KEY ODORANTS OF ALMOND MILK AND THEIR CONTRIBUTION TO ALMOND MILK ODOR IMAGE

A Project Paper

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ABSTRACT

The number of odorants can be identified by human in a mixture is limited to four. Only some key odorants in food are essential for human to recognize the object. Our GC-O experiments discovered that hexanal, 1-octen-3-ol, 2-acetyl-1-pyrroline (trimethylpyrazine) are key odorants for a commercial almond milk. In addition, we found that key odorants might be different between people. One of key odorants for subject A is different from other subjects, yet the odor image created by mixing key odorants for subject A was similar to other subjects' results.

Starting with measuring the threshold for different subjects, we found that threshold for hexanal and 1-octen-3-ol are similar among subjects. To determine how key odorants interact in the binary mixtures and even tertiary mixture, we conducted the experiment based on each subject's threshold. Suppression effects were found in EOR analysis, in which hexanal suppressed other two compounds for subject A while 2-acetyl-1-pyrroline suppressed other two compounds subject B. In addition, subject B's predicted EOR is close to measured EOR while subject A's predicted EOR is different from measured EOR results.

Finally, the almond milk odor image was successfully simulated by mixing three key odorants for subject B. We observed that only by mixing the exact ratio of three key odorants, that almond milk odor image can be simulated. If the concentration of a key odorant is too high or too low, it has a dramatic effect on the configural odor of the mixture.

BIOGRAPHICAL SKETCH

Xianjia Zeng is a master's student in Terry Acree's lab in the Food Science Department at Cornell University. Her research investigates the olfactory perception and binary mixture interaction. She is a member of Institute of Food Technology (IFT), American Chemical Society (ACS), and Association for Chemoreception Sciences (AchemS). In April 2018, she and her advisor presented the poster "Temporal effects of odorants on mixture perception" at Achems meeting.

She previously graduated from the Pennsylvania State University with a B.S. in Food Science and a B.S. in Agribusiness Management in 2015. After that, she worked as a key account HSE manager at Compass Group in China.

Outside of academics, Xianjia is very involved in volunteer work. She is a global translator at Coursera.org, and has helped to translated several courses from English to Chinese, including "Six sigma and the organization (Advanced)" and "Six sigma advanced define and measure phases".

To my wonderful mother: for her love and measureless support

&

To my amazing advisor Terry: for your care and all the funny stories

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This work would not have been possible without the help of my advisor, Terry Acree, who have been so supportive of my research and shared his stories to bring happiness in my life. With his endless help and guidance, I become more and more interested in olfaction world every day, and be able to discover the beauty in every experiment.

I am grateful to all of those with whom I have had the pleasure to work during this and other related projects, Candice Zheng, Kaifeng Ding, Xiaoyuan Wang, and all my subjects, who provide unending inspiration.

At last, I would thank to my family who gave me enormously mental support. My mom, dad, brother, aunt, and uncle, you are truly amazing.

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LIST OF ABBREVIATIONS

ORN Olfactory Receptor Neuron

OE Olfactory Epithelium

ppb parts per billion, = ng/L

ASTM American Society for Testing and Materials

ppm parts per million, =mg/l

CHARM Combined Hedonic of Aromatic Response Measurement

RI Retention Index

EOR Equal Odd Ratio

ms millisecond

AM almond milk

SO Sniff Olfactometer

GC Gas Chromatography

GC-O Gas Chromatography-Olfactometry

OAV Odor Activity Value

2A1P 2-acetyl-1-pyrroline

1O3O 1-octen-3-ol

jnd Just-Noticeable Difference

LIST OF SYMBOLS

I Stimulus Intensity

k Constant

n Power

S Sensation intensity

ΔI Change in Energy/Intensity

CHAPTER 1

INTRODUCTION

1.1 Psychophysics of olfaction

Humans have an exquisite ability to distinguish things by their smell. It has been shown that people can identify at least 1 trillion olfactory stimuli, perhaps there is no upper bound (Bushdid, Magnasco, Vosshall, & Keller, 2016). However, the mechanism of how humans process olfactory information remains unknown. To investigate this, perceive odors we must use psychophysics to quantify the psychological experience of smelling at various odorant mixtures and concentrations. Traditional psychophysics uses a detection probability experiment to determine the threshold of an odorant while odor intensity measurements use cross-modal matching to a scale e.g. a line length, force of squeezing a cylinder, the pitch of a tone, etc. with the perceived intensity of a smell. Discrimination measurements are based on the probability of recognition not on the scaling of a sensation.

Detection thresholds, described as the lowest level of a stimulus that a subject can respond to reproducibly are noisy limiting their ability precinct behavior. Recognition threshold is the odorant concentration at which subjects just recognize the odorant are more stable but still too noisy because they require the subject to recognize an odorant at very low intensity. However, when subjects are required recognize an odorant in the presence of another odorant well above its threshold (e.g. 7% ethanol) the results are much less noisy and in the case of alcoholic beverages more relevant. However, the ratio of such odorant thresholds cancel the effect of ethanol and predict the effect of the two odorants in a mixture. In this project the binary predictions of the most potent odorants in the headspace were used to predict the tertiary behavior of the three potent odorants detected in the headspace of almond milk. The three that produced a convincing simulation of almond milk were then tested for their ability to simulate almond milk headspace.

Quantification and measurement of sensory experiences can be traced back to nineteenth century. One of the earliest psychophysics models is Weber's Law of Just Noticeable Differences based on Bernoulli model. He noted that the increase in a stimuli ($\triangle I$) to produce a change in sensory intensity that is just barely noticeable is at a constant percent change (k).

$$\Delta I/_I = \mathrm{k}$$

K is called the "Weber fraction" or j.n.d, indicates how well the stimuli determines sensory system to detect changes. Later in 1860, G.T. Fechner found that the relationship of perceived intensity to physical magnitude can be determined by accumulating j.n.d, which can yield a psychophysical model.

$$\mathrm{S} = \mathrm{k} \log I$$

The intensity measurement or magnitude estimation procedure was later developed by Steven. He found that sensation grows as a power function such that

$$S = kI^n \text{ or } S = n \log I + \log k$$

This new model takes stimulus and sensation into consideration, and predicts how measures of subjective experience is predicted by stimulus concentration. Estimates for the value of n for olfaction varies from 0.8 to 0.3 for different odorants. However, these differences may be just an artifact of choosing concentrations at random points on an exponential psychophysical function. Recently, a large collection of sensory data and estimates of their corresponding chemical features were used by 21 teams from 49 individuals called the "DREAM Olfaction Prediction Challenge" to predict human olfactory perception based on chemical features of odor molecules. The model successfully predicted odor intensity and pleasantness, but not odor quality (discriptors) (Keller et al., 2017). In this project we attempt to predict odor perception from the interaction of it's component odorant psychophysics.

1.2 Perception of odor mixtures

Human are exposed to mixtures of odorants in foods every day. In many cases, the individual odorants produce unique perceptions on their own and they can be recognized when you smell a mixture. For example, when smelling a mixture of odorants A and B, odorant A can be recognized, and followed by odorant B. This does not seem to happen simultaneously. First, you smell A because there is more of it in the mixture, or it has a lower threshold than B. Then the subject can consciously analyze the odor of a mixture and decide it contains B. However, if the mixture of A and B is characteristic of some other thing, called D, then they may also recognize this sniff as D before they recognize A and B. The process of detecting the element of (A, B) is called "elemental perception" and the process of detecting of (A, B) as D is called "configural perception". It is also possible that both of the perception can be presented simultaneously, sequentially when an organism switches from elemental to configural almost instantaneously. This switch depends on the odorants similarity and the number of recognizable odorants (Jinks & Laing, 2001). Interestingly, a recent study has shown evidence for elemental odor processing with the analysis of peanut butter odorant components, in which they found the decreasing orbitofrontal cortex and amygdala response are associated with satiety-related response (Howard & Gottfried, 2014). On the other hand, configural processing of odorants was identified in the PPC.

Similar phenomenon is also found in psychophysical experiment level. Barkat examined the perceptual blending in odor mixtures and concluded odor blending in human exists with odor mixture composed of 2 or 3 odorants and account the result for configural processing of odor mixtures. However, olfactory expertise has the ability to prevent perceptual odor blending and impose elemental processing as is common for sommeliers.. (Barkat, Le Berre, Coureaud, Sicard, & Thomas-Danguin, 2012)

If psychophysical function of two odorants in a mixture is known and human processes these odorants in an elemental way, the intensity of that mixture can be predicted from the vector sum of the two respective aroma compounds but only under elemental perception. Synergetic effect

and suppressive effect are observed in several experiments when people mix two odorants in a mixture (Berglund & Olsson, 1993). Unfortunately, adaption and suppression modulate recognition in unpredictable ways.

