

Chapter 7

Wine Preference and Wine Aroma Perception

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7.1 Introduction

An odor (or scent) is the sensation that results when the olfactory receptors in the nose are stimulated by specific chemical compounds in gaseous form (volatile compounds). Broadly speaking, in English language there are many terms that one can use to refer the sense of smell and different types of smells (aroma, fragrance, perfume, odor, scent). However, there are some subtle differences among them. While the term odor means a clearly recognizable smell, normally issuing from a single source, that can be both pleasant and unpleasant, the terms aroma and fragrance are used primarily by the food and cosmetic industry to describe a pleasant odor, and are sometimes used to refer to perfumes. In the scientific literature, odor and aroma are indistinctively used and during this chapter both terms will be used as synonyms.

Wine is a special complex matrix, which contains a wide array of inorganic and organic constituents which contribute to its unique aromas, tastes, and oral sensations. Already in the 1990s, it was acknowledged that wine contains on the order of 600–800 volatile aroma compounds (Rapp 1990). It is also recognized that aroma is the major contributor to overall flavor perception (Polaskova et al. 2008) and it is one of the most important intrinsic factors that influence wine quality and consumer preferences (King et al. 2010). Thus, it is not strange that the characterization of wine aroma compounds, the elucidation of their odorant characteristics, but also the understanding of the impact of different viticultural and enological practices on the wine aroma profile have been the aim of a large piece of research recently reviewed (Robinson et al. 2014b).

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The complex aroma of wine is derived from many sources, including the grape, yeast, and microbial fermentations and post-fermentation treatments such as oak storage and bottle aging. The origin of these compounds allows us to classify wine aroma compounds in (a) primary or varietal aroma coming from the grape, (b) the secondary aroma produced during alcoholic or malolactic fermentation, and (c) the tertiary aroma or bouquet, which results from the transformation of the aroma during aging (Rapp and Mandery 1986). There are several excellent revisions providing accurate information about the chemical components involved in wine flavor (Ebeler 2001; Etievant et al. 1986; Polaskova et al. 2008; Rapp and Mandery 1986). These volatile compounds are highly heterogeneous and include alcohols, esters, aldehydes, ketones, acids, terpenes, phenols, and sulfur compounds present in variable concentrations from milligram to nanogram per liter. The total content of aroma compounds in wine is approximately 0.8 to 1.2 g L⁻¹ (Rapp 1990; Rapp and Mandery 1986). However, there are many differences in the amount and type of wine aroma compounds among wines depending on both viticultural (climate, soil, water, cultivar, grape-growing practices) and enological (condition of grapes, fermentation, post-fermentation treatments) factors.

The great development of analytical techniques and instruments has allowed to advance from the first studies focused in the analysis of major volatile compounds to the analysis of compounds present in very low concentrations (even at levels below of ng L⁻¹) but with very low odor thresholds. Due to the great complexity of the wine matrix, the analysis of some minor, but *key* aroma compounds might require pre-concentration steps, the use of stable isotopic dilution analysis, and multidimensional gas chromatography coupled to the most modern powerful detectors such as time-of-flight mass spectrometers to obtain reliable results. Several authors have published interesting revisions on advances in the aroma extraction, concentration, separation, and detection methods applied for wine volatile analysis (Ebeler 2001; Munoz-Gonzalez et al. 2011; Robinson et al. 2014a, b).

These important studies on wine aroma composition have highlighted the complexity of the wine volatile fraction, but we already know that not all of these compounds have sensory relevance for wine aroma, or in other words they might not have an impact on wine aroma perception. To try to elucidate the sensory relevance of wine volatiles, gas chromatography-olfactometry (GC-O) studies have been then performed. In the last years several reviews in food flavor analysis have been published about the olfactometry technique (d'Acampora Zellner et al. 2008; Ferreira et al. 2009; Plutowska and Wardencki 2007). All of these GC-O methodologies have in common the combination of instrumental and descriptive sensory techniques to determine the odor activity (compounds present at concentration below or above the sensory detection threshold) and description (smell) as well as the time of odor activity and the intensity of the odor of volatile compounds. Following the GC-O screening for impact odorants, the odor activity value (OAV) of each compound can be calculated by dividing the concentration of a compound by its odor threshold. In these studies, compounds with OAVs >1 are considered of sensory relevance for wine aroma.

Nonetheless, few wine research studies have included the sensory validation of GC-O data and/or OAVs to determine the real impact of key odorants, taking into consideration synergistic, enhancement, and suppression effects of different odorants as well as other wine matrix components (Villamor and Ross 2013) (Villamor and Ross 2013). These studies are currently known as omission-reconstitution tests and involve the preparation of a recombinant aroma by addition of the target aroma compounds selected on the basis of their OAVs or dilution factors (DFs) to a synthetic wine. The aroma models are then compared with the original wine for similarity or difference using triangle or duo tests (Aznar et al. 2001; Pineau et al. 2009). Other studies include omission or addition experiments to evaluate the aroma models when one compound is eliminated (Ferreira et al. 2002a; Guth 1997) or added (Escudero et al. 2004) to the model.

But in spite of these necessary studies, this information is still not enough to completely understand the flavor of a wine, and wine consumer preferences. Interactions among odorants, interactions between sense modalities, and matrix effects can all impact odorant volatility, aroma release, and the overall perceived flavor (or aroma) intensity and quality. Besides this, the effect of human physiology and specifically oral physiology on wine aroma release during wine consumption and its relationship with wine aroma perception will open a new and challenging topic of research for wine flavor scientists in the following years. An overview on all of these aspects is provided in the following sections.

