WINE-RELATED AND HUMAN-PHYSIOLOGICAL FACTORS AFFECTING AROMA RELEASE DURING WINE CONSUMPTION

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Abstract

Aroma is an important driver for consumers liking and preferences. This has led to an abundance of research focused on elucidating the role played by wine odorants in aroma perception. However, it is unknown what happens with these compounds during wine intake and the factors influencing retronasal aroma during consumption. These types of studies require specific analytical methodology to monitor aroma compounds in closer consumption conditions. In recent years, the research carried out at the Instituto de Investigación en Ciencias de la Alimentación (CIAL) has been focused on the development on *–in vitro* (artificial mouths) and *–in vivo* methods (*-in nose* or *–in mouth* aroma sampling) to evaluate the transformation that these compounds can undergo in the oral cavity and the wine compositional (physicochemical characteristics of aroma compounds, matrix composition) and human physiological factors (saliva composition, oral microbiota) that can be involved in the retronasal aroma experienced during wine tasting. Our final goal is to better understand the link between the differences in individual oral physiology, aroma perception and consumer preferences

Key words:

Wine, retronasal aroma, oral physiology, wine composition, sensory perception, consumer preference.

Introduction

The importance of wine aroma as a driver of consumer preferences and choices has led to important research work aimed at understanding the impact that odorant compounds have on the sensory characteristics of wine. (Francis & Newton, 2005; Polášková et al., 2008; Robinson et al., 2014). These studies have provided an important background of knowledge, but it is still insufficient in explaining the relationship between wine volatile composition and aroma perception. To fully understand wine aroma perception, as well as the wine aroma characterisation, other related aspects such as the study of the perceptual interactions among wine volatiles (synergisms, antagonisms) or about the physicochemical interactions between volatile and non-volatile wine matrix compounds is also necessary (Pozo-Bayón et al., 2016). In addition, the changes that odorant molecules might experience in the oral cavity during wine consumption is an incipient research that is providing new and valuable findings.

During wine consumption, three phases can be distinguished by which volatile compounds can enter the body. A first phase corresponds to the pre-ingestion stage, in which the volatile compounds released from the hydroalcoholic matrix enter with the breathing air via the orthonasal route towards the olfactory receptors. This is called “odor”. This is followed by a second phase of wine ingestion, which corresponds to the release of volatile compounds into the oral-pharyngeal cavity and their transport by the retronasal exhalation flows to the olfactory receptors. It is the first exhalation taking place immediately after swallowing that produces the greatest aromatic intensity. Finally, a post-ingestion phase can also be distinguished. This starts once the wine has been swallowed and it is due to the sweeping of aromatic compounds adsorbed to the oral and pharyngeal mucosa surfaces containing adsorbed aroma molecules and residual

wine matrix. These retained aroma compounds can be released with the successive saliva swallowing/exhalation episodes once the wine has disappeared from the oral cavity (Pozo-Bayón et al., 2016). This process is responsible for the long lasting aroma perception, also known as aroma persistence.

Analytical methods for aroma analysis during wine consumption

To study the changes that aroma compounds might experience in the oral cavity during wine consumption, specific analytical methodology that considers the physiological conditions in which aroma compounds are released into the oral cavity is necessary, i.e. at the ingestion (immediate aroma) and post-ingestion (prolonged aroma) stages (Muñoz-González et al., 2011).

Figure 1. Different methodologies for aroma analysis during wine consumption. (a) artificial-mouth-PTR-ToF-MS; (b) intra-oral SPME-GCMS; (c) in-nose PTR-ToF-MS; (d) RATD-CIS-GCMS; (e) in-mouth-HSSE-TDU-GCMS

These types of methods can be divided into -*in vitro* methods, which employ systems that simulate the consumption process, such as artificial mouths and throats; and -*in vivo* methods, in which the retronasal aroma is monitored in the oral (*in-mouth* aroma sampling) or nasal cavity (*in-nose* aroma sampling) and therefore, in real physiological conditions. In the case of -*in vitro* methods, an example is the artificial mouth (**Figure 1a**). This device works as a bioreactor in which wine and human saliva are introduced in a glass vessel. The system incorporates an input for an inert gas (N_2) simulating the respiratory flows and the entire system is under stirring and temperature control. The reactor has an outlet through which the aromatic compounds of the wine are released. The released aroma compounds can be monitored off-line by incorporating a solid-phase microextraction fiber (SPME) at the system output (Muñoz-Gonzalez et al. 2014b), or on-line, using a mass spectrometer (Proton Transfer Reaction Mass Spectrometry) that allows the detection of the ion corresponding to the aroma compound at real time (Muñoz-Gonzalez et al., 2015a). In this second approach, an aroma release curve can be obtained and different kinetics parameters (area under the curve, Imax, etc.) can be acquired, which are very useful for comparing, for example, the behavior of a given aromatic compound in wine matrices of different chemical composition (Muñoz-Gonzalez et al, 2015 a).

