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Volatile Compounds in Wine before and after Imbibition

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ABSTRACT

Flavor is created in a person's brain as an image combining their senses of *taste* and *smell*. The sense of smell detects volatile chemical compounds that reach the neurons in the nasal cavity. These volatile compounds can be inhaled through the nose (*orthonasal smell*), or they can be exhaled from the mouth, throat and trachea (*retronasal smell*). With regard to sensing the flavor of wine, I hypothesize that, while the wine is warmed in the mouth to 37°C and interacting with both salivary enzymes and the oral microbiome, the wine and its volatile compounds will be chemically altered so that the retronasal smell of the wine will be different than its orthonasal smell. In this research, volatile compounds from wine samples and samples of exhaled breath pre- and post-imbibition were measured gas chromatographically (F.I.D.) to detect new compounds in the exhaled post-imbibition samples relative to the wine samples and the exhaled pre-imbibition samples. Multiple new compounds were detected. This research supports the hypothesis that the retronasal smell of wine is an important contributor to its perceived flavor.

Keywords: *volatile, orthonasal, retronasal, gas chromatography, wine, flavor, aroma, taste, imbibition*

INTRODUCTION

Wine is a big industry; flavor is a wine's most important distinguishing characteristic. Premium wine is a "craft" product, not a "commodity" and so variation in sensory profiles and chemistry are expected. (Sacks 2018). Flavor is the overall impression of wine, consisting of both taste and aroma. The most important tastes in wine would be bitter, sweet, and acidic. "Smell or aroma is another component in the flavor of wine. Although there are tens of thousands of volatile compounds in wine, it only takes two or three dozen of those volatiles to create the aroma" (Sacks 2018).

"The most mysterious aspect of wine is the endless variety of flavours that stem from a complex, completely non-linear system of interactions among many hundreds of compounds" (Lambrechts 2000). Flavor is wine's most important distinguishing characteristic creating the flavor refers to the overall impression of aroma and taste components. Aroma is associated with odorous, volatile compounds of wine We are most aware of the sensory sensations of wine tasting. "All sensations created by the brain are due to the movement of the wine in our mouth and throat and the movement of volatile molecules released into the air in our respiratory tract." (Shephard 2017, p.7). When the person drinking wine and breathing at the same time this is the relationship between orthonasal (breathing in) which is the aroma and retronasal (breathing out) smell breathing out to carry aroma from the wine in the mouth and throat to the nose during expiration, to add retronasal smell to the total sensation of flavor. The movement of breathing and swallowing generates movements that stimulates the brain.

Fluid dynamics has been studied in the movement of wine. "Fluid dynamics – the flow of liquids and gases in nature" (Shepard 2017, p.7). When drinking wine moving the wine requires muscles in the mouth and throat to move called propulsion. If we know and understand the muscle movement our wine experience can be greater. These muscles are involved in

Orthonasal and retronasal olfaction have similarities and differences. "Orthonasal air comes exclusively from the environment and consists of the gases nitrogen, oxygen, and carbon dioxide, together with evaporated water varying with temperature and humidity" (Shepard 2017, p. 10). These compounds can vary depending on environment such as the pollution, pollen, dust, and lent. Orthonasal air contains many different types of volatile molecules, depending on many our many different activities throughout ones day. Activities like where you work, being inside or outside, mealtimes sampling food and drinks. These external conditions can distort flavor perception.

"Retronasal air, in contrast, reflects the characteristics of the expiring air that contains contributions from inside the body after it is filtered by the upper airway" (Shepard, 2017 p.10). Just like orthonasal air there is nitrogen, oxygen, and carbon dioxide, but there is also high humidity because of the moisturizing by the airway mucus membranes and a warm temperature because of body temperature. There are also some trace elements of volatile compounds that have been breathed in. These volatile compounds inter the retronasal smell through many different routes. "One way is by diffusing from the lung capillaries into the air in the tiny pockets called alveoli that make up the lung" (Shepard, 2017 p.11). Another way is by seepage into the airway within the lung, trachea, and pharynx. Lastly is from the wine in the mouth.

The stages of moving wine through the mouth end with swallowing the wine. The swallowing forces the wine from the mouth into the throat and through the throat into the esophagus. “This leaves a coating of wine on the mouth and throat, whose volatiles are swept up by the respiratory system and transported to the nasal cavity” (Shepard, 2017 p. 12).