7.2 Wine Aroma Perception

Aroma perception from foods and beverages is a sequential process that starts when we smell the food and the volatiles travel through the nose to the olfactory epithelium where they are perceived (orthonasal route). However, during eating and drinking odorant compounds are also released into the mouth during oral food processing and travel through the nasopharynx route to reach the olfactory epithelium. This route is usually called retronasal route (Fig. 7.1). Whichever their route, orthonasal or retronasal, volatile molecules released from foods or beverages interact with the olfactory epithelium. Here, there are sensory cells with receptors to which odorant molecules can bind reversibly as a first step towards the generation of an electric signal. Sensory cells are neurons. A receptor neuron has a dendritic pole bearing fine cilia immersed in the nasal mucus. The ciliary membrane hosts receptor macromolecules. The cells possess an axon that projects the olfactory bulb and conveys electrical signals elicited by receptor activation. In the olfactory bulb, axons synapse with second-order neurons that in turn project to the primary olfactory cortex. From there, the olfactory message is sent to many other areas in the brain for a complex processing (Holley 2006).

In the 1980s, Rozin suggested that olfaction can be seen as two functionally distinct senses: one sense for identifying objects at a distance (orthonasal percep-

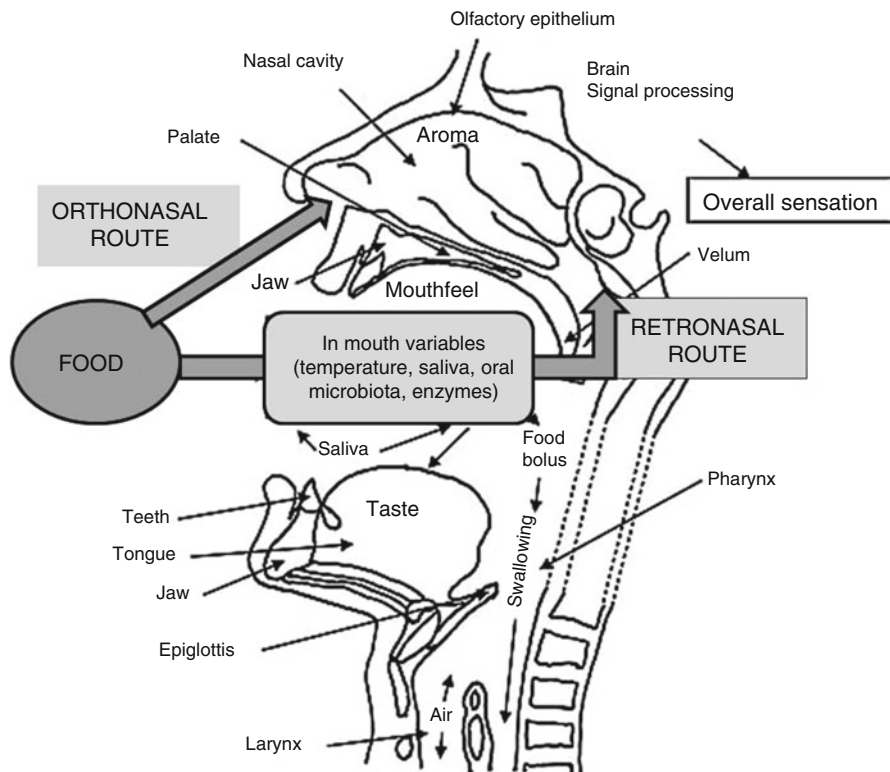


Fig. 7.1 Mechanism of release and perception of sensory stimuli during food consumption. Modified from Gierczynski and co-workers (Gierczynski et al. 2011)

tion) and another sense that contributes to flavor and hence food identification in the mouth (retronasal perception) (Rozin 1982). While these “two senses” physiologically differ perhaps only in the efficiency of delivery of odors to the olfactory epithelium (Voiron and Daget 1986) the information delivered by each may differ in its cognitive impact (Prescott 1999). Thus, it has been postulated that the identification of foods is the combination of the food’s qualities (taste and retronasal odors) into a unitary perception. In agreement with Prescott, consumer’s initial response to foods suggests that this is how sensory properties are perceived. Although we perceive through multiple senses, sensory information is commonly integrated to produce a whole percept. In fact, when food is in the mouth, taste, olfactory, chemesthetic, and tactile senses are concurrently stimulated (Prescott 1999).

This multisensory perception of food has brought the concept of flavor as the most appropriate to define the sensorial experience perceived during drinking or eating. The olfactory components of a food (such as wine), together with tastes and other sensory properties, identify the foods located in the mouth. Flavor could be considered as a distinct sense which is cognitively constructed from the integration of distinct physiologically defined sensory systems (mainly olfaction and taste).

There is scientific evidence that shows that tastes and odors are encoded in the brain as part of a unique perceptual system, in the form of distinct flavor entities (Prescott 1999).

7.3 Perceptual Aroma Interactions

The processing of complex stimuli by the olfactory system is a central issue in the understanding of odor perception in natural conditions because the odors we perceive come mostly from complex mixtures of odorants. The perception of single odorants and mixtures is a product of both interactions at the level of olfactory receptors and interactions during neural processing of olfactory information. In the case of a mixture of odorants, competition may occur at the olfactory receptor level as well as inhibitory interactions at the neural level. Therefore, the perception of an odorant mixture is not a simple sum of the percepts of the unmixed components (Laing and Jinks 2001). The impact of perceptual interactions on perceived flavor has been summarized in excellent revisions (Auvray and Spence 2008; Delwiche 2004; Stevenson et al. 1995).

As already stated (Barkat et al. 2012), most of the studies concerning odor mixture processing have been conducted in animal models (Coureaud et al. 2008; Derby et al. 1996). From them, it has been demonstrated that a binary mixture can be perceived in at least two ways. First, each component of the mixture remains separate and identifiable. This type of perceptual processing has been called dissociative, analytical, or elemental (Derby et al. 1996). In the second type of perceptual processing, the mixture is perceived as an entity, conveying a unique quality not present in its single components. This phenomenon has been called associative, synthetic, or configural processing (Derby et al. 1996). It has been shown that compared with the olfactory systems of naive subjects, the specific training and exposure to odors experienced by expert subjects (flavorists, perfumists, oenologists) lead the olfactory system to engage more readily an elemental processing of odor mixtures (Barkat et al. 2012).

