ODORANTS INTERACTION IN TERTIARY ODOR MIXTURES

MFS Project Report

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Master of Food Science

by

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ABSTRACT

Madeleine M. Rochelle tested equal odds ratio of binary mixtures about three key food odorants (KFOs) in potato chips to explore the mechanisms of organisms decoding odor mixtures published in 2017. The goal of this project is testing odorants interactions in tertiary mixtures. In other words, how the change of concentration of third odorants affects the perception of binary mixtures. The selection of three odor compounds are based on the Rochelle's research, three key odorants in potato chips including methional (MAL) with baked potato nots, methanethiol (MOL) with rotten cabbage notes, and 2-ethyl-3,5-dimethylpyrazine (2E3,5DP) with toast notes. Sniff Olfactometry (SO) was used to measure threshold of each odorant, EOR of binary mixtures, and EOR of tertiary mixtures after 70ms stimulations for 4 subjects. However, three subjects were screened out , because they did not pass the pre-testing or recognition test. Even though the number of subjects is limited, the project set up an initial to proceed the experiment procedures, so that future research could repeat more successfully by recruiting more subjects with fully training and applying different odor compounds to investigate odorants interactions in tertiary mixtures. The results indicated odor perception is not a linear process in tertiary mixtures

BIOGRAPHICAL SKETCH

Sirui Chen will graduate in May 2023 from her Master of food science (MFS) degree, at Cornell University. She was in Terry Acree's lab, working on the perception of food odorants. She received a Bachelor of Science degree in Food Science from the University of Florida, and her first two years of undergraduate in Food Science were from Michigan State University. She is interested in improving food sensory and flavor.

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Thank my parents for their financial and emotional support during the project.

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
MAL	Methional
MOL	Methanethiol
2E3,5DP	2-ethyl-3,5-dimethylpyrazine
EOR	Equal odds ratio
PEG 400	Polyethylene Glycol 400
SO	Sniff Olfactometer

1.Introduction

People are surrounded by multiple mixtures of odorants in daily life. No matter the smell from air, foods, or something else, all perceptions of odorants begin with the activation of human olfactory receptors and these percepts are invisibly changed by our memories and past experiences.

A paper published in 2017 explored how psychophysical functions of human odor perception of binary odorant mixtures of the three key odorants in potato chips *(Madeleine M. et al., 2017).* In other words, how human perception changes as they are asked to smell very brief puffs of binary odorant mixtures as a function of their concentration ratios. These ratios ranged from concentrations dominated by one odorant to those dominated by the other. Olfactory perceptions of the components of a binary mixture are encoded simultaneously and dominate the perception based on their ratios. However, the decoding process that follows the binding of odorants to certain sets of olfactory receptors (ORs) in the olfactory epithelium *(Huang et al., 2022)* is affected by suppression and adaptation depending on the similarities and differences in their odor qualities. For example, C8, C10, and C11 n-aldehydes cross-adapted each other due to their similar odor quality, “citrus” while they were suppressed by C6 aldehyde, hexanal due to its different odor quality, “green smelling”, even though all four compounds have very similar chemical structures *(Kurtz et al., 2010) (Kurtz et al., 1970).*

A dramatic decline has been observed in the capacity to accurately identify all the odor components of a mixture if it contains more than 3 odorants *(Laing et al., 2003)*. In addition, Laing found that the types of odorants have no effect on the total number of odorants detected, but that training for familiarity will affect which odorant in a mixture is detected.

