The Effects of Swallowing and Expectorating on Basic Taste Perception, Flavor Perception, and Concentration of Volatile Compound in the Nasal Headspace

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Amanda Peck

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Zata Vickers, Ph.D.

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Chapter 1

The Swallowing Mechanism

Three phases make up the swallowing mechanism; the oral phase, the pharyngeal

phase, and the esophageal phase. Some believe when a liquid is placed in the

mouth the "pre-oral" phase begins (Donner and Jones 1991). After the pre-oral

phase, the oral phase begins as outlined below:

> "During the oral phase, the lips, teeth, tongue, and jaw muscles mix the
> food with saliva to create the proper consistency for ingestion. The bolus
> is propelled into the oropharynx by the tongue, completing the oral phase
> of swallowing. Once the bolus reaches the tonsils, the pharyngeal phase of
> the swallowing reflex is triggered. This phase is automatic and lasts
> approximately one second. At this phase the palate closes separating the
> oral cavity from the nasal cavity, the throat muscles constrict pushing the
> food downwards, and the upper esophageal sphincter relaxes allowing the
> bolus to enter the esophagus. Finally, the food is actively transported
> down to the stomach during the esophageal stage. This is complete when
> the bolus passes the lower esophageal sphincter and into the stomach.
> However, the three phases of swallowing are not carried out in a uniform
> manner across the population; this impacts perception when swallowing a
> food product" (How You Swallow 2010).

Land (1996) proposed that the closure of the tongue and velum border during the

pharyngeal stage made the coating of the throat and "swallow breath" crucial to

perception since no retronasal aroma could be perceived during the pharyengeal

stage.

However, all people swallow in slightly different ways based on their personal anatomy and medical factors. This has led many authors to account the swallowing mechanism as a source of bias in sensory testing and retronasal headspace measurement. Interindividual variability has been noted in aroma release profiles across subjects and may be attributed to these anatomical and physiological differences (Deleris and others 2011, Buettner and others 2002, Normand and others 2004). The authors of these studies also noted that these individual differences in swallowing mechanism and aroma release profile present significant challenges to relating sensory perceptions to instrument measurements.

Swallowing and Expectorating

Without any background knowledge on sensory testing, one would assume products are tested in the same way they are eaten, by putting the food in the mouth, chewing (if necessary) and swallowing. Since the swallow condition must increase the total number of receptors stimulated, the perceived intensity might be expected to increase substantially with swallowing (Bartoshuk and others 1981). The effects of swallowing and expectorating on perception vary greatly depending on the products being tested and methods being used (Burdach and Doty 1987). Informal studies indicate that flavor intensity of food substances tend to increase during swallowing (Burdach and Doty 1987). Various studies focusing on

perception, discrimination, sensation dominance, and duration of perception have been conducted in an attempt to determine whether there is a difference in perception for panelists when swallowing compared with expectorating.

Burdach and Doty (1987) examined the influences of active oral processes on the retronasal perception of odor intensity. They hypothesized that mouth movements would lead to more intensely perceived flavors and that swallowing would lead to more intensely perceived flavors. In order to evaluate these hypotheses, subjects made magnitude estimates of flavor intensity of rum extract solutions and orange extract solutions. The evaluation period lasted 30 seconds with evaluations made five seconds after placing a solution in their mouth with their nose held, five seconds later with their nose open, five seconds later with either mouth movements or no mouth movements, and ten seconds later after either swallowing or expectorating the solution. Through this study, they found that mouth movements resulted in a higher perceived intensity than no mouth movements for the rum and orange extracts. When comparing expectorating and swallowing, there was no difference in perceived intensity of the orange extract solutions. However, swallowing yielded a significantly higher perceived intensity than expectorating for the rum extract solutions. When the hypotheses were combined, they were supported for both flavor solutions during the mouth movement

procedure and for the rum extract solution during the swallowing and expectorating procedure.

One limitation to this study was that ratings were made along a set time line. Swallowing or expectorating was always evaluated after the mouth movement portion of the study. The results may have been different if mouth movements and swallowing/expectorating data had been analyzed in a more independent manner. It would seem that because the mouth movements were proven to cause a significantly higher perceived intensity for both the orange and rum extract solution, these movements may have been impacting the swallowing and expectorating perceived intensity. Had Burdach and Doty instructed the panelists to swallow and expectorate without the preliminary mouth movements or analyzed the data independently of the mouth movements, a significant difference may have been observed in those two methods because the subjects wouldn't have been focused on the mouth movements. Also, one may note from the data that although they were not significant, the perceived intensities of the rum and orange extracts appeared greater when swallowing than when expectorating.

Kelly and Heymann (1989) conducted a study to determine whether swallowing and expectorating had an effect on panelists' ability to discriminate. The authors used milk with added milk fat and kidney beans with added salt to represent liquid and solid food systems. On each system, judges performed a triangle test and a

paired comparison test. The panel of nine judges was trained on basic procedures

and product characteristics prior to the testing sessions. Kelly and Heymann

concluded that there was no significant difference in discrimination abilities

between swallowing and expectorating for either the milk fat levels or the salt

levels. However, the confidence intervals they produced suggested a better

discrimination rate for ingestion than expectoration. By only manipulating the

sodium level of the kidney beans, Heymann and Kelly merely tested differences

in swallowing and expectorating when discriminating a change in basic taste. In

addition, the milk discrimination tests were focused on the texture of the milk. In

both cases, the manipulation of the product may not have caused changes to the

volatile flavors. The majority of texture and basic taste perception occurs in the

mouth, which would not be impacted by the act of swallowing or expectorating.

A more recent study conducted by Deleris and others (2011) showed that

swallowing resulted in more complex perceptions, but decreased the dominance

rate of aromatic compounds. A ten member panel that participated in four training

sessions was used. The solutions used were commercially available flavored

vodkas served at room temperature. After placing the solution in their mouths, the

panelists were instructed to hold it there for 10 s while performing predetermined

mouth movements. Panelists made ratings using the temporal dominance of

sensations method. Panelists chose the dominant attribute during the drinking

process which remained the same until another attribute was chosen as dominant. The authors found that when the product was expectorated, a fruity note was the only dominant sensation. However, when the product was swallowed, perceptions became more complex and lasted for various durations. For example, when the product was expectorated, a fruity note was perceived and remained dominant for 47 s. However, when the product was swallowed, a fruity note was perceived for 10 s, then a warm sensation was perceived for 12 s, and a green note was perceived for 5 s. One limitation to this research was the ethanol based product. Even with a 2-fold dilution, the ethanol still may have played a role in flavor perception because of the trigeminal sensations combining with flavor perception. Also, the trained panel consisted of members of the lab from which the researcher worked; by using panelists that were familiar with the products, the trigeminal sensations from the ethanol may have been more easily ignored and the true flavor profile experienced. An untrained or unfamiliar panel may not have perceived the volatile flavor as strongly over the ethanol sensations.