In the specific case of wine, the presence of perceptual odor interactions is favored because of the simultaneous presence of many different odorants (chemical compounds) provoking that the final perception will be the result of a complex brain processing in which some odors are integrated into a single perception. In wines, some odorant compounds might act in competitive or even destructive way (Atanasova et al. 2004) while others interact to form a new and different perception. As previously stated (Ferreira and Cacho 2009), the presence in the wine of whole sets or aroma chemical members of a chemical homologous series displaying similar odors makes that the role of some chemicals should be considered as a part of a combination and the final role of each of them can only be determined via different sensory experiments (such as omission-reconstitution tests).

In an early work, it was shown that when the woody character of a wine increases, the flavor complexity decreases, and the intensity of fruity and floral notes is also

reduced. This suggested an interaction between the fruity and woody notes of wine (Moio et al. 1993). In a later study, Atanasova and co-workers showed this effect in more detail using three binary mixtures of wine aroma compounds (Atanasova et al. 2004). The two first mixtures involved whisky lactone (woody note) that was mixed separately with two esters (fruity note), ethyl butyrate and isoamyl acetate. For the third mixture, guaiacol was mixed with ethyl butyrate (fruity note). The results of this study confirmed the presence of perceptual quantitative interactions between fruity and woody odorants. In fact, they also suggested that quantitative mixture interactions observed at low but suprathreshold intensity levels might be different from those observed at higher intensity levels. Moreover, they stated that hyper-addition could occur when mixing low iso-intense fruity and woody odors.

Besides the abovementioned aroma compounds, perceptual interactions among wine aroma compounds have been described for furanones (furanol and homofuranol), C13 norisoprenoids such as β -damascenone, sulfur compounds such as dimethyl sulfide or diacetyl, and acetoin, acetic acid, and γ -butyrolactone, which might indirectly contribute to fruity expression in red wines (Lytra et al. 2013). These examples emphasize the importance of perceptive interactions on the intensity and quality of red wines' fruity aromas. Pineau and co-workers demonstrated that in some complex mixtures in dearomatized red wines, very small variations in the concentrations of some ethyl esters were perceived even at concentrations far below their individual olfactory thresholds and affected their red and blackcurrant aromas (Pineau et al. 2009). They demonstrated that ethyl propanoate, ethyl-2-methylpropanoate, and ethyl-2-methylbutanoate were involved in blackberry aromas, whereas ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl 3-hydroxybutanoate impacted red berry aromas.

More recently Lytra and co-workers investigated the role of 12 esters present in a mixture at the average concentration usually found in red wines, especially on fruity character (Lytra et al. 2013). They performed omission tests in the aromatic reconstitutions that were prepared in hydroalcoholic solutions and they investigated the occurrence and nature of interactions and their origins from chemical, physicochemical, and psychophysical points of view. Their results revealed the indirect impact of ethyl propanoate, ethyl 3-hydroxybutanoate, butyl acetate, and 2-ethylpropyl acetate present at subthreshold concentrations, on fruity aroma expression (red and blackberry fruit aromas). The presence of ethyl-3-hydroxybutanoate and 2-methylpropyl acetate in the mixture led to a significant decrease in the olfactory threshold of the fruity pool demonstrating their synergistic effect in increasing the overall intensity. Employing sensory tests they showed that besides ethyl-3-hydroxybutanoate, the omission of each of these compounds had a significant attenuating effect on blackberry and fresh fruit aroma intensity. The compounds with similar chemical structures participate, both quantitatively and qualitatively, in modulating fruity aromas and, specifically, naturally enhancing blackberry and fresh-fruity aromas.

Besides the existence of odor-odor interactions, there are some works in the literature that have shown interactions between taste and some typical wine aroma compounds (Dufour and Bayonove 1999b; Welge-Lussen et al. 2005). These interactions have been proven to affect an important quality of wines as it is the astringency.

gency. In an interesting work, Saenz-Navajas and collaborators showed that the addition of a white wine aroma extract (mainly described as fruity) to a reconstituted and deodorized red wine determined a decrease in astringency and bitterness and an increase in sweet perception, which is mainly produced because of the inverse relationship between astringency and bitterness to fruity aroma (Saenz-Navajas et al. 2010b).

7.4 Wine Matrix-Aroma Interactions

One of the most important factors that can limit the rate of release of aroma compounds during wine consumption could be the interaction between aroma and non-volatile matrix components. Aroma compounds can physically or chemically interact with wine matrix components such as polyphenols, glycoproteins, and polysaccharides. This can change the distribution of aroma compounds between the aqueous solution and the vapor phase (partition coefficient), therefore altering the odorant volatility, and might influence headspace partitioning of volatiles producing two opposite effects: a retention effect, therefore decreasing the amount of aroma in the headspace, or a “salting out” effect, provoking an increase in the headspace concentration of a volatile compound because of the increase in the ionic strength of the solution (Jouquand et al. 2004). Interesting reviews on the impact of wine matrix composition on wine aroma release have been recently published (Munoz-Gonzalez et al. 2011; Pozo-Bayón and Reineccius 2009; Villamor and Ross 2013).

