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Sip volume affects oral release of wine volatiles

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ABSTRACT

In this study, the influence of sip volume on *Falanghina* white wine aroma release was studied *in vitro* by simulating in-mouth conditions and using human saliva. Our results indicate the wine sip volume as a significant parameter affecting the volatiles released from wine and likely, the in-mouth olfactory perception. Simulating the intake by large wine sips, a significant increase in benzyl alcohol, 2-phenylethanol, TDN, and hexanoic acid was observed. Differently, a significantly higher release of ethyl acetate, 2-methyl-1-propanol, 3-methyl-1-butanol, 1-hexanol, β-damascenone, and benzaldehyde was detected miming the wine assumption by smaller sips. The observed behaviors have been related to the air/water partition coefficient of wine volatiles, and to the presence of saliva. Both these variables play a significant role in the distribution of odor active compounds among the different phases involved in the oral process. The release of some volatile markers, involved in the fruity and oxidative characters of wine, was mainly affected by the sip volume after wine–saliva interaction. All changes and their sensory impact need to be tested by additional *in vivo* assays in order to confirm these results suggesting that, during wine sensory assessment, it is important to control/measure the sip volume in order to reduce/take into account inter-individual variability.

1. Introduction

Wine aroma is defined as an olfactory stimulation perceived during tasting, when wine is in the mouth. Its odorants interact with olfactory receptors by moving from the mouth to the nasal cavity via nasopharynx (Munoz-Gonzalez, Rodriguez-Bencomo, Moreno-Arribas, & Pozo-Bayon, 2011). Both the release and the retention of aroma compounds from wine, depend on their concentration and chemical nature (Polaskova, Herszage, & Ebeler, 2008). Various properties such as molecular size, shape, volatility, and polarity can determine a high or a low availability of a specific aroma compound to the olfactory system, always depending on its concentration (Linforth & Taylor, 2000; van Ruth, O'Connor, & Delahunty, 2000). Also other factors, such as the oral physiology and anatomy, are involved in the retronasal aroma perception. One of the main oral physiological factors affecting aroma release in the mouth is saliva (Linforth, Martin, Carey, Davidson, & Taylor, 2002; Salles et al., 2011). In a previous paper (Genovese, Piombino, Gambuti, & Moio, 2009), we investigated the retronasal aroma perception of wine. The results showed differences between orthonasal and retronasal volatile composition due to an important influence of saliva on aroma release from white and red wines. The interaction of wine volatiles with salivary components (mainly proteins and enzymes) and other non-volatile wine components were hypothesized to be responsible for the detected differences. These evidences were recently supported by Munoz-Gonzalez, Feron, et al. (2014). These authors found differences depending on the use of human or artificial saliva, proving that mucins and other proteins seem to have an important role in wine aroma release. Together with salivary composition, other factors such as salivary secretion rate, breath flow, oral volume, processing modality affect in-mouth volatile release from foodstuffs (Buettner & Beauchamp, 2010; Hodgson, Linforth, & Taylor, 2003; Piombino et al., 2014; Rabe, Krings, & Berger, 2004; Salles et al., 2011). To enhance retronasal detection, tasters frequently aspirate wine and then the air with a consequent swirling in the mouth. This procedure should favor the volatilization of molecules by increasing the free surface (analogous to the wine swirling into the glass) (Jackson, 2009). So far, one of the wine tasting factors received little attention: it is the sip volume during wine assessment. Generally, some professional handbooks of wine tasting reported that the level of wine into a glass should be around one-quarter to one-third full (Jackson, 2009). All glasses should be identical in the shape and filled-in at the same level to allow each wine to be sampled in the mouth under equivalent conditions and facilitate vigorous swirling enhancing the release of aroma compounds in the air. Then, a taster should take a sip into the mouth. Any previous investigation aimed to understand the effect of the sip volume on the in-mouth wine aroma release. As stated above, during wine assessment the service conditions are standardized (e.g.: glasses shape and capacity, wine quantity and temperature) but usually it is not the same for parameters defining the taking modality such as the sip volume. A recent study on espresso coffee demonstrated that the headspace concentration for some key aroma compounds was significantly affected by the sip volume (Genovese, Caporaso, Civitella, & Sacchi,

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2014). In the same manner, we question if the sip volume could be one of the factors explaining differences among repetitions when the same wine is assessed by the same taster at different times. It is well known that individual tasters often perceive different flavors from a given food sample tested more times (Laboure, Repoux, Courcoux, Feron, & Guichard, 2014; Ruijschop, Burgering, Jacobs, & Boelrijk, 2009). Some of these variations may be due to changes in flavor release as a consequence of assumption of different capacities, as already verified in vanilla and chocolate custard desserts (Prinz & de Wijk, 2007; Ruijschop et al., 2011). Humans show wide variation in this parameter e.g. males usually make larger bites than females (Foster et al., 2011). Therefore, the sip volume may be a crucial factor affecting the retronasal aroma release during wine tasting and ultimately sensory perception.

In this context, the aim of this study was to investigate the impact of the sip volume on the retronasal aroma release of white wine by a model mouth system (RAS: Retronasal Aroma Simulator). This kind of *in vitro* analysis was previously applied by several authors (Benjamin, Silcock, Beauchamp, Buettner, & Everett, 2012; Foster et al., 2011; Rabe et al., 2004; Roberts & Acree, 1995; van Ruth & Roozen, 2000) because of its good correlation (>99%) with *in vivo* breath-by-breath measurements (Deibler, Lavin, Linforth, Taylor, & Acree, 2001).