In order to evaluate the roles swallowing and expectorating play in the time intensity perception of basic tastes, Ott and Palmer (1990) used nine trained panelists to evaluate sucrose, sodium chloride, citric acid, and caffeine solutions. Panelists placed 10 mL of liquid sample in their mouths, held it there for twelve seconds and either swallowed or expectorated. The subjects began rating the

intensity of the solutions at the end of the twelve seconds and continued to make

ratings until the taste sensation had disappeared. With the exception of sodium,

Ott and Palmer found that there were no differences in aftertaste perception of the

basic taste solutions when swallowed vs. expectorated. They also found the

subjects were less consistent in their intensity ratings when the solutions were

expectorated than when the solutions were swallowed. Because of the less

consistent intensity ratings, they suggested that panelists may be having

difficulties making ratings while doing something unnatural such as

expectorating. Although Ott and Palmer did make conclusions about swallowing

and expectorating, the method used may have influenced the results by forcing the

panelists to hold the solution in their mouths for twelve seconds and then swallow

or expectorate and continue to make ratings. This initial time period may have

caused some degree of adaptation or some degree of unresponsiveness under

conditions of constant stimulation (Lawless and Heymann 2011). In addition, the

long holding times in the mouth would have caused the solution to become

diluted with saliva leading to lowered perception of the intensity. However, this

method did standardize the time the panelists were exposed to the product which

made it more precise than previous studies.

Retronasal Olfaction

 Physiological Process

To be smelled, flavor molecules need to reach the olfactory epithelium, which is located in the nasal cavity; "this can be achieved through orthonasal (sniff) or retronasal (mouth) airways," (Diaz 2004). Flavor is the combination of sensations from taste stimuli dissolved in saliva, and retronasal odor stimuli in air delivered backwards into the nose from the mouth upon chewing, but mainly on swallowing (Land 1996). Many times, the components of flavor can be confused when a panelist is attempting to separate taste and aroma. "The overall perceived flavor of a food is impacted by the way in which volatile aroma compounds are released in the mouth and transported to the olfactory receptors in the nose during food consumption, making retronasal olfaction an important factor in sensory evaluation," (Deleris and others 2011).

In 2001, Buettner and others conducted a study to determine the effects of swallowing on retronasal aroma stimulation using real time magnetic resonance imaging and videofluroscopy. They hypothesized that physiological barriers could retain volatiles in the oral cavity during the eating process. To test this hypothesis, a trained panel of five subjects took 25 mL of helium into their mouths without swallowing, breathed into a glass nose piece that was connected to a helium detector while their lips were closed. The collection lasted for one minute of

normal respiration. During this time, the pharyngeal performance was being monitored with videofluoroscopy. The same experiment was also repeated with subjects' heads bent towards their chest to ensure that the helium was collecting at the velum-tongue border. When the subjects refrained from swallowing, there was no detectable helium in the expired air from the nose. However, the researchers were able to detect an opening at the velum-tongue border when subjects performed a swallowing motion. As a result of this opening, an output of helium could be detected in the expired air from the nose. This demonstrated that closure of the velum is an effective barrier for volatile gases. The demonstration of the closure proved that when swallowing liquids, a direct connection of odorant loaded areas to the nasal cavity, exists at the instant of the swallowing breath (Buettner and others 2000).

In addition to closing the velum barrier when swallowing liquids, Buettner and others (2002) found the same closure process occurred when chewing semi solid and solid foods. They noted that during the mastication process, there was no transfer via the retronasal route to the nasal cavity. However, after swallowing semi-solid and solid foods, they observed the formation of a viscous salivary coating on the back of the tongue that may have contained particles of food along with odorants.

Buettner and others (2002) found that the transport of aroma via the retronasal route depends greatly on the texture and amount of food material present. It is noted that "when consuming a semi solid or solid food the first opening of the velum border occurs when the fork or spoon introduces the food into the mouth, allowing the person to have the first perception of retronasal aroma" (Buettner and others 2002). They also noted that the more liquid a food is, the greater the volume in the mouth. A solid food can become liquid through mastication, resulting in a phase change as well as a volume change. A more liquid phase and a greater volume present result in fewer velum border openings during the processing of the sample. However, after mastication, numerous intermittent swallows of the food sample occur, in turn creating many small swallow breaths prior to the larger and final swallow breath. Buettner and others noted the importance of differentiating the retronasal aromas produced by the intermittent swallows and the larger aroma pulse by the main swallow breath.

This research is critical to sensory research focusing on swallowing and expectorating because it shows that retronasal olfaction is influenced by the mastication and swallowing process. Although it isn't known how the expectoration mechanism works, there will not be much retronasal olfaction possible if the velum-tongue border remains closed.

In order to determine the effects of swallowing on ion concentration in the nasal headspace, Deleris and others (2011) used a Proton Transfer Reaction Mass Spectrometer (PTR-MS). The researchers hypothesized that the amount of ion present in the nasal headspace would be different when subjects swallowed the sample versus when they expectorated the sample. A ten member panel that participated in four training sessions was used. The solutions used were commercially available flavored vodka served at room temperature. Prior to sampling the nasal headspace air, the air in the test room was collected for 10 seconds. After the initial 10 seconds, the panelists positioned themselves on the nosepiece and breathed regularly for 30 seconds. The panelists then placed the entire sample in their mouth and held it there while making mouth movements for 10 seconds. After the ten seconds, they either swallowed or expectorated and continued to breathe for three minutes. This allowed the researchers to collect ion release data during the oral phase and after the swallowing or spitting protocol was followed. They concluded for certain ions that swallowing produced a greater aroma concentration in the nasal cavity than spitting. In addition, the highest concentration of ion was present at an earlier time point when the subjects swallowed than when they expectorated. These two observations led the authors to conclude that swallowing led to higher and earlier release of aroma compounds than did spitting, but only for some, not all ions.

Buettner and others (2002), aimed to determine the exact timing of odorant transfer to the nose and the oral mucosa adsorption level in regards to retronasal aroma perception. They used videofluroscopy, real time MRI and the spit off odorant measurement technique (25 mL of the solution was rinsed in the mouth of the panelist while the lips were closed, after 10 minutes it was spit into saturated $CaCl_2$ solution) to determine the process and timing odorants undergo when transferred retronasally from the mouth to the nose. The authors used four compounds that were distilled immediately before use. Panelists were instructed to brush their teeth and rinse their mouth with aroma free products. Following the cleaning procedures, they rinsed with deionized water several times and waited fifteen minutes before beginning the experiment. Panelists took 25 mL of each solution into their mouths, and swished it carefully in the mouth with the lips closed. The panelists made sure not to swallow and not to open the velum tongue border prior to expectoration (Buettner and others 2002). After ten minutes of rinsing, the panelist spit the solution into a saturated CaCl2 solution to inhibit enzymatic reactions. The authors concluded that the major amount of odorants lacking in the spit off solutions were bound in the oral mucosa. Odorants should also be bound to the oral mucosa when a product is swallowed. Based on the idea that there is a coating on the throat of volatile flavor compounds when swallowing, the amount of volatile compounds still present in the oral cavity would be greater when swallowing than when expectorating because of the throat

coating and the volatiles bound to the oral and throat mucosa. From the conclusions that no continuous aroma release in the oral cavity is possible once a food is placed into the mouth, we can assume that swallowing would be necessary for the greatest amount of volatile compound to be perceived retronasally.