The extent of odorant-matrix interactions can be measured by analyzing the concentration of the analyte in the headspace above the solution, typically by using gas chromatography procedures. As it has been indicated in some revisions on this topic (Polaskova et al. 2008; Pozo-Bayón and Reineccius 2009), in general, much more work has focused on studying aroma release under equilibrium conditions as opposite to dynamic conditions. Other methodologies such as the equilibrium dialysis (Lubbers et al. 1994) or spectroscopy methods such as RMN have also been used to evidence these interactions (Dufour and Bayonove 1999b; Jung and Ebeler 2003). The interactions between aroma compounds and wine matrix components produce different effect on wine aroma and they have been summarized in Table 7.1.

One important aspect to consider in these studies is that in most of them the effect of wine matrix has been studied by using one or several aroma compounds and a much reduced number of wine matrix components. Although very valuable, these works do not consider the whole complexity of the nonvolatile wine matrix. In one of these scarce studies, Robinson and co-workers (Robinson et al. 2009) carried out a factorial design to determine the role of some important wine matrix components (ethanol, glucose, glycerol, catechin, and proline) on 20 representative wine aroma compounds. Their results showed an important effect of ethanol followed by glucose and a very small effect of catechin, glycerine, and proline. More recently, Villamor and co-workers studied the combined effect of ethanol, tannin, and fructose through the use of HS-SPME-GCMS (Villamor and Ross 2011).

Table 7.1 Major wine matrix compounds and their effects on wine aroma determined in different studies using different analytical approaches

Wine matrix compound	Effect on wine aroma	Tested aromas	Analytical approach	References
Ethanol	<ul style="list-style-type: none"> - Contributes to wine aroma “per se” - Increases wine viscosity - Masking/enhancing aroma perception - Increases aroma solubility (decreasing aroma release) - “Marangoni effect” (improves aroma transfer from the bulk to the surface of the hydroalcoholic solution) 	Alcohols, esters, pyrazines, terpenes, ketones, aldehydes, C13 norisoprenoids, acids	Sensory analysis GC-O Static HS (HS-SPME); APCI-MS Dynamic HS (APCI-MS, Purge & Trap)	Jones et al. (2008), Le Berre et al. (2007), Petrozziello et al. (2014) Pineau et al. (2007) Ferreira et al. (2002b), Guth and Grosch (1998), Villamor and Ross (2011) Athes et al. (2004), Aznar et al. (2004), Camara et al. (2006), Conner et al. (1998), Hartmann et al. (2002), Le Berre et al. (2007), Petrozziello et al. (2014), Robinson et al. (2009), Villamor and Ross (2013), Whiton and Zoecklein (2000) Aprea et al. (2007), Le Berre et al. (2007), Tsachaki et al. (2005), Tsachaki et al. (2009)
Glycerol	<ul style="list-style-type: none"> - Contributes to wine sweetness and viscosity - Does not contribute to aroma volatility 	Alcohols, esters	<ul style="list-style-type: none"> - Sensory analysis Static HS (HS-SPME)) Dynamic HS(Purge & Trap) 	Jones et al. (2008), Lubbers et al. (2001), Nurgel and Pickering (2005) Robinson et al. (2009) Lubbers et al. (2001)
Phenolic compounds	<ul style="list-style-type: none"> - Hydrophobic or π-π interaction with aroma compounds reducing or increasing compound volatility - Effect depending on the polyphenol and aroma compound structure and polarity and on the concentration assayed 	Aldehydes, pyrazines, esters, terpenes, mixture of many different chemical classes	Sensory analysis GC-O Static HS (HS-SPME, LC-SH) Dynamic HS (exponential dilution, HS-SPME) RMN	Aronson and Ebeler (2004), Goldner and Zamora (2010), Lorrain et al. (2013), Petrozziello et al. (2014) Villamor and Ross (2013) Escalona et al. (2001), Goldner and Zamora (2010), Mitropoulou et al. (2011), Petrozziello et al. (2014), Robinson et al. (2009), Rodriguez-Bencomo et al. (2011), Villamor et al. (2012), Villamor and Ross (2011) Aronson and Ebeler (2004), Dufour and Bayonove (1999b), Jung and Ebeler (2003) Dufour and Bayonove (1999b), Jung et al. (2000)

Polysaccharides	<ul style="list-style-type: none"> - Different effects depending on the type of polysaccharide and aroma compound, concentration and conformational status - Decreasing aroma release due to hydrophobic interactions of some volatile compounds with mannoproteins 	Esters, alcohols, terpenes, acids, aldehydes, ketones, mixture of many different classes	Sensory analysis GC-O Static HS (HS-SPME, LC-SH) Dynamic HS (exponential dilution, equilibrium dialysis) RMN	Comuzzo et al. (2006), Chalier et al. (2007), Jones et al. (2008) Comuzzo et al. (2006) Comuzzo et al. (2006), Chalier et al. (2007), Mitropoulou et al. (2011), Rodriguez-Bencomo et al. (2011) Chalier et al. (2007), Dufour and Bayonove (1999b), Langourieux and Crouzet (1997), Lubbers et al. (1994) Dufour and Bayonove (1999a)
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GC-O gas chromatography-olfactometry, HS-SPME headspace SPME, LC-SH liquid calibration-static headspace

Although most of these studies have monitored changes in wine aroma composition by using analytical approaches (GC-MS analysis), the importance of these interaction in the sensory characteristics of wine has been highlighted in the work of Pineau and co-workers. They showed that the detection threshold of an important wine aroma compound, such as β -damascenone, was 1000 times higher in a reconstituted red wine than in a hydroalcoholic solution (Pineau et al. 2007). Based on these findings, authors suggested the revision of the odor activity values (OAV) calculated for different types of wine aroma compounds. In fact, a later and wider study was carried out by Rodríguez-Bencomo and collaborators. In this work, authors used an aroma mixture composed of 40 representative wine aroma compounds to aromatize at different levels of concentration five types of de-aromatized and reconstituted wines (white, sparkling, sweet, red, and aged red wines) (Rodríguez-Bencomo et al. 2011). They showed significant differences in the headspace concentration of aroma compounds when compared with reconstituted and synthetic wines (hydroalcoholic solution, pH=3), the latter without matrix effect. This study revealed a great “matrix effect” that in general provoked the retention of most of the aroma compounds assayed, reducing their release from wine. This effect might be able to produce a sensory impact on wine aroma perception, as it has also been shown (Saenz-Navajas et al. 2010a).

