Analysis of volatile aroma-active compounds from a headspace of a novel whiskey glass

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Ville Jalo

Ville Jalo

UNIVERSITY OF TURKU
Department of Biochemistry

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In the literature part of this thesis the human olfactory sense is explored. Also, individual differences in the sense of smell are studied. Since the study focuses on whiskey the anesthetic effects of ethanol on the olfactory system are explored. The different drinking vessels commonly used with whiskey are introduced and reviewed.

A novel whiskey glass was studied for its aroma enhancing effects. The glass was designed to lessen the effect of ethanol anesthesia while nosing a whiskey from the glass. A method for headspace solid-phase microextraction (HS-SPME) was developed which adsorbed volatile compounds from the whiskey glass. The HS-SPME method developed was used to analyze whiskey volatile aroma-active compounds. The compounds were identified and quantitated using gas chromatography-flame-ionization detector (GC-FID).

For comparison a whiskey sample is quantitated by direct injection and analyzed using gas chromatography-mass spectrometry (GC-MS). Ethanol evaporation experiments were conducted to verify the working principle of the novel whiskey glass. Gas chromatography-olfactometry (GC-O) was used to recognize that the aroma-active compounds of whiskey extracted from the novel glass can be perceived.

The HS-SPME method from the whiskey glass was developed to simulate the natural whiskey nosing conditions to ensure relevant results. Several matters regarding the HS-SPME method were considered. These included duration, timing, closed or open headspace and temperature. With the developed method whiskey odorants known to be in the whiskey sample could be identified. Quantitation of these odorants was more challenging and subsequent analyses showed variation in quantities. GC-O also did not perfectly correlate with quantitation's indicating that some odor-active compounds were under the detection limit.

Keywords: Whiskey, HS-SPME, Olfactory, GC-MS, GC-O, Aroma, Glass

Contents

ABREVIATIONS

AEDA	Aroma extraction dilution analysis
DVB-CAR-PDMS	Divinylbenzene-carboxen-polydimethylsiloxane
GC	Gas chromatography
GC-FID	Gas chromatography-flame-ionization detector
GC-MS	Gas chromatography-mass spectrometry
GC-O	Gas chromatography-olfactometry
SPME	Solid-phase microextraction
HS-SPME	Headspace solid-phase microextraction
KOC	Key odor compound
OT	Odor threshold
PDMS	Polydimethylsiloxane
PDMS–DVB	Polydimethylsiloxane–divinylbenzene
PCA	Principal component analysis
RI	Retention indices

1. Introduction

Whiskey is an alcohol beverage with aficionados around the world. Whiskey enthusiasts often research different whiskey styles and what gives them their unique attributes. An attribute that many consider essential for the whiskey experience is aroma. Drinking vessel shape contributes a lot to the whiskey aroma and many enthusiasts have their preferred whiskey glass.

There have been studies focusing on the volatile aroma-active compounds of whiskey. (Poisson & Schieberle 2008; Mall, Veronika & Schieberle 2018) Often these studies have focused on identifying different volatile compounds of different whiskeys. Less studies have attempted to quantitate these compounds. Few studies have also focused on the drinking vessel's impact on whiskey's attributes namely aroma. As of writing this, no efforts have been made to quantitate aroma-active compounds from a drinking glass.

Some whiskey drinking vessels have been developed to enhance the aroma qualities of whiskey. These glasses often rely on a tulip or bowl shape which condense the volatile aroma-active compounds into the mouth of the vessel. A problem with glasses shaped like this is that the ethanol vapor is also being condensed to the mouth of the glass. Ethanol is an anesthetic substance which in high doses hinders the human olfactory system. So even though tulip and bowl-shaped whiskey vessels condense the aroma compounds they also contribute to more anesthetic effect.

The aim of this study was to research a novel style whiskey glass. This glass, referred to as Savu glass, was developed to enhance the whiskey nosing experience. The glass achieves this by lessening the amount of ethanol vapor during nosing, therefore lessening the effect of ethanol anesthesia. A HS-SPME method which adsorbs aroma-active compounds from the Savu glass had to be developed. The aim was to be able to identify certain volatile compounds and possibly quantitate them using this method. Additionally, GC-O was conducted to recognize that the volatile compounds could be perceived. This in turn would indicate that the compound odor thresholds were exceeded.

1.1. Olfactory system

Olfactory system, better known as sense of smell, is the sensory system used in smelling. Olfaction sense has directly associated contributing organs. For many animals, olfaction has an influence on behavior, reproduction, and daily survival, but with humans the olfactory system mainly contributes to the perception and selection of food. Humans possess two nasal cavities with variations appearing between individual's nasal anatomy. (Doty 2015) A human nasal cavity can be seen in figure 1.

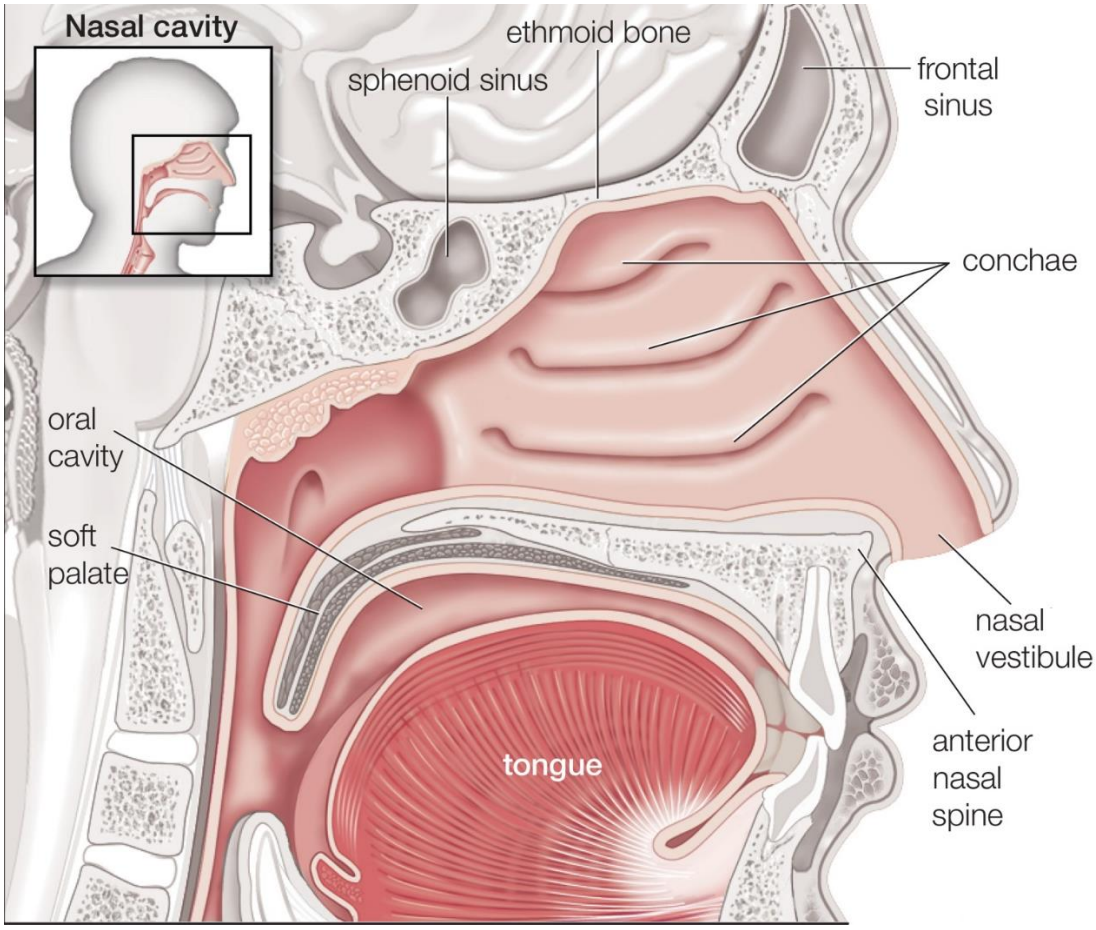

Figure 1. Nasal cavity anatomy. (Encyclopaedia Britannica, Inc 2022)

When humans inhale through the nose, airflow first arrives to the vestibule and the respiratory track, both of which contribute to temperature adjustment, filtering and humidification of the air humans breath in. (Craven et al, 2007) Odor compounds travel with the inhaled airflow to the nasal cavity. Gaseous odor

chemicals emit to the air from various sources. Vestibule is filled with hair like vibrissa which filters large airborne substances as well as pathogens. Naturally some of the airflow's odor compounds are stopped by the vestibule. Inferior and middle concha saturate and condition airflow which contains odor compounds. The airflow enters the lower respiratory airways from which oxygen travels to the lungs. Before this the olfactory epithelium collects odor compounds from the airflow. Variation in airflows movement and speed effect how saturated the air is when arriving to the olfactory epithelium. Thus, the olfactory sense of an individual is influenced by biological as well as physiological factors of the olfactory system. (Peterlin et al, 2008)

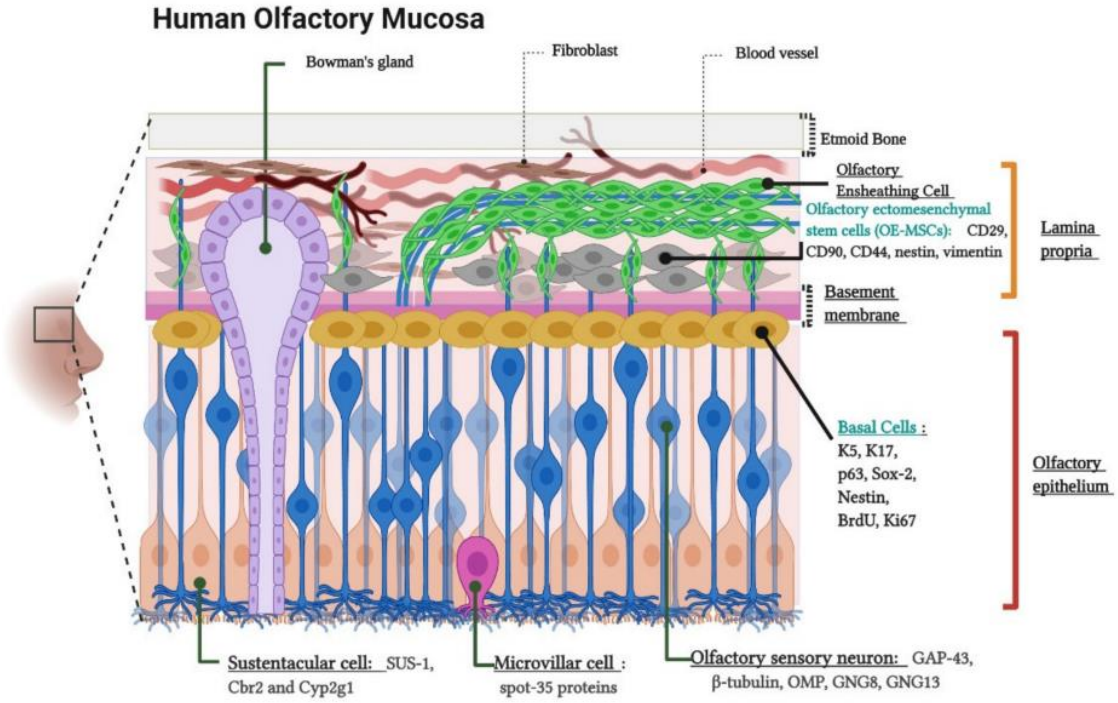

Figure 2. Cellular components of the olfactory mucosa. (Gómez-Virgilio et al. 2021)

Nasal cavity houses the olfactory cells, which are specialized for stimuli caused by odor active compounds. They form the olfactory epithelium, an epithelial tissue inside the nasal cavity. Olfactory epithelium along with other cellular components can be seen in figure 2. Microvillar cells increase the surface area where odor compounds are absorbed. The olfactory sensory neurons carry the odor compound stimulated signal to the olfactory bulb. Through the olfactory bulb the signal travels to the limbic system as seen in figure 3. (Ghatpande & Reisert 2011) The brains limbic system is responsible for behavioral and emotional

responses. (Queensland Brain Institute 2019) The olfactory epithelium is high in the nasal cavity which reduces the availability of perceptible odor active compounds. (Imamura ja Hasegawa-Ishii 2016) Yet, 400 receptor genes inside human's both nasal cavities enhance the olfaction sensitivity. Olfactory epithelium receptor cells have a thermal knob with cilia fibers attached to it. The function of these fibers is to increase the surface area of the receptor cells. The receptor cells send axons through cribriform plate, which is a sieve-like structure near the nasal cavity, to the synapses. From there the stimuli message is delivered to the olfactory bulbs' mitral cells forming synapses from which the stimuli are processed in the brain. (Lawless ja Heymann, 2010)