1.3 Stochastic model of odor mixtures perception

Olsson and Cain conducted series experiments measuring the odor identification in a mixture as a function of the relative intensity of the mixture (Olsson, 1994). They later proposed a theoretical model predicting the intensity of a mixture from the intensity of the component odors (OLSSON, 1998). The equation is written in the form of

$$P(a) = \frac{R_A^2}{(R_A^2 + R_B^2)}$$

P(a) is the probability of identifying the odorant, R(A) and R(B) are odor intensity for two odorants perceived. Later, Hettinger and Frank (Hettinger & Frank, 2018) adjusted the model to the following equation:

$$P(a) = \frac{(1 - R_B)^2}{R_a^2 + (1 - R_A^2)}$$

However, measuring the relative intensity of an odorant in a mixture takes is tedious and noisy. Thus, we developed a new stochastic model to measure only the quality of the odorants and their relationship with perceptual recognition instead of measurements of perceived odor intensity. This model was first tested in a paper in 2017, and proved to generate reproducible and reliable results (Rochelle, Prévost, & Acree, 2017). For example, in order to identify the threshold of single odorant, each experiment is comprised from 4 sub-experiment containing 12 different concentration of solutions. Three solutions are randomly tested in each single threshold analysis sub-experiment. Subjects are asked if they smell one odorant in the mixture or the other. The subjects have a binary recognition choice instead of cross-model scaling task. The resulting metric is calculated from the stochastic result of repetitive choices. Recognition is the only cognitive process used by the subject make an unscaled choice.

1.4 Key Odorants and Odor Image

Although there are many odorants in a food, most of them are at a very low concentration and often below the threshold which we can smell. Additionally, it has been found that human has limited capacity to identify odors in mixtures, which indicates only several key odorants combined give food its odor image. (Laing & Francis, 1989)

Key odorants are defined as the volatile chemical compounds that contribute the most to the perception and recognition of a mixture. They either have high concentration which result a high intensity of their aroma, or their odor quality is clear enough for people detect and recognize even if their odor potency is very low. An experiment based on apple model mixture indicated that three chemical compounds (hexyl acetate, trans-2-hexenal and 1-hexanol) effect apple aroma despite their different impact (Bult et al., 2002). The author also pointed that low impact components are suppressed by high impact component on intensity ratings on apple attribute. In a characterization of the key odorants in light aroma type Chinese liquor paper, the author successfully simulated its aroma by combining 27 important odorants. However, omission experiments confirmed that only two chemicals, beta-damascenone and ethyl acetate are key contributors to the liquor's fruity and floral notes (Gao, Fan, & Xu, 2014). More evidence is shown in the experiment of simulating grenadine by mixing six odorants that were previously proved all to have contribution of grenadine. (Romagny, Coureaud, & Thomas‐Danguin, 2018) Furthermore, it was found by the author in a potato chip odor image study, in which methional, methanethiol, and 2-ethyl-3,5-dimethylpyrazine combined at a certain ratio produced a potato chips odor, meaning the subjects smelled the mixture and recognize it as potato chips even though the subjects perceived the components differently (Rochelle et al., 2017).

Recently, there are increasing studies using GC-O, quantitative measurements, aroma recombination, and omission studies to construct a wine odor. However, the key odorants they chose is often between 4 and 9 odorants, which is outside the range Laing's odor recognition limit.

We expect to reconstruct the odor image of the food by identifying the key odorants and their psychophysical function. Thus, we plan to study the threshold of the key odorant in order to observe the sensory response related to a reference (7% ethanol in water). Odor perception is highly variable among humans because it is modulated and informed by the subject's ecological history. So we collect data on individual subjects who have been conditioned to associate their perceptual identification with a common lexicon. To eliminate the effect of threshold noise, we tested subject responses at a concentration between 3 and 30 times the subject's own threshold to standardize the experiment.

1.5 Thesis Statement

The purpose of this project is to find the key odorants of almond milk and their contribution to the almond milk odor by using a stochastic psychophysical model.

CHAPTER 2

SNIFF OLFACTOMETER

2.1 Sniff Olfactometry

The Sniff Olfactometry is a device which delivers equilibrium odor head-space in less than 70 ms to an actively inhaling subject. It is composed of several parts, an actuator box, a sniff port with Teflon bottles, and a monitor. Three Teflon™ bottles at different concentration of odorants are installed on the sniff port at different positions. A subject sits in front of the machine with her chin on a rest. The sniff port is 1 cm below the nares of the subject , where odorants can be delivered in a short puff to the subject. During an experiment, the screen on the SO cues the subject to inhale just before a puff. Once the subject is ready and hit the mouse, a puff is delivered to the subject from a sample bottle by a 70ms compression by an actuator controlled by Arduino board (Adafruit Inc.) and a python program (PsychoPy).

2.2 PhycoPy Software

PhychoPy is an open source software that gives instruction to the SO and collecting a subject's responses. It is used in threshold, binary, and product-simulant comparison experiments that present stimuli (odorants) and record responses. This software delivers visual cues on monitor while collect responses when subject click the mouse to pick their answers. Threshold tests, binary odorant tests, and product-simulant comparison tests all use A-B force choice method. The step-by-step instruction and experiment design are shown in the appendix.

CHAPTER 3

EXPERIMENT 1: DETERMINATION OF KEY ODORANTS IN ALMOND MILK

3.1 Aim

This experiment is conducted to identify key odorants in almond milk by using GC-O, dilution analysis, and Charm Analysis. The key odorants will be used to conduct threshold analysis, EOR analysis, and construct odor image of almond milk in the next experiment.

3.2 Materials and methods

3.2.1 Samples and reagents

1.89L cartons of three different brands of almond milk (Ingredients: almond milk [filtered water, almonds], calcium carbonate, sea salt, potassium citrate, sunflower lecithin, gellan gum, vitamin A palmitate, vitamin D2, D-alpha-tocopherol) were purchased locally. Great Value Original Unsweetened Almond Milk was chosen for this experiment because it is made from almond, water, preservative additives without added flavors.

Hexanal (CAS: 66-25-1)>99%, 1-Octen-3-Ol (CAS: 3391-86-4) >99%, 2,3,5-trimethylpyrazine (CAS: 14667-55-1) were obtained from Sigma Aldrich (St Louis, USA). 2-Acetyl-1-pyrroline (CAS: 85213-22-5) (10% w/w in Toluene) was obtained from Santa Cruz Biotechnology Inc. (Texas, USA). All of them were used as standards to determine odorants in almond milk.

3.2.2 Human subjects

Two female and two male non-smoker subjects with age range from 23-26 at Cornell University, passed the screening test and were chosen for participating in GCO experiment. All subjects were students from Cornell University department of food science. None of the subjects were smokers or report olfactory defects. Before the experiment, the subjects are trained for 15 minutes to learn how to respond to stimulants eluting from the GCO.

3.2.3 Solid phase microextraction extraction (SPME) of aroma compounds

Pérez-González and his team determined optimal headspace SPME GC-MS methodology for the analysis of processed almond beverages” in his paper that using DVB/CAR/PDMS fiber for 2 mL sample with saturation level of NaCl, extracted at 60℃ at 700 rpm for 60 minutes (Pérez-González, Gallardo-Chacón, Valencia-Flores, & Ferragut, 2015). We adjusted some of their methods. A Carboxen/DVB/PDMS (2 cm), 50/30 um three phase SPME fiber were purchased from Agilent Technologies (Santa Clara, CA) and assembled into a manual SPME holder. Before the experiment, the fiber was conditioned at 225℃ for 1 hour according to Agilent SPME Assemblies manual and allowed to cool down to room temperature for 10 minutes. 10 mL sample of almond milk was taken out from fridge and put under room temperature (23℃) for 30 minutes before the extraction. During the extraction, 10 mL sample of almond milk was placed into a 50 mL glass bottle with a magnetic stir bar rotation at 300 rpm cycles per minute. SPME fiber was exposed in the head space for 60 minutes and desorbed at 225℃ in the injector port of the GC-O.

3.2.4 Gas chromatography-olfactometry (GC-O)

Gas chromatography system used in this experiment is comprised of a 6890 Gas Chromatography (Agilent Technologies Inc., Santa Clara, CA) with Flame Ionization Detector (GC-FID), and a sniff port (DATU Technology Transfer, Geneva, NY) and coupled an olfactometer. Each extract is injected into a DB5 column 35℃ to 225℃ at a rate of 6 ℃/min. The initial and final hold time were both 3 minutes. Carrier gas was helium at a constant rate at 2 mL/min. The olfactometer air flow was 1 L/min at 23℃. During an experiment, subjects sat in front of the GCO and smelled for odorants coming from the sniff port. When subject smells an odorant from the sniff port, they click the mouse and hold until they couldn't smell that aroma anymore and after the mouse click is released. Odor descriptor previously chosen were presented on the monitor and subjects associated one with the perceived odor by checking a label on the screen with the mouse. The software will automatically recorded start retention index, stop retention index, and odor descriptor. The total running time per session was 40 minutes.