Nonetheless, most of these studies have been performed in static conditions, which although very valuable to determine the chemical nature of these interactions do not represent the retronasal delivery of volatiles during a real wine consumption situation. To overcome this drawback, very recently Muñoz and co-workers have evaluated the role of wine matrix composition on the *in vivo* aroma release during the consumption of different types of wines (Muñoz-Gonzalez et al. 2014b). For this study authors used a retronasal aroma-trapping device (RATD) that was previously optimized and validated (Muñoz-González et al. 2014c). The system incorporated a tenax polymer to entrap the exhaled breath of the panelists during the consumption of a total of 100 mL of wine that was further desorbed and analyzed by GC-MS. During this work five wines of different wine-making technology (young and aged red wines, sweet wine, white wine, and a sparkling wine) were employed. All of them were adjusted to the same ethanol level (except the sweet wine) and aromatized with a mixture of four target aroma compounds at the same aroma concentration. Results showed that the aroma released during wine intake was different depending on the type of wine consumed. It was found that red wines released higher amount of aroma compared to white and sweet wines. A further correlation analysis using many wine compositional parameters showed a direct relationship between wine polyphenols and aroma release. It is worth noting that a complementary study with the same wine types but following an *in vitro* approach using an artificial mouth coupled on line with a PTR-ToF-MS also confirmed differences in the real-time aroma profiles depending on wine matrix composition (Muñoz-González et al. 2015b). In agreement with the *in vivo* study, red wines showed higher AUC and I_{max} values after 30 s of monitoring time (Muñoz-González et al. 2015b). The higher aroma release determined during the real or simulated red wine intake could be related to the formation of complexes between

human saliva proteins (in the surface of the throat or in the saliva added to the wine in the in vivo or in vitro experiments, respectively) and polyphenols (more abundant in red wines) as might also occur in in vitro conditions (Mitropoulou et al. 2011).

7.5 Physiological Interactions and Aroma Perception

Once the food or beverage is introduced into the oral cavity, it will be submitted to an oral processing more or less intense depending on the type of food material. During this process, different physiological factors such as the breathing flows, the temperature of the oral cavity, the saliva flows and composition, the adsorption of odorants into the oral mucosa, and the impact of oral microorganisms might determine differences in the aroma release pattern which in turn might affect wine aroma perception. The role of physiology factors and more specifically the impact of oral physiology on wine aroma release is a scarcely studied aspect and only some recent works have dealt with it to explain wine aroma perception and consumer preferences.

7.5.1 Respiratory Flows

The exhaled air during wine consumption sweeps the volatiles retained in the mucosa layer of the throat and mouth helping aroma compounds to be transported till the aroma receptors in the olfactory epithelium (Buettner and Beauchamp 2010). In an early work, Voirol and co-workers showed how the aroma perception was affected by the air volume that reaches the olfactory receptors (Voirol and Daget 1986). In the case of liquid foods such as wine, the highest amount of aroma is released as a unique pulse after swallowing causing the so-called exhalation breath. Once the highest pulse of aroma released has been produced, very little amount of aroma is released in the subsequent expiration episodes (Rabe et al. 2004). Therefore, in this case, the breath capacity could be an important parameter limiting aroma release. However, different studies have found different results. For example, it has been suggested that a greater respiratory rate could contribute to bring more volatiles to the upper airways, and consequently more volatiles could be present in the expired air of the panelists (Hanaoka et al. 2001; Pionnier et al. 2004). Nonetheless, in another study performed in vivo and in vitro using an artificial throat, Weel and co-workers found that an increase in the flow rate resulted in a decrease in aroma release due to a dilution effect (Weel et al. 2004). Recently, Muñoz-González and co-workers showed that individuals with higher breathing capacity (estimated as forced vital capacity and vital capacity) released higher amount of aroma during wine consumption compared to individuals with lower breathing capacity (Fig. 7.2) (Munoz-Gonzalez et al. 2014b). These results seem to support the idea that the higher the breathing flow, the higher the amount of aroma available to reach the olfactory system.

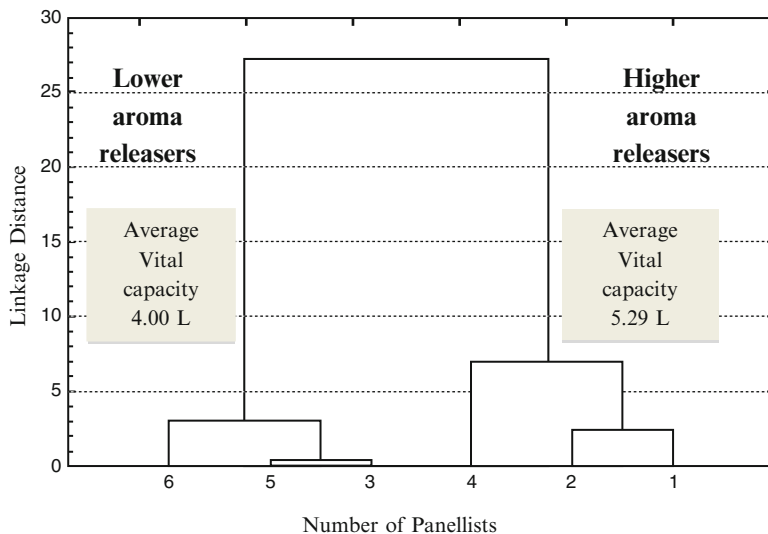


Fig. 7.2 Dendrogram showing the clustering of panelists in two groups: lower and higher aroma releasers obtained with the total aroma release data after the consumption of 100 mL of five types of wines using a retronasal aroma-trapping device. For more details see reference (Muñoz-González et al. 2014b)