1.2. The sense of smell

Olfactory system, commonly known as the sense of smell, is used to detect, identify, and discriminate volatile aroma compounds from the environment. This sense directly affects survival by the perception of e.g. smoke indicating a fire hazard or a food spoilage indicating a danger of food poisoning. (Sorokowska et al, 2013; Rawson 2006) From a volatile aroma compound three properties affect the detection by olfaction sense: odor detection threshold, intensity function and variation in individual sensitivity. (Delahunty et al, 2006; Rawson 2006)

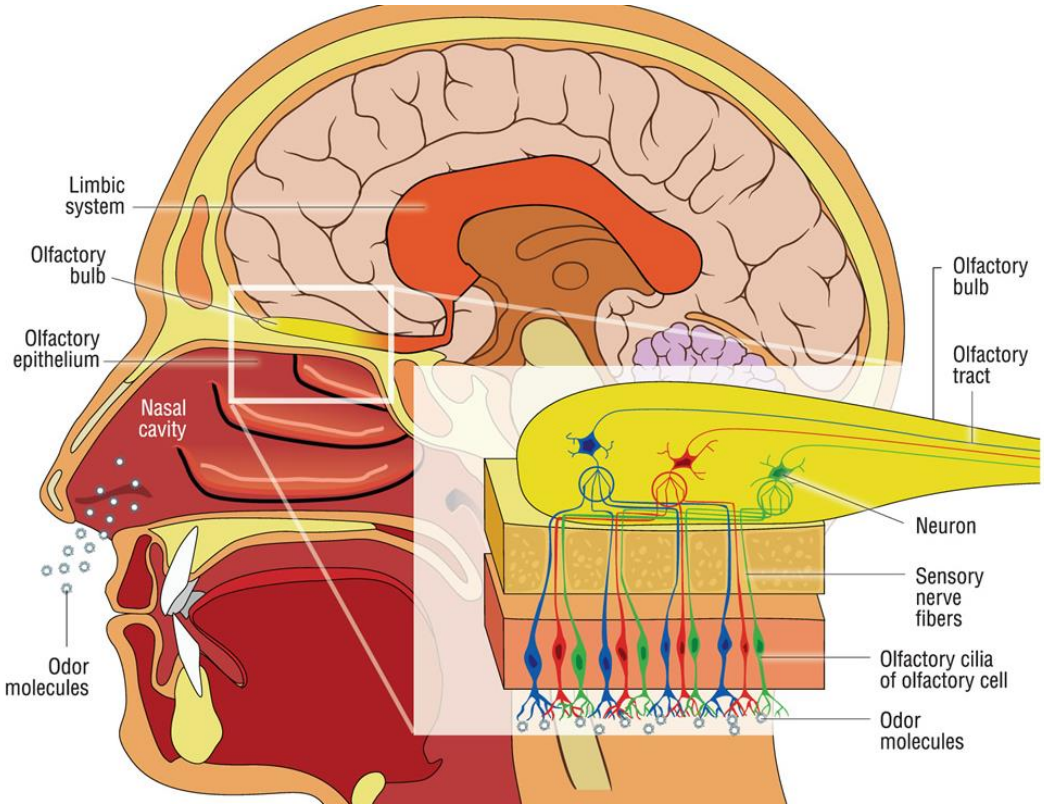

Figure 3. Human olfactory system detecting odors. (Coker 2013)

Odor detection threshold, or just odor threshold (OT), is the minimum concentration of a volatile aroma compound that can be perceived by human olfactory system. As seen in figure 3 odor molecules absorb and stimulate the olfactory cilia. The stimulation signal travels through the sensory nerve fibers to the neurons. Neurons in turn send signals to the brains limbic system which interprets the signal as an odor. (Ghatpande & Reisert 2011) Humans are able to detect odor-active compounds in concentrations of parts per billion (ppb). Yet, such extremely low OT volatile odors do not occur often. An example of these intense odors is the pungent and irritating sulfur with a minimum odor threshold of 670 ppb. Higher odor threshold volatiles are usually more common in human daily life. As an example, ethanol has an odor threshold of approximately 80 ppm. The lower the odor threshold goes the easier it is to detect the odor. In food substances, only the volatile compounds which concentration is higher than their odor threshold contributes to the aroma profile of the food. (Lawless & Heymann, 2010)

Different compounds' odor thresholds can be tested with different olfactory tests. Although, no certified standard tests for odor threshold determination have been

developed, the simplest test is to evaluate ascending odor concentrations from lowest to highest. This evaluation test can be performed with different olfactometers, gas chromatography olfactometry (GC-O) systems or sniffing sticks. The determined odor threshold values usually differ in literature because of these different test methods. Additionally, in these determinations fluctuating variables often go unmonitored. These variables include odor delivery, order of evaluated volatiles, pressure and temperature as well as the medium in which the odor active compound is presented. Furthermore, individuals performing these evaluations usually differ in their personal standard of detectability of different stimuli. (Lawless & Heymann 2010)

1.2.1 Aroma

The aroma of a food subject derives from all volatile compounds which emit perceivable odor. All volatile compounds which contribute to the perceived aroma are considered aroma compounds. Aroma is perceived through orthonasal route and gives information about the smell and quality of the food. Volatile compounds which contribute to aroma are formed by chemical or microbial reactions in the food or during food processing. There can be near 1000 different volatiles in some heated foods like tea or coffee. Yet, the combined concentration of volatiles present in food items can be very low at approximately 10-15 mg/kg. Additionally, only some of these volatiles contribute to the food's aroma. Thus, food's perceived aroma is dependent on the properties and amount of the odor active volatile compounds as well as human's sensory system. Key odor compounds (KOC) are volatiles with the characteristic aroma of certain foods. As an example, for a very smoky whiskey the KOC would be Guaiacol. (Belitz et al, 2009)

In modern food chemistry the focus is on characterizing and identifying the volatile compounds which affect aroma rather than identifying all the present volatiles of the food item. For this, sensory evaluations and analytical methods must be combined since a volatile compounds effect on the aroma cannot be known without sensory evaluations. Still, human's olfactory perception is poor at analytical work. (Ache & Young 2005) Recognition of several specific volatile compounds from a complex mixture is difficult, but with less than ten different volatiles it is feasible. Yet, a food item's aroma can be formed from tens or even hundreds of different volatiles. A food's aroma is affected by these volatiles

varying concentrations and structures as well as interactions between them. There is also interest to identify volatile aroma compounds which are produced during different food processing methods. One such method is fermentation which has been used for centuries but is still rather unknown in its contribution to aroma. (Belitz et al, 2009)

1.3. Individual differences

Several factors affect an individual's olfactory perception capabilities like age, genetics, experience, education, pregnancy and smoking. (Reed & Knaapila 2010) Also, many illnesses and poisonous substances can cause harm to an individual's olfactory perception. Additionally, the olfactory genes impact olfactory capabilities. Yet, environmental individual differences are believed to impact olfactory perception more than genes although there have been few studies on this subject. (Doty et al, 1984)

Olfactory perception differences in individuals can be compound or structure specific. An odorant's detection threshold can vary majorly between individuals. (Stevens & Cain 1987) Also, different odor thresholds, discrimination tests and descriptive analysis tests are hard to compare since the analyses are usually performed in different places with different intensities and the studies often have different goals. This means that these results are regularly research and location specific. Evaluation space should possess controlled lighting, be free of disturbances, be rid of excess distractive odors and be comfortable for evaluators. Usually, study locations do not possess these perfect conditions and vary in different fields. (Stone 2020)

Food's odor is thought to be more impactful for its flavor than taste. Odor perception has a direct influence on an individual's appetite. (Lisiewicz et al, 1966) Olfactory dysfunctions have been discovered to lower the pleasure derived from eating. (Ramaekers et al, 2014) Perceived odors affect the appetite towards similar products which means that dysfunctions in the olfactory system can change an individual's entire diet.

1.3.1. Age

Old age has a noticeable negative effect on a person's sensory perception. Age affects olfaction more affected than taste. Age related olfactory perception loss is called presbyosmia. Yet, some aged humans can maintain their olfactory sense on the same level as younger age groups. Multiple complex interactions in humans contribute to this phenomenon so it is difficult to find a definitive reason for it. There is high variation in studies related to presbyosmia, but it seems noticeable differences in olfactory sense occur at 50 years of age. (Schubert et al, 2013) Many studies on prebyosmia attempt to clarify whether the decline of olfactory function is in fact a result of aging or age-related illnesses like Alzheimer's for example. (Doty et al, 1984; Koskinen et al, 2003; Kremer et al, 2007)

Degenerative aging processes affect the olfactory epithelium and olfactory bulb. Olfactory bulbs volume can decrease as well as the epithelium thickness. This in return eliminates receptor cells affecting the olfaction sense. Additionally, the quantity of glomeruli and mitral cells decrease, and mesial temporal lobe area shrinks with age leading to reduced brain activity. This results in weakened perception or anosmia. With age, oral health also decreases resulting in weaker chewing. This results in fewer volatiles vaporizing from the mouth by retronasal routes affecting an individual's olfactory perception. (Malaty & Malaty 2013)

1.3.2. Gender

It is commonly believed that females outperform men in olfactory sense, and this has been confirmed in several studies. Women excel over men at odor identification, recognition, discrimination, detection and memory. Female olfactory sensitivity is thought to be linked to maternal behavior. (Brand & Millot 2001)

The difference in olfactory sense between genders is believed to be hormone based. It is thought that female hormones such as estrogen and progesterone enhance olfactory abilities, while with men, declining olfactory abilities have been linked with androgens. (Nováková et al, 2013) Estrogen, estradiol, estrone and estriol ration olfactory neurogenesis in the hippocampus which in return escalate

olfactory receptor neuronal cell propagation and decrease apoptosis. (Barker & Galea 2008)

1.3.3. Experience and learning

An individual's normal eating habits are mostly based on routines, so analytical sensory evaluation isn't normal regarding everyday food selection. Therefore, learning and experience plays a vital role in sensory evaluation. Analytical sensory evaluation differs largely to everyday eating habits. Since normal eating habits are based on routines there is a comparison point with previously purchased products. As with sensory evaluations, the evaluators often lack a comparison point. Additionally, with analytical sensory evaluation, the participants may not be given the history, producer, or origin of the evaluated product. Also, the product must solely be evaluated by the instructed properties and in the given order. In addition, the portion sizes in sensory evaluations are very small compared to normal day food portions. Even the location is controlled in analytical sensory evaluations, since they usually take place in plain booths with good lighting to present the sample as clearly as possible. Although nearly every aspect of a sensory evaluation is controlled, the evaluators answers are still established by the evaluators own pas and preferences. (Sidel & Stone 1993)

Evaluators usually have varying levels of experience. Experience in analytical sensory evaluations can have a positive or negative effect on the evaluations. In sensory evaluations the information from the evaluator is given in words. This leads to people with wider vocabularies being able to give more accurate and detailed answers. (Lawless & Engen 1977) This also applies to individuals with more experienced in analytical sensory evaluations. Yet, the same experience can lead to repetition of certain attributes from earlier evaluations. Thus, the experience difference leads to variation in results. (Lawless & Heymann 2010)

Experience and learning effects the human olfaction in several ways. Experienced individuals have a memory bank of odors to help in recognition and comparison. In evaluations the answers are usually based on previously learned information. Familiar stimuli, such as odors, evoke memories and emotions due to the olfactory neurons direct connection to the limbic system. The memory of a certain odor can result in two different outcomes. The sensing of an odor can be

adapted to familiar odors, leading to a decrease in intensity, or familiar odors with emotional background can be sensed as more intense. (Sidel & Stone 1993)

1.4 Sensory evaluations of aroma

With sensory evaluations several external and subjective factors affect the objectivity and accuracy of the results. With aroma sensory evaluations some volatiles contribute more to the overall aroma of the food item than others. These KOCs are usually associated with particular smells. KOCs can be identified with GC-O by injecting the extracted sample material to the GC. The compounds, including the KOCs, are separated in the GC column and are presented to the evaluator separately. Yet, GC-O gives only directional results of the aroma since the aroma is largely affected by the interactions with other volatiles.

1.4.1. Problems with aroma evaluations

Humans are individuals with their own experiences and capabilities when it comes to sensory evaluation. As humans are not analytical instruments, they usually sense what they expect to sense in evaluation situations. This is because several physical and chemical as well as mental issues affect a human's judgement. Comparison of odors comes much more naturally to evaluators than straight measuring of an attribute. As an example, it is easier for people to find the smokiest sample from a set of samples than it is to measure a samples smokiness on a numeral scale.