The goal of this work was to determine the optimum odorant concentration ratios, conditioning processes, and behavioral paradigms to test subjects for their ability to recognize the key potato chip odorants. Once subjects and procedures are in place that produce robust psychophysical functions for all three binary mixtures we will be able to investigate methods to further explore psychophysical function of human perception in tertiary mixtures based on the odorant interaction in each binary mixture in combination with the missing third key odorant. The key food odorants in potato chips, includ methional (MAL) with baked potato notes, methanethiol (MOL) with rotten cabbage notes, and 2-ethyl-3,5-dimethylpyrazine (2E3,5DP) with toast notes *(Madeleine M. et al., 2017).*

2.Material and Methods

2.1 Materials

2-ethyl-3,5-dimethylpyrazine CAS Registry No. 27043-05-6 (>95%) (2E3,5DP), methanethiol (MOL) CAS Registry, and methional CAS Registry No. 3268-49-3 (>97%) (MAL) were obtained from Sigma-Aldrich (St. Louis, MO, USA) while Polyethylene Glycol 400 (PEG 400) were obtain from CAS Registry No. 9002-88-4, JT Baker®, Avantor Performance Materials, Inc, (>99.5%). Solutions were made in distilled water containing 10% v/v PEG 400.

2.2 Subjects

4 subjects, including 1 male and 3 females from Cornell University (22-24 years old), were recruited. One subject was eliminated after pre-testing while two subjects were eliminated after recognition tests. Only one subject finished all tests.

2.3 Equipment and software

Sniff Olfactometer (SO) with high-speed actuators (9cm/s) was used to squeeze 250 mL Teflon bottles, so that odor in the headspace of bottles can be puffed out from a sniff port. PsychoPy® (v2021.2.3) was operated automatically while R (version 4.1.3 – “One Push-Up”) was used to analyze data.

2.4 Methods

10% PEG 400 Preparation and Deodorization

3600 mL of water was mixed with 400 mL PEG 400. The mixture was added 20g of charcoal powder and was shaken. The deodorized 10% PEG was obtained by setting for a week, and charcoal powder was removed by using vacuum filtration.

Sample Preparation

10 PPM stock solution for MOL in 10% PEG 400 and 1000 PPM stock solution for MAL and 2E3,5DP in 10% PEG were prepare separately in three 50 mL amber bottles.

Test Sample Solution Preparation

Each odorant was diluted into different concentrations by using 10% PEG solution. The mixture solutions were prepared in 50 mL amber bottles 1 day before the experiment. After overnight shaking, the solutions were transferred from amber bottles into 250 mL Teflon bottles 15 mins before experiments.

Threshold Measurements

One subject was trained and conditioned three odorants, MAL, MOL, and 2E3,5DP with baked potato, rotten cabbage and toast descriptors. Three bottles containing ascending concentrations (the ascending concentrations were based on the rule “mutual difference between concentrations greater than $\Delta C/C \geqq 0.33$”) of one odorant with 1 to 3 labeling were puffed 4 times randomly. The other three subjects were recruited for threshold measurements after pre-testing. In the pre-testing, one bottle was prepared with highest concentration (according to the threshold determination from that one subject) of one of odorants while another bottle was prepared with 10% PEG as a blank. The thresholds were detected by calculating the concentration where detection probability equals 0.5, also called recognition thresholds. One subject was screened out after pre-testing while the remaining three subjects’ thresholds for each odorant were obtained.

Table 1
The selection of concentrations for testing MAL, MOL, and 2E3,5DP recognition thresholds.

Samples	MAL in PPM	MOL in PPM	2E3,5DP in PPM
Bottle 1	0.01	0.05	0.5
Bottle 2	0.1	0.1	10
Bottle 3	0.5	0.2	30

Recognition test

The highest concentrations from testing recognition threshold of three odorants were placed into SO at once, and each odorant was puffed randomly to test if subjects were able to distinguish each odorant. Subjects were asked to choose “baked potato”, “rotten cabbage” or “toast” from question: “What did you just smell?” Subjects who are unable to distinguish three odorants could not finish the experiments.