Saliva is another important factor in wine tasting it has many functions. “Saliva keeps the taste buds moist, enhancing the ability of taste molecules and ions (tiny electric charges) to stimulate the taste cells” (Shepard, 2017 p.21). Saliva is warm and the warming of the wine enhances the release of volatiles to retronasal smell. Lastly the secretion of saliva contains enzymes that help break down components in the wine. Saliva changes the characteristic of wine from the time it enters the mouth to the time it exits. “Most flavor comes from the aroma as sensed by smell. This flavor only happens when breathing out through your nose” (Shepard, 2017 p. 42).

After swallowing, some of the wine is left coating the mouth and throat. The coating sticks to the throat walls and allows the volatiles to be exhaled. “The concentration of volatiles is of course at its peak with the first expiration after swallowing” (Shepard, 2017 p.53). This gives a strong sensation of the wine aroma that is with a strong perception of the wine taste this could be the strongest contribution to the taste of wine.

Flavor analysis using a variety of methods has been conducted for many years in the development of new products, to understand the nature of existing products, to study shelf-life, and to maintain quality of foods and beverages (Chambers 2016). Using chemical analytical instruments as a method for examining flavor can provide feedback about individual compounds. These methods are used in different ways but they all involve separation, identification, and quantification of compounds. These different methods of analysis can be achieve in a headspace. Headspace sampling avoids the introduction of involatile or high-boiling contaminants from the sample matrix.,

Testing samples of volatile compounds using the gas chromatography of two different red wines. Then comparing those volatile peaks with exhaled breath before and after imbibition, to see if there is an observational change in volatile peaks.

This brings more knowledge about the flavor of wine at a volatile level. What is unknown is, does the composition of wine change before and after imbibition and if it does change how much does it change and why is the composition changing.

In this research I find out observationally if peaks are changing and the different reasons for the changes in composition of wine before and after imbibition.

Red wine volatile compounds were analyzed vs exhaled breath with red wine volatile compounds. To analyze the effect that being imbibed had on the volatile compounds that contribute their aroma to the flavor of wine.

MATERIALS AND METHODS

Wine

The wines used were a 2015 Barrel Reserve Red and a 2015 Norton, from Grace Hill Winery in Newton, Kansas.

Sampling method

An aliquot of 10ml of wine was placed in a 22 ml Perkin-Elmer headspace screw top vial. A headspace sample of the volatile compounds from the wine was withdrawn using a Hamilton 500 µL gas-tight sample-lock syringe for injection into the gas chromatograph (GC). Retronasal exhaled breath was sampled from the nasal cavity using a 5mL B-D plastic syringe that was filled while the subject was exhaling. A 500 µL aliquot was then withdrawn from the B-D syringe into the Hamilton syringe. This was then done three different times successively.

Instrumentation and gas chromatography conditions

Each sample (500µL) was injected into the Perkin Elmer Clarus 500 GC with flame ionization detector (injector port temperature 150˚C, column temperature isothermal at 40 ˚C, detector temperature 180˚C, N_2 carrier at 30 mL/min, H_2 at 30 mL/min, zero Air at 30 mL/min, column is 6 feet and 1/8 inch Porapak-Q 100/120 mesh). The detector’s mV signal output was recorded and analyzed using an Iworx 214 data acquisition system with LabScribe3 software.

Retronasal Aroma Trapping Procedure during Wine Consumption

Three volunteers (2 males and 1 female) participated in this study. They were instructed not to eat, drink, or smoke 2 hours before the experiment. Before each experiment, the panelists had to clean their mouths and rinse with a bicarbonate solution.

Analysis of Volatile Compounds

For each different type of wine, three different sets of measurements were made in triplicate. Three replicates measurements were made for exhaled breath before wine imbibition, three replicate measurements were made of wine volatile compounds in the headspace vial, and three different measurements were made of exhaled breath after wine imbibition.

Data Analysis

The data were collected using Labscribe functions. I did an observational analysis, seeing if there are changes to peaks and their presences and absences before and after imbibition.

RESULTS

Observationally, peaks changed in retention time in seconds. Peaks were detected from the flame ionization detector. This sensitive detector burns any organic molecules. The injected mixture of volatile compounds are separated based on physical and chemical properties. As the organic molecules burn the GC produces peaks. These different molecules are burned at different times. Once they are burned they are detected and the GC then produces a visual peak.