3.2.5 GC-O dilution analysis

GC-O dilution analysis was performed on 1:2 v/v dilution using GC-O conditions described above and various split ratios at the injector (Deibler, Martin Llesca, Lavin, & Acree, 2004). The series dilution experiments ended when subjects couldn't smell any aroma compound coming out from GCO in a single session. There are 4-5 sessions depending on the subject's threshold.

3.2.6 Charm Analysis and key odorants identification

To calculate the Kovats retention indexes, C6 – C20 aliphatic hydrocarbon standards (Sigma-Aldrich, St. Louis, MO, USA) was injected to GC and used as standards. Although we've found ~20 odorants from almond milk, to save time, only odorants that were identified as key odorants were quantified using CharmAnalysis. CharmAnalysis uses following equation

$$Charm\ value = \int_{peak} F^{n-1}\, di$$

F, n and di represents dilution factor, number of dilution, and detection time respectively. To generate peak area in the chromatogram, which shows the individual odor concentration.
Next, the compound is compared with reference RI on Flavornet.com and aroma compounds that were previously measured by Erten & Cadwallader (Erten & Cadwallader, 2017) and Perez-Gonzalez (Pérez-González et al., 2015) to confirm key odorants. Four chemical standard solutions were used to validate the key odorants.

3.3Results

The four subjects were yielded different GC-O Chromatograms shown in figure xx at the same retention index (RI) scale (750-1200). The key odorants for each subject are summarized in the table below.

Table 1. Summary of the key odorants from subjects

Odor Descriptor	Chemical	RI	Subject A	Subject B	Subject C	Subject D
Green	Hexanal	790	x	x		x
Nuts	2-Acetyl-1-pyrroline	925		x	x	x

Mushroom	1-Octen-3-ol	979	x	x	x	x
Nuts	Trimethylpyrazine	1005	x			

Each subject smell different odorants from the last dilution series experiment session. Subject A apparently has a high threshold of 2A1P since all other subjects can smell this odorant from the last dilution but not this subject. However, subject 1 can smell trimethylpyrazine from the last dilution others couldn't smell. Interestingly, both 2-acetyl-1-pyrroline and trimethylpyrazine are described by subjects having "nuts" or "almond" aroma.

Thus, we concluded that key odorants in almond are different for each subject. Hexanal, 1O3O and trimethylpyrazine are identified as key odorants for subject 1 while hexanal, 1O3O and 2A1 are identified as key odorants for subject 2.

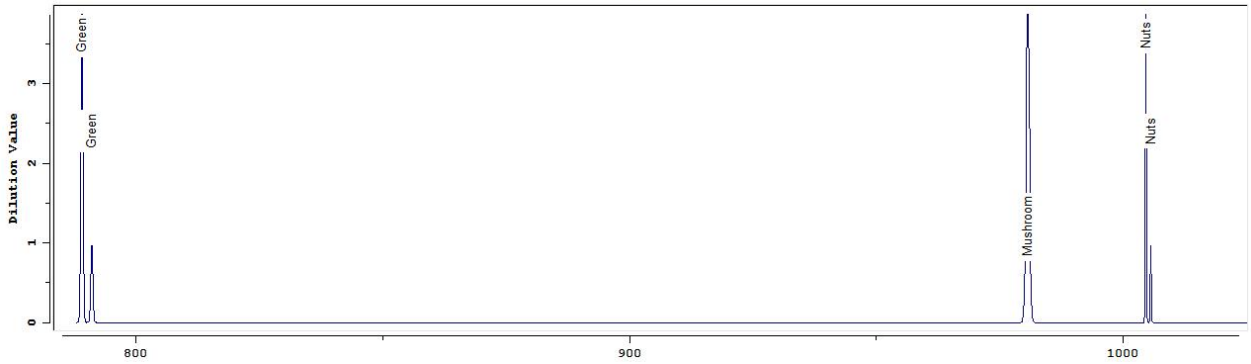

Figure 1. Subject A GC-O chromatogram

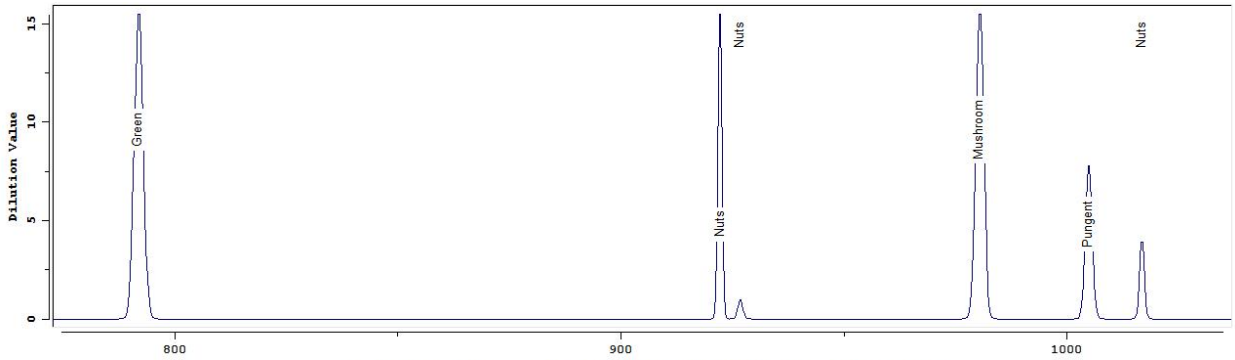

Figure 2. Subject B GC-O chromatogram

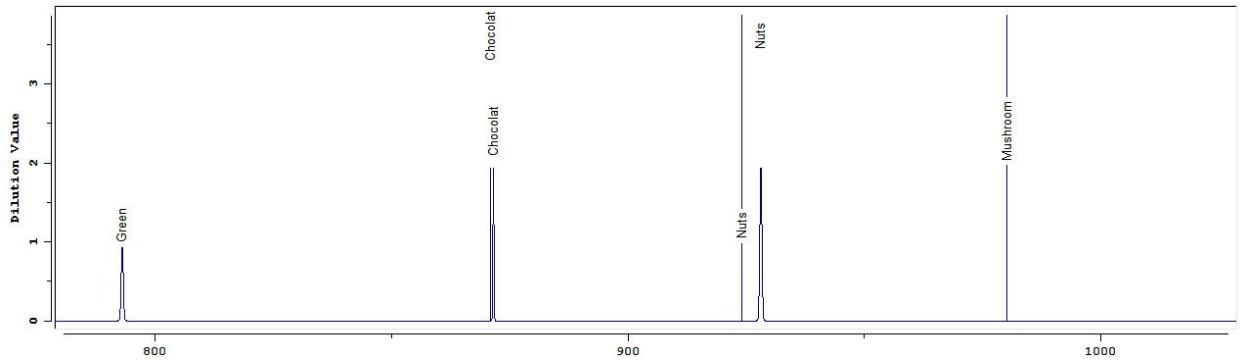

Figure 3. Subject C GC-O chromatogram

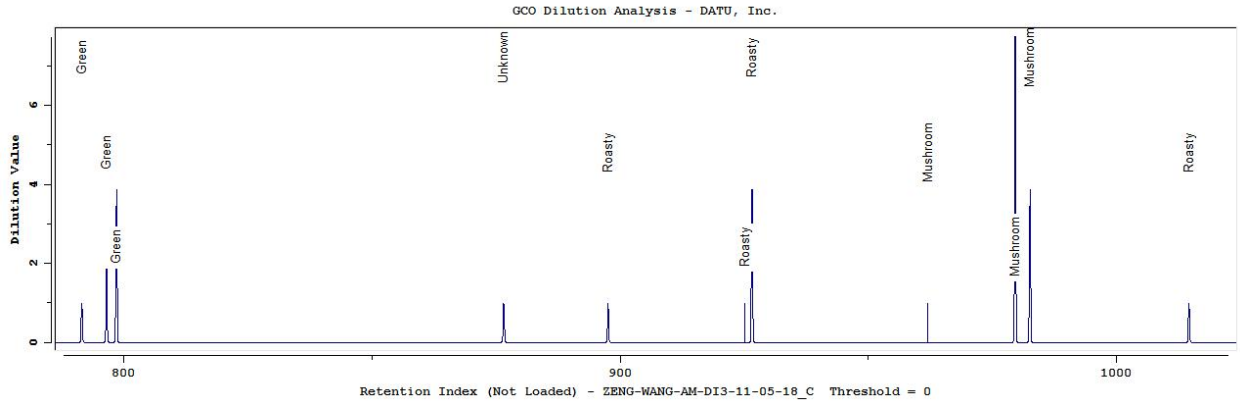

Figure 4. Subject D GC-O chromatogram

CHAPTER 4

EXPERIMENT 2: THRESHOLD AND EOR DETERMINATION

4.1 Aim

This experiment is conducted to determine the threshold of three key odorants and investigate the binary mixture blending for each pair of the key odorants. It sets a basis for reconstructing almond milk experiment.