Determination of EOR of binary mixtures

After determining each subject's threshold for each odorant, three-times threshold concentration for each odorant was used as constant-concentration odorant mixing with six ascending concentrations of another two odorants. The three combinations in this experiment included three-time threshold of MAL as constant-concentration odorant with six ascending concentrations of MOL from 0 to 1 PPM and 6 varying concentrations of 2E3,5DP from 0 to 50 PPM, and three-times threshold of 2E3,5DP as constant-concentration odorant with 6 ascending concentrations of MOL from 0 to 1 PPM. Subjects were asked to answer questions, for example, "What did you just smell?" while they are forced to choose either "baked potato" or "rotten cabbage". The EORs of binary mixtures were obtained by calculating the concentration where the detecting probability of either varying-concentrations odorants or constant-concentrations odorants equals 0.5.

Table 2
The selection of concentrations for testing binary mixtures, MAL and MOL

MAL at constant in PPM	Varying MOL in PPM
0.3	0
	0.08
	0.15
	0.25
	0.5
	1

Table 3
The selection of concentrations for testing binary mixtures, MAL and 2E3,5DP

MAL at constant in PPM	Varying 2E3,5DP in PPM
0.3	0
	5
	10
	15
	30
	50

Table 4
The selection of concentrations for testing binary mixtures, 2E3,5DP and MOL

2E3,5DP at constant in PPM	Varying MOL in PPM
15	0
	0.08
	0.15
	0.25
	0.5
	1

Determination of EOR of tertiary mixtures

Similar procedure with determination of EOR of binary mixtures, six ascending concentrations of each odorant were used to mix with binary mixtures at EORs. The descriptors for combination of MAL and MOL, MAL and 2E3,5DP, MOL and 2E3,5DP were called "chips", "fries", and "peanuts" separately. Subjects were trained to learn the smell of binary mixtures with new descriptors. The concentration range for 2E3,5DP used in mixing with binary mixtures is from 0 to 50 PPM; MOL is from 0 to 1 PPM; and MAL is from 0 to 3 PPM. Subjects were asked to answer questions then, for example, "What did you just smell?" while they are forced to choose either "chips" or "toast". The EORs of tertiary mixtures were obtained by calculating the concentration where the detecting probability of either a binary mixture or third odorant equals 0.5.

Table 5
The selection of concentrations for testing tertiary mixtures, MAL&MOL combination and 2E3,5DP

MAL in PPM + MOL at constant in PPM	Varying 2E3,5DP in PPM
0.3+0.25	0
	5
	10
	15
	30
	50

Table 6
The selection of concentrations for testing tertiary mixtures, MAL&2E3,5DP combination and MOL

MAL in PPM + 2E3,5DP at constant in PPM	Varying 2E3,5DP in PPM
0.3+15	0
	0.08
	0.15
	0.25
	0.5
	1

Table 7
The selection of concentrations for testing tertiary mixtures, MOL&2E3,5DP combination and MAL

MOL in PPM + 2E3,5DP at constant in PPM	Varying MAL in PPM
0.25+15	0
	0.1
	0.3
	0.5
	1
	3

3.Results and Discussion

3.1 Threshold measurements

Three subjects were tested for their detection thresholds of compounds, MAL, MOL and 2E3,5DP. The concentration range for MAL is from 0.01 to 0.5 PPM; the concentration range for MOL is from 0.05 to 0.2 PPM; the concentration range for 2E3,5DP is from 0.5 to 30 PPM. But, because one of subject did not pass the recognition test, the threshold results of that subject were not included in Table 8.

Table 8

Recognition thresholds for odorants MAL, MOL, and 2E3,5DP for three subjects

Subjects / Samples	1	2
MAL in PPM	0.09	0.033
MOL in PPM	0.07	0.08
2E3,5DP in PPM	5	1.989

Three subjects' threshold for MAL, MOL, and 2E3,5DP were measured by SO and operated by R. The detection thresholds of MAL were measured for subject 1 and subject 2 is 0.09 and 0.033 PPM respectively; the detection thresholds of MOL were measured for subject 1 and subject 2 is 0.07 and 0.08 PPM respectively; the detection thresholds of 2E3,5DP for subject 1 and subject 2 is 5 and 1.989 PPM respectively (Table 8).