In the case of *-in vivo* aroma monitoring methods, as explained before, distinction should be made between *-in nose* methods, in which the aroma is monitored in the nasal cavity, and *-in mouth* methods, in which the aroma is sampled in the oral cavity. Among the first types of methods, we developed the RATD (Retronasal Aroma Trapping Device) (Muñoz-González et al., 2013; Muñoz-Gonzalez et al., 2014a) (**Figure 1d**). This tailored-made system allows trapping of the individual's breathing (exhalation) during the consumption of 100 mL of wine using traps of an adsorbent polymer (Tenax). The whole system is connected to a vacuum pump that favors the extraction of the aroma from the nostrils to the polymer. Subsequently, the aroma retained in the trap is eluted with a mixture of organic solvents and after concentration of the breath aromatic extract, the volatiles are analyzed using gas chromatography with detection by mass spectrometry (GC-MS). This technique has been applied, for example, to evaluate differences in aroma release between wines with different non-volatile composition (Muñoz-González et al., 2014a).

More recently, we have developed a system based on real-time *in vivo* monitoring of the aroma released during wine consumption by means of PTR-ToF-MS (Proton Transfer Reaction Time of Flight Mass Spectrometry) (Muñoz-González et al., 2019) (**Figure 1c**). This technique is based on the monitoring of aroma compounds in the nostrils. To do so, two cannulas attached to a hull are introduced in both nostrils. The breath with the aroma is transported through a hot transfer line to the mass spectrometer, which provides a real-time time profile of the ion corresponding to a specific aroma in each swallowing/exhalation episode. This procedure can be applied to determine the impact of wine matrix components (i.e. oenological tannins) on the aroma persistence in the wine post-ingestion phase (Muñoz-Gonzalez et al., 2019).

On the other hand, we have also developed different types of *-in mouth* systems, which allow the monitoring of the release of the aroma from the oral mucosa after the exposure to wine (after mouth rinsing with wine and expectoration). One of these systems is the intra-oral SPME (Solid Phase Microextraction) method (Esteban-Fernandez et al., 2016) (**Figure 1b**). This methodology is based on the extraction of the aroma released into the oral cavity using a SPME fiber after spitting-off the wine. Previously, a gentle rinse of the mouth with the wine (30s) is done. The fiber with the adsorbed aromatic compounds can be directly desorbed into the gas chromatograph injector. With this technique, it has been possible to determine the differences in the oral persistence of chemically different odorant molecules (with different hydrophobicity, boiling points and/or chemical structure) (Esteban-Fernandez et al., 2016) (**Figure 2**). More recently, we have also used this technique to determine the effect of the composition of the wine matrix (amino acids, polysaccharides, polyphenols, etc.) on the oral aroma persistence after the consumption of aromatized wines (Esteban-Fernandez et al., 2018). However, this type of technique requires manual desorption of the fibers in the GCMS, which limits the number of trials and participants in this type of study. To solve this problem, more recently, we have designed the *in-mouth* head space sorptive extraction technique (in-mouth HSSE) (Pérez-Jiménez & Pozo-Bayón., 2019a) (**Figure 1e**). This technique is based on the use of a twister, which is a magnetic bar covered by a polymer (polydimethylsiloxane) introduced into a perforated glass tube. The tube with the twister inside is introduced into the oral cavity of the volunteer at different times after rinsing and spitting-off the wine. The twisters with the aroma compounds must be desorbed and concentrated before GCMS analysis by using a thermo-desorption unit (TDU) and a cryogenic trap (CIS). The advantage of this technique is its greater sensitivity due to the greater thickness of the polymer in the twister, and therefore the possibility of using very short extraction times in the mouth (30s). This allows us to use several twisters for the same sample of wine, and thus, to obtain the kinetics of aroma release of the oral mucosa that is experienced after wine consumption in shorter periods (<2 min), which can be related to the aromatic persistence by sensory time-intensity studies. In addition, another advantage of the *in-*

mouth HSSE method is that the entire aroma desorption process is fully automated, allowing for a greater number of trials with a greater number of volunteers.