4.2 Materials and methods

4.2.1 Samples and reagents

The following chemicals were used in threshold and EOR analysis. All of the odorant or binary mixture were diluted into distilled water containing 7% v/v ethanol (food grade).

Table 2. Summary of chemicals used in experiment 2

	CAS	Odor	Supplier	Concentration
Hexanal (>99%)	66-25-1	Green	Sigma Aldrich	0.025 – 2.5 ppm
1O3O (>99%)	3391-86-4	Mushroom	(St Louis,	0.01 – 5 ppm
Treimethylpyrazine (>99%)	14667-55-1	Nuts	USA)	
2A1P (10% w/w in	85213-22-5	Nuts	Santa Cruz	0.12 – 0.38 ppb
Toluene)			Biotechnology	
			(Texas, USA)	

4.2.2 Threshold determination

In this experiment, threshold of three key odorants for different subjects were analyzed using Sniff Olfactometry. Right before experiment, 50 mL of each diluted sample was transferred to a 250 mL Teflon bottle. Starting from the highest three concentration, three Teflon bottles are randomly installed on sniff port. During the experiment, the bottle that is punched by the actuator

is randomly determined by the program in a double blind experiment. After the subject is ready for the experiment, the program instructs the subject and begins with two training and conditioning session. Next, subjects received a total 12 replicates of three random puffs, and are asked question “What do you smell? A or B”. The subject has to choose between odorant identifier or ethanol. Once finished, the software thanks subject for participating experiment. Next, three Teflon bottles (still at same concentration) rotate three times to minimize error.

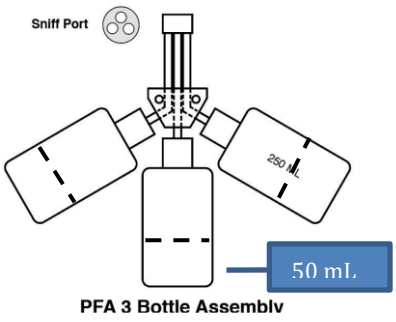

Figure 5. Teflon bottles with sniff port

Table 3. Summary of solution and bottle positions

Session/Position	1	2	3
Rights bottles	High concentration	Medium concentration	Medium concentration
Center bottles	Medium concentration	High concentration	Low concentration
Left bottles	Low concentration	Low concentration	High concentration

4.2.3 EOR determination

EOR (equal odds ratio) is defined as the concentration ratio of two aroma compounds that subject can detect at an equal frequency. EOR analysis was performed after threshold of all three key odorants were determined. To be more specific, the starting concentration of the binary mixture for EOR analysis is based on five times the threshold of two odorants. One of the odorants is fixed while the concentration of another odorant is adjusted. The method is same as the threshold determination experiment described before except the binary mixture is tested instead of single odorant verses ethanol at 7%. In total, three pairs of EOR were measured.

4.2.4 Data analysis

Data was analyzed by Microsoft Excel and Wolfram Mathematica software. Since the response results are binary data, the ‘ethanol’ response is assigned to 0, all other response is assigned to 1

in the threshold experiment. In the EOR experiment, one odor descriptor was assigned to 0 and the other one was assigned to 1.

Threshold analysis: For each odorant, there are total 12 (concentrations) ×3 (position switch) ×12 (replicates) =432 binary response. These data were organized in one excel file sheet and was fit into generalized linear model with 95% prediction interval generated by Wolfram Methematica model. The logistic function model yiealds a psychophysical function showing the relationship between the probability of choosing the odorant and the log concentration of this odorant. The intersection of probability 0.5 and corresponding concentration is the threshold of that odorant.

Binary EOR analysis: Similar to the threshold data analysis, each odorant is assigned to either 0 or 1 in each binary pair, as the table shown below. The data is plotted in generalized linear model to generate the EOR function. The intersection of probability 0.5 and the fitted function is defined as the ratio that the subject can detect at an equal potency.

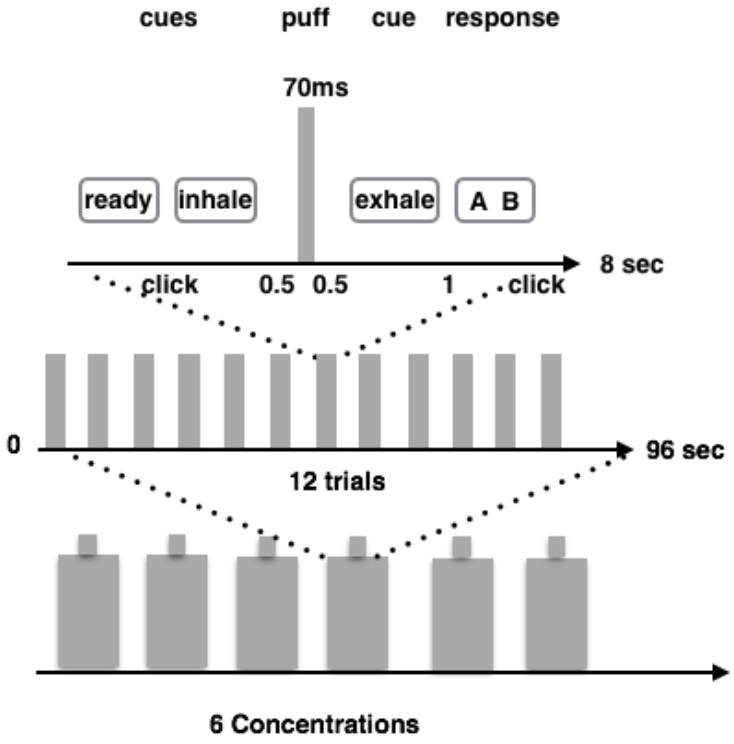

Figure 6. Example of puffs, concentrations, and sessions

Table 4. Number assign for each pair of binary mixture for Subject A

Binary mixture	0	1
Hexanal + 1O3O	1O3O	Hexanal
1O3O +trimethylpyrazine	Trimethylpyrazine	1O3O
Hexanal + trimethylpyrazine	Hexanal	Trimethylpyrazine

Table 5. Number assign for each pair of binary mixture for Subject B

Binary mixture	0	1
1O3O + 2A1P	1O3O	2A1P
1O3O + Hexanal	1O3O	Hexanal
2A1P + Hexanal	2A1P	Hexanal

After obtaining all the thresholds and EOR values, correlation tests were conducted between the predicted EORs by the threshold and the measured EORs, and predicted EORs by the other EOR values and the measured EORs to understand the interaction between the mixtures.

4.3 Threshold results

4.3.1 Hexanal threshold

The subjects were found to have close threshold for hexanal, with 0.157 ppm and 0.181 ppm respectively. The fitted logistic curve is shown in the figures below. The 95% predictive interval is shown in the shaded area.

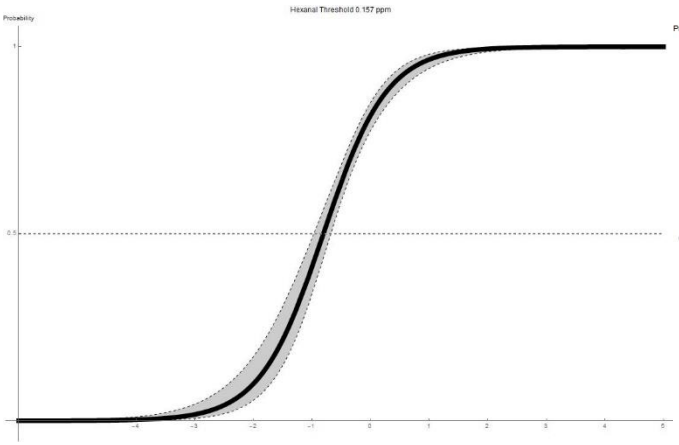

Figure 7. Hexanal threshold for Subject A

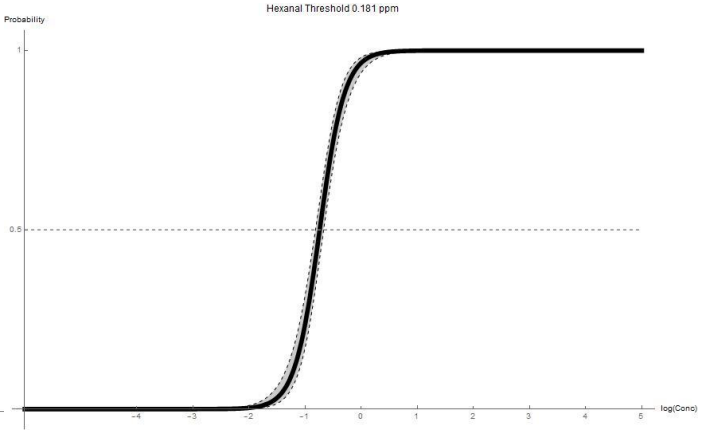

Figure 8. Hexanal threshold for Subject B

4.3.2 1-Octen-3-Ol threshold

The subjects were found to have similar 1O3O threshold result.