Measurement Process via Atmospheric Pressure Chemical Ionization Mass Spectrometry

The atmospheric pressure chemical ionization mass spectrometer was first introduced as an analytical technique for analysis of trace compounds in the gas phase (Taylor and others 2000). It utilizes an inlet and an ionization source to detect very minimal amounts of volatile compounds, typically down to parts per trillion. One of the main goals of using APCI in research is to allow panelists the ability to eat and drink normally (Taylor and others 2000). The process used to by the APCI-MS to analyze volatile compounds in the nasal headspace is outlined below:

> "The APCI uses a small plastic tube or glass nose piece to collect air from the subjects' nostrils upon swallowing, then the air is pulled through a heated nitrogen venture with a set flow rate. It then crosses over a point to plane corona discharge that performs ionization. The ions are then sampled into a standard quadrupole MS maintained under vacuum. The reactant ion can then transfer charge to any ion molecule with a higher affinity," (Taylor and others 2000).

Taylor and others (2000) noted that acetone is tracked along with the compound being studied to ensure that panelists are breathing at a normal rate through their noses. The authors also went into detail about data analysis stating that a linear calculation is necessary to determine instrumental response and concentration. They used a calibration port to inject small volumes of a compound diluted with hexane into the heated flow of the nitrogen venture. Injecting this stock solution in different volumes allowed Taylor and others to make a calibration curve to relate the instrument response to the concentration of ion in the sample stream. The authors concluded that APCI-MS provides a technique for measuring volatile release in vivo for breath by breath analysis. They also note that through this technology, rate of release has been determined to play a major role in sensory perception. Unfortunately, the nature of the volatile compound being studied determines its behavior within the APCI-MS so it may not be the solution for evaluating all the compounds in a food.

Normand and others (2004) published a study with the goal of correctly incorporating the physiology of eating and drinking into the APCI-MS collection. In order to do this, they used an APCI-MS to evaluate volatiles present in the nose space when a panelist performed 3 swallowing protocols:

- "Protocol 1 freely…,"

- "Protocol 2: (i) hold mouth shut without swallowing for 2 min; (ii) inject 8 ml of flavoured solution into closed mouth; (iii) hold solution in mouth for 30s; (iv) swallow all the solution; and (v) breath normally through the nose, keeping mouth shut…,"

- "Protocol 3 identical to protocol 2 except that the subjects were asked to breathe at a given frequency…,"

They assumed that sensory perception would vary greatly depending on the breathing factors. The flow rate and composition of exhaled air were measured simultaneously. The authors found that patterns of flavor release varied greatly depending on the way people swallowed and breathed; a faster breathing rate mimicked the trend of having a higher concentration of volatile in the nose, so differing breath rates were a cause of potential error in data analysis.

Many have concluded that the most aroma rich air passing from the mouth to the nose occurred in the swallow breath (Land 1996, Buettner 2002, Normand and others 2004, Rabe and others 2004). Normand and others effectively took into account breathing rate, swallowing mechanism and saliva/mucosa volatile absorption to suggest analytical models for eliminating panelist breathing rate differences when obtaining volatile concentration from nose space using the APCI-MS.

Although there are differences in panelist breath flow rates and swallowing mechanisms, direct collection from panelists will give us the most insight into the relationship between concentration of volatile compound in the nasal headspace and sensory perception. The options of PTR-MS and the artificial nose are viable ones, but the APCI-MS remains the most effective means (Shojaei and others 2006) to measure the differences in aroma delivery between products when swallowed and expectorated by the panelist.

RESEARCH OBJECTIVES AND HYPOTHESES

Part I: Consumer Perception of Basic Taste and Flavor Intensity Upon Swallowing and Expectoration

Objective 1: To determine if basic taste intensity perception changes when the subject swallows the sample versus when the subject expectorates a sample.

> *Hypothesis 1*: Basic taste intensity will not change in swallowing versus expectoration.

Objective 2: To determine if ethyl butyrate intensity perception changes when a subject swallows a sample versus when a subject expectorates a sample.

> *Hypothesis 2:* Flavor intensity will be perceived to be higher when the panelist swallows the sample.

Part II: Trained Panelists' Perception of Flavor Intensity via the Time-Intensity Method upon Swallowing and Expectoration

Objective 1: To determine whether trained panelists perceive flavor more intensely when an aqueous solution, a pudding or a cookie was swallowed versus when an aqueous solution, a pudding, or a cookie was expectorated.

> *Hypothesis 1:* Flavor will be perceived more intensely in all three products when the subject swallows the sample compared to when the subject expectorates the sample.

Objective 2: To compare the time intensity profiles when the products were swallowed versus when the products were expectorated

> *Hypothesis 2:* Flavor will be more intensely perceived for a longer time when the subject swallows the sample; this will be true for all three products.

Part III: Determination of Aroma Compound Concentration in the Retronasal Headspace Using an Atmospheric Pressure Chemical Ionization-Mass Spectrometer.

Objective 1: To determine the concentration of aroma compound present in the nasal headspace when a subject swallows a sample versus when a subject expectorates the sample.

> *Hypothesis 1:* The nasal headspace will have more ethyl butyrate present when the subject swallows the sample than when the subject expectorates the sample.

Chapter 2

Part I: Consumer Perception of Basic Taste and Flavor Intensity upon Swallowing and Expectoration

Overview

Part I of this study includes experiments 1 and 2. The objective of experiment 1 was to determine whether consumers perceive basic tastes more intensely when they swallow the solution versus when they expectorate the solution. The objective of experiment 2 was to determine whether consumers perceive volatile flavors more intensely when they swallow the solution versus when they expectorate the solution. I hypothesized that consumers will not perceive basic tastes as more intense when they swallow than when they expectorate and that they will perceive volatile flavors more intensely when they swallow than when they expectorate.

Experiment 1

Methods

Panelists

Thirty-two panelists participated in the sweet and sour basic taste sessions, while twenty-eight participated in the bitter and umami taste sessions. The panelists

were students and staff of the University of Minnesota. All panelists were recruited through the Sensory Center at the University of Minnesota's panelist database of over 800 people. The panelists were selected based on their availability, absence of food allergies and willingness to participate (Screener, Appendix A, Figure A.1). Subjects were compensated $5 for each session they completed. Some subjects participated in more than one basic taste session. All research and procedures were approved by the Institutional Review Board at the University of Minnesota (Consent Form, Appendix B, Figure B.1).