With practice and repeated evaluations, the OT of a compound may decrease for some people. Also, individuals may experience a sensitivity increase to some odors with increased exposure. Some volatiles possess suppressing, enhancing or synergistic effects to a food item's aroma. Different volatile compounds can either inhibit or suppress other compound's odors. These volatiles lower or increase the OT of other compounds. Therefore, changing aroma characteristics and making identification more difficult. (Lawless & Heymann 2010)

Anosmia is the loss of olfaction sense or complete inability to smell. Anosmia occurs more commonly among older people. It is estimated that around 5% of the world's population suffers from some degree of anosmia. (Brämerson 2004) Anosmia can affect only certain odors or a wider range of different odors.

Anosmia symptoms progress alongside with neurodegenerative diseases but anosmia can occur immediately from birth. This inability to smell from birth is called congenital anosmia. (Huart et al, 2011)

Human sense of smell is highly adaptive. Continued exposure to an odor can lead to decreased sensitivity towards that odor. This phenomenon can easily be noticed when changing environments. For a while the new environment's characteristic aroma is highly perceivable but with time the aroma perception vanishes. Adaptation of olfaction sense occurs because no new information of the environment occurs. This phenomenon can lead to the carry over effect in sensory evaluation. This means that certain odors or properties from an earlier sample get carried over to the next sample. (Kemp et al, 2011; Lawless & Heymann 2010)

1.4.2. Ethanol effects on the olfactory system

With whiskey, as well as other alcohol beverage's aroma evaluation, ethanol anesthesia is a major hindering effect. This effect is more severe with beverages with a high alcohol concentration. Ethanol is the most abundant odorant in all distilled alcohol beverages, such as whiskey. Ethanol ingestion modifies an individual's olfactory sensitivity to ethanol and can influence odor discrimination. (Manska 2018)

Ethanol causes negative anesthetic effects on human olfactory bulb sensory synapses. Abundant ethanol concentration can cause pain to olfactory receptors. This effect is rather individual and occurs more often with sensitive noses. Mucous congestion can occur due to ethanol irritating the olfactory epithelium which hinders odor detectability. Ethanol can be introduced to the bloodstream through the pulmonary system which leads to cognitive impairment. Ethanol anesthesia can affect all of the 636 different olfactory receptors. Yet, more research is needed for identification and quantification of the anesthetic effects of ethanol receptor binding. (Manska 2018)

1.5. Whiskey odorants

Different whiskeys have a plethora of odorants ranging from a sweet caramel to a peated smoky scent. Whiskey odorants derive from different stages of

manufacturing. A heavy peat or smoke odor arrives from the amount and quality of used peat in drying the malts. Many sweet berry-like odors derive from the barrels used in aging the whiskey. For example, former sherry casks are widely used in whiskey aging which contribute berry-like odorants commonly found in wines. Yet, the most abundant odorant in any whiskey is ethanol.

Table 1. Odorants and their concentrations found in a peaty whiskey as found in a study by Veronika Mall and Peter Schieberle. (Mall & Schieberle 2018)

Odorant	**Concentration (µg/l)**
2-Ethylphenol	870
3-Ethylphenol	537
2-Methylphenol	4120
Creosol	1790
4-methylphenol	2900
4-ethylphenol	2740
m-Cresole	1400
o-Guaiacol	2600
2-Methoxy-5-methylphenol	122
Guaiacol	97,6
4-ethylguaiacol	1370
Whiskey lactone	2000
Eugenol	139
Vanillin	3140

Ethanol has an anesthetic effect on the human olfactory system. This in return affects odor discrimination. Additionally, ethanol causes pain to olfactory receptors, and irritates and enflames the olfactory epithelium. Ethanol is also the first odorant to reach the olfactory system due to its high volatility, high vapor pressure, and a low boiling point and surface tension.

1.5.1. Whiskey glasses

There are several different glasses used for whiskey nosing and tasting. These glasses differ significantly in their size and shape. Both size and shape of the

glass contribute to the overall experience of whiskey nosing and tasting. Both attributes also differ largely depending on the whiskey glass. Some of the glasses to be discussed are more widely used for other strong spirits, such as cognac, but can and are still used also with whiskey.

Tulip and copita style whiskey glasses are most widely used worldwide. The traditional copita glass dates to the early 1800s when these shaped glasses were more widely used for 20 alcohol by volume spirits. In the 1800s the copita style glass received a reputation of quality and has since become the standard for strong spirits. Copita glass has distinctive characteristics such as tall bowl height, narrow bowl diameter, small rim diameter and mostly straight or convergent sides. These characteristics have endured from the 1800s to this day. Two characteristics most subject to change are the level of converging sides and the rim diameter. In Figure 4 is a copita shaped glass.

From the copita glass, the more tulip shaped Glencairn glass was developed in Scotland in the early 2000s. This glass was designed by Raymond Davidson, the managing director of Glencairn Crystal Ltd. The glass was designed with the aid of master blenders from five large whiskey companies in Scotland. The Glencairn glass rapidly became the gold standard glass for whiskey and is endorsed by the Scotch Whiskey Association.

Copita style glasses, although being the most used, have severe drawbacks to their whiskey nosing experience. The small rim diameter prevents the nose from properly entering the glass. Additionally, the narrow rim diameter results in concentrating ethanol on the rim which crowds other aroma compounds. Furthermore, the convergent, vertical or nearly vertical walls contribute more ethanol concentration to the rim. The bowl diameter is too small for efficient swirling when comparing to the glass tall headspace which results in fewer aroma compounds reaching the rim. Further, the tall rim height prevents the detection of some large-mass aroma compounds.

The tumbler whiskey glass is much used among whiskey drinkers. Tumbler is widely used when ice cubes are added to the housed spirit. Tumbler glass has a wide rim (≈80 mm) with a similar inside height and vertical or slightly convergent

sides. The tumbler came to existence in 1600s and later in 1700s became the most used whiskey glass due to its low price.

The tumbler has some good qualities when it comes to whiskey sniffing. The wide and slightly rounded bottom allows for reasonably good swirling. The wide rim allows for some ethanol dissipation lessening the effect of ethanol anesthesia. Additionally, the wide rim allows the nose to get closer to the whiskey allowing more aromatic compounds as well as large-mass aromatic compounds to be detected.

A less popular glass among whiskey enthusiasts is the snifter. Snifter is widely used with cognac but can equally be used with whiskey. Snifter has a very wide bowl diameter (≈100 mm) which allows for great swirling. However, snifter glass has highly convergent walls and a rather narrow rim, like copita glass. These attributes result in massive ethanol concentration and minimal ethanol dissipation causing severe ethanol anesthesia.

A whiskey glass that is based on science is the NEAT glass. Its name is an abbreviation of Naturally Engineered Aroma Technology and as the name implies this glass was designed to provide a better whiskey nosing experience. NEAT glass has been studied at the University of Nevada, where it was compared to other spirits glasses such as Glencairn. NEAT glass came to be in 2002 as a result of a glass blowing mistake creating its signature shape. Later in the 2010s the shape was refined and since NEAT glass was released. It has won several awards and is used in some spirits competitions by judges. NEAT whiskey glass can be seen in Figure 4.

Figure 4. A. NEAT whiskey glass. B. Copita glass. C. Snifter glass.

The NEAT whiskey glass has short walls which allow the perception of large mass aromatic compounds. It has a similar bowl diameter as tumbler glass (≈80 mm) which allows for sufficient swirling. NEAT glass has converging walls which would concentrate ethanol, but closer to the rim the glass has flared walls which dissipate ethanol away from nose.

1.5.2. Different glass shapes influence on aroma

There aren't many studies conducted on the influences on aroma that different whiskey glasses have. Yet, there have been studies which focus on different alcohol and non-alcohol beverages and their distinct glass's effects on aroma as well as taste. These beverages include wine, beer, vodka, lemonades as well as coffee and tea.

Many studies have been made with and regarding wine. Some of these studies focus strongly on different glasses' effects on taste and aroma. Research named "*The effect of glass shape on the concentration of polyphenolic compounds and perception of merlot wine"* by K. Russel focuses on three different glass shapes and their influence on aromas perceived from wine. These glass types are Flute, Bordeaux and Martini. In this study a 12-member panel could not perceive differences over aroma regarding the glass type. (Russell et al, 2005) Another study focusing on glass shape influence on wine aroma researched four glasses. Three of these glasses were similar in shape (bulb-shape) with differences in opening diameters and one glass as square-shaped straight wall glass. Study

had 30 panelists doing blindfolded testing on the different glasses. The results showed the Bordeaux glass having a significantly lower rating for aroma intensity. Therefore, the study showcased that vessel shape can have an impact on the perceived aromas of beverages. (Delwiche & Pelchat 2000)

Figure 5. A. Flute glass. B. Bordeaux glass. C. Martini glass.

A study by Carvalho and Spence researched the impact of cup shape on coffee taste and aroma. The study noticed differences in three different cup styles regarding taste attributes and aroma intensity. (Carvalho & Spence 2018)

Figure 6. Cup shapes used in Carvalhos' and Spences' study. (Carvalho & Spence 2018)

The study had a large pool of participants. 276 evaluators in total with over half of them professionals from different fields linked to coffee. The study concluded that split and open style cups contributed to different taste attributes, while the tulip shape contributed mostly to aroma intensity. (Carvalho & Spence 2018) Tulip shaped vessels have also been widely considered to intensify aromas with whiskey. Scotch single malt whiskeys are often served from different tulip shaped glasses to intensify the aromas of the spirit.

1.5.3. Sensory analysis of whiskey

There are several ways of evaluating whiskey's different attributes. Several whiskey enthusiasts, hobbyists, distillers, as well as tasting professionals have different methods to whiskey evaluation. Several different whiskey's tasting and nosing methods have been described in different whiskey related literature and media. There has even been research on multisensory environment's effect on whiskey tasting and nosing. Creating different atmospheres be it by for example room décor has an effect on the whiskey experience. (Velasco et al, 2013) Due to the several different opinion-based evaluation methods, there is a popular saying *"Tasting whiskey is as much art as it is science"* which seems to ring true.

Following tasting, nosing and visual sensory analysis methods arrive from *Cyril Mald* and *Alexandre Vingtier* written book "*Iconic Whiskey: Tasting Notes & Flavour Charts for 1,500 of the World's Best Whiskies*".(Cyrille & Alexandre 2016) Mald is a whiskey and spirits reporter, as well as the ambassador for Scotch Malt Whiskey Society in France. Vingtier is the head of selection at France's leading whiskey importer and distributor Maison du Whiskey, as well as a freelance spirits consultant. Additionally, he is the former Editor-in-chief of Whiskey Magazine & Fine Spirits, a regular editor to France's leading wine and spirits magazine La Revue du Vin de France and the founder of Rumporter magazine.