3.2 Measurements of EOR of binary mixtures

Applying three-times concentration of threshold for odorants, MAL and 2E3,5DP, and keeping them at constant concentrations, then to vary the concentration of another odorant. To be specific, threshold of MAL was estimated as 0.1 PPM (Table 8) for subject 1 and 2 while 0.3 PPM of MAL was used and kept constantly, and the concentrations of MOL varied from 0.08 PPM to 1 PPM. 0 PPM of MOL, or to say the bottle only with 0.3 PPM of MAL was used as a control. The concentrations of MOL in 6 bottles were ascending, including 0, 0.08, 0.15, 0.25, 0.5 and 1 PPM of MOL. Same procedures ware repeated for binary mixture of MAL and 2E3,5DP. The concentration of MAL was set at constant 0.3 PPM while the concentration range of 2E3,5DP was from 5 PPM to 50 PPM, including 0, 5, 10, 15, 30 and 50 PPM. In addition, threshold of 2E3,5DP was estimated as 5 PPM for subject 1 and 2 (Table 8). Therefore, the experiment used 15 PPM of 2E3,5DP as a constant concentration odorant, and 6 concentrations of MOL were varied at 0, 0.08, 0.15, 0.25, 0.5 and 1 PPM.

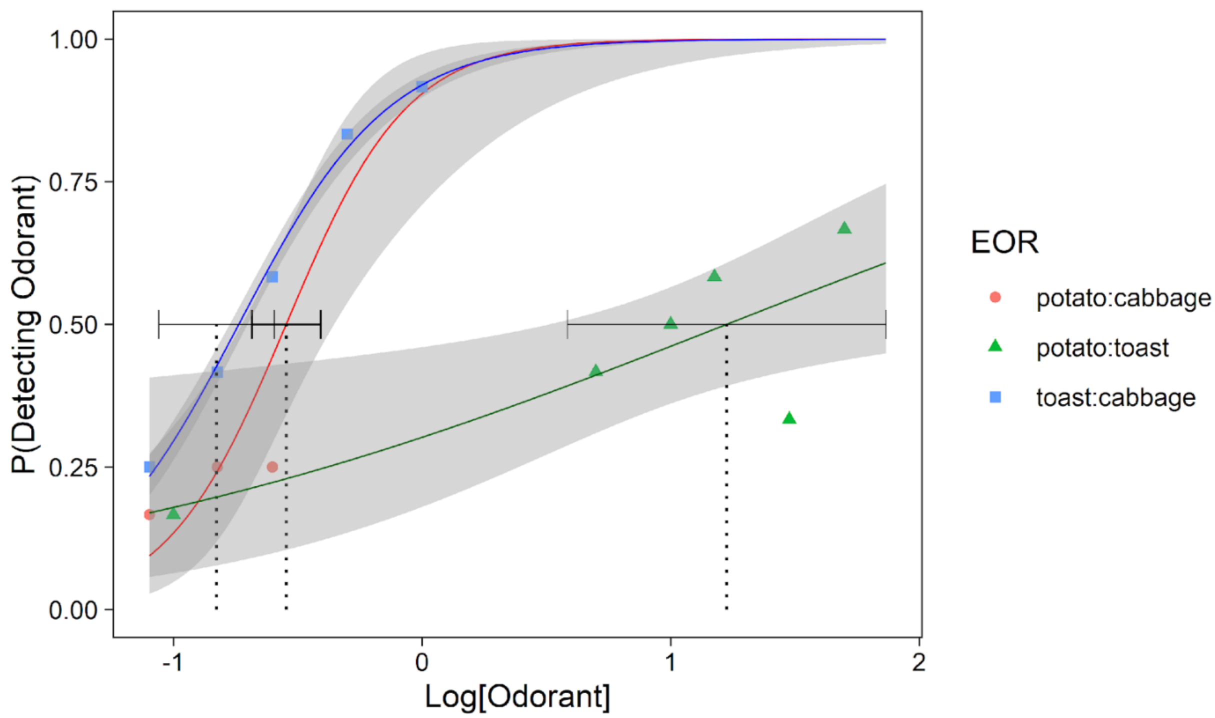

Figure 1. The concentrations of binary mixtures EOR, including MAL&MOL mixture, MAL&2E3,5DP mixture, and 2E3,5DP&MOL mixture, for subject 1