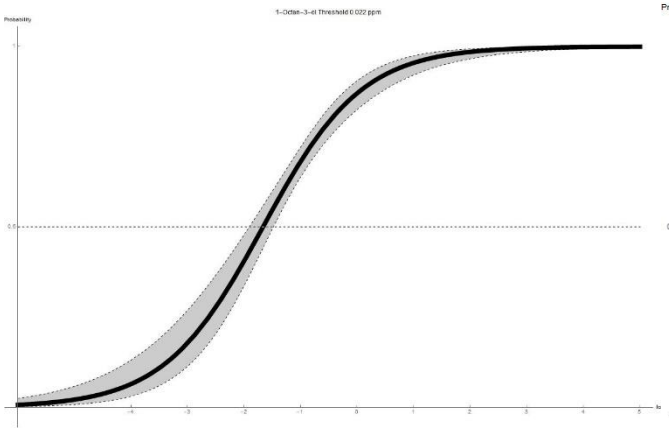

Figure 9. 1-octen-3-ol threshold for Subject B

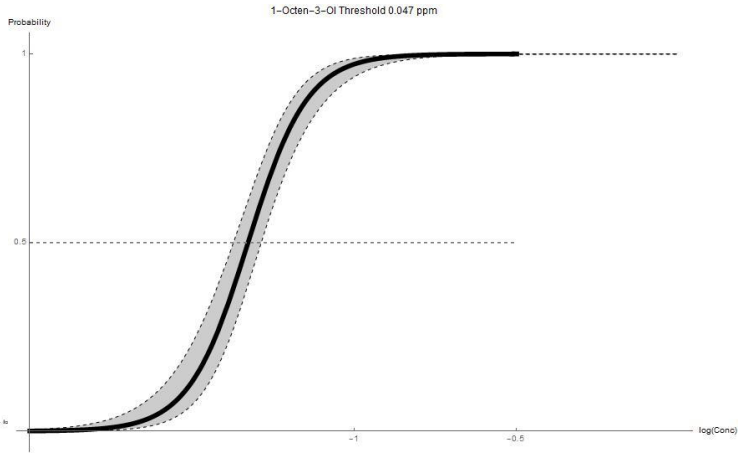

Figure 10. 1-octen-3-ol threshold for Subject B

4.3.3 Trimethylpyrazine threshold

The trimethylpyrazine threshold for subject A is 1.320 ppm.

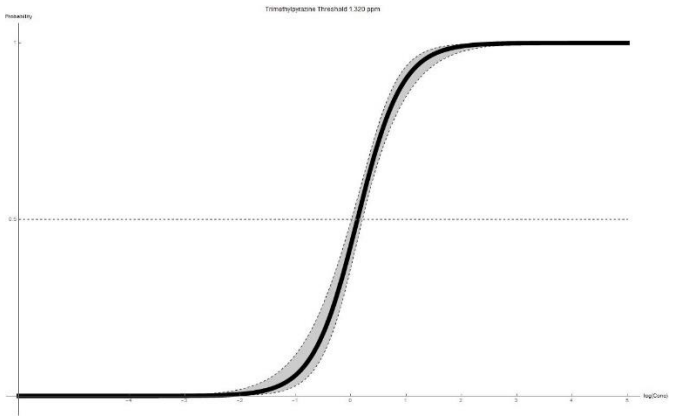

Figure 11. Trimethylpyrazine threshold for Subject A

4.3.4 2-acetyl-1-pyrroline threshold

The threshold of 2A1P for subject B is calculated at 224 ng/L. Comparing to other chemical compound, the threshold of 2A1P is extremely low. In addition, its nutty and roasty aroma is the most like to almond milk aroma.

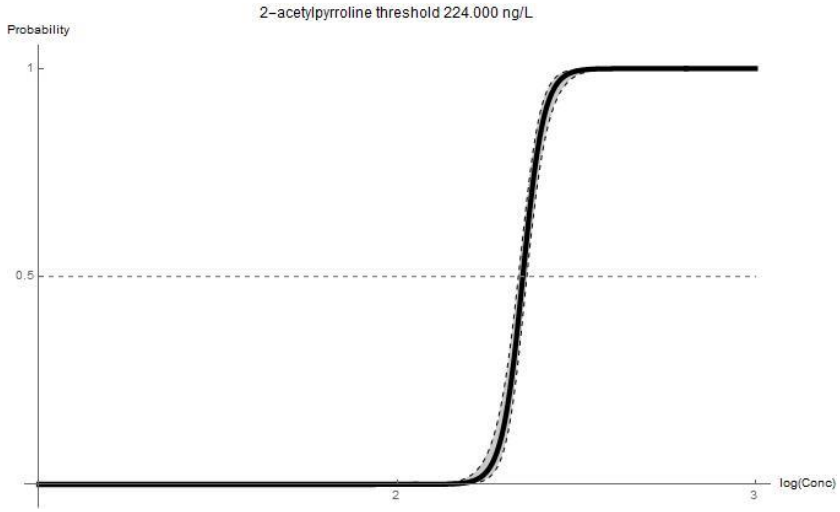

Figure 12. 2-acetyl-1-pyrroline threshold for Subject B

4.4 EOR results

4.4.1 Subject A binary comparison

The binary EOR comparison for three mixtures for subject A are shown below. The orange, blue, and black curve represents binary mixture of 1O3O+trimethylpyrazine, hexanal+1O3O, and hexanal+trimethylpyrazine respectively.

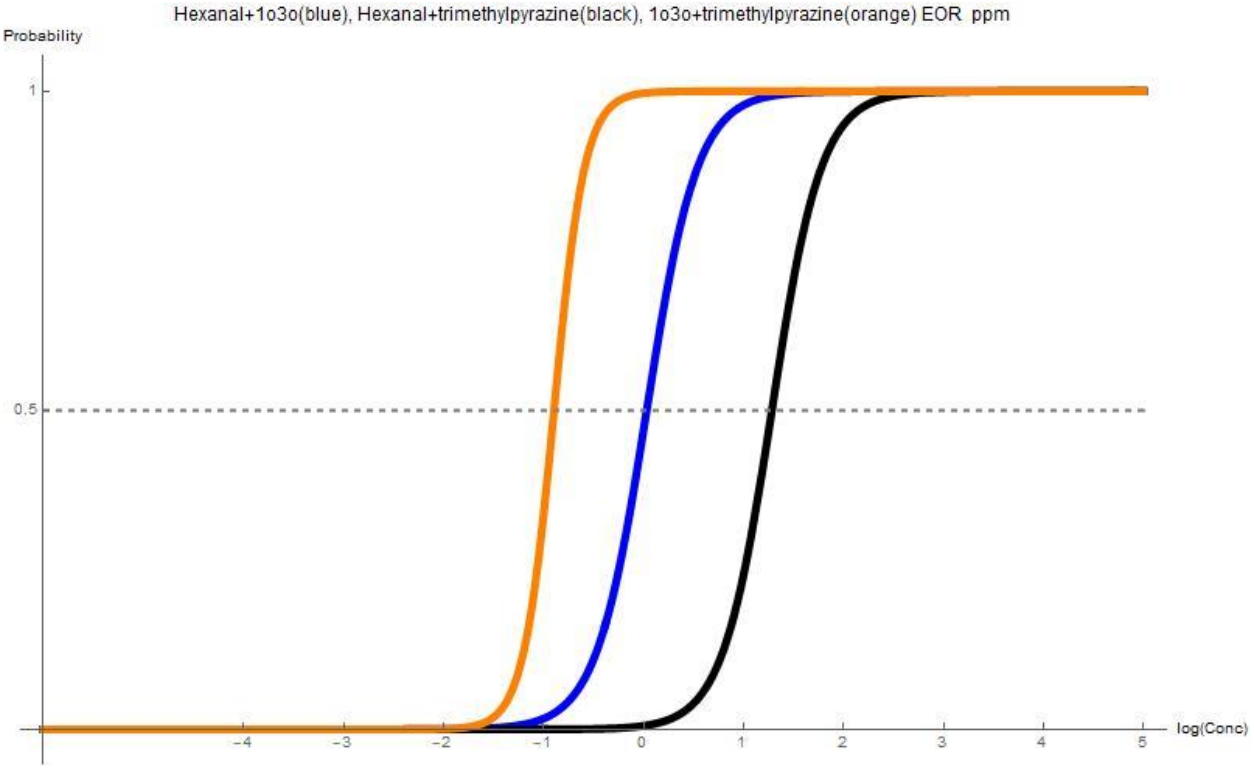

Figure 13. Hexanal+1O3O, hexanal+trimethylpyrazine, 1O3O+trimethylpyrazine EOR comparison for subject A

3.4.2 Subject B binary comparison

The binary EOR comparison for three mixtures for subject A are shown below. The orange, blue, and black curve represents binary mixture of 1O3O+2A1P, hexanal+1O3O, and hexanal+2A1P respectively.