Factors affecting aroma release during wine consumption

The above mentioned methods have allowed us for the first time to approach the study of the main factors that can affect aroma release during wine consumption. These factors are related to the physical-chemical characteristics of the aroma compounds, to the chemical composition of the wine matrix, or they can be factors associated with the individual oral physiology.

We have verified that the physical-chemical characteristics of the aroma compounds (volatility, polarity, hydrophobicity, etc.) are important in explaining the release behavior of the aroma compounds in the oral cavity. We found that some odorants, such as esters responsible for pleasant fruity aromas, are quickly released in the first moments after wine swallowing, but they are also quickly lost (**Figure 2**). However, more hydrophobic compounds such as β-ionone are slowly and progressively released (**Figure 2**), so they may be more related to the greater persistence of the aromatic notes associated with these compounds (e.g. "violet") (Esteban-Fernandez et al., 2016). We have also found that within the same chemical group, as in the case of esters, those with a longer hydrocarbon chain (ethyl octanoate, ethyl decanoate) are less lost, and are still found in a high proportion in the oral cavity once the wine has been swallowed, compared to short chain esters (ethyl butanoate, ethyl pentanoate, ethyl hexanoate) (Pérez-Jiménez et al. 2019b). This indicates a greater oral persistence of the more hydrophobic esters, which may be related to their greater ability to bind saliva proteins in the oral mucosa (Ployon et al., 2017).

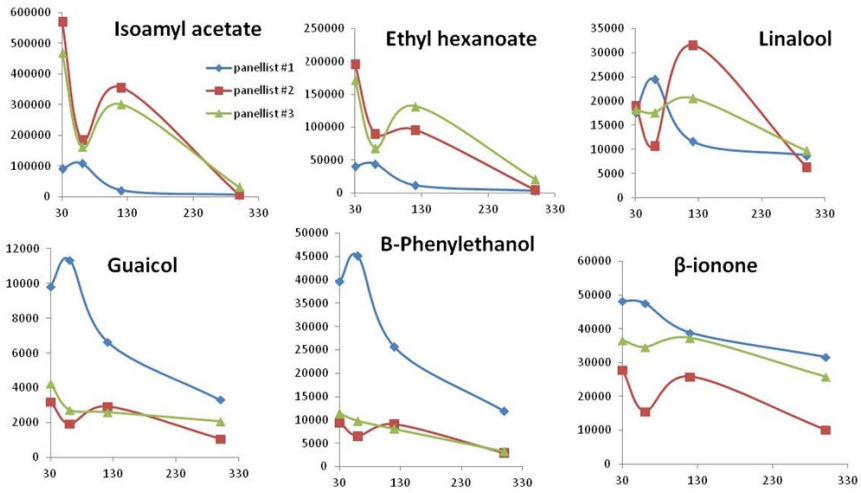

Figure 2. Kinetics of aroma release of three panelists obtained using intra-oral SPME-GCMS at different times after wine expectoration. *Y* axis represents absolute peak area; *X* axis represents the sampling points after wine expectoration (in seconds).

On the other hand, we have found that wine matrix composition can greatly affect retronasal aroma during wine consumption. In a study in which we used twelve commercial wines from different winemaking technology (whites, rosés, young reds, reds aged in wood, etc.), we found that from all the chemical parameters analyzed (proteins, amino acids, polysaccharides, total polyphenols, etc.), total polyphenols were the wine chemicals more related to oral aroma release. A lower release of some aroma compounds such as esters were found when the wine polyphenol content increased. A further detailed study of the polyphenolic composition of these wines using UPLC-MS/MS indicated that procyanidins were the most and negatively correlated phenolic compounds with the oral release of some aroma compounds (isoamyl acetate, ethyl hexanoate) (Eteban-Fernandez et al., 2018) (**Figure 3**). To assess whether the impact of wine polyphenols would be perceptible at the sensory level, we designed a study in which the same type of rosé wine was flavored with six target aroma compounds. From this wine (control wine), three wines types were made by adding three types of different commercial polyphenolic extracts; two of them from grape seeds corresponding to the oligomeric and monomeric procyanidins fractions, and the other wine was supplemented with a commercial extract from a red wine rich in anthocyanins (Pérez-Jimenez et al., 2019c). For this study a double experimental approach was used. On the one hand, a group of eight individuals was recruited to carry out the aroma release study, using intra-oral SPME, at two different times, immediately after rinsing with the wine (t=0m) and five minutes later (t=4 m). On the other hand, using the same panel of volunteers, they were trained for several months in the recognition and evaluation of the intensity of the aromatic descriptors associated with the target compounds used in the aromatization of wines. The results confirmed other previous results since we observed how some odorant compounds, such as esters, were more released after rinsing with control wines than with wines supplemented with polyphenolic extracts, and this happened immediately after wine expectoration and four minutes later. In addition, the most interesting fact of this study was the decrease in the aromatic intensity of some descriptors in wines supplemented with polyphenolic extracts that agreed with the lower oral release of the compounds associated with these descriptors. This confirmed the good congruence between the *-in vivo* aroma analysis (intra-oral SPME) and the sensory findings.