1.5.3.1. Visual analysis

Sight is the first sensation simulated when evaluating a whiskey. Optimal temperature for evaluating whiskey is that of room temperature, between 18 ºC and 22 ºC. In evaluation, a normal whiskey sample quantity is between 2 to 4 cl. In whiskey evaluation, it is common to swirl the glass in a circular motion. This increases the oxidation of the surface, which can result in obtaining dry residue as well as bringing out aromas from the bottom of the glass. (Cyrille & Alexandre 2016)

Attributes which can give information about the whiskey sample are color, clarity and viscosity. Color can determine information such as what type of cask was used in whiskey ageing, as well as how long the whiskey has been matured. This can in return lead to predetermined assumptions of the flavor and aroma of the sample. Whiskey can also be artificially colored with caramel (E 150) which can

lead to false visual evaluation. In addition to giving whiskey a richer brownish color, the caramel can also have a negative impact on the whiskey's aromatic profile. (Cyrille & Alexandre 2016)

The clarity of a whiskey can reveal if chill-filtration was used during manufacturing. Chill filtration is used to clean defects and balance out a whiskey. Yet, chill filtration can result in the whiskey losing fatty acids, proteins and esters, which effects its aromatic profile. Thus, it deprives the whiskey of complexity and richness. A non-chill filtered whiskey will become cloudy when it drops below a certain temperature, or when water is added to the whiskey. This is not a defect, nor does it say anything about the quality of the whiskey. The cloudiness happens due to certain compounds being soluble only above 46 % of alcohol. (Cyrille & Alexandre 2016)

Whiskey's viscosity can allow an assessment of its alcohol percentage. A whiskey's viscosity is influenced by the difference in surface tension between water and alcohol. Additionally, the surface tension leads to the Marangoni effect, which is the visible mass transfer along an interface between two fluids. This is visible after swirling as "tears" along the inner surface of the glass. More and slower tears result in a higher alcohol content. Also, the thicker these tears are the more fatty acids the whiskey contains. (Cyrille & Alexandre 2016)

1.5.3.2. Olfactory analysis

In their book *C.Mald* and *A.Vingtier* divide the olfactory analysis of whiskey into six stages. These stages differ largely by the position of the nose towards the whiskey while analyzing. Through these different position differences between the nose and whiskey different aroma compounds will be detected. (Cyrille & Alexandre 2016)

In the first stage of olfactory analysis the whiskey glass is positioned in an upward position with the nose directly above the glass. Importantly, the whiskey won't yet be swirled at this stage to avoid the aroma compounds getting concentrated. This stage allows the perception of the lighter volatiles. This stage also serves as an adjusting period for the ethanol, preventing the ethanol from "burning" the olfactory sense. (Cyrille & Alexandre 2016)

In the second stage of sniffing the glass vessel is turned sideways so it is perpendicular to the evaluators face. While evaluating aromas the glass is moved vertically up and down. Different aromas from heavier to lighter compounds should be sensed this way. The heavier aromas stay closer to the bottom of the glass while the lighter more volatile aromas are sensed higher up the glass. Heavier aromas include smoky, earthy and woody aromas while the lighter aromas include floral, fruity and berry-like aromas. In the middle part are the medium weight aromas which include spicy and malty aromas. In the third stage the evaluators nose should be a centimeter above the glass rim while sniffing. This technique isolates the lightest and most volatile compounds from the heavier aromas. This way the heavier more powerful aromas do not interfere with the lighter aromas such as floral aromas. (Cyrille & Alexandre 2016)

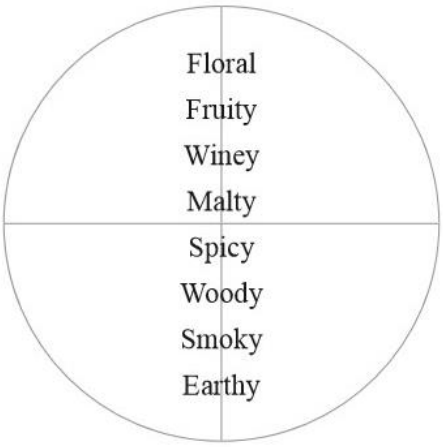

Figure 7. Aroma strata of second stage olfactory evaluation. (Cyrille ja Alexandre 2016)

The fourth and fifth stages involve techniques to use during the three different methods described in stages one through three. Fourth stage involves changing the rate of inhalation during evaluation. This will diversify the detection of different odor compounds by their binding ability to the olfactory mucus. Low binding capability molecules can be more easily detected when inhaling slower, while high binding capability molecules can be easily detected when inhalation is more rapid. This is due to the high binding molecules saturating the first part of the epithelial zone before having enough time to stimulate the entire olfactory surface. Fifth stage involves varying the nostril used for sniffing in evaluation. Generally, one nostril is responsible for 80 % of inhalation while the other is obstructed by the swelling of the inner nasal concha. Therefore, the two nostrils

inhale at different rates and one of them leans towards conveying volatile aromatic compounds more efficiently. Thus, both respiratory nostrils contribute a different olfactory perception. (Cyrille & Alexandre 2016)

The sixth stage is determining the perceived aromas by referring to the aromatic wheel. Many individuals can perceive the same aromatic compound in different ways, yet the element doesn't change. The aroma wheel has different compounds classified to the same family if their structural similarities reflect their aromatic similarities. The aroma wheel has the advantage of providing a unified grammar regarding different whiskey aroma characteristics to both new and experienced evaluators. Additionally, for novice evaluators the aroma wheel is a great tool for learning. (Cyrille & Alexandre 2016)

Figure 8. Whiskey aroma wheel. (Aromaster.com 2022)

The seventh stage involves drinking as well as sniffing water every now and then when evaluating. This “neutralizes” the senses which can in turn help detect different stimuli previously missed in initial evaluation. In the eight stage the whiskey is tasted in small doses of a few milliliters at a time. Small tasting doses accustom the palate to the high amount and potency of alcohol. To stimulate salivary glands, the whiskey should be circulated in the mouth. Mastication releases aromatic compounds into the oral cavity and will enhance the retro-nasal stimuli. It also increases the aromatic molecules released when swallowing. These aromatic molecules are released to the back of the throat reaching the olfactory mucosa through there. The ninth stage involves this retro-nasal experience and how to enhance it. After swallowing a small amount of whiskey, one should exhale deeply through the nose. This increases the airflow from the throat to the olfactory system. (Cyrille & Alexandre 2016)

The tenth stage involves the final stage of olfactory evaluation of whiskey. This takes place after the whiskey glass has been emptied. An empty whiskey glass contains a brownish dry extract from the non-volatile and very low volatile compounds of the whiskey. The dry extract aromas can emerge for minutes or up to hours. These aromas are usually described as woody. These aromas can further be enhanced by closing the whiskey glass after emptying it and letting the aromas build up inside the glass. (Cyrille & Alexandre 2016)

1.6. Gas chromatography-olfactometry (GC-O)

GC-O is a method of gas chromatography where a willing participant is the detector used to discover odorants. Odorants are commonly analyzed with gas chromatography coupled with mass spectrometer. However, this type of analysis often cannot detect some lower concentration volatiles in food items. The advantage of GC-O is that it gives the information of human sensory perception even if the odor compounds aren’t detected with other instruments. This occurs when a volatiles concentration in the food item is higher than its OT. However, information on an odorant’s behavior in a complex mixture cannot be analyzed through GC-O. A layout of the GC-O instrument is shown below in Figure 9. (Davoli et al, 2003)

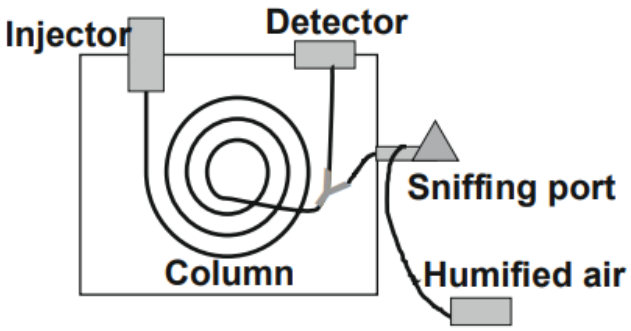

Figure 9. Layout of a GC-O instrument.

A GC-O evaluation is performed by the evaluator sniffing the GC eluate flow. Y-Connector is used to split the eluate flow to the flame-ionization detector (FID) and the odor port in equal measure. Evaluators give a signal for as long as they detect an odor which results in a retention time and intensity. The retention times can be used to identify compounds with GC-MS analysis. Also, evaluators give descriptions of the smelled odor compound. These descriptions can be connected to the corresponding odor-active compound.

There are also other alternative GC-sniffing techniques to GC-O. Some of these include aroma extraction dilution analysis (AEDA), detection frequency methods and Charm-analysis also known as combined hedonic response measurements. These procedures are based on the OT principle. With AEDA and Charm-analysis a dilution series of the sample in question is analyzed with GC attempting to determine the lowest perceivable concentration. The difference of these analyses resides in the sample presentation order. With AEDA the diluted samples are presented in decreasing order, while with the Charm-analysis the sample dilutions are presented in a randomized order. (Brattoli et al, 2013) Also, AEDA measures only the highest concentrated sample dilution while the Charm-analysis also measures the duration of odor-active compounds. These two analyses are very time consuming and because of that the sensory analyses are performed with few evaluators. (van Ruth 2001)

In perception frequency methods the same sample is evaluated multiple times and the accumulative response towards the stimulant is the acquired result. GC-O evaluations give the participants only a single chance to detect an odor from one compound. Detection frequency methods use evaluators effectively since no training is required. Intensity ratings acquired from detection frequency methods

have been proven to correlate with the real sensory attributes. Yet, the results can be ambiguous because the used intensities don't always match with the intensities of the real-life samples. (Plutowska & Wardencki 2008)

GC-O has been used successfully used in studies concerning whiskey odorants. Some studies have been successful for example in comparing perceptible aroma compounds from matured and un-matured whiskeys. Studies have also compared the perception of aromas against their known quantities. Some variation has been found with compounds odor thresholds not always being perceived using GC-O. (Wiśniewska et al, 2015)

1.7. Headspace solid phase micro-extraction (HS-SPME)

SPME is a solvent free sample preparation technique where the sample material is adsorbed or absorbed to a solid phase such as fused silica fiber. Three different extraction methods exist: headspace, direct and membrane protected extraction. This study utilized the headspace extraction method since the compounds of interest were volatiles. The fiber used in SPME is enclosed with a liquid or solid stationary phase. SPME can be used to extract gaseous compounds with the headspace method or non-volatile compounds with direct and membrane coated extractions. The three SPME extraction methods are illustrated in Figure 10.

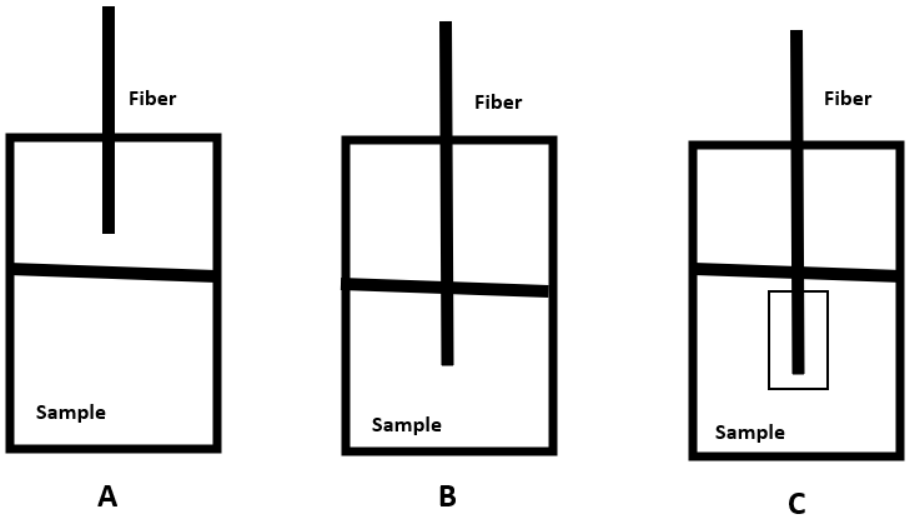

Figure 10. Three SPME methods: a) headspace extraction; b) direct extraction; c) membrane coated extraction.

There are multiple aspects to consider and optimize when using the SPME method. These include parameters such as fiber coating, sample volume, extraction time, desorption conditions and temperature. There are several more aspects which can be considered but the aforementioned parameters can be considered the most important regarding this study. Simplified, The aim of SPME is to extract the target analytes from the sample matrix. The challenging part is the preparation and exposure of the sample for SPME. The sample preparation can take a long time. Yet, the positive feature is that with a defined system the method parameters and preparation stages are repeatable and comparable to results as well as occurring errors. Ideally the sample preparation steps should be minimized to save time and reduce errors. (Pawliszyn 2012)

SPME has several positive features. Usage of SPME is easier since it requires no solvents and because of this it prevents pollution. SPME procedures can be automated, and little sample is needed in the process. Additionally, SPME sample preparation techniques couple well with modern GC instrumentation such as injectors and liners. The sample phase doesn't matter because qualitive and quantitative results are achievable from gaseous, liquid and solid phases. (Souza Silva et al, 2013)

HS-SPME has been widely used in other studies involving whiskey and other spirits. In these studies, volatile compounds have been characterized as well as quantitated. With these studies there are many variables to consider. Therefore, there is also a lot of time-consuming trial and error. (Poisson & Schieberle 2008) No universal guidelines for HS-SPME of whiskey can be determined since different volatiles and semi-volatiles found in whiskey act differently in different conditions. For example, different fibers are developed to absorb or adsorb different compounds more efficiently. Temperature increases the vapor pressure of different compounds in the headspace and can increase the diffusion rate into the fiber. Yet, too high temperature decreases highly volatile compounds adsorption into the fiber. (Câmara et al, 2006)

1.7.1 HS-SPME instrumentation

In HS-SPME the sample is usually placed in a sealed container. There an equilibrium is established between the sample material and air space. In HS-SPME the sample's temperature can be adjusted to a desired level using a heater

and a water bath for example. When equilibrium is reached inside the vessel, the fiber is placed inside the container for the desired extraction time. (Ramos 2012)

SPME analytical instrumentation focuses primarily on the development of new higher sensitivity coatings by utilizing selective sorbents as the coating materials. There are several coatings available, but the most utilized are polydimethylsiloxane (PDMS), polydimethylsiloxane–divinylbenzene (PDMS–DVB) and divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS). Different coating materials affect the quantity of extracted volatiles as well as the thickness of the coating. The quantity of extracted materials can be increased with thicker coating material since it possesses a higher surface area. Yet, a thicker coating requires longer extraction times leading to more time consumption. (Ramos 2012)

Relatively speaking fibers used in SPME have low operating temperatures (240 ºC-280 ºC). SPME fibers are known to crack or break due to excessive usage, temperature exposure and mechanical impact. A more porous coating material with higher operating temperature (320 ºC) has been developed using sol-gel technique. (Chong et al, 1997) Additionally, unbreakable SPME fibers are under development consisting of steel and titanium wires. (Bagheri et al, 2012)

1.8. Aim of the practical work

The aim of the work is to study the behavior of whiskey's aromatic volatile compounds in a novel whiskey glass, Savu glass. The Savu glass will be compared to a highly praised and universally used Glencairn whiskey glass. HS-SPME will be utilized to adsorb aromatic volatile compounds. HS-SPME hasn't been widely used to study the behavior of volatile compounds in drinking glasses. Therefore, a method for adsorbing aromatic volatile compounds from the two whiskey glasses must be developed.