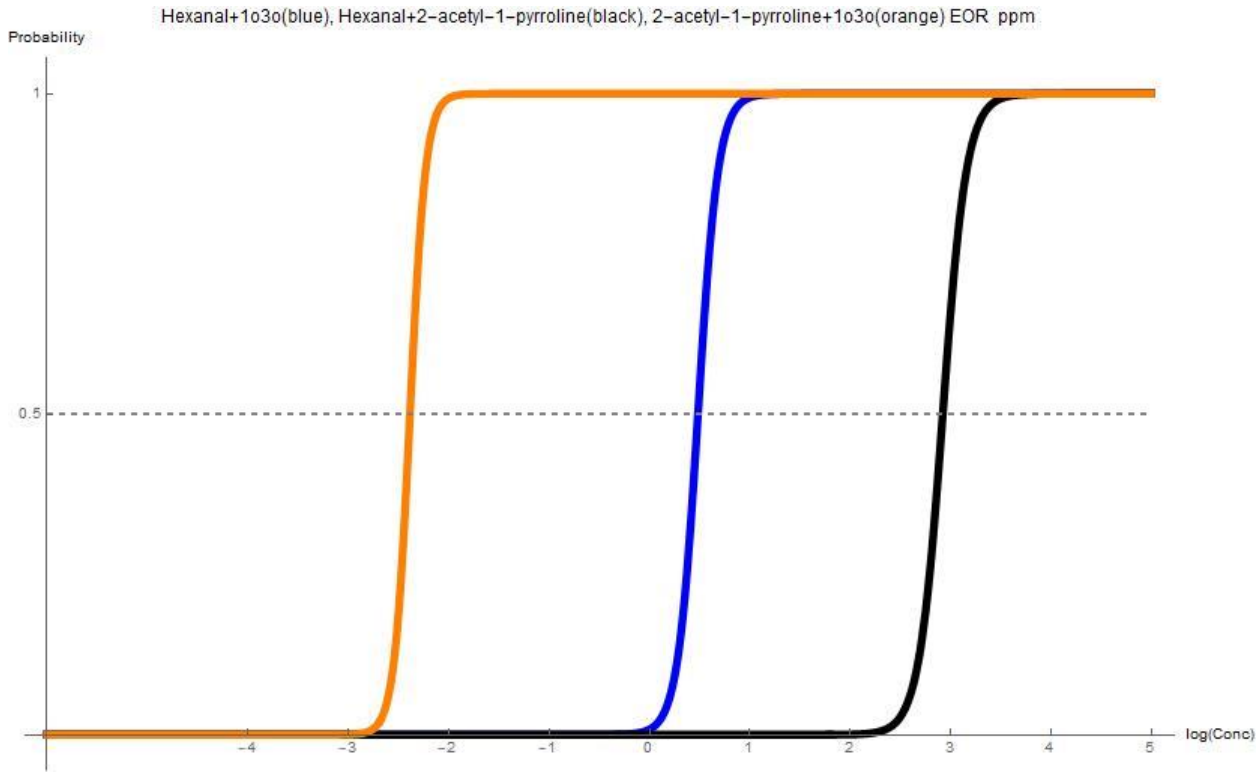

Figure 14. Hexanal+1O3O, hexanal+2A1P, 2A1P+1O3O EOR comparison for subject B

CHAPTER 5

EXPERIMENT 3: RECONSTRUCTING ALMOND MILK ODOR IMAGE

5.1 Aim

This experiment was conducted to reconstruct odor image of almond milk by using the EOR from previous experiment. Additionally, we wanted to analyze the relationship between the EOR of three key odorants and the final concentration which may deliver almond milk odor image.

5.2 Materials and methods

5.2.1 Samples and reagents

5 mL 70% ethanol was put into 45 mL of Great Value Original Unsweetened Almond Milk (Ingredients: almond milk [filtered water, almonds], calcium carbonate, sea salt, potassium citrate, sunflower lecithin, gellan gum, vitamin A palmitate, vitamin D2, D-alpha-tocopherol) which matches other testing solutions.

The following chemicals were used in this experiment:

Table 6. Summary of chemicals used in experiment 3

	CAS	Odor	Supplier	Concentration
Hexanal (>99%)	66-25-1	Green	Sigma Aldrich	
1O3O (>99%)	3391-86-4	Mushroom	(St Louis,	
Treimethylpyrazine (>99%)	14667-55-1	Nuts	USA)	
2A1P (10% w/w in Toluene)	85213-22-5	Nuts	Santa Cruz Biotechnology (Texas, USA)	0.12 – 0.38 ppb

Solutions were made in distilled water containing 7% v/v ethanol (food grade). The concentration of the test solution ranges was based on the EOR ratio and corresponding concentration from the previous experiment. The binary mixture ratio of 1O3O and hexanal was fixed for subject A based on her EOR ratio, and different concentration of trimethylpyrazine was added into eight binary mixture containing 1O3O and hexanal. For subject B, hexanal and 1O3O

ratio is fixed based on his EOR and different concentration of 2A1P was added into eight binary mixture containing hexanal and 1O3O. The concentration gradient of the 2-Acetyl-1-Pyrroline is from 1.0 ppb to 4.2 ppb.

5.2.2 Methods

In this experiment, the method is used similar to the threshold and EOR experiment described above except some minor changes. The first change is every session includes 2 different mixture solutions and one almond milk standard sample instead of three testing solutions. The second change is the question. Right before experiment, 50 mL of each diluted sample was transferred to a 250 mL Teflon bottle. Starting from the lowest two concentration, three Teflon bottles are randomly installed on sniff port including a control sample, and rotate three times for the first experiment. During the experiment, the bottle that is punched randomly. After the subject was ready for the experiment, the program gave instruction for two training sessions. Next, the subject smelled 12 replicates of three random puffs, and were asked “Does it smell like almond milk?”. The subject has to choose between Yes or No. Once finished, the software thanks subject for participating experiment. Next, three Teflon bottles were rotated three times to minimize error.

5.2.3 Data analysis

Data analysis is similar to the method used above. Except the graph is made by using Matlab except mathematica. Two different models include polymorphic and normal distribution were used to fit the results from experiments.

5.3 Results

By plotting the data into different models, different shape of curves was created. The data was first plotted into order three model, which generated a tail curve. Next, the data was plotted to the normal distribution model, which doesn’t have the tail curve.

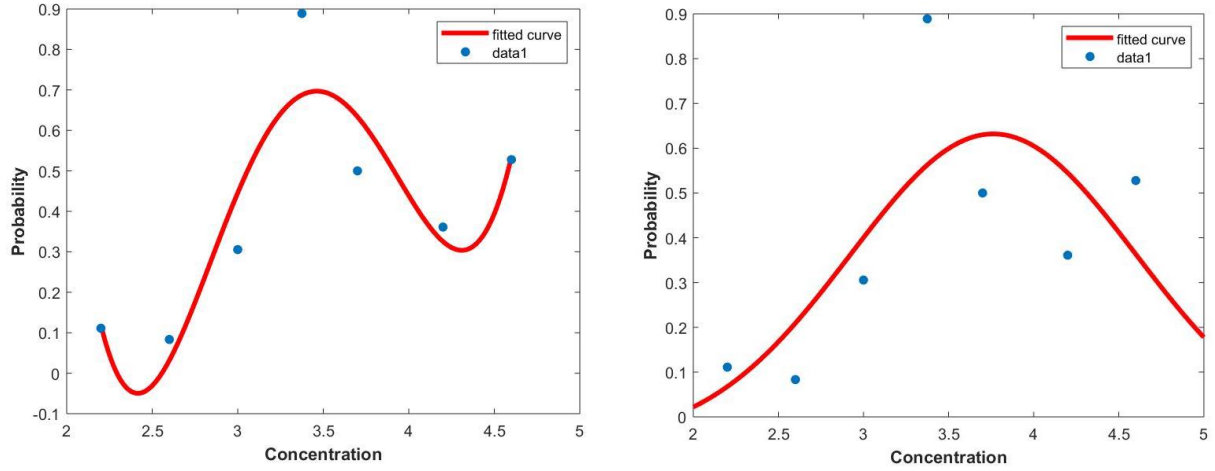

Figure 15. Probability of identifying mixture as almond milk vs trimethylpyrazine concentration