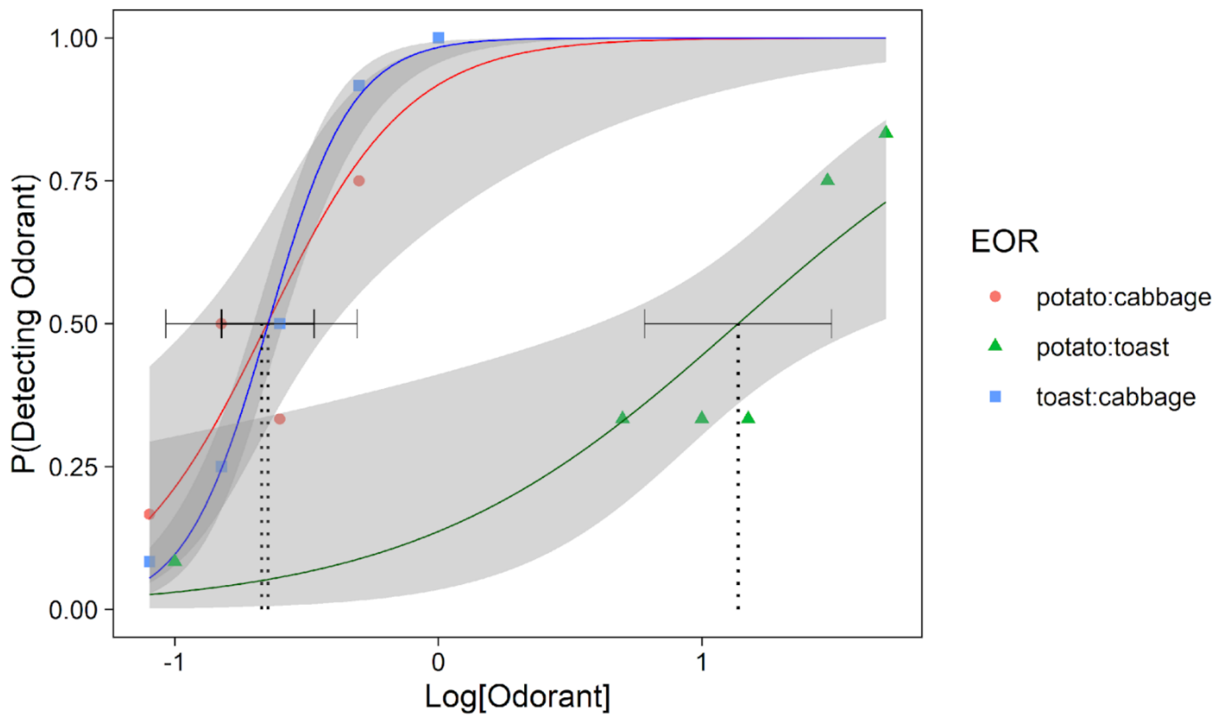

Figure 2. The concentrations of binary mixtures EOR, including MAL&MOL mixture, MAL&2E3,5DP mixture, and 2E3,5DP&MOL mixture, for subject 2

Table 9

Three binary mixtures EOR concentration for 2 subjects

Samples \ Subjects	1	2
MAL + MOL in PPM	0.284	0.225
MAL + 2E3,5DP in PPM	16.797	13.733
2E3,5DP + MOL in PPM	0.149	0.213

The results of concentrations of binary mixtures EOR for three combinations of MAL and MOL, MAL and 2E3,5DP, and 2E3,5DP and MOL for subject 1 are 0.284, 16.797 and 0.149

respectively while the concentrations of binary mixtures EOR are 0.225, 13.733 and 0.213 respectively (Table 9).