Jim Beam rye whiskey will be used as sample in studying the volatile aromatic compounds behavior in the whiskey glasses. Thus, the important aromatic volatile compounds of the sample whiskey will be identified by their retention indices (RI) values and compared to other studies obtained RI values. To obtain

the RI values, a mixture of n-alkanes will be analyzed using the same GC method as used when analyzing the aromatic volatile compounds from whiskey glasses.

The rye whiskey odorants adsorbed from the whiskey glasses using HS-SPME will be quantified using external standards. 12 different standards are available which all contribute important aroma characteristics to whiskey. These standards will be used to prepare a mixture diluted in ethanol. The standard mixture will be further diluted with ethanol in stages. The standard mixture dilutions will be analyzed in the GC to obtain calibration curves for the 12 standard compounds and the curves will be further utilized to calculate the compounds quantifications in rye whiskey samples.

The Savu glass's ethanol emission reducing effect will be studied by SPME from the glass without sealing its headspace. The starting time for the open headspace SPME will be determined by an ethanol evaporation experiment. In this experiment, the sample whiskeys evaporation over time will be monitored by its weight.

GC-O evaluations will be held to determine the sensibility of whiskeys important odorants as well as the used standard compounds. GC-O evaluations will be held for the Savu glass with the rye whiskey sample. The volatile aroma compounds will be adsorbed with the developed HS-SPME method.

Sensory evaluations will be held to compare the Savu and Glencairn glass's ability to emit important whiskey odorants as well as to compare their sensing of ethanol. The hypothesis is that Savu glass will have little or no ethanol to sense and therefore will not cause anesthetic effects on the orthonasal sense. Furthermore, the Savu glass would still retain the emission of different important whiskey odorants.

2. Materials and methods

The work consisted of five interconnected sections. These include the HS-SPME from glass method development, identification and quantitation of important whiskey odorants, Savu glass ethanol emission experiment, GC-O evaluations, and data handling. The research was started by optimizing the HS-SPME from Savu glass. After the method optimization was finished the identification and quantification of important whiskey odorants was done while simultaneously carrying out some of the GC-O evaluations. After finishing the GC-O evaluations, the Savu glass ethanol reducing effect experiments were conducted. All the research sections were conducted in the laboratory and other facilities of the Department of Life Technologies in Turku University.

2.1. Sample and external standards

The whiskey sample used in the work was Jim Beam Rye Whiskey Pre prohibition style. The external standards used in this work were acquired from Sigma Aldrich. The standards and their odor descriptions, as acquired from literature, are listed in table 2.

Table 2. Used external standards and their odor description.

External standard	Odor description
Ethyl butyrate	Fake fruit, marker
Ethyl isovalerate	Sharp, fake fruit
Ethyl hexanoate	Fake fruit, sharp, sweat, marker
Guaiacol	Smoke, sweet
2-phenylethanol	Floral, rose
4-ethylguaiacol	Floral, sweet, rose, berry
Whiskey lactone	Coconut, stale
Eugenol	Spicy, smoky, vanilla
Vanillin	Marshmallow, vanilla
β-Damasceone	Apple
β-Ionone	Violet

2.2. Savu glass

The newly developed whiskey glass, which was studied in this work, was given the working name of Savu glass. The Savu glass can be viewed in Figure 11.

Figure 11. Savu glass.

The Savu glass was developed by Jari Tuominen of Kenzen ltd. Tuominen was also the initiator of this work.

2.2.1. Working principle of Savu glass

Savu glass is developed to enhance the experience of nosing a whiskey. The Savu glass negates the effect of ethanol on the nose, therefore allowing the easier sensing of different volatile aromatic compounds of whiskey. The working principle of the Savu glass can be viewed in Figure 12.

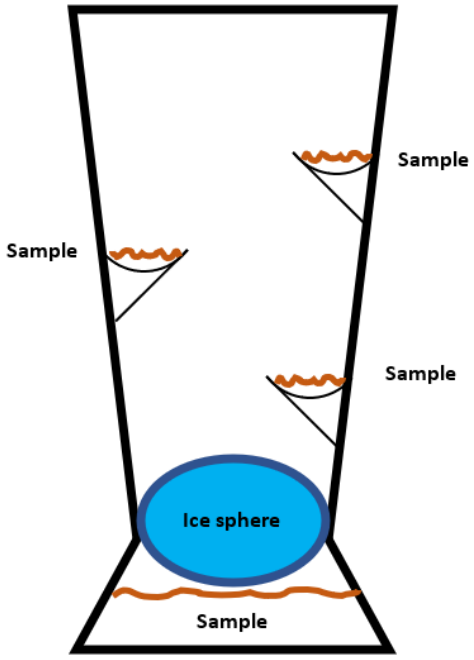

Figure 12. Working principle of the Savu glass.

The Savu glass works by trapping most volatile aromatic compounds along with ethanol in the space between the whiskey and the ice sphere. Above the ice sphere in the glass wall are three levels of 0,1 ml volume each. These levels fill with whiskey when pouring. It is from these levels that the volatile aromatic compounds evaporate to the nose. However, before nosing the glass should be swirled to allow the evaporation of ethanol from the levels. The time of swirling required for the evaporation of ethanol is determined in this work. After the ethanol has evaporated the whiskeys volatile aromatic compounds evaporate and can be sensed without ethanol interference.

2.3. Glencairn glass

The Glencairn glass is a widely used and considered a top tier whiskey glass. Its tulip shape allows for concentration of whiskey aromatics to the rim of the glass allowing for a wholesome nosing experience. This glass can be seen in Figure 13.

A:  B: 

Figure 13. A: Glencairn glass. B: Tumbler glass

Yet, its signature tulip shape also concentrates the ethanol evaporating from whiskey. This complicates the nosing experience as the pungent ethanol crowds the sensing of whiskey aromatics.

2.4. Tumbler glass

The tumbler is a classic style glass for enjoying whiskey. This glass has a wide diameter and either vertical or slightly, few millimeters, narrowing walls. Additionally, tumbler glasses often have a high glass base, which does not affect the whiskey drinking or nosing experience but is an interesting characteristic. The Tumbler is widely used as an “on the rocks” glass, meaning that this glass is often used when enjoying whiskey with ice cubes. The tumbler glass can be seen in Figure 13.

The tumbler has a wide rim which dissipates ethanol rather well which in turn mitigates the effect of ethanol anesthesia while nosing from a tumbler glass. Unfortunately, the wide rim also allows the important pleasant odorants to escape.

2.5. GC column selection

Several different GC columns were considered for use in qualification and quantitation. Few of these columns were tested for qualification of important whiskey odorants. The capillary column DB1-MS (30 m length; 0,25 mm diameter; 0,25 µm film thickness) was considered since several studies had used this column to quantify volatile aromatic compounds from high alcohol content spirits. Nevertheless, though DB1-MS gave clear sharp peaks for the lighter compounds, the heavier compounds were showing problems with the separation. The heavy compound peaks were hardly showing. Even with standard mixtures of these compounds the DB1-MS required excessive amounts of standard for a small response in the chromatograph.

SPB-624 (60 m length; 0,25 mm diameter; 1,4 µm film thickness) column was tested for better responses for the heavier aromatic compounds. This column is characterized to be slightly polar which can also assist in better separation for some of the compounds. SPB-624 has not been largely utilized in the separation of these compounds so reference data was scarce. Additionally, the column became prone to clogging.

The last tested and best confirmed column was HP5-MS (30 m length; 0,25 mm diameter; 0,25 µm film thickness). This column gave great responses for the lighter aromatic compounds exactly like DB1-MS column. Also, HP5-MS presented rather good response for the medium weight and heavy weight compounds. This column has been used in several liquor and alcohol spirit studies, meaning that reference data was readily available.

2.6. SPME fiber selection

Several different SPME fibers were considered for this study. Different fibers possess largely varying extraction efficiencies. Fibers have molecular weight ranges, and they also differ in polarity efficiency. The extraction is largely dependent on the fiber coating. Additionally, different coatings and mixtures of coatings possess varying extraction efficiencies towards different volatile compounds. Also, the physical properties of the fiber can affect the adsorption or

absorption. These physical properties consist of surface volume, pore size and more.

Four fibers were tested to determine if one or the other has better adsorption capabilities for whiskey odorants. These four fibers were 100 µm PDMS (Supelco, Bellefonte, USA), 75 µm CAR/PDMS (Supelco, Bellefonte, USA), 85 µm PA (Supelco, Bellefonte, USA) and 50/30 µm DVB/CAR/PDMS (Supelco, Bellefonte, USA). Fibers were tested by conducting SPME of a standard solution of whiskey volatiles. This standard solution and concentrations can be found in table 3. 10 µl of standard solution was pipetted to a 5 ml autosampler bottle which was sealed and the SPME duration for each fiber was 15 min. Before each SPME the fibers were conditioned in the GC inlet with the following parameters: PDMS 250 ºC 30 min, CAR/PDMS 250 ºC 30 min, DVB/CAR/PDMS 270 ºC 60 min and 270 ºC 30 min. The conditioning for fibers was conducted with RTX-5MS (15 m length; 0,2 mm diameter; 0,25 µm film thickness) column.

Table 3. Whiskey volatile standards in 40 % ethanol / water.

Standard	**Concentration (mg/l)**
Ethyl butyrate	0,875
Ethyl isovalerate	0,864
Ethyl hexanoate	0,869
Guaiacol	1,129
2-phenylethanol	1,02
4-ethylguaiacol	1,063
Whiskey lactone	0,952
Eugenol	1,067
Vanillin	10
β-Damasceone	0,8
β-Ionone	0,945

2.7. GC-O method

The GC-O was conducted by only two panelists. The column used with the chromatography was the HP5-MS column noted in chapter 2.5. Analysis conditions for the oven temperature were as follows: initial oven temperature 40

°C; 40-220 °C at 5 °C/min and 220-240 °C at 10 °C/min with a final time of 5 min. The HS-SPME method used in GC-O differed slightly from the one used with quantitation. Difference was that the whiskey sample was only pipetted to the Savu glass's layers for a total sample size of 0,3 ml. Leaving out the 2 cl whiskey sample from the bottom of the glass. Additionally, the ice sphere was not needed since there was no sample to lock out from the bottom of the glass.

2.8. Data analysis

The GC-O audio data was edited using Audacity (The Audacity Team, 2018, v. 2.3.0). Further data handling and chart building was performed using Excel. The peak identifications and areas as well as chromatograms in general were analyzed with UniChrom™ V (v. 5.1.12.258). Statistical analysis was conducted using Unscrambler X (v. 10.4.1).

3. Results

Several different experiments related to studying the principle of the Savu glass were conducted. Jim Beam Rye was used as a whiskey sample for all the experiments where a whiskey sample was needed.

This study was mostly a development project of new methods and techniques. Below is a list of goals to accomplish with this study:

- Develop a novel HS-SPME method from a glass drinking vessel.
- Identification and quantitation of volatile odor-active compounds from a drinking vessel.
- GC-O evaluations of odorants emitting from Savu glass.
- Demonstrate the ethanol anesthesia lowering effect of Savu glass in the nose.
- Conducting sensory evaluation using the Savu glass.
- Comparing quantitative whiskey odorant results and sensory evaluation results to other glasses commonly used with whiskey.