Solutions

Sixteen solutions were used in each session of Experiment 1, four for each of the concentrations of each basic taste evaluated (sweet, sour, bitter, and umami). All solutions were prepared on a weight/volume basis. All basic taste solutions were prepared with purified water (Premium Waters, Minneapolis, Minnesota). The sweet solutions were prepared with sucrose (American Crystal Sugar, Chaska, Minnesota), in four different concentrations: 7.96 g/L, 51.0 g/L, 103.7 g/L, and 156.4 g/L. The sour solutions were prepared with citric acid (Fisher Scientific, Waltham, MA), in four different concentrations: 0.31 g/L, 0.84 g/L, 1.65 g/L, and 2.90 g/L. The bitter solutions were prepared with caffeine, anhydrous (Sigma Aldrich, St. Louis, Missouri), in four concentrations: 0.19 g/L water, 0.76 g/L water, 1.49 g/L water, and 2.68 g/L water. The umami solutions were prepared with Monosodium Glutamate (Ajinomoto, Chicago, Illinois), in four

concentrations: 0.03 g/L water, 0.35 g/L water, 3.62 g/L water, and 8.32 g/L water. The solution concentrations were selected based on the basic taste intensity scales used by the University of Minnesota, and will be referred to by their scale numbers (Table 2.1). All solutions were served at room temperature in a volume of 10 mL per sample in a coded 1-oz plastic soufflé cup.

Table 2.1: The taste compound concentrations (g/L) and the taste intensity scale values used throughout this thesis for the sucrose, citric acid, MSG, and caffeine solutions.

Taste Intensity (scale number)	Tastant			
	Sucrose (Sweet)	Citric Acid (Sour)	MSG (Umami)	Caffeine (Bitter)
2	7.96	0.31	0.03	0.19
5	51	0.84	0.35	0.76
8	103.7	1.65	3.62	1.49
11	156.4	2.9	8.32	2.68

Taste Sessions

During one session, the panelists evaluated the sample set for one of the basic tastes; there were four sessions total, one for each of the basic tastes. The samples were presented to the panelists in a Williams Latin Square design balanced for order and carryover effects (Williams 1949). This presentation balanced the orders in which the samples were served as well as the orders in which they were swallowed and expectorated. Regardless of the order, all panelists swallowed two samples of each concentration and expectorated two samples of each concentration. All sixteen samples in the specific basic taste session were

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presented on one large tray. Upon arriving at the test session, panelists were

prompted to enter their panelist code into the computer and follow all instructions

as prompted through the ballot. Panelists rated the taste intensities on computers

equipped SIMS 2000[©] (Sensory Computer Systems, Morristown, NJ, USA). Each

panelist was prompted to place the first sample in their mouth, hold it still until

they had perceived the taste then either swallow or expectorate (Ballot, Appendix

C, Figure C.1). After swallowing or expectorating, panelists used a 20-point line

scale to rate the taste intensity of each solution. Intensity was scaled from "0"

(None) to "20" (Intense) (Figure 2.1). The specific taste to be rated was provided

in the instructions to the subject. The scale was the same for the swallowed

samples and the expectorated samples. This process was repeated for all sixteen

samples.

Figure 2.1: Example of the line scale used to rate the perceived sweetness of a sugar solution.

Rate the sweetness intensity you perceived after SWALLOWING the sample

None   Intense

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
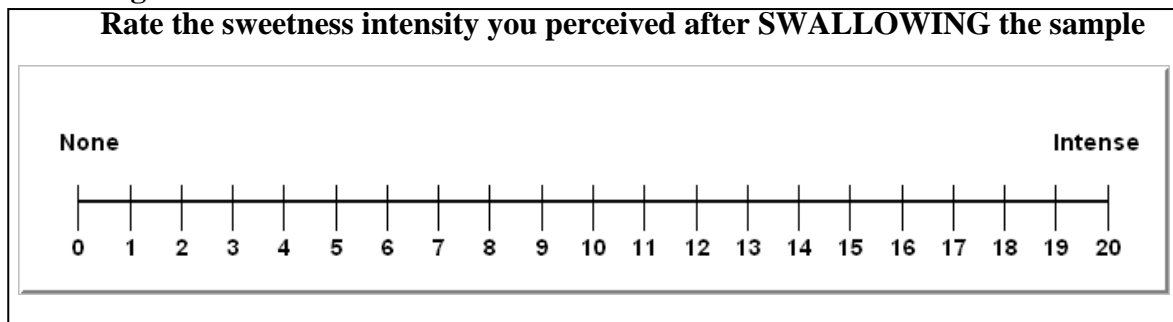
Data Analysis

The data analyses were conducted using SAS version 9.2. A univariate analysis of variance model (proc GLM) was run to determine if there was any difference in the taste intensity when the sample was swallowed vs. expectorated. The dependent variable in the model was specific taste intensity and the predictor variables were panelist, rep, concentration, swallow and concentration*swallow. This analysis was run for all concentrations combined first. Next, this analysis was run on each of the concentrations individually to evaluate the effect of swallowing and expectoration at each concentration level.

Example SAS code for all concentrations together:

```
proc glm data=xxx.sweet outstat=glmsweet;
class judge rep swallow concentration;
model overallaftertaste= judge rep concentration swallow
concentration*swallow;
random judge;
means rep swallow concentration/snk;
run;
```

Example SAS code for one concentration:

```
data xxx.concentrationsweettwo;
set xxx.sweet;
if concentration=2;
run;

proc glm data=xxx.concentrationsweettwo outstat=conctwosweet;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
```

This data analysis was carried out for all basic tastes and concentrations (Part 1 SAS Code, Appendix D).

Results

Sweet Solutions

No difference was found in the perceived taste intensity when the sweet solutions were swallowed versus expectorated for any of the concentrations (Table 2.2). No difference was found when the averages were evaluated overall on the basis of swallowing versus expectoration by combining all concentrations (Table 2.2 Figure 2.2).

Table 2.2: Average perceived intensities, F, and p values for each concentration and all judges (N=30) when the sample was swallowed and when it was expectorated. Small letters indicate a significant difference between swallowing and expectorating at that row's concentration (p<.05)

Concentration	Swallow	Expectorate	F-value	p-value
2	1.1	1.0	0.43	0.52
5	6.8	6.8	.0010	0.98
8	11.1	11.0	0.009	0.93
11	13.4	13.3	0.08	0.78
All Concentrations	8.0	8.0	0.07	0.79

Figure 2.2: Shows the mean perceived intensity for all judges (N=30) for sweet solutions overall when swallowed and expectorated
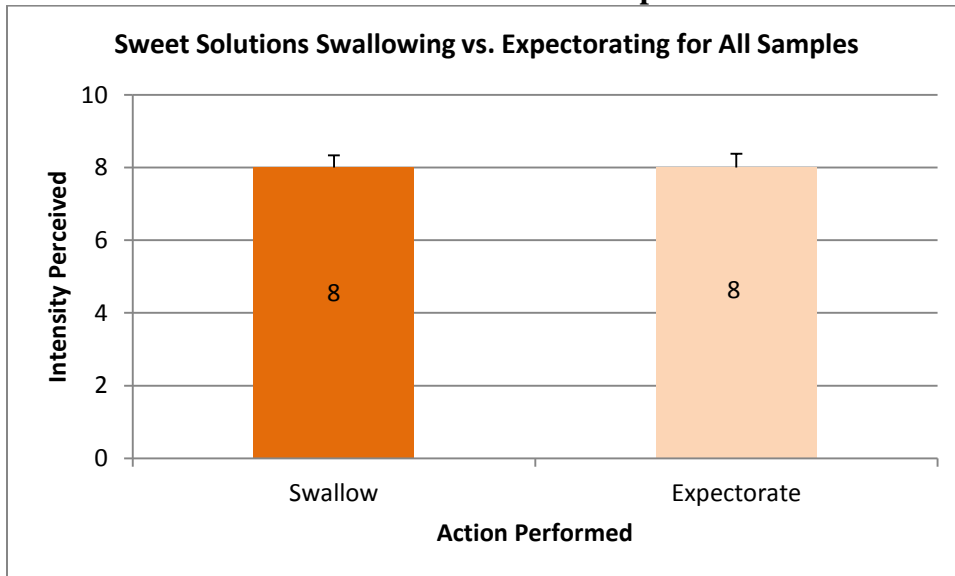
Sour Solutions