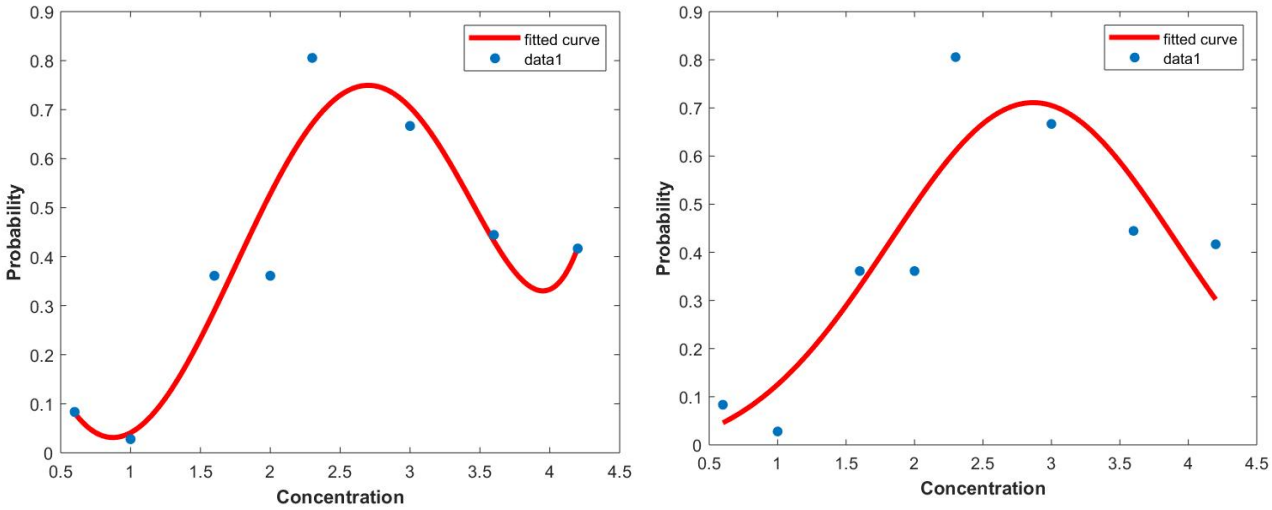

Figure 16. Probability of identifying mixture as almond milk vs 2A1P concentration

CHAPTER 6

DISCUSSION AND CONCLUSION

6.1 Differences key odorants among subjects in almond milk

Almond milk tends to have weak flavor since only about 4% w/w almond was actually in the beverage. Besides different dilution analysis is done by subjects, the key odorants obtained by using GC-O, dilution analysis and Charm analysis also showed different key odorants among people. All of the subjects can smell 1O3O and most of the subjects can smell hexanal. Hexanal is considered as a good indicator of nuts oxidation (Pastorelli et al., 2007). 1O3O is also a common oxidation product (Rosso et al., 2018). It is reasonable that subjects could smell these two compounds. However, the aroma of these two compounds, which smells like green and mushroom, doesn't have a nutty or roasty aroma easily detectable in almond milk.

According to the results from GC-O analysis, subject B, C, D had 2A1P as their key odorants. 2A1P was identified as a predominant aroma component of raw almond by Erten and Cadwallader (Erten & Cadwallader, 2017), but not as key odorant because it didn't appear in the highest dilution. 2A1P has a roasty or nutty aroma, that subjects described as "similar to almond milk aroma".

Subject A is different from other subjects since she smelled trimethylpyrazine from the highest dilution instead of 2A1P. Thus, trimethylpyrazine was by subject A as a key odorant for almond milk. It is interesting to find that trimethylpyrazine also has a nutty odor in which subject described as "somehow similar to almond milk". In some papers, the same key odorants were used for all subjects to simulate a certain food aroma. This is the first time that we found subjects use different key odorants to recognize the same food product. If our finding are widely seen among people, then we should always identify key odorants for subject before we simulate any food aroma.

6.2 Comparison of threshold and EOR between subjects

Although two subjects have one different key odorants for almond milk, both of them have hexanal and 1O3O as their key odorants and their thresholds of these two compounds are close. One interesting thing is that subject A often had a flatter threshold and EOR logistic curves, while subject B's curve was relatively steep. This indicates that when doing the experiment, subject A has more random choices among two odorants. Whereas subject B is certain that one odorant is smelled over the other. In EOR analysis, we can see a flatter curve for subject A and a steeper curve for subject B. We estimated the EOR ratio among two odorants by using the equation:

$$Predicted\ EOR_{(A,B)} \frac{Threshold_A}{Threshold_B}$$

For EOR analysis, the solution is made based on each subject's threshold and very near their threshold thus intensity expected not to play a role in this experiment. Thus, our model is different from the intensity response model that was proposed by Cain and other scientists (Ferreira, 2012). If there is no suppression between odorants, measured EOR should be equal to equal to predicted EOR. If the measured EOR is larger than the predicted EOR, it means that one of the odorant is suppressed by another since larger amount of this odorant is required in the binary mixture to reach equal potency. For example, in the binary mixture of trimethylpyrazine and hexanal, it requires more than twice the predicted trimethylpyrazine to reach equal potency than hexanal. It means that trimethylpyrazine is largely suppressed by hexanal in the mixture. In this case, for subject A that hexanal suppressed both trimethylpyrazine and 1O3O and trimethylpyrazine suppressed 1O3O. For subject B, 2A1P suppressed both hexanal and 1O3O while hexanal suppressed only 1O3O. Another thing than should be noticed is subject B's measured EOR how close it is to the predicted EOR while subject A's measured EOR deviates a substantially from the predicted EOR. More threshold and EOR are needed to from different subjects to explain the reason for this phenomenon.

Table 7. Summary of measured EOR and predicted EOR for subject A

	Measured EOR	Predicted EOR
Hexanal+1O3O	1.08	7.14
Trimethylpyrazine+hexanal	19.20	8.41
1O3O+trimethylpyrazine	0.127	0.017

Table 8. Summary of measured EOR and predicted EOR for subject B

	Measured EOR	Predicted EOR
Hexanal+1O3O	3.05	3.85
Hexanal+2A1P	826	808
2A1P+1O3O	0.004	0.005

6.3 Reconstruction of almond milk odor image

After investigating the threshold of odorants and their interactions among binary mixtures, we tried to reconstruct almond milk odor image by mixing three key odorants. By adjusting only one key odorant, the probability of identifying mixture as almond milk changed with concentration. The highest probability can nearly reach 90% similarity of almond milk. In addition, we notice that only at a certain mixture ratio that the odor mixture could be identified as having an almond milk aroma. This observation was also mentioned by Romagny's experiment. (Romagny et al., 2018) He stated that only a certain ratio of the key odorants could generate a similar odor image of that food. Because we fixed the concentration of two key odorants and adjusting the concentration of the third key odorants, we can clearly see that its impact on probability of make the solution smell like almond milk.

6.4 High impact key odorants and low impact key odorants

During the reconstruction of almond milk odor image experiment, we noticed that some odorants have a greater impact than others. To be more specific, adding one component can largely suppress other odorants in the mixture. For subject A, she noted that adding just little hexanal could highly suppress 1O3O and trimethylpyrazine. For subject B, adding tiny amounts of 2A1P could make the solution smell like popcorn. This can be also seen in reconstructing almond milk experiment results. Although we didn't have time to prove our assumption, we did observe that one key odorant has a greater impact than other two key odorants.

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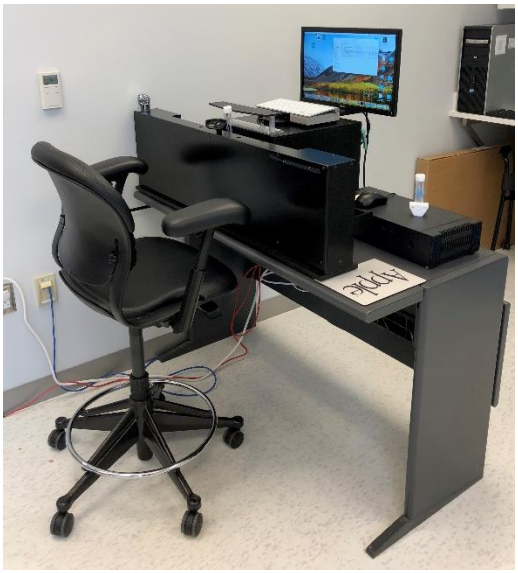
APPENDIX

Appendix 1. Sniff Olfactometer set up a. SO setup b. Teflon bottles positioning c. Subject doing experiments d. Inside of SO e. one-puff experimental timeline

a.

