling-Abstammung. Vitis 32: 171.

Razungles A. 2010. Extraction technologies and wine quality. In Volume II of Managing Wine Quality, Andy Reynolds, Ed., Woodhead Publishing Limited. 589.

Ribéreau-Gayon P., Glories Y., Maujean A. and Dubourdieu D. 2005. Traité d'oenologie 2. Chemie du vin-Stabilisation et traitements, ed. Dunod, Paris (France).

Robinson A., Boss P.K., Solomon P. S., Trengove R.D., Heymann H. and Ebeler S. E. 2014. Origins of Grape and Wine Aroma. Part 1. Chemical Components and Viticultural Impacts. American Journal of Enology and Viticulture 65: 1, a.

Robinson A., Boss, P.K., Solomon P. S., Trengove R.D., Heymann H. and Ebeler S. E. 2014. Origins of Grape and Wine Aroma. Part 2. Chemical and Sensory Analysis. American Journal of Enology and Viticulture 65:25, b.

Roland A., Vialaret J., Razungles A., Rigou P. and Schneider R. 2010. Evolution of S-Cysteinylated and S-glutathionylated thiol precursors during oxidation of Melon B. and Sauvignon blanc musts. Journal of Agricultural and Food Chemistry 58: 4406.

Roland A., Schneider R., Razungles A. and Cavelier F. 2011. Varietal thiols in wine: Discovery, analysis and applications. Chemical Reviews 111 (11): 7355.

Roujou de Boubée D., Cumsille A.M., Pons M. and Dubourdieu D. 2002. Location of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon grape bunches and its extractability during vinification. American Journal of Enology and Viticulture 53: 1.

Roujou de Boubee D., Van Leeuwen C. and Dubourdieu D. 2000. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines effect of environmental conditions on concentrations in grapes during ripening. Journal of Agricultural and Food Chemistry 48: 4830.

Ryona I., Leclerc S. and Sacks G. L. 2010. Correlation of 3-isobutyl-2-methoxypyrazine to 3-isobutyl-2-hydroxypyrazine during maturation of bell pepper (*Capsicum annuum*) and wine grapes (*Vitis vinifera*). Journal of Agricultural and Food Chemistry 58: 9723.

Ryona I., Pan B.S., Intrigliolo D.S., Lakso A.N. and Sacks G.L. 2008. Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. Cv. Cabernet Franc). Journal of Agricultural and Food Chemistry 56: 10838.

Ryona I., Reinhardt J. and Sacks G.L. 2012. Treatment of grape juice or must with silicone reduces 3-alkyl-2-methoxypyrazine concentrations in resulting wines without altering fermentation volatiles. Food Research International 47: 70.

Sala C., Busto O., Guasch J. and Zamora F. 2004. Influence of vine training and sunlight exposure on the 3-alkyl-2-methoxypyrazines content in musts and wines from the *Vitis vinifera* variety Cabernet Sauvignon. Journal of Agricultural and Food Chemistry 52(11): 3492.

Sala C., Busto O., Guasch J. and Zamora F. 2005. Contents of 3-alkyl-2-methoxypyrazines in musts and wines from *Vitis vinifera* variety Cabernet Sauvignon: Influence of irrigation and plantation density. Journal of the Science of Food and Agriculture 85(7): 1131.

Scheiner J.J., Sacks G.L., Pan B., Ennahli S., Tarton L., Wise A., Lerch S.D. and Van den Heuvel J.E. 2010. Impact of severity and timing of basal leaf removal on 3-isobutyl-2-methoxypyrazine concentrations in red wine grapes. American Journal of Enology and Viticulture 61: 358.

Schmitt P. Conetech could take out wine pyrazines, The Drinks Business, 2014, 18 December. URL (http://www.thedrinksbusiness.com/2014/12/conetech-could-take-out-pyrazines-in-wine/) (April, 13, 2015).

Sefton M.A. 1998. Hydrolytically-released volatile secondary metabolites from a juice sample of *Vitis vinifera* grape cvs Merlot and Cabernet Sauvignon. Australian Journal of Grape and Wine Research 4: 30.

Silva M.A., Jourdes M. and Teissedre P.-L. 2011. Impact of closures on wine post-bottling development: a review. European Food Research and Technology 233: 905.

Vidal S. and Aagaard O. 2008. Oxygen management during vinification and storage of Shiraz wine. Wine Industrial Journal 23 (5): 1.

Vinsonneau E., Escaffre P., Crachereau J.C. and Praud S. Evaluation du procédé de vinification par flash déténtedans le bordelaise, ITV France Bordeaux – Blanquefort, 15/05/2006, 1-23, http://www.matevi-france.com/uploads/tx_matevibase/Evaluation_du_procede_de_vinification_par_flash_detente_dans_le_Bordelais.pdf
Waterhouse A. L. and Laurie F. 2006. Oxidation of wine phenolics: a critical evaluation and hypotheses. American Journal of Enology and Viticulture. 57: 306.

Wyngaard E., Brand J., Jacobson D. and Toit W.J. 2014. Sensory interaction between 3-mercaptohexan-1-ol and 2-isobutyl-3-methoxypyrazine in dearomatised Sauvignon blanc wine. Australian Journal of Grape and Wine Research 20: 178.

Yang C.X., Wang Y.J., Liang Z.C., Fan P.G., Wu B.H., Yang L., Wang Y.N. and Li S.H. 2009. Volatiles of grapes berries evaluated at the germplasm level by headspace-SPME with GCMS. Food Chemistry 114: 1106.

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