No differences were found in the perceived taste intensity when the sour solutions were swallowed versus expectorated for any of the concentrations (Table 2.3, Figure 2.3). No difference was found when the averages were evaluated overall on the basis of swallowing versus expectoration by combining all concentrations (Table 2.3, Figure 2.4).

Table 2.3: Average perceived intensities, F and p values for each concentration and all judges (N=30) when the sample was swallowed and when it was expectorated.

Concentration	Swallow	Expectorate	F-value	p-value
2	2.4	2.6	0.61	0.44
5	5.7	5.6	0.18	0.68
8	9.7	8.9	1.53	0.22
11	11.5	11.6	0.007	0.93
All Concentrations	7.3	7.2	0.26	0.61

Figure 2.3: Shows the mean perceived intensity for all judges (N=30) and all concentrations of sour solutions. The light orange dashed line represents the perceived intensity when swallowed while the dark orange line represents the perceived intensity when expectorated. Each point represents the perceived intensity for the specific concentration of citric acid.
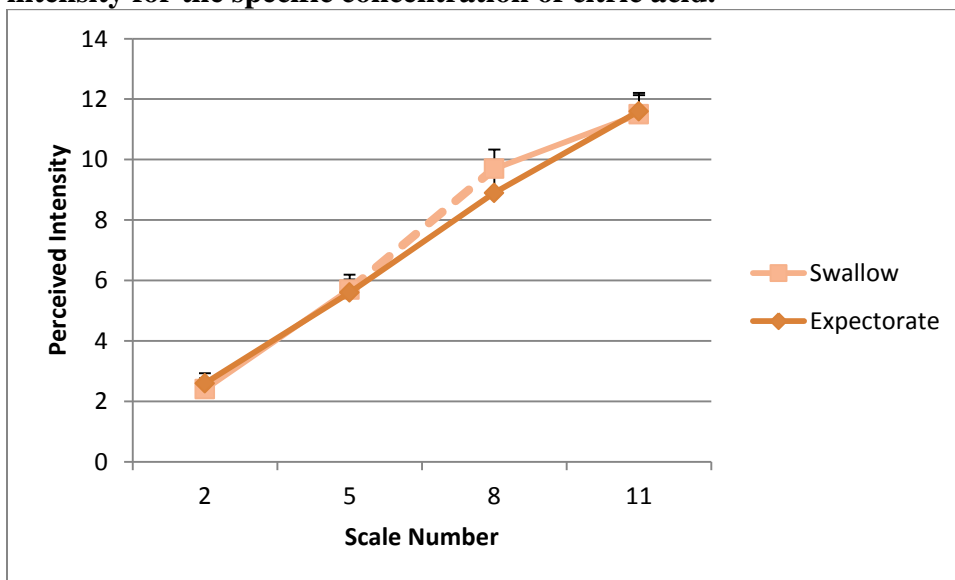
Figure 2.4: Shows the mean perceived intensity for all judges (N=30) for sour solutions overall when swallowed and expectorated.
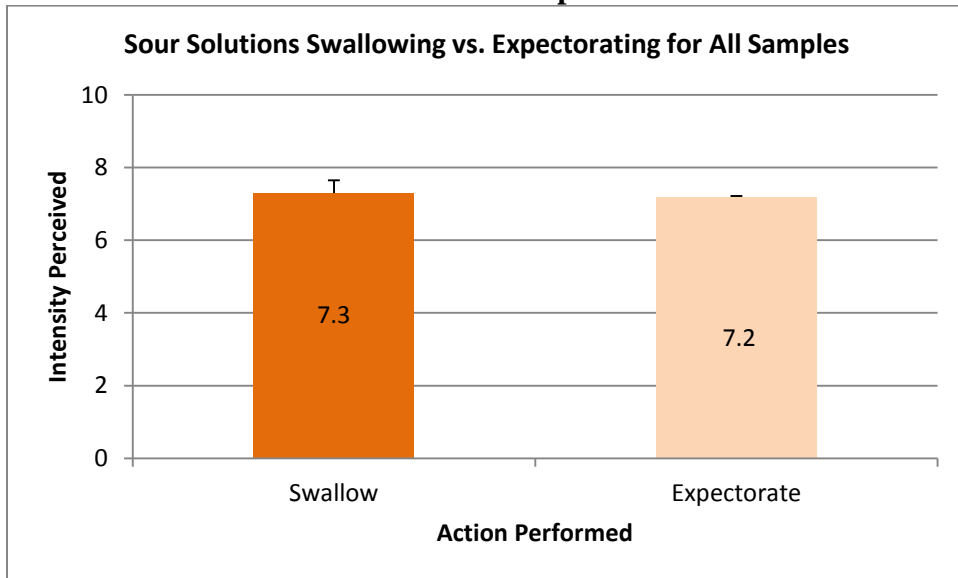
Umami Solutions

No difference was found in the perceived taste intensity when the umami solutions were swallowed versus expectorated for concentrations 2, 5, and 8. However, taste intensity of concentration 11 was perceived to be significantly higher when swallowed than when expectorated (Table 2.4, Figure 2.5). No difference was found when the averages were evaluated overall on the basis of swallowing versus expectoration by combining all concentrations (Table 2.4, Figure 2.6).