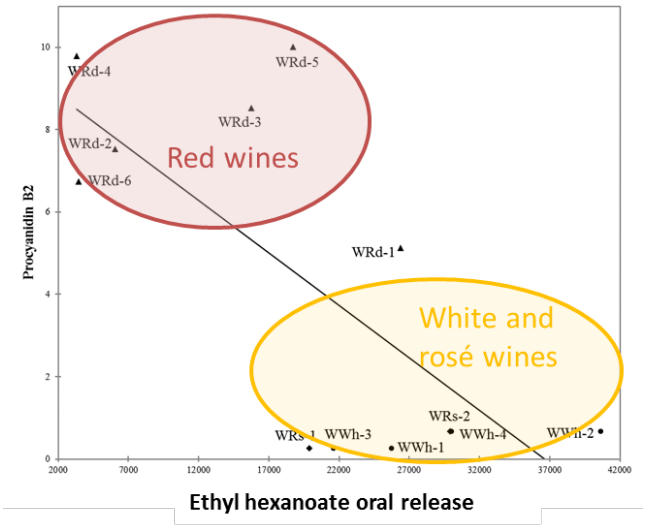

Figure 3. Negative correlation between the content of Procyanidin B2 in different types of wine and oral release of ethyl hexanoate.

In addition to aspects linked to the volatile and non-volatile composition of wines, aroma release during wine consumption is affected by physiological factors. Saliva flow and composition (proteins, enzymes, pH, etc.) might influence the aroma transfer to the respiratory flow that transports odorant molecules to the olfactory receptors. We have recently found that unstimulated saliva total protein content can influence the release of aroma in the post-swallowing phase after wine consumption (Muñoz et al., 2019). Also, salivary flow affects the perceived intensity of specific aroma attributes associated with wine esters at different times after wine consumption when applying dynamic sensory techniques (Criado et al., 2018). In addition, the interaction between saliva proteins and aroma compounds can determine the amount and type of odorant molecules available for perception. These types of interactions (hydrophobic, hydrogen bridges) depend on the physical-chemical characteristics of the odorant molecules, and on the presence of other non-volatile components of the wine. Using an -*ex vivo* approach we have recently verified that salivary enzymes can hydrolyze wine aroma esters giving rise to an increase of the corresponding carboxylic acids with very different aromatic characteristics and odor thresholds (Pérez-Jimenez et al., 2019b). We are currently researching the existence of this metabolism under -*in vivo* conditions. In addition, this metabolic capacity seems to be individual-dependent, since many of these enzymatic activities are differently expressed in individuals. On the other hand, oral microbiota may also have an effect on aroma generation. In one of the first papers on this topic and using glycosidic aroma precursors isolated from grapes and oral microbiota isolated from the saliva of healthy individuals, we confirmed the ability of these microorganisms to hydrolyze odorless precursors from grapes and to produce different types of odorant molecules at different concentrations, which also depended on the individual (Muñoz-Gonzalez et al., 2015b).

CONCLUSIONS

The results of all these studies have allowed us to begin to understand how the aromatic fraction of wine behave in the oral cavity during consumption and how they can be affected by human physiological and wine compositional factors. The –*in vitro* (artificial mouths) and –*in vivo* analytical techniques developed though these studies can improve the know-how on the behavior of aromatic compounds in the oral cavity and it could contribute to a better understanding of consumer's preferences and attitude towards wine types. Also, the results of this research might help in the implementation of winemaking techniques to improve the aroma release in the mouth, making wines with higher aroma quality and in the development of new oenological additives that improves the oral release of pleasant aroma compounds reducing or minimizing the release of negative aroma notes (*off-flavors*). These methods can also be of interest for wine/food pairing studies, helping in understanding the role of specific ingredients in wine aroma release. Finally, the knowledge generated about the impact of different factors affecting retronasal aroma will contribute to a better design of wines more directed towards target consumer groups and with typical physiological traits (seniors, young consumers, etc.).

ACKNOWLEDGEMENTS

Authors greatly thank all the volunteers who have participated in these studies and CSIC and MINECO for funding (I-link1049-CSIC and AGL-2016-78936-R projects respectively).