Table 4. Volatile compound chromatograph relative peak areas from Glencairn glass, Savu glass without ice sphere, and Savu glass with the ice sphere.

Compound	Glencairn	Savu without ice	Savu with ice	Odor description
Ethanol	97,6403 %	99,3373 %	98,1063 %	
2-methyl-1-propanol	0,0011 %	0,0002 %	0,0057 %	Damp
Acetic acid	0,0003 %	0,0040 %	0,2088 %	Vinegar
Ethyl propanoate	1,4039 %	0,0022 %		Fake fruit, marker
3-methyl-1-butanol	0,0009 %	0,0069 %	0,0262 %	Fusel, almond, chocolate
Ethyl butyrate	0,0001 %	0,0016 %	0,0021 %	Fake fruit, maker
Isovaleric acid	0,0014 %	0,0032 %	0,0148 %	Cheesy, fecal
Ethyl isovaleriate	0,0005 %	0,0002 %	0,0342 %	Sharp, fake fruit
Isoamyl acetate	0,1428 %	0,3051 %	0,9652 %	Fake banana
Ethyl hexanoate	0,2553 %	0,1947 %	0,4079 %	Fake fruit, sharp, sweat, marker
Guaiacol	0,0013 %	0,0010 %	0,0037 %	Smoke, sweet
Phenylethyl alcohol	0,0022 %	0,0003 %	0,0094 %	Floral, rosy
4-ethylphenol	0,0004 %	0,0008 %		Bandage
Phenylethyl acetate	0,0009 %	0,0021 %	0,0067 %	Floral
Ethyl guaiacol	0,0012 %	0,0008 %	0,0031 %	Spicy, woody
p-vinylguaiacol	0,0046 %	0,0015 %	0,0033 %	Curry, ruinous
cis-Whisky lactone	0,5324 %	0,1295 %	0,1683 %	Coconut
2,6-dimethoxyphenol	0,0004 %	0,0010 %	0,0027 %	Spicy, smoky, vanilla
γ-nonalactone	0,0015 %	0,0018 %	0,0043 %	Creamy, peach, strawberry
Eugenol	0,0022 %	0,0005 %	0,0096 %	Spicy
Vanillin	0,0007 %	0,0016 %	0,0071 %	Marshmallow, vanilla
cis-B-damascenone	0,0003 %	0,0006 %	0,0004 %	Apple
Ethyl cinnamate	0,0002 %	0,0004 %	0,0010 %	Fruity, fake
Phenylacetic acid	0,0027 %	0,0022 %	0,0040 %	Bad rose

Ethyl vanillate	0,0032 %	0,0025 %	0,0070 %	Spicy, bad, cinnamon
β-ionone	0,0006 %	0,0010 %	0,0014 %	Violet

Table 4 shows results from a previous study conducted on the Savu glass. The HS-SPME from Savu glass was conducted using a similar method described in chapter 3.2.1 of this study, with the difference of the SPME duration only being 7 minutes. Also, the analysis was conducted using a DB1-MS column in the GC-FID. From the results represented in table 4, a principal component analysis (PCA) was conducted with Unscrambler X and can be seen in figure 14.

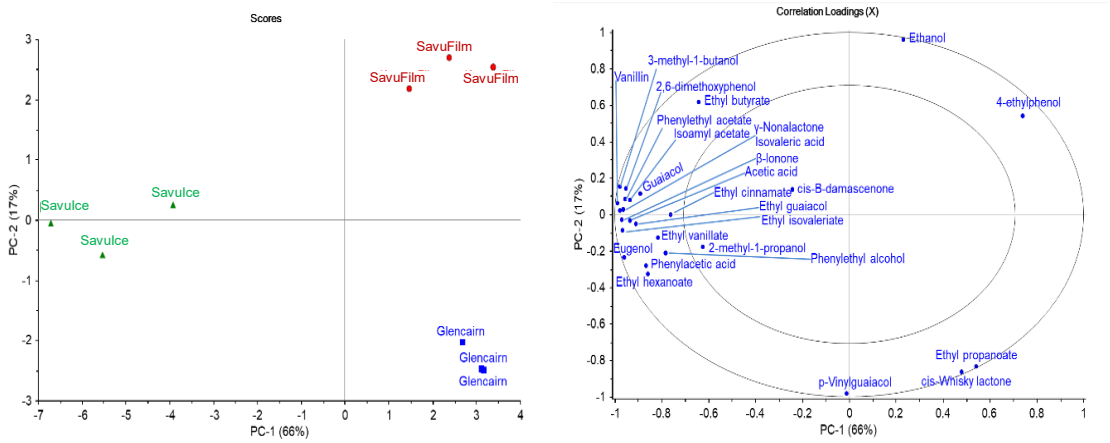

Figure 14. Scores and correlation loadings plot of all the aromatic volatile compounds relative peak areas.

From figure 14 we can see that the Savu glass with the ice sphere samples correlate with most of the aromatic volatile compounds. Glencairn samples correlate with three compounds and Savu glass without the ice sphere (SavuFilm) samples correlate with one compound 4-ethylphenol. Interestingly, in the relative peak area plots SavuFilm samples correlate more with ethanol than Glencairn glass samples. One could argue that Glencairn glass, with its ethanol concentrating tulip shape and smaller headspace, would correlate with ethanol more than SavuFilm samples.

3.1. Quantitation of important rye whiskey odorants

To enable quantitation of rye whiskey's odorants, standards were analyzed in different concentrations. The standards used are listed in table 2. All standard

concentration mixtures were diluted with 40 % ethanol in water. The concentrations listed in table 3 were diluted as 1:10, 1:100 and 1:1000. The analysis of these dilution series was used as external standard in quantitation of odorants from rye whiskey.

3.1.1. Direct injection of volatile aromatic compound standard mixtures

The dilution series of whiskey odorant standards were analyzed by direct injection method gas chromatography. Additionally, a rye whiskey sample was analyzed. These were analyzed and the odorants of the whiskey sample were quantitated using the dilution series as external standard. These quantitated odorants can be seen in table 5.

Table 5. Whiskey odorants quantitated from direct injection analysis.

Compound	**Concentration (mg/l)**
Ethyl butyrate	55,100
Ethyl isovaleriate	0,335
Ethyl hexanoate	1,783
Guaiacol	0,219
4-ethyl guaiacol	0,270
Whisky lactone	0,302
Eugenol	0,070
Damasceone	9,616

3.2. Whiskey sample HS-SPME from Savu glass analysis

HS-SPME was conducted from Savu glass with the goal of quantitating important volatile aromatic compounds generally found in whiskeys. External standard mixture of volatile aromatic compounds was also analyzed from Savu glass for quantitation.

3.2.1. HS-SPME method optimization

When optimizing the HS-SPME method from a whiskey glass there were several factors to consider. These include duration, timing, closed or open headspace and temperature. Temperature was decided to be kept as close to room temperature as possible, to simulate the whiskey nosing experience as closely as possible. Higher temperature could have yielded more odorants. However, it would also have yielded more ethanol and therefore increase the anesthetic effect. Also, higher temperature would have resulted in the ice sphere melting faster which in turn would have shortened the HS-SPME duration.

The melting time of the ice sphere in the Savu glass affects the maximum extraction time. In room temperature the ice sphere closed the headspace for an average of 28 minutes before dropping into the bottom of the glass. Therefore, the maximum HS-SPME time could be 28 minutes. Additionally, the extraction was not wise to begin immediately after inserting the ice sphere. This is due to the ice sphere not yet properly set to close the headspace. While the sphere was not properly set, excessive amounts of ethanol vapor could enter through crevices to the headspace. Thus, a setting time of 3 minutes was needed. This in turn dropped the maximum HS-SPME duration to 25 minutes. Additionally, the ice sphere often melted at different rates, so a long HS-SPME duration was deemed risky. A 15-minute HS-SPME was determined to be sufficient. This was because the fiber seemed to be saturated before 25 minutes had passed.

HS-SPME timing and duration can impact which odorants are mostly adsorbed. If the SPME is started early the lighter molecular mass odorants will be adsorbed, while if the SPME starts much later heavier molecular mass odorants will get adsorbed. When starting early with a long HS-SPME duration lighter odorants were adsorbed more. If the SPME was started later and the duration was shorter the response for lighter odorants decreased while the heavier odorants response increased slightly. Yet, the increased heavy odorant response was marginal at best.

Open glass SPME was considered and experimented with in this study to emulate nosing experience. When doing open glass experiments the SPME was conducted in a laminar flow cabinet to ensure minimum unwanted compounds

from the air getting adsorbed. Yet, the airflow of the cabinet also carries the wanted whiskey odorants away faster resulting in bad adsorption amounts. Therefore, the laminar flow was shut down when starting the SPME. This yielded slightly better results. Some lighter odorants, which were more abundant, were detected but the heavier odorants were not adsorbed at all. So, it was deemed more efficient to close the whiskey glass headspace with parafilm.

Final method used in quantitation was determined with aforementioned factors in mind. After the whiskey and ice sphere were inserted to the glass there was a 3-minute period where the headspace was still open. This was done to let some ethanol evaporate from the glass layers. After the wait time the glass's headspace was sealed with parafilm and the HS-SPME was conducted with a 15-minute duration. The chromatogram from using this HS-SPME method can be viewed in Figure 15.

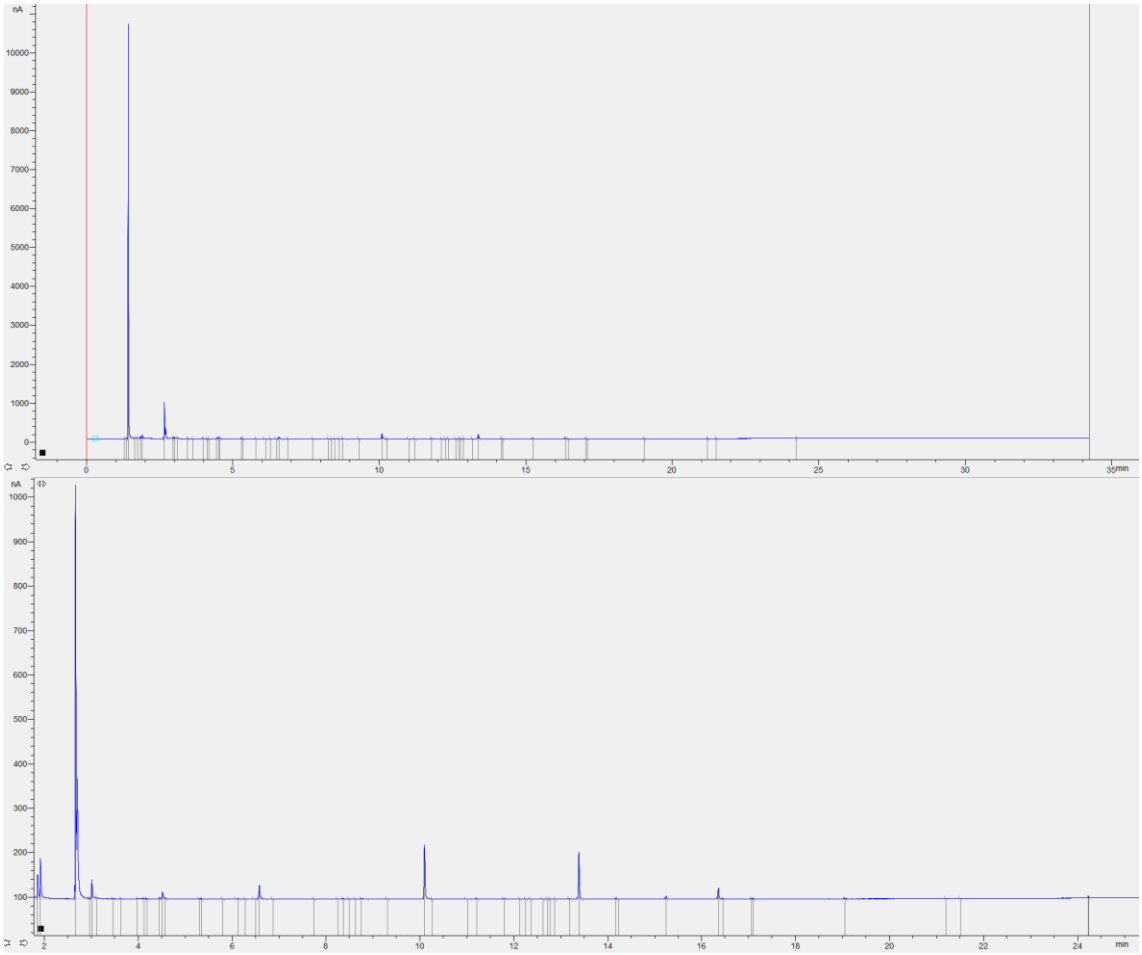

Figure 15. Chromatogram of HS-SPME from Savu glass and a zoomed in version which excludes ethanol spike.