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Table 2.4: Average perceived intensities, F and p values for each concentration and all judges (N=28) when the sample was swallowed and when it was expectorated. Small letters indicate a significant difference between swallowing and expectorating at that row's concentration (p<.05)

Concentration	Swallow	Expectorate	F-value	p-value
2	1.3	1.3	0.04	0.84
5	1.9	1.6	0.66	0.42
8	7.8	7.5	0.23	0.64
11	10.5[a]	9.2[b]	5.28	0.02
All Concentrations	5.3	4.9	1.06	0.30

Figure 2.5: Shows the mean perceived intensity for all judges (N=28) and all concentrations of umami solutions. The dark orange dotted line represents the trend of the swallowed solutions and the light orange line represents the trend of the expectorated solutions

Figure 2.6: Shows the mean perceived intensity for all judges (N=28) for umami solutions overall when swallowed and expectorated
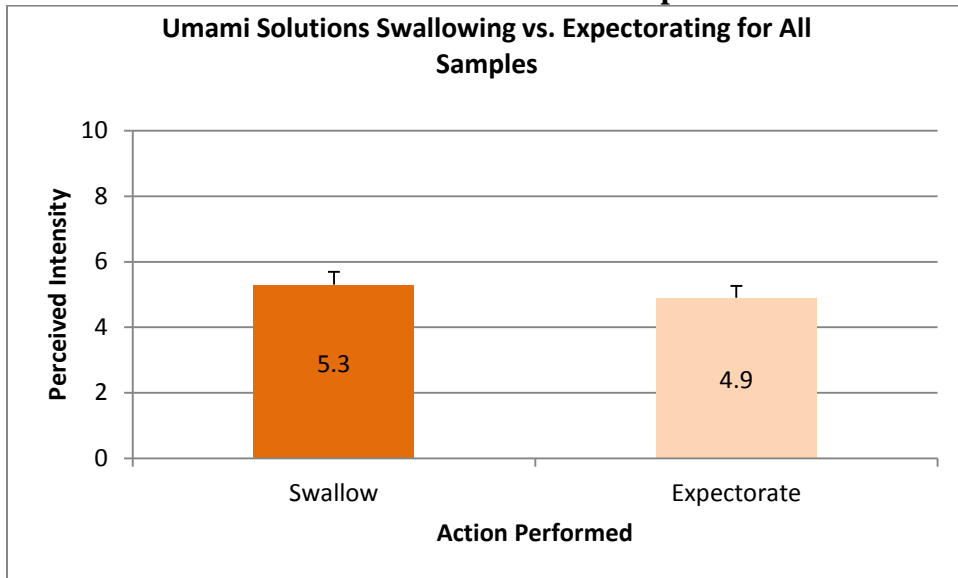
Bitter Solutions

No difference was found in the perceived taste intensity when the caffeine solutions were swallowed versus expectorated for concentration 2. However, taste intensity was perceived to be significantly higher when swallowed for concentrations 5, 8, and 11 (Table 2.5, Figure 2.7). Taste intensity was perceived to be significantly higher when swallowed for all bitter solutions overall (Table 2.5, Figure 2.8)

Table 2.5: Average perceived intensities, F and p values for each concentration and all judges (N=28) when the sample was swallowed and when it was expectorated. Small letters indicate a significant difference between swallowing and expectorating at that row's concentration (p<.05)

Concentration	Swallow	Expectorate	F-value	p-value
2	1.8	1.6	0.99	0.32
5	6.3[a]	4.8[b]	6.37	0.01
8	9.9[a]	7.1[b]	18.1	<.0001
11	12.6[a]	10.5[b]	11.2	0.001
All Concentrations	7.7[a]	6.0[b]	27.6	<.0001

Figure 2.7: Shows the mean perceived intensity for all judges (N=28) and all concentrations of bitter solutions. The dark orange dotted line represents the trend of the swallowed solutions and the light orange solid line represents the trend of the expectorated solutions
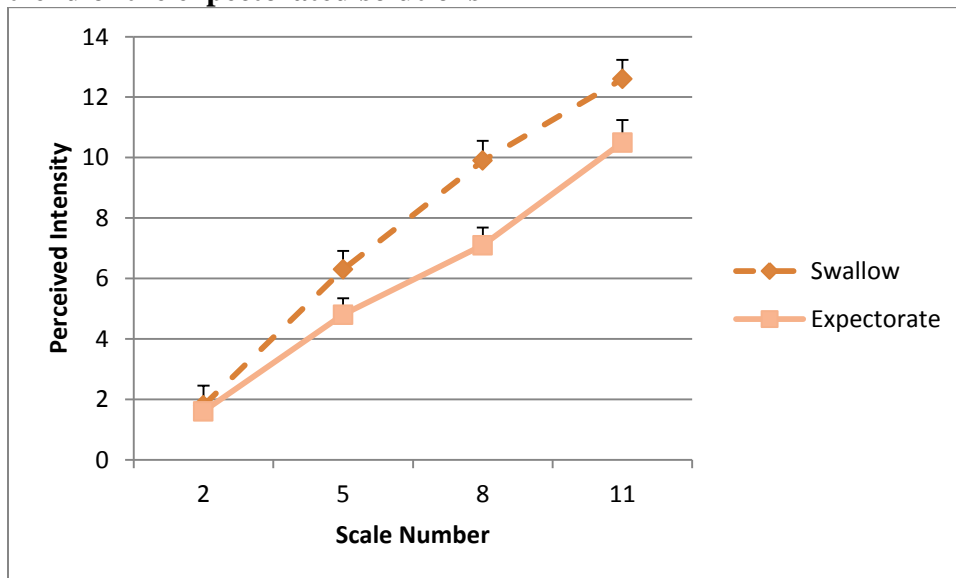
Figure 2.8: Shows the mean perceived intensity for all judges (N=28) for bitter solutions overall when swallowed and expectorated.
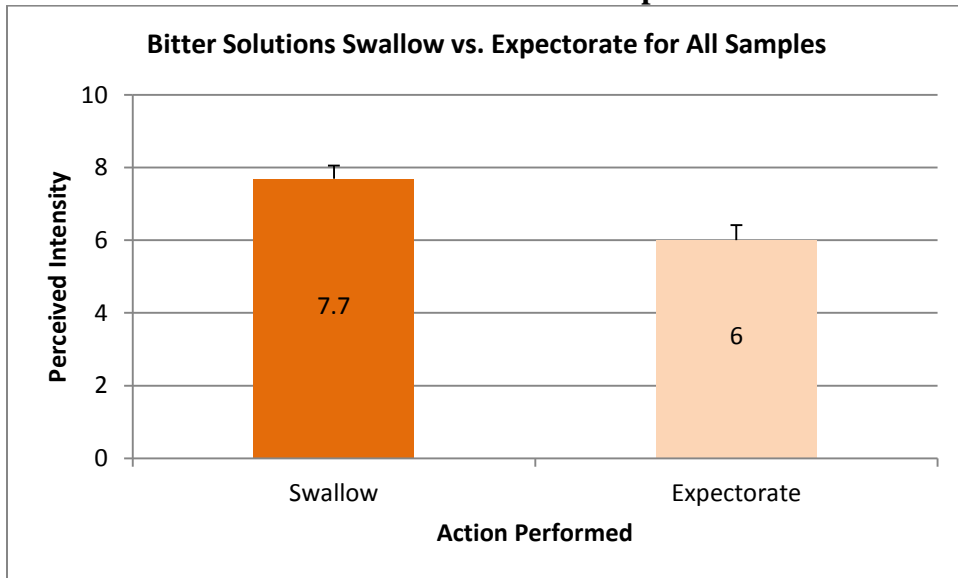
<center>*Experiment 2*</center>

Methods

Panelists

Twenty-nine people participated in the intensity rating portion of experiment 2.
Some of the panelists participated in Experiment 1 while others did not. The
subjects were students and staff of the University of Minnesota. The subjects were
recruited through the Sensory Center of the University of Minnesota's panelist
database of over 800 people. The subjects were selected based on their
availability, absence of food allergies and willingness to participate (Screener,
Appendix A Figure A.1). Panelists were compensated $5 for participation in this
session. All procedures were approved by the Institutional Review Board at the
University of Minnesota (Consent Form, Appendix B, Figure B.1).