Figure 1 has shown that subject 1 was unable to distinguish odorants, MAL and 2E3,5DP, due to unfamiliarity of these two odorants while subject 2 may have same issue on distinguishing MAL and 2E3,5DP . Another potential reason that affects the curve for subject 2 could be the concentration differences among 3 of 6 concentrations of 2E3,5DP are not distinguishable for subject 2.

According to the poor performance of subject 1 on distinguishing odorants, subject 1 discontinued to finish the measurement of tertiary mixtures EOR.

What should be mentioned is lowest concentration, 0 PPM, in each binary mixture were dropped to reflect on Figure 1. and Figure 2. The reason for this is there is a conflict between experiment designs and PsychoPy running system. The system does not expect to log 0. Therefore, 0.01 (the number as less as possible to approach 0, and 0.01 is small enough in this case) was used to replace 0 to make the PsychoPy system work successfully. However, 0.01 PPM concentration never existed in the experiment as the lowest concentration. To keep the accuracy of the results, the lowest concentration, 0 PPM were dropped.

3.3 Measurements of EOR of tertiary mixture

Subject 1 discontinued this round to test tertiary EORs, because subject 1 was unable to distinguish each odorant, especially for MAL and 2E3,5DP. Subject 2 was asked to learn the smell of binary mixture of MAL and MOL at EOR, and the binary mixture at EOR was named “chips”. Same procedures were repeated to teach subject 2 the smell of binary mixtures at EOR of MAL and 2E3,5DP named “fries”, and the mixture of MOL and 2E3,5DP named “peanuts” (Figure 3).