3.2.2. Quantitation of important whiskey odorants from Savu glass

Analyses were performed with a HP 6890 gas chromatograph coupled with an FID. The capillary column equipped to the GC was a HP5-MS. Analysis conditions for the oven temperature were as follows: initial oven temperature 40 ^{o}C; 40-220 ^{o}C at 5 ^{o}C/min and 220-240 ^{o}C at 10 ^{o}C/min with a final time of 5 min. The injection was done manually and in pulsed split mode. The injected fiber is thermally absorbed with the injector at 250 ^{o}C during 3 min, with the pulsed-split injection inlet temperature of 290 ^{o}C, pressure 40 psi for 0,3 min and 50:1 split.

HS-SPME for quantitation of the whiskey odorants was conducted with the method described in the previous paragraph. The whiskey sample was analyzed three times and quantitation was calculated from each analysis. Quantitation was performed with external standard curves. The external standards in different dilutions were also analyzed using the same method.

Table 6. Whiskey odorant compounds quantitated from Savu glass.

Compound	**Concentration (mg/l)**	**Odor threshold (mg/l)**
Ethyl butyrate	0,502	0,001
Ethyl isovaleriate	0,297	0,240
Ethyl hexanoate	1,703	0,001
Guaiacol	0,730	0,021
2-phenylethanol	0,645	1,880
4-ethyl guaiacol	0,237	0,050
Whisky lactone	2,244	0,210
Eugenol	0,383	0,030
Damasceone	12,519	0,00002
Vanillin	17,262	0,200
β-ionone	0,0874	0,00009

All the quantitated compounds were above their odor thresholds as can be seen in table 6. Odorants such as Damasceone and Vanillin even showed excessive amounts. It can be argued that these have been falsely quantitated since the direct injection quantitation of this whiskey shows that the HS-SPME nearly yielded a perfect adsorption result. Also, the concentration from the HS-SPME

experiment for Damasceone and Eugenol was higher than in the direct injection experiment.

As an attempt to improve the quantitation results a new technique was attempted. In this the odorants were split into three groups: light, medium and heavy weight compounds, categorized by their molecular mass. The theory was that the light compounds would evaporate earlier hindering the evaporation, and in turn adsorption, of the heavier compounds. This method attempted to improve the adsorption efficiency of different weight compounds by letting lighter compounds evaporate away. The headspace was sealed right before beginning the HS-SPME to allow lighter compounds to evaporate. The HS-SPME duration was set to three minutes for these experiments. The light compound HS-SPME was started after three minutes of setting the ice sphere, medium weight compound HS-SPME started after seven minutes and heavy weight compound HS-SPME started after 11 minutes. The analyses were conducted with GC-FID.

The experiment of quantitating different weight compounds didn't work. The peak areas did not show up higher for their respected weights. Instead, the adsorption seemed to be rather even with different weight compounds even when the HS-SPME was conducted at different time windows. Additionally, the shorter SPME duration diminished the adsorption of some heavier compounds to not appear in the chromatograms at all.

Ethanol was not quantitated in this experiment. Still analysis using the same HS-SPME method was conducted on a Glencairn whiskey glass. This allowed to compare the ethanol peak areas between Savu and Glencairn glasses. Three analyses were conducted with both glasses. Savu glass showed an average ethanol peak area of 1980 while Glencairn glass showed an average peak area of 162875 for ethanol. This shows that if ethanol would have been quantitated it would have most positively been much lower with the Savu glass.

3.3. Ethanol evaporation experiments

Experiments were conducted to determine at what rate 96,6 % ethanol, 40 % ethanol in water solution and whiskey evaporates at room temperature. This

experiment would determine the point in time when ethanol would stop hindering the evaluation of whiskey's other aromatic compounds.

0,1 ml of sample was pipetted to a 70 mm watch glass, which was weighted on a laboratory scale to acquire the initial weight. A stopwatch was started when the solution was first weighted. New weightings were conducted every two minutes. Between the weightings the watch glass was twirled in a circular motion to increase the surface area of evaporation. This also simulates the swirling of a whiskey glass.

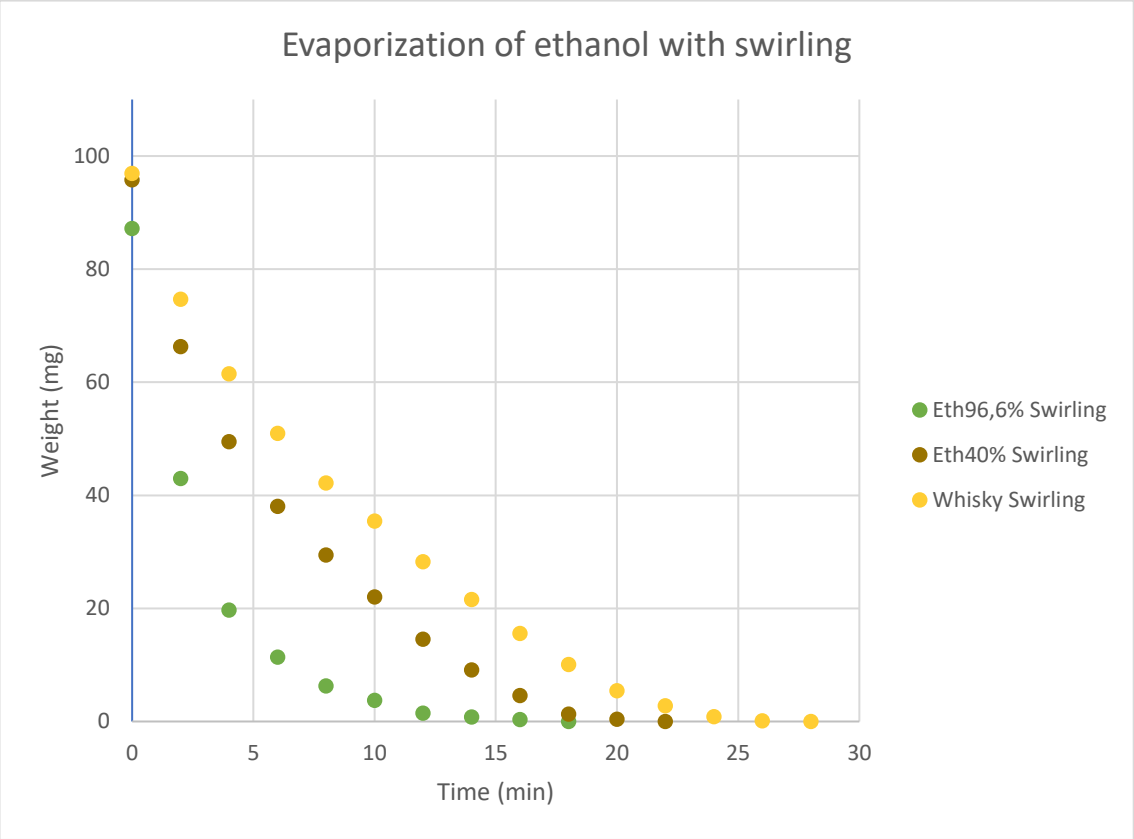

Figure 16. Evaporation of 96,6 % ethanol, 40 % ethanol in water solution and whiskey.

As seen in Figure 16 the evaporation rate is fastest with 96,6 % ethanol while slowest rate was with the whiskey sample. The goal was to determine the proper time to start nosing the whiskey from Savu glass. To avoid ethanol anesthesia near completely, the nosing should begin after 20 minutes. Yet, the ice sphere usually melted after 25 minutes. Additionally, the lighter volatile compounds will have evaporated after 20 minutes.

With 96,6 % ethanol we can see that 77,4 w% evaporated in 4 minutes. With 40 % ethanol the evaporation was slower with only 48,4 w% evaporated in 4 minutes. The whiskey sample had even slower evaporation with 36,5 w% evaporated in 4 minutes.

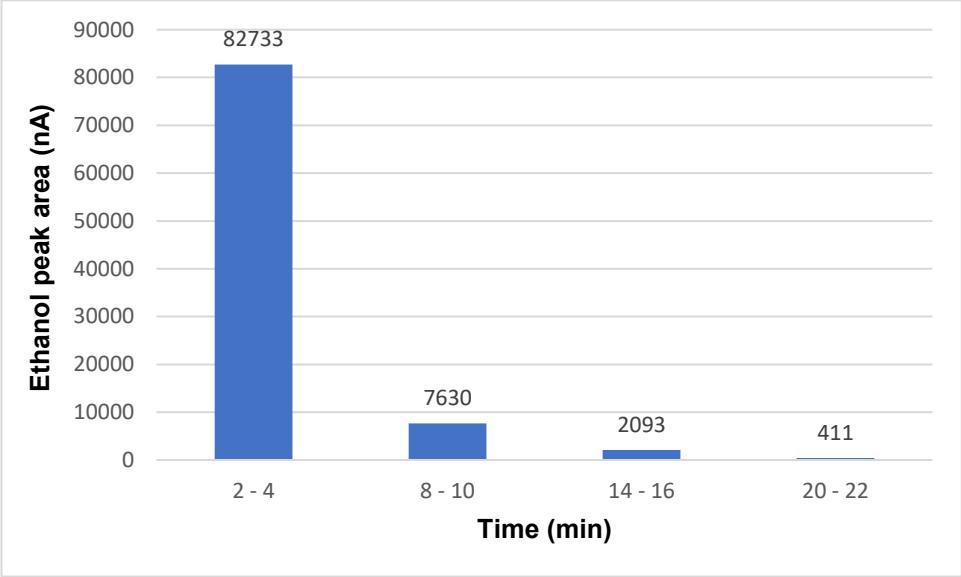

Figure 17. Ethanol peak areas in different time stages of Savu glass nosing.

Figure 17 shows how ethanol's adsorption by HS-SPME decreased from the Savu glass over time. In this experiment 0,1 ml of rye whiskey sample was pipetted on each of the glass's three layers and 2 cl of the same sample was pipetted to the bottom of the glass. The bottom was the sealed with an ice sphere. The timing began when the ice sphere was placed to seal the bottom sample. After this SPME for the duration of two minutes was conducted at four different time frames. The results were the analyzed by GC-FID.

Figure 16 shows how the ethanol peak area decreased over time. Ethanol evaporates rapidly from the Savu glass's layers. Within ten minutes ethanol peak area had decreased over tenfold. At 22 minutes the ethanol peak area had decreased to only 0,5 % of the original peak area. After 22 minutes there were still clear volatile odor-active compounds emitting from the glass. The author of this study could perceive aromas of vanilla, peat, and smoke. This indicates that the ethanol evaporates from the glass while other odorants remain to be perceived.

3.4. Open glass SPME experiments

SPME was conducted from an unsealed Savu glass to see if some or any aromatic compounds could be qualified from the chromatograms. SPME from an open container is rather unreliable. Unwanted volatile compounds can be adsorbed from the air. Additionally, volatile compounds from the sample escape and majority of them may not be adsorbed.

To lessen the effect of unwanted volatile compounds from the surrounding air, the SPME was conducted inside a laminar flow cabinet. The cabinet was on for 30 minutes before the SPME started to purify the air inside the cabinet. The cabinet was turned off for the duration of the SPME as to not hinder the adsorption of the sample.

3.4.1. Analysis and chromatograms

Figure 18 shows a chromatogram from the open Savu glass SPME experiment.

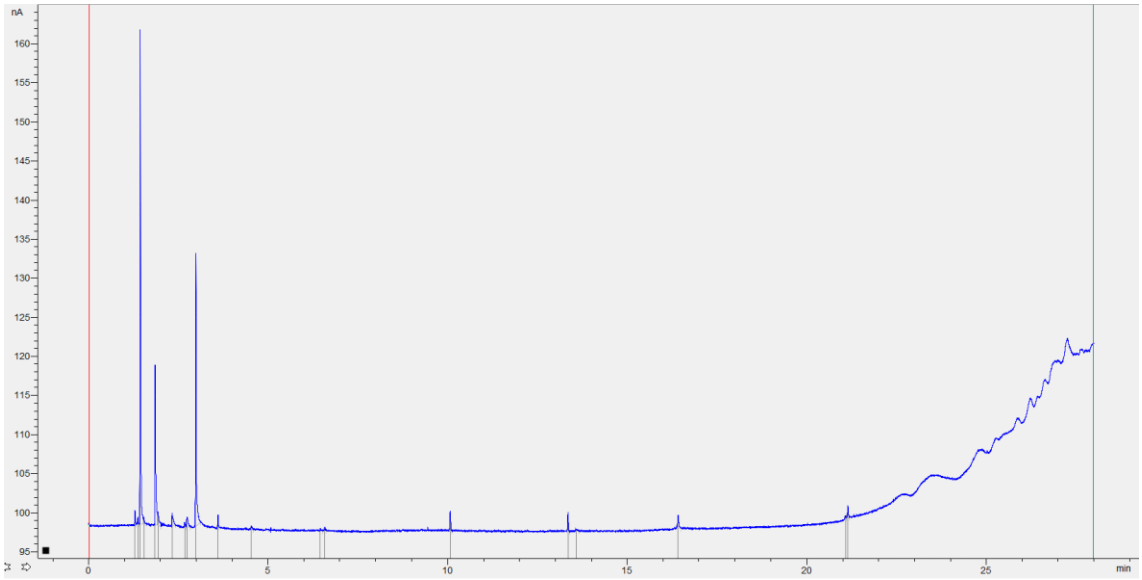

Figure 18. Chromatogram of open Savu glass experiment.