7.5.2 Oral Temperature

The average body oral temperature is 37 °C, and in the mouth above 36.8 °C ± 0.4 °C is being considered as stable without much differences among individuals. However, oral temperature might vary during the consumption of different foods (e.g., ice cream vs. soup). This fact will affect the partition of volatiles between the gas and liquid phase. In fact, it has been shown that for many volatile compounds aroma release increases with an increase in food temperature (Linthorpe et al. 2002) which could be due to the higher mass transfer of volatiles into the gas phase as a result of an increase in the partition coefficients of the volatile compounds and a major matrix viscosity (Lubbers and Butler 2010).

From a sensory point of view, a possible sensorial consequence of the food/liquid heating within the mouth could be a higher aroma intensity because of the release of higher amount of a specific molecule, or even the detection of some compounds that occurred in the sample at concentration below their threshold (Delwiche 2004). However, some studies have proven that an increase in sample temperature (20, 40, 60 °C) influenced the orthonasal ratings of beef-type flavorings, but not retronasal ratings (Voirol and Daget 1986). Other studies on odor-temperature interactions in sweetened fruit beverages failed to find a temperature influence when aroma was presented retronasally (Cliff and Noble 1990; Noble et al. 1991). This apparent contradiction might be explained by the fact that once a liquid is placed in the mouth, it is rapidly brought to body temperature. Then, temperature differences in such stim-

uli would be rapidly nullified, making differences in odor intensity fleeting. Nonetheless, in the case of solid foods (such as beef steak), flavor ratings increase with temperature, suggesting that it could be due to the less rapid change in temperature of solids that would occur in the mouth (Delwiche 2004).

Although in the case of wine consumption the effect of wine temperature on aroma release has not been studied so far, in the work of Muñoz and co-workers using *in vitro* conditions simulating the dynamic conditions accounting for during drinking, an important effect of “in-mouth” temperature on wine aroma release was shown (Munoz-Gonzalez et al. 2014a). In this work authors used a saliva bioreactor cell that allowed the incorporation of a gas flow and saliva with a digital temperature control. Aroma release was monitored by means of HS-SPME at two sampling points. A first sampling corresponded to the introduction of the wine in the mouth (18 °C) in the sampling vessel with saliva. In this case, a first aroma extraction that accounted for 2 min, started at 25.5 °C and finished at 32.3 °C, was performed. These conditions might better represent the oral phase when the wine is introduced in the mouth and the temperature of the wine: saliva mixture is lower than physiological temperature. During the same experiment the sampling was also performed 10 min later when the temperature was already stable (36 °C), which better matched a postoral phase after swallowing which might be important to explain aroma release from the wine depots remaining in the mouth. With this experiment authors proved that a relatively small change in “in-mouth” temperature as a consequence of the introduction of the wine (cooler than the mouth) dramatically affected the release of most of the tested aromas (45 aroma compounds), increasing their release.

7.5.3 *Saliva*

Saliva is a complex dilute aqueous solution with different compositions depending on the respective physiological status, types of food consumed, oral hygiene, etc. (Neyraud et al. 2012). Saliva contains numerous inorganic salts (sodium, calcium, potassium, chloride, phosphate, and bicarbonate) and organic components such as enzymes (amylase, lipases, proteases, etc.) (Buettner 2002a; Buettner 2002b; Neyraud et al. 2012) and proteins (mucins, proline-rich proteins, histidine-rich proteins, etc.) (McRae and Kennedy 2011; Salles et al. 2011).

Previous studies have shown that saliva might exert an important role on aroma release through different physicochemical (dilution of aroma due to the aqueous phase of saliva, changes in the pH of the food, hydration of the food which favors aroma release, interaction with salts causing a salting out effect, interaction with proteins), chemical (degradation of odorants), biochemical (degradation of odorant or release from aroma precursors), or even physiological effects (impact on velum-tongue seal formation and swallowing performance), which form part of many previous works performed on this topic (Buettner 2002a, b; Friel and Taylor 2001; van Ruth and Buhr 2003; vanRuth et al. 1996).

In the case of wine, the effect of saliva has been mainly studied because of its involvement in wine astringency (Cala et al. 2012; de Freitas and Mateus 2012; Mateus et al. 2004; Rinaldi et al. 2012) among others. However, there are very few studies focused on the role of saliva on wine aroma release (Genovese et al. 2009; Mitropoulou et al. 2011). One could objectively think that the relatively short intra-oral period of consumption of liquid foods seems to support the idea of a limited action of saliva on wine aroma release during wine consumption. However, the formation of an intraoral (and pharyngeal) aroma reservoir (Buettner et al. 2001) and the fact that natural swallowing of saliva is continuously performed make the idea that saliva might exert an important role in the perception of wine aroma during consumption perfectly viable. Indeed, it could be possible that saliva composition and flows could affect the persistence of aroma perception during the postoral phase of wine consumption. Very recently, using *in vivo* conditions, it was shown that enzymatic degradation of palm wine odorants due to saliva was not noticeable among pyrazines, pyrrolines, and most alcohols but was quite pronounced among aldehydes, esters, and thiols (Lasekan 2013).