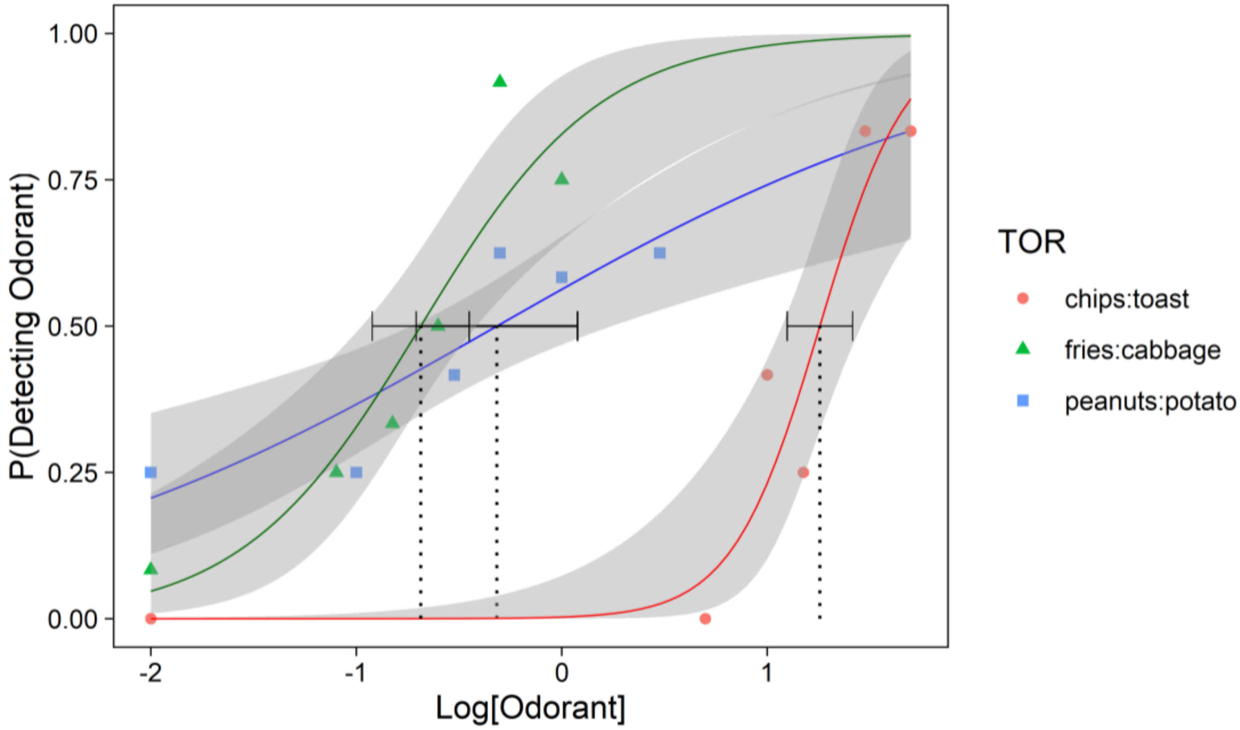

Figure 3. The concentrations of tertiary mixtures EOR, including MAL&MOL with addition of 2E3,5DP mixture, MAL&2E3,5DP with addition of MOL mixture, and 2E3,5DP&MOL with addition of MAL mixture, for subject 2. The binary mixture of MAL and MOL was named as “chips” descriptor; the binary mixture of MAL and 2E3,5DP was named as “fries” descriptor; the binary mixture of 2E3,5DP and MOL was named as “peanuts” descriptor.

Table 10
Three tertiary mixtures EOR concentration for subject 2

Test round / Samples	1	2	3
MAL in PPM	0.3	0.3	0.483
MOL in PPM	0.25	0.206	0.25
2E3,5DP in PPM	18	15	15

In the test round 1, while MOL and MAL were mixed as a binary mixture at EOR, the concentrations of 2E3,5DP varied from 0, 5, 10, 15, 30 and 50 PPM (Table 5). The result from test round 1 showed the tertiary mixture EOR for the addition of 2E3,5DP was 18 PPM. In the test round 2, at the binary mixture EOR of MAL and 2E3,5DP with a "fries" descriptor, the binary EOR was estimated as 0.3 PPM and 15 PPM respectively, the tertiary EOR of the addition of MOL was 0.206 PPM. The tertiary EOR of MAL, MOL and 2E3,5DP in test round 3 was 0.483, 0.25 and 15 PPM (Table 10). Three combinations of any two of three odorant mixtures were measured for their binary EORs. At the binary EORs, the possibility that humans are able to percept either odorant A or odorant B should be 50 percent, which means that the odorant potency of odorant A or B is same at binary EOR. However, the results showed that odor perception is not a linear process. Concentrations that need to reach EOR in a tertiary mixture are not significantly different from the concentrations needed for binary mixtures in three test rounds.

4.Conclusion

Overall, the project set an initial plan for starting to explore odorant interaction in tertiary mixtures. The threshold concentrations of three compounds, MAL, MOL and 2E3,5DP, as key odorants in potato chips were determined by using PEG 400 as a base solution. Based on the threshold, three binary EORs of each subject were determined as well. What should be mentioned is threshold concentrations of three odorants for two subjects are not significantly different in this case. However, the determination of concentrations for testing binary or tertiary EORs should be based on the specific subject's threshold. For measuring tertiary EOR, the kind of questions asked during the test and descriptor names of binary mixture were established. These are also potential reasons that may affect the results of experiments. The results demonstrate that multiple measurements of EOR generated reproducible results, which means that only one combination of odorants is necessary to generate trustworthy EOR. Also, the results further confirmed that odor perception is not a linear process. The concentration needed to reach EOR in a tertiary mixture is not significantly different from concentration needed for a binary mixture.

Before we can proceed with more subjects in a full blown experiment, we need to optimize training and conditioning to prepare subjects to have clear discrimination between and recognition of all stimuli. The subjects who are unable to pass pre-testing and recognition testing must be excluded or retrained before binary and tertiary EORs are tested.

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