It is clear to see from Figure 18 that the chromatograms' largest spike is from ethanol as can be expected. When zooming closer some lighter compounds can be seen. Many of the heavier compounds didn't show up in the chromatogram. Additionally, the chromatograms had excessive noise which can hinder the identification of the heavier compounds' spikes. As a positive note there didn't seem to be additional spikes from pollution in the air.

3.5. Sensory evaluations GC-O

The GC-O experiments were conducted by only two people, the author of the work and the supervisor of the work. The GC-O was performed with rye whiskey. HS-SPME was performed by the method described in chapter 3.2.1 in this study. GC-O results can be seen in table 7.

Table 7. GC-O odorant descriptions and times of whiskey analyzed by HS-SPME from Savu glass.

Time (min)	Description
1:50	Something
2:34	Forest
3:58	Flowery
6:08	Peat
6:27	Fresh, leafy
6:36	Fruity mild
7:26	Roast
8:58	Something
13:17	Stuffy mild
15:42	Smoke
19:04	Something
19:47	Salty licorice
20:41	Something
23:00	Roast

Some of the descriptions could correlate to some of the odorants commonly found in rye whiskey. Smoke, peat and roast descriptions correlate to Guaiacol and can also correlate to Eugenol. Flowery correlates to 2-phenylethanol and 4-ethylguaiacol. Additionally, forest and leafy descriptions could correlate to these two odorants. Fruity can correlate to Ethyl butyrate, Ethyl isovalerate and Ethyl hexanoate. These three odorants have fruity odors, but they are often described as fake fruit which indicates a sharper industrial odor to be associated with these. Stuffy description could correlate to Whiskey lactone since it is often described as stuffy. Unfortunately, none of these descriptions match to their respected odorants retention times. This could indicate that the odorants described in the GC-O experiments are completely different odorants than the commonly found odorants from rye whiskey.

3.6. Possible future development areas

Developing the perfect whiskey nosing glass is a challenge to say the least. Additionally, analyzing volatile aromatic compounds from any drinking glass is a challenge. It is immensely challenging to develop an analytic method to replicate what the human olfactory sense can experience. SPME is currently one of the best analytic method for this.

3.6.1. Optimizing the Savu glass

Savu glass has a wonderful core principle for the enjoyment of whiskey, focusing on the scent of a whiskey instead of the taste. The use of layers on the sides of the glass to house smaller amounts of whiskey is a working concept to alleviate the effect of ethanol anesthesia. Currently the Savu glass houses three plates each with a 0,1 ml volume. A possible way for faster ethanol evaporation could be to increase the number of plates while slightly decreasing individual plates volumes. Unfortunately, this would also make it harder to pour whiskey evenly on all the plates.

Savu glass uses an ice sphere to separate the bulk of whiskey from the bottom of the glass. This is to block the excessive amount of ethanol from evaporating and causing anesthetic effect. Unfortunately, most whiskeys are not best enjoyed chilled and are watered down too much with the use of ice. The consensus is to not use ice especially with high end whiskeys which also possess the most rich and varied aromatics.

Some use whiskey coins, plates of metal or clay to trap aromatics inside the headspace of a whiskey glass and removing the coin when nosing the whiskey. This also traps the ethanol in the headspace and when the coin is removed the massive amount of ethanol is released to the nose causing ethanol anesthesia. Yet, this type of system would help with the Savu glass. If the ice sphere would be replaced with some other material sphere it would not cool and dilute the whiskey in the bottom of the glass.

3.6.2. Optimizing analysis from Savu glass

The biggest challenge in analyzing volatile aroma compounds from a drinking glass is quantitation. Drinking glasses have a large headspace volume and different compounds can migrate to different parts of the headspace. Thus, the large headspace volume welcomes another challenge, SPME needle positioning. If the needle is positioned in the upper part of the headspace, it will undoubtedly be saturated with the lighter volatile compounds. Yet, if the needle is placed lower in the headspace the adsorption of heavier volatile compounds might improve at the cost of the adsorption of lighter compounds. Three different layers could be analyzed to attempt better adsorption for light, medium and heavy weight volatile compounds as illustrated in figure 19.

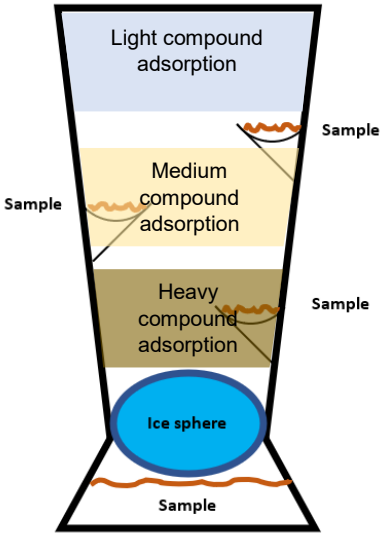

Figure 19. Different levels for HS-SPME of different molecular weight volatile compounds.

In addition to difficulties with headspace volume, the SPME adsorption efficiency is also a challenge. As stated, the SPME first adsorbs the lighter volatile compounds. With a large amount of sample, the needle can easily be saturated with the lighter compounds before enough of the heavier compounds has been evaporated to the headspace for efficient qualification and quantitation. One solution for this is to use different SPME needles for heavier compounds and lighter compounds as experimented with in this work. The problem is that the lighter compounds cannot be extracted from the headspace. While there are SPME needles designed for heavier compounds, they still adsorb some lighter

compounds as well. With an abundance of volatiles in the headspace the lighter compounds can still hinder the adsorption of the heavier compounds.

4. Discussion

Savu glass is a novel whiskey glass specifically designed to enhance the aroma experience of whiskey while decreasing the effect of ethanol anesthesia on the nose. Some whiskey glasses and glass shapes, such as tulip shape, are though to enhance whiskey aroma. Like figure 4 shows most commonly whiskey glasses are tulip shaped. This shape condenses all volatiles including ethanol. So, it can be argued that the anesthesia caused by ethanol nullifies the benefit of the tulip shape while nosing a whiskey from these shaped glasses. With the Savu glass's objective of alleviating the effect of ethanol anesthesia the tulip and bowl shapes are clearly not beneficial shapes to use.

Straight walled glasses such as the Tumbler glass shown in figure 13 B can result in less ethanol on the nose but is hard to argue if different aroma compounds can be better perceived from this shaped glass. Inverted cone shaped glasses are commonly used with other spirits, such as a Martini glass seen in figure 5 C, but not with whiskey. Whiskey aromatics could possibly benefit from this shape more than clear spirits since clear spirits often do not have as rich odor profiles as whiskeys do. With these shaped glasses the ethanol dissipates from a wider area decreasing the ethanol anesthesia. Savu glass takes inspiration from a variety of glass shapes to alleviate the ethanol's anesthetic effects to provide a unique whiskey nosing experience.

Different whiskey glasses have not yet been researched whether they truly enhance the odor perception of whiskey. This is likely due to lack of method with which to prove the glasses' hypotheses. Odor compound identification and quantitation from a drinking vessel has not been attempted before. HS-SPME was the obvious selection for odor adsorption from whiskey sample. Several factors had to be considered when developing the HS-SPME method from the Savu glass.

Several key odorants of whiskey could be identified using the HS-SPME method developed for the Savu glass. GC-O showed some success with just two panelists. Quantitation from Savu glass shows some problems with consistency. Ethanol evaporation experiment demonstrated the novel glass's working principle.

There are many challenges with developing a new method of volatile compound qualification and quantitation. The quantitation from Savu glass still needs further development to verify the results. Should a method for proper quantitation be developed it could be used to research other glass shapes influence on the whiskey aroma as well. This in turn would quite possibly lead to more innovating glass designs and research on different glass’s working principal hypothesis.

GC-O evaluations should be continued with a larger evaluator panel. The panel should consist of both experts in the field of whiskey and / or other spirits as well as non-professionals. Same evaluators could be used in sensory evaluations where the Savu glass is compared to other whiskey glass shapes. With sensory evaluations the odor perception could be evaluated at different time stages similarly to this studies chapter 3.3 SPME experiment. This should result in interesting results with different odorants being perceived at different stages. Additionally, this evaluation could also show the decreasing ethanol effect on the olfactory sense.

For the two evaluators none of the times matched up with both identifying a scent at the same timeframe. This is truly odd since the HS-SPME, and chromatography methods were identical with both GC-O evaluations. This could be an indication of the challenges of using HS-SPME from a glass with a large volume headspace. It could be that the odorants of the whiskey distributed and circulated the headspace differently in the two extractions. This would result in varying amounts of different odorants to be adsorbed to the fiber explaining the different GC-O results.

Below is a summary of this studies goals, what was accomplished and what still needs further work:

- Develop a novel HS-SPME method from a glass drinking vessel.

  - An HS-SPME method that adsorbs volatile compounds straight from the glass was successfully developed.
- Identification and quantitation of volatile odor-active compounds from a drinking vessel.
  - Odor-active compounds were successfully identified from the Savu glass. Those compounds were also quantitated. Yet, with some compounds the quantitation didn't properly correlate with the whiskey samples direct injection compound concentration. Therefore, some further testing is recommended.
- GC-O evaluations of odorants emitting from Savu glass.
  - The GC-O evaluations were only conducted with two participants. GC-O evaluations showed promise, but they should be conducted with a larger trained panel. The oven program used with GC-O in this study lasted for 20-minutes. This is a rather long time for a panelist to do olfactometry evaluation, so the length of the GC-O evaluation should be trimmed down if possible.
- Demonstrate the ethanol anesthesia lowering effect of Savu glass in the nose.
  - Through the ethanol evaporation experiments it was confirmed that with Savu glass the ethanol concentration decreases fast. Quite possibly the ethanol concentration drops below its OT in just few minutes. The ethanol concentration should be quantitated from the Savu glass at different time timeframes to see when ethanol drops below its OT.
- Conducting sensory evaluation using the Savu glass.
  - Sensory evaluations were not conducted in this study due to time restrictions. Sensory evaluations with a trained panel consisting of whiskey hobbyists and professionals would possibly yield great results. The panel should try to identify as many odor perceptions as possible and some way of measuring whether ethanol anesthesia is occurring with the panelists should be developed. One such way could be to rate some odorants intensity before and after the Savu glass sensory evaluation. With this way it should also be kept in mind that sensory evaluation can cause fatigue with the

panelists which in return can affect the intensity rating. This odor intensity test should also be used with other whiskey glass sensory evaluations to give an interesting comparison.

- Comparing Savu glass quantitative whiskey odorant results and sensory evaluation results to other glasses commonly used with whiskey.
    - Quantitative results from Savu glass were not compared to other glasses quantitative results due to time restrictions. Savu glass quantitative results should definitely be compared to different whiskey glasses quantitative results. Glasses such as Glencairn and Tumbler would be good comparing subjects. Ethanol concentration adsorbed from these different glasses should also be compared, since it has a major anesthetic effect on the human's olfactory sense. Sensory evaluation results of Savu glass could also be compared against the results of Glencairn and Tumbler glass. Odor perception results could show that odorants are much more easily perceived from Savu glass compared to other glasses, since with Glencairn and Tumbler the abundance of ethanol harms the olfactory sense.

5. Conclusions

This study has showed the potential of a novel whiskey glass ability to enhance the whiskey experience. Olfactory sensation is an essential part of enjoying whiskey and development of a glass focusing on the odor sensations of whiskey is a great concept. To prove the scientific concept of the novel whiskey glass is a very time consuming and difficult operation. There is still much more work to be done with the Savu glass. The Savu glass shows great commercial potential with its novel concept.

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