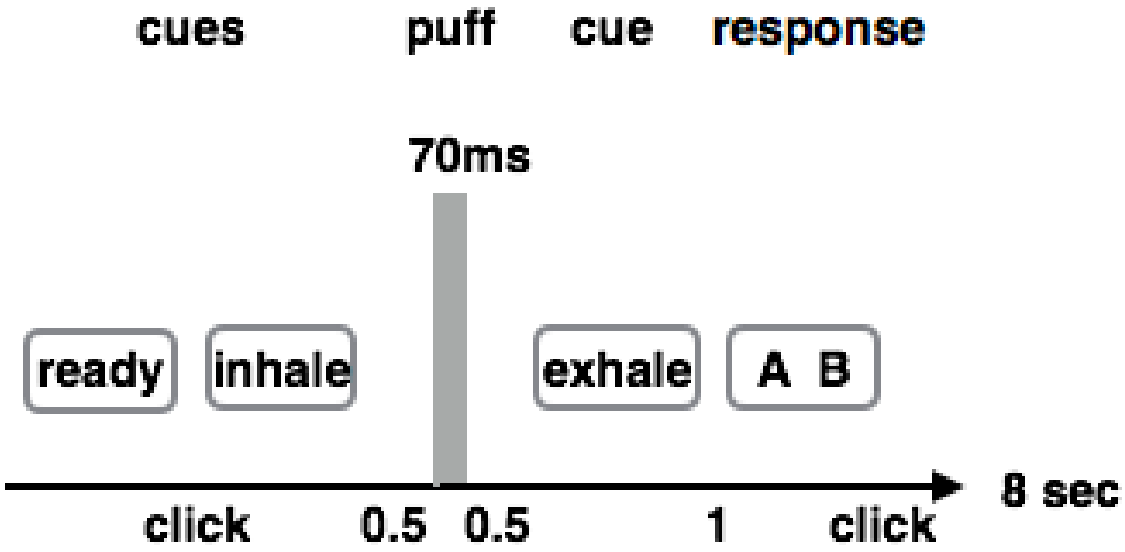


b.

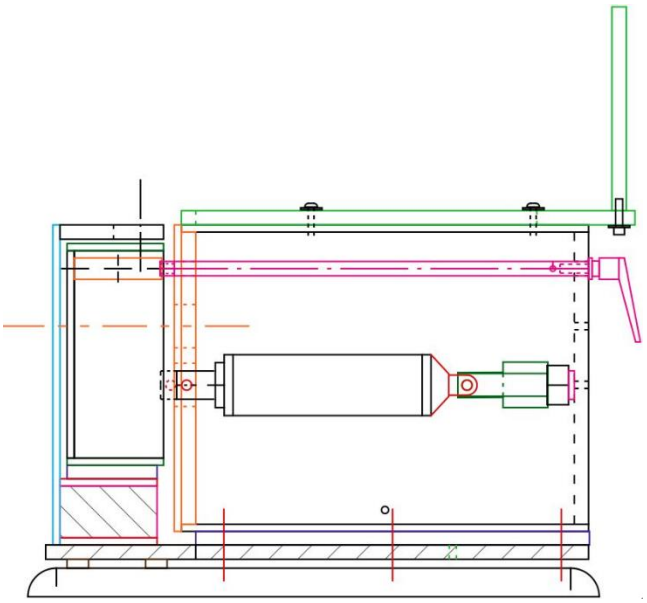
c.

d.

e.



Appendix 2. Side seeing of sniff olfactometer



Appendix 3. PsychoPy software experiment screenshots

Welcome to the Sniff Olfactometer

Each time, you will have to identify the odor you smell in the bottle.

We will start with a training test.

Ready?

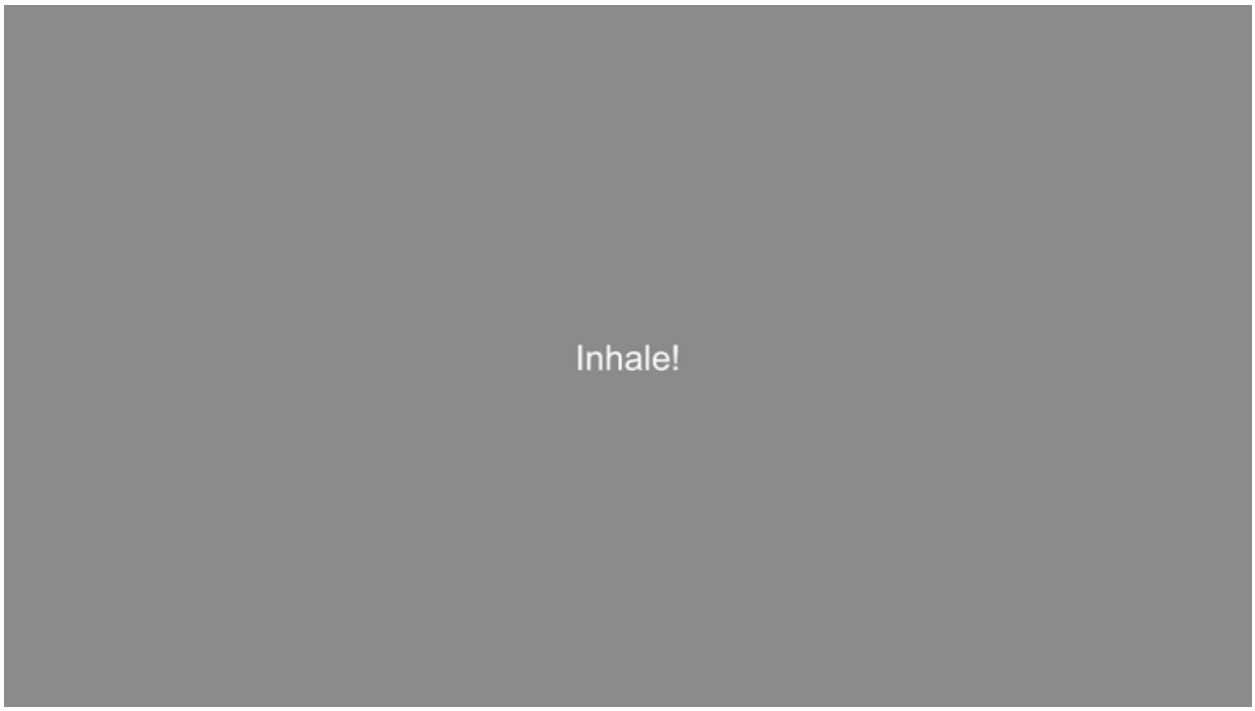
Perfect!

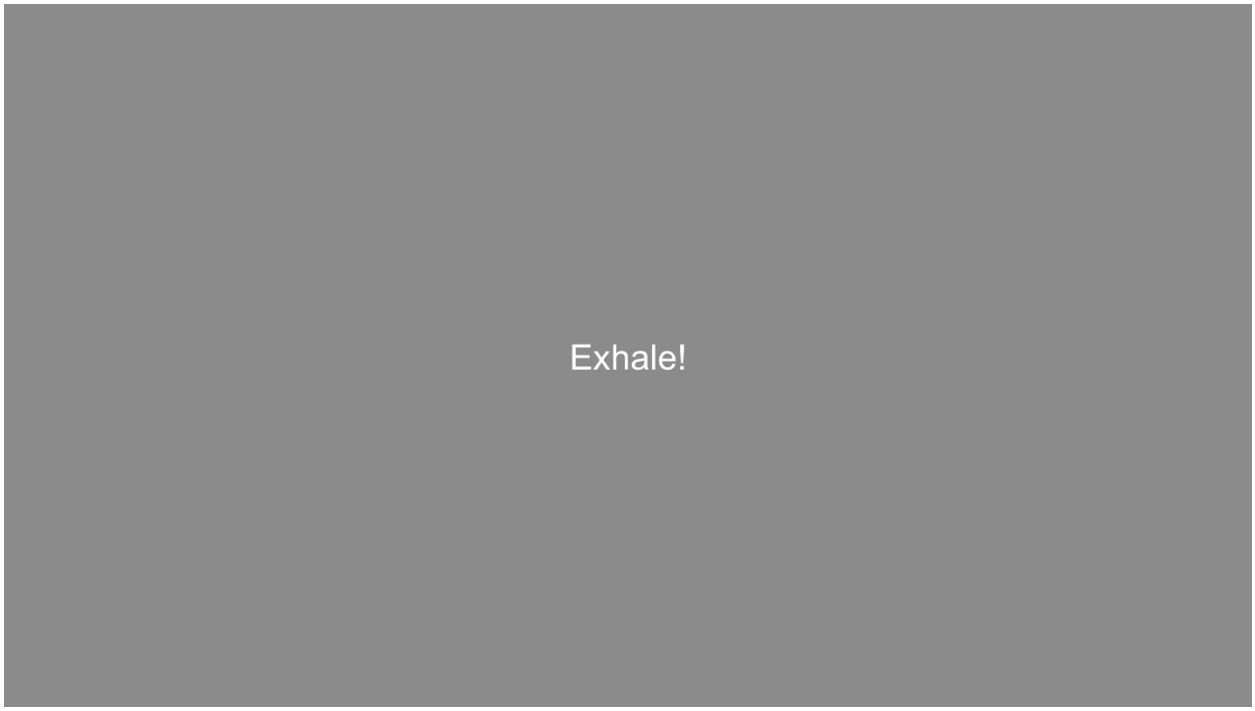
Let's continue with the real experiment

You will have to smell 36 samples

Click to continue

When you are ready to inhale click


Inhale!


Exhale!