Solutions

The solutions in experiment 2 were all prepared in a base solution of 7.96 g/L w/v
sucrose (American Crystal Sugar, Chaska, Minnesota) and 1 Liter of purified
water (Premium Waters, Minneapolis, Minnesota). Ethyl butyrate (Sigma Aldrich,
St. Louis, Missouri) was added in concentrations of 1 ppm, 2 ppm, 3 ppm and 4
ppm. We served 10 mL of each sample at room temperature in a coded 1-oz
plastic soufflé cup.

<center>32</center>

Test Session

The test procedure was the same as in Experiment 1. Each panelist evaluated sixteen solutions (Ballot, Appendix C, Figure C.1).

Data Analysis

The data in this experiment were analyzed in the same way as in Experiment 1 (Part 1 SAS Code, Appendix D.).

Results

Overall, the ethyl butyrate solutions were perceived to have a more intense flavor when swallowed (Table 2.6, Figure 2.9). The ethyl butyrate solutions were perceived to have a more intense flavor when swallowed for the 1 ppm, 2 ppm, and 3 ppm concentrations. However, there was no significant difference in perceived flavor intensity for the 4 ppm concentration (Table 2.6, Figure 2.10).

Table 2.6: Shows average perceived intensities, F and p values for each concentration and all judges (N=29) when the sample was swallowed and when it was expectorated. Small letters indicate a significant difference between swallowing and expectorating at that row's concentration (p<.05)

Concentration	Swallow	Expectorate	F-value	p-value
1 ppm	4.99^a	4.19^b	5.41	0.02
2 ppm	5.61^a	4.82^b	4.51	0.04
3 ppm	6.57^a	5.70^b	4.79	0.03
4 ppm	6.25	6.06	0.37	0.54
All Concentrations	5.86^a	5.19^b	13.67	0.0002

Figure 2.9: Shows the mean perceived intensity for all judges (N=29) and all concentrations of ethyl butyrate solutions. The dark orange dotted line represents swallowing, the light orange solid line represents expectorating.
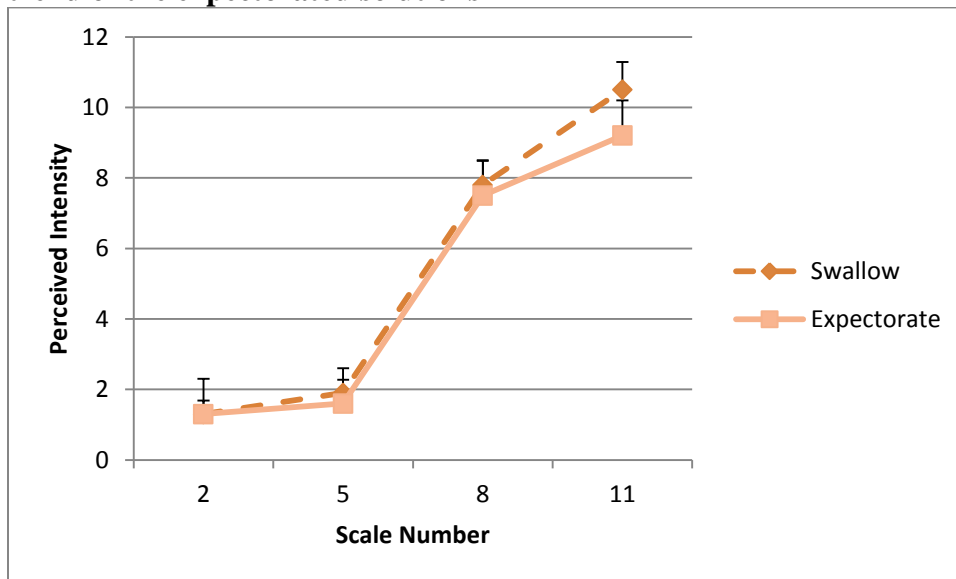

34
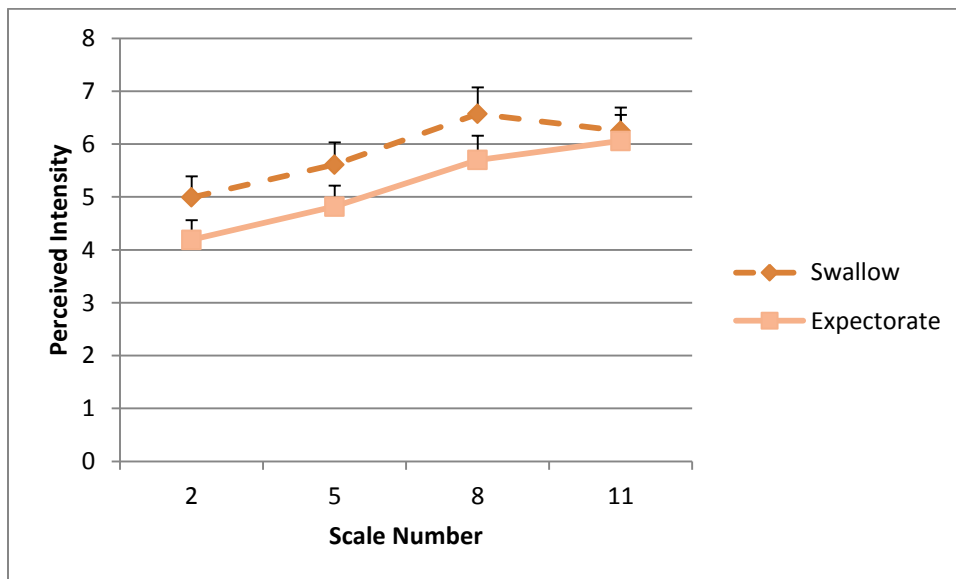
Figure 2.10: Shows the mean perceived intensity for all judges (N=29) for ethyl butyrate solutions overall when swallowed and expectorated.