2. Material and methods

2.1. Standards and wine sample

Pure reference standards of ethyl butanoate, 3-methylbutyl acetate, ethyl hexanoate, ethyl lactate, ethyl decanoate, ethyl succinate, 2-methyl-1-propanol, 3-methyl-1-butanol, 1-hexanol, benzyl alcohol, 2-phenylethanol, furfural, benzaldehyde, β-damascenone and 2-octanol were supplied by Aldrich (Steinheim, Germany). 2-Phenylethyl acetate, was supplied by Fluka (Buchs, Switzerland). Hexanoic acid was supplied by Sigma (St. Louis, USA). Ethyl acetate was supplied by Carlo Erba (Milan, Italy). Ethyl octanoate, was supplied by Lancaster (Karlsruhe, Germany). Ethanol was supplied by J.T. Baker (Deventer, Holland).

In this experiment, a blend (50%) of two *Falanghina* white wines (Benevento, Campania, Italy) from two different vintages (gap: 4 years), was analyzed in order to have samples containing the key volatile compounds of both young and aged *Falanghina* wines.

2.2. Human saliva

Mixed whole resting saliva (150 mL) was separately collected from 13 male subjects (21 to 46 years old) after 1.5 h from toothbrushing (12.00 a.m.) as previously described by Piombino et al. (2014). Subjects were recruited among students, researchers, and professors from the University of Naples Federico II. They were non-smoking volunteers, exhibiting no known illnesses at the time of examination and with normal olfactory and gustatory functions. Immediately after collection, samples were vortexed, split into several aliquots and stored at −20 °C until the subsequent analyses.

2.3. Release of aroma compounds in the model mouth system

The in-mouth dynamic conditions were simulated by using a Retronasal Aroma Simulator (RAS) device equipped with an SPME fiber (Solid Phase Micro Extraction; Supelco Co., Bellefonte, USA), as previously described (Genovese et al., 2009). Two different sample matrices were analyzed: wine (W) and wine added with saliva (WS). For WS, 30 and 40 mL of *Falanghina* white wine (pH 2.75) together with 6 and 8 mL of whole resting saliva (pH 7.60) respectively, and 200 μL of an alcoholic solution of 2-octanol (50 mg into 250 mL of ethanol) as internal standard, were transferred into the model mouth flask (100 mL), which was kept at 37 °C into a water bath. The two different volumes of added saliva were necessary to maintain the proportion between wine and saliva volumes unchanged. The pHs were measured by a CRISON pH-Meter Basic 20+. As controls W, 30 and 40 mL of wine without saliva were analyzed in the same conditions. In this case, little glass balls were added in the RAS flask in order to maintain the same headspace volumes (64 and 52 mL, respectively) as in the previous experiments without changing dilution. All samples were analyzed in the same conditions. The SPME fiber was inserted into the sample container through a septum and then exposed to the headspace. A purified nitrogen flow (20 mL/s) passed through the wine/saliva mixture for 10 min, while volatile compounds were trapped on a conditioned (250 °C for 3 h in a GC injection port) SPME fiber (DVB/CAR/PDMS; 50/30 μm thickness; coating phase; 2 cm length). The absence of extraneous/residual molecules on the fiber was checked before each analysis.

2.4. High resolution gas chromatography–mass spectrometry (HRGC/MS)

Volatiles adsorbed on the SPME fiber's coating phase were desorbed in split–splitless mode (split valve opened at 11 min and closed at 25 min) at 250 °C for 10 min in the injection port of a GC/MS-QP2010 quadrupole mass spectrometer (Shimadzu, Shimadzu corp., Kyoto, Japan) equipped with a DB-WAX column (60 m, 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific Inc., Folsom, CA 95360, USA). The temperature program used was 40 °C for 5 min, raised at 2 °C/min to 220 °C, and held for 20 min at maximum temperature, starting immediately after exposure of the SPME fiber in the RAS device. The injector port and the ion source were maintained at 250 and 230 °C, respectively. The carrier gas used was helium (1.3 mL/min). Electron impact mass spectra were recorded with ion source energy of 70 eV and peak areas were measured using a GC/MS solution program Shimadzu version 2.30 (Shimadzu corp., Kyoto, Japan). Compound identification and concentration measurement were performed as previously reported (Genovese et al., 2009). In a few cases the pure chemical standard was not available, and the compounds were labeled as tentative $^{(t)}$.

2.5. Estimation of physicochemical properties of the volatile compounds

The logarithm of air/water partition coefficient (log *P*) of the volatile compounds was empirically estimated considering atom/fragment/group/bond contribution, for all molecules, using EPI Suite v.4.1 software, U.S. Environmental Protection Agency and Syracuse Research Corp.

2.6. Statistical treatment of data

Significant quantitative differences among the two sample matrices W and WS and between the same sample matrix at different volumes (30 and 40 mL) were determined for each compound by performing a one-way analysis of variance (ANOVA). Tukey's test was used to discriminate among the mean values of the variables. Differences were considered significant at $p < 0.05$. In order to better understand the influence of sip volume, saliva, air/water partition coefficient as well as their interactions on the release of volatile compounds from wine, a multifactor ANOVA with second-order interactions was carried out. Principal component analysis (PCA) was performed on the volatile compound concentration to observe how volatiles associated best with 30 or 40 mL of wine samples. Data elaboration was carried out using XLStat (version 2009.3.02), an add-in software package for Microsoft Excel (Addinsoft Corp., Paris, France).

3. Results and discussion

In total, 22 volatile compounds were identified in the dynamic headspace of white wine: 10 esters, 6 alcohols, 3 C_{13}-norisoprenoids, 2 aldehydes and 1 acid. The identified volatile compounds, grouped into chemical classes with their log $P_{a/w}$, the relative changes in headspace concentration using 30 respect to 40 mL of wine, without and with saliva addition, are given in Table 1. The first column (W) expresses