Nonetheless, there are very few studies concerning the effect of saliva on wine aroma release and results are also contradictory. In the work of Genovese and collaborators, saliva induced, in general, a decrease on aroma release for most of the wine volatiles, and this effect seemed to be more important in white than in red wines (Genovese et al. 2009). On the contrary, Mitropoulou and co-workers observed an enhancement on the release of hydrophobic compounds from model wines and a decrease in the release of the most hydrophilic compounds in the presence of saliva, although this effect was dependent on the concentration of tannins and polysaccharides (Mitropoulou et al. 2011). Both works were, however, performed in very different conditions: by using dynamic conditions in the work by Genovese et al. (2009), and by using a static headspace approach in the work of Mitropoulou et al. (2011). The dynamic conditions are advisable to achieve more realistic conditions to that accounting for during food consumption; however, the static conditions have been shown to be better suited for the study of interacting effects that otherwise might be underestimated with the first approach (Fabre et al. 2002; Friel and Taylor 2001). More recently, Muñoz-Gonzalez and co-workers carried out a large systematic study in order to elucidate the influence of saliva on wine aroma release by using static and dynamic headspace SPME conditions (Munoz-Gonzalez et al. 2014a). Reconstituted wines (previously deodorized and lyophilized) with different nonvolatile wine matrix composition (red and white) and a synthetic wine (without matrix effect) were used. All the wines were aromatized with a mixture of 45 volatiles representative of the wine aroma profile and adjusted to same ethanol level. In addition, two types of saliva (human and artificial) and control samples (with water) were used to better understand the different mechanisms that saliva might induce on the release of aroma compounds from wine. Results of this work showed that in static conditions most of the aroma compounds were equally affected by the type of saliva and matrix composition. The addition of saliva (artificial or human) provoked a significant decrease of aroma release for most of the tested compounds. However, the extent of this effect was not just depen-

dent on the wine type (red or white), but also on the aroma chemical class (higher retention and lower release for the most hydrophobic compounds). For instance, it was observed that red wines with human saliva showed the lowest values of aroma release. Authors suggested that the higher amount of polyphenols and neutral polysaccharides in red wines might favor the formation of complexes involving salivary proteins (e.g., PRPs) and wine polysaccharides, which could “encapsulate” hydrophobic compounds resulting in a reduction on aroma release (Mitropoulou et al. 2011). However, in dynamic conditions a minor effect of saliva compared to wine matrix composition was evidenced, which could be linked to the limitation of the dynamics conditions (displacement of the equilibrium), which might reduce the retention effect produced by proteins (Fabre et al. 2002) or by other wine matrix components (e.g., polyphenols, polysaccharides).

7.5.4 *Oral Mucosa*

As previously stated, during the consumption of liquid foods as a wine, the major part of aroma compounds reaches the olfactory receptors after swallowing like a pulse of aroma usually called “swallowing breath” (Buettner and Schieberle 2000). This is due to the formation of a thin layer of the liquid sample on the surface of the pharynx acting as an aroma reservoir ready to be released by the expiration flows. The existence of this liquid layer after liquid (or semisolid) food consumption has been visualized employing physioanalytical techniques such as videofluoroscopy (Buettner 2002a). However, additional aroma peaks could be perceived by further actions of saliva since a proportion of the aroma containing liquid remains in the mouth and pharynx as a film coating. This, indeed, provides insights that two modes of aroma release and perception following food and beverage intake can be distinguished: the immediate aroma impression when liquid food is just swallowed and the prolonged retronasal aroma perception after swallowing, often called after-odor (Buettner 2004). This type of aroma perception resulting in the long-lasting aroma perception of some odors following wine intake is a wine feature of special importance during wine tasting and it is an important characteristic to assess wine quality. In spite of that, the number of scientific works focused on the chemistry behind this phenomenon is largely scarce, and these studies have been carried out from a sensory point of view. For example Goodstein and co-workers performed a time-intensity study and they observed differences in the persistence of some aromatic notes in model white wine (Goodstein et al. 2014). They observed that fruity notes are less persistent than coconut, mushrooms, or floral notes, which is also in agreement with other recent works (Baker and Ross 2014a, b).

The chemistry behind the aroma persistence phenomena has been very little explored. In some of these works, the persistence of aroma compounds after the intake of two types of wines or from a palm wine was measured and these data were compared with the intraoral aroma release (Buettner 2004; Lasekan 2013). In these works authors showed differences on aroma release depending on the type of aroma

compound (physicochemical properties and on the type of studied wine). Recently, Muñoz-González and co-workers have suggested that wine red intake produced higher aroma release compared to white wine intake due to differences on the non-volatile wine matrix composition, and specifically on the amount of total polyphenols in the wines (Munoz-Gonzalez et al. 2014b). The explanation they gave is related to the formation of polyphenol-aroma complexes that might bond to oral (and throat) mucosa layer, which favors aroma release. Although this hypothesis needs to be confirmed, there are scientific evidence that supports the idea that saliva can increase the “stickiness” to oral surface of polyphenols and their prolonged retention in the oral cavity (Ginsburg et al. 2012). This could be explained by the interaction of these compounds with proteins forming part of the mucosal pellicle that covers the oral (and throat) surfaces. The mucosal pellicle is a protein-rich bacteria-free adsorbed film that assembles on all surfaces within the oral cavity and it is formed by the selective adsorption of salivary proteins derived from whole saliva (Ash et al. 2013). Ginsburg and co-workers showed that polyphenols in beverages held in the mouth for short period of times (30 s) might also be retained in the oral cavity for long periods despite a constant saliva flow (Ginsburg et al. 2012). Even if the existence of these mechanisms is the origin of this aroma release dynamics, the role of aroma compound properties and the molecular mechanisms involved need to be established.

7.5.5 Oral Microbiota

Oral microbiota is one of the most complex bacterial communities associated with the human body and it is formed by more than 700 bacterial species (Tian et al. 2010). The different microenvironments in the oral cavity (cheeks, palate, tongue, tooth surface, gingival areas, and saliva) have their own microbiota (Requena et al. 2010). Therefore, oral microbiota varies in composition on distinct surfaces (e.g., tooth, mucosa), and at sites on a specific surface (e.g., fissures, gingival crevice), which shows the adaptation capacity of these microorganisms. However, most of them belong to genera *Gemella*, *Granulicatella*, and *Streptococcus* y *Veillonella* (Aas et al. 2005). In addition other factors such as diet, age, and type of diet might affect the bacterial diversity.