The peaks were analyzed based on their retention time how fast or slow they were detected and produced by the GC. The volatile compound samples that were injected into the GC were the two different wines, Norton and Barrel Reserve at 25˚C and 37˚C, and exhaled breath samples taken before and after imbibition from three different volunteers. After analyzing the peaks, differences were observed. There were six new peaks that were detected in the exhaled breath after drinking the Norton when compared to the exhaled breath pre imbibition, 25˚C, and 37˚C Norton samples. Those new peaks can be observed by looking at Table 1. For the Barrel Reserve exhaled breath after imbibition there were five new peaks observed, compared to the post imbibition with Barrel Reserve 25˚C, and 37˚C and pre imbibition exhaled breath. These new peaks can be observed by looking at Table 2.

DISCUSSION

There are over 1300 volatile compounds in red wine (Ebeler 2001). For this experiment I analyzed red wine volatile compounds' different peaks. I was not looking for any specific peaks, rather I was looking to see if the resulting peaks changed. When comparing the peaks from just the volatile compounds in red wine with the exhaled breath, after imbibition. I analyzed the different peaks produced from red wine volatile compounds and I compared these peaks with the volatile compounds of exhaled breath of three volunteers before and after imbibition of the red wine. In my experiment I analyzed two different red wines. 2015 Barrel Reserve and 2015 Norton from Grace Hill Winery. I anticipated that some peaks will be different while others will stay the same. These peaks will be different because of the process of being in a mouth. In the mouth the wine will be at a different temperature than what the red wine it was originally at before entering the mouth.

Saliva also plays a role in the breaking down process of wine. When wine comes in contact with saliva the breakdown process begins. In red wine, treated with human saliva, only a few esters decrease, with a reduction of 22–51% due to protein-binding ability of polyphenols that are able to inhibit the activity of the saliva (Lisanti, 2008). Saliva also keeps the taste buds moist which enhances the ability of taste molecules. The temperature changes in the mouth have an effect on the changes on volatile compounds,

Table 1. This is the Norton volatile compound wine sample. There are three different exhaled breaths post imbibition that were compared to the pre imbibition exhaled breath, Norton volatile compounds at 25˚C and 37˚C. The N's indicate the new peaks that were found.

Samples R/T in sec.	50	130	140	160	170	235	250	280	300	360	395	450	460	470	520	535	545	660
Norton @ 25˚C	X		X			X				X								
Norton @ 37˚C	X					X			X									
A. Before Imbibition	X	X		X					X									
A. After Imbibition	X	X			N				X									
B. Before Imbibition	X							X					X			X		
B. After Imbibition	X								X	X	N							
C. Before Imbibition	X		X			X						X			X		X	X
C. After Imbibition	X				N		N					X		N	X	N		X

Table 2. This is the Barrel Reserve volatile compound wine sample. There are three different exhaled breaths post imbibition that were compared to the pre imbibition exhaled breath, Norton volatile compounds at 25˚C and 37˚C. The N's are the new peaks that were found.

Samples R/T in sec.	50	100	110	130	170	200	235	270	285	300	315	375	400	500	700	735
Barrel Reserve @ 25˚C	X						X						X	X		
Barrel Reserve @ 37˚C	X									X						
A. Before Imbibition	X			X					X	X					X	
A. After Imbibition	X				N					X						X
B. Before Imbibition	X		X	X								X				
B. After Imbibition	X				N	N										
C. Before Imbibition	X		X								X					
C. After Imbibition	X	N						N								N

and saliva activity in the mouth will produce different peaks when analyzing the exhaled breath of the aroma red wine compounds.

The flavor of wine dates back to the ancient Mediterranean and has evolved and is still evolving. (Ployon, et al., 2017). Aroma perception is an important factor driving food acceptance. Volatile organic compounds are released and soon after they reach the receptors located in the nasal cavity. These receptors then lead to their perception of the aroma. (Ployon, et al. 2017). These steps are closely dependent on the physicochemical properties of the volatile compounds and the food matrix, but also on human physiology. Among the different physiological parameters involved, the literature reports that saliva has many different effects on volatile organic compounds and therefore appears as a major actor impacting the perception of aroma.

From my results I can conclude that there are volatile compound changes before and after imbibition. These changes are due to warming of the wine, saliva, enzymes, and different bacteria in someone's mouth. Changes were seen observationally by retention time. There were new peaks seen after imbibition of the wine when comparing it with the before imbibition exhaled breath and the wine held at 25˚C and 37˚C.

What can further be looked at for this study is using a more sensitive GC will help the output of volatile compound peaks be more accurate. Future researchers can analyze the peaks in different ways other than just analyzing them at their retention times.

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