These microorganisms can adhere to oral surfaces and form an organized multispecies community known as biofilms (Kuramitsu et al. 2007). The main sources of nutrients for oral microbiota include saliva, crevicular fluid, and host diet. Although saliva is the main nutrient source, due to its chemical composition and continuous production, food is rich in a wide variety of components which could be used by the microbiota to generate secondary products. Initial adhesion invariably involves the interaction of bacterial surfaces with the acquired pellicle derived from salivary constituents adsorbed onto the surfaces of the oral cavity, which serves as a substratum for the adhesion of the so-called early colonizers (*Streptococcus*, *Actinomyces*, *Veillonella* y *Neisseria*) (Aas et al. 2005). The anaer-

obic conditions produced by the early colonizers favor the adhesion of secondary colonizers such as *Fusobacterium spp.*

The metabolic impact of oral microbiota on typical wine aroma compounds such as polyphenols has been previously described (Kamonpatana et al. 2012; Walle et al. 2005). Even it has been suggested that in liquid and semisolid foods the role of oral microbiota could be even higher since the absence of a solid food matrix might facilitate the release of these compounds and the action of oral microbiota (Walle et al. 2005).

Besides polyphenolic compounds, Starkenmann and co-workers showed the ability of some oral anaerobic bacteria to hydrolyze odorless cysteine-S-conjugates from onion, bell pepper, and grapes into their corresponding odorant thiols (Starkenmann et al. 2008), which might be related to a delay in aroma perception, as was already observed by Peynaud and collaborators after the consumption of Golden Sauvignon grapes (Peynaud and Jacques 1996). More recently, in vivo degradation of phenolic volatile precursors has been found, which are associated to unpleasant aromatic nuances such as “toasted” and “burnt”, in which they suggested that oral microbial could be involved (Mayr et al. 2014).

In addition to these studies, it has also been shown that oral microbiota can hydrolyze odorless glycosidic aroma precursors into odorant aglycones. For this study, Muñoz-González and co-workers followed two methodological approaches involving the use of representative oral bacteria (*Streptococcus sanguinis*, *S. oralis*, *S. mutans*, *Actinomyces naeslundii*, *Veillonella dispar*, *Fusobacterium nucleatum*, *Staphylococcus aureus*, *Enterococcus faecalis*) or the whole oral microbiota isolated from human saliva. In the latter, fresh saliva was incubated in aerobic and anaerobic conditions in the presence of the grape glycosidic aroma precursor (Muñoz-González et al. 2015a). In addition, fresh saliva was submitted to different thermal treatments in order to obtain sterile (without microorganisms or enzymes) and nonenzymatic (without enzymes) saliva samples. Odorant aglycones released in the culture broths were isolated and analyzed by HS-SPME-GC/MS.

Results from this study showed the ability of all the oral bacteria tested to hydrolyze grape aroma releasing different types of aglycones (terpenes, benzenic derivatives, and C6-alcohols). This capacity was dependent on the type of bacteria, *A. naeslundii* being the highest aroma producer. In the second approach, using the total microbiota isolated from human saliva, two experiments were performed. In the first one, a pooled saliva sample was submitted to different growing conditions in the presence of the grape glycosidic extract, and linalool release was monitored. Interestingly, this compound was only detected in the saliva samples growing in anaerobic or aerobic conditions, but not in the sterile and nonenzymatic saliva. In a second experiment, the saliva from the three individuals was independently incubated in aerobic and anaerobic conditions. In this case, large interindividual differences that were not related to quantitative differences in oral microbiota were observed; thus authors suggested that they could be due to differences in oral bacteria composition.

Although the large incubation time employed in this study (till 48 h) is far from wine consumption conditions, this work has provided valuable information about the

capacity of oral microbiota to hydrolyze grape glycosides, which are important odorant compounds on the basis of their low odor threshold and in general pleasant aroma nuances. Another consideration that could be pointed out from the abovementioned study is the relatively short residence time of wine within the oral cavity, which might suggest a limited effect of oral microbiota on wine aroma perception. However, as previously stated, results from recent research suggest a possible interaction of some wine matrix nonvolatile compounds with oral and pharyngeal mucosa which might increase the residence time of aroma precursors and free aroma compounds in the oral/pharyngeal cavities, thus increasing their susceptibility to oral parameters (saliva, oral microbiota, etc.) (Munoz-Gonzalez et al. 2014b). Anyway, these types of works invite us to think in the role of oral microbiota on wine aroma generation and they pointed out the necessity of new studies in order to determine the meaning of this effect on retronasal aroma perception during wine consumption.

7.6 Conclusions

It is clear that aroma is a main actor when one tries to explain wine consumer preference. It is because of this that a great amount of work has been focused on the chemical characterization of wine aroma compounds and on trying to determine their sensory meaning. In spite of this, the correlation between sensory and analytical studies is far to be understood. This will require complementary human-centered approaches, in order to monitor what happens with wine aroma compounds once they interact with the human body during consumption. The incorporation of oral physiology factors in this scenario is necessary to understand the aroma transformation of the original wine aroma composition (“wine in the glass”) into the “active” aroma delivered to the olfactory receptors. This task will require a multidisciplinary approach combining analytical, physio-analytical, and sensory techniques and new methodologies using in vivo experiments or the development of in vitro representative physiological setups. Therefore, in the following years we will attend to this new scenario, in which human physiology will be taken into consideration to better understand wine aroma perception and consumer preferences and wine choices.

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