Ethyl Butyrate Solutions Swallow vs. Expectorate for All Samples

Perceived Intensity

Swallow — 5.9
Expectorate — 5.2

Action Performed
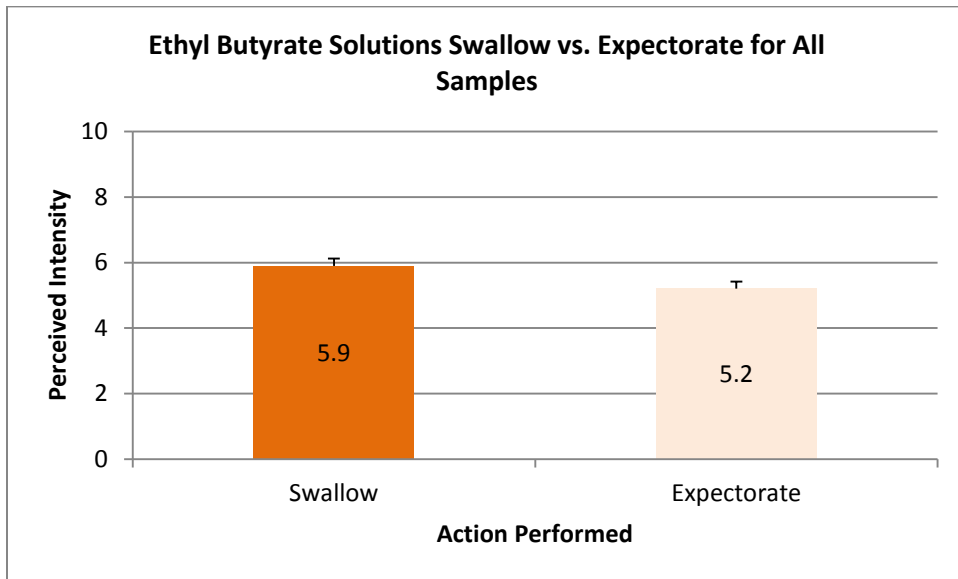
Chapter 3

Part II: Trained Panelists' Perception of Flavor Intensity via the Time-Intensity Method

Overview

Part II of this study contains experiment 3. Experiment 3 was broken into three sessions in order to evaluate flavor intensity in different food systems. The objectives of experiment 3 were to (1) determine whether trained panelists perceive flavor more intensely when the solution, pudding or cookie was swallowed versus when the solution, pudding or cookie was expectorated, (2) determine whether flavor was perceived more intensely for a longer time when the products were swallowed versus when the products were expectorated and (3) validate the untrained panelist data from experiment 2 by using a trained, calibrated panel. I hypothesized that the flavor of all three products would be perceived more strongly when swallowed than when expectorated and that the flavor would be perceived for a longer time when swallowed versus when expectorated.

Experiment 3

Method

Panelists

Eleven panelists from the trained panel from the Sensory Center at the University

of Minnesota participated in experiment 3. Panelists were calibrated to citric acid

and butanol twenty point scales. Each member of the panel had at least fifteen

hours of training. Panelists were selected based on their willingness to participate.

Panelists were compensated $10.00 for the training session and $13.00 an hour for

each of the three testing sessions. All research and procedures was approved by

the Institutional Review Board at the University of Minnesota (Appendix B,

Consent Form, Figure B.1).

Products

The first products were the four concentrations of ethyl butyrate solution used in

Experiment 2. The ethyl butyrate solutions were prepared in the same way as the

solutions in Experiment 2.

The second products were three flavor concentrations of milk-based pudding. The

base pudding was made with 948 mL of whole milk (Market Pantry, The Target

Corporation, Minneapolis, Minnesota). 9 g Xanthan Gum (Bob's Red Mill

Natural Foods, Milwaukee, Oregon), and 48 g sugar (American Crystal Sugar, Chaska, Minnesota). It was made by heating the milk and sugar to a simmer, and then adding the xanthan gum. It was immediately removed from the heat and beaten with a whisk for 10 minutes until it was free of lumps. After beating, the pudding was flavored with Pure Almond Extract (McCormick & Co., INC., Hunt Valley, Maryland). In total, three batches of pudding each with a different flavor concentration (.5%, 1.0%, and 1.5%) were made; all concentrations were prepared on a by weight basis, so 2.7 g, 5.5 g and 8.2 g of almond extract were added to the puddings respectively. All puddings were served at room temperature in an approximate weight of 4.5 g per sample, presented in a coded 2-oz plastic soufflé cup. Panelists received four samples of each concentration, twelve puddings in total.

The third products were three flavor concentrations of cookies. They was made following a basic butter cookie recipe including 440 g of butter (Market Pantry, The Target Corporation, Minneapolis, Minnesota), 206 g of sugar (American Crystal Sugar, Chaska, Minnesota) and 584 g of all-purpose flour (Market Pantry, The Target Corporation, Minneapolis, Minnesota). The cookies were made by creaming the butter and sugar with an electric hand mixer for five minutes. Then the flour was added to form cohesive dough. After the dough was formed, the flavor was added to a well in the middle and the dough was beaten with an

electric hand mixer for another minute. The cookies were flavored with Pure

Orange Extract (McCormick & Co., INC, Hunt Valley, Maryland). Three batches

of cookies were made according to the process above. In total, three cookie

treatments each with a different flavor concentration (.5%, 1.0%, and 1.5%) were

made; all concentrations were figured on a by weight basis, so 6.2 g, 12.3 g and

18.5 g of orange extract were added to the cookie batches respectively. The

cookies were baked at 300°F for 20 minutes. All cookies were served at room

temperature in a weight of 4.5g per cookie, presented in a coded 4-oz plastic

soufflé cup. Panelists received four cookies of each concentration, twelve cookies

in total.

Time Intensity Sessions

Training

Panelists attended one group training session. In this session, they were presented

with an aqueous solution, a pudding and a cookie that were representative of the

products being tested. During this session, they were trained on the mouth

movements, swallowing and expectorating procedures. They were also instructed

on the use of the time-intensity questionnaire and were given practice samples to

familiarize themselves with using the computer interface. Panelists were

encouraged to ask questions and were observed during training to ensure the

procedure was properly followed.

Experimental Procedure

Time Intensity

All computers were equipped with SIMS 2000© (Sensory Computer Systems, Morristown, NJ, USA). The samples were balanced and presented to panelists in the same way as in Experiment 1 (each product concentration was swallowed and expectorated twice). Upon arriving at the test session, panelists were prompted to enter their panelist code into the computer and follow all instructions as prompted through the ballot. This procedure was followed for all three test sessions; first the session for aqueous solutions, then the session for puddings and finally the session for the cookies.

Aqueous Solutions

The same sixteen solutions were used in this experiment as in Experiments 1 and 2. The panelist was prompted to place the sample in their mouth, hold it still until they perceived the intensity and either swallow or expectorate. Immediately after swallowing or expectorating, panelists rated the perceived flavor intensity. The panelists continued to rate the perceived flavor intensity every ten seconds for a minute after swallowing or expectorating. This process followed ASTM Time-Intensity procedure (ASTM E1909-11). The panelists rinsed their mouths with water three times between samples while taking a three minute break. The same method was used for all sixteen samples.

Puddings

The panelist was prompted to place the entire sample in their mouth using a plastic spoon, hold it still hold it still until they perceived the intensity and either swallow or expectorate. Immediately after swallowing or expectorating, panelists rated the perceived flavor intensity. The panelists continued to rate the perceived flavor intensity every ten seconds for a minute after swallowing or expectorating. This process followed ASTM Time-Intensity procedure (ASTM E1909-11). The panelists rinsed their mouths with water three times between samples while taking a three minute break. The same method was used for all twelve samples.

Cookies

The panelists were prompted to place the entire cookie in their mouth, chew until a bolus was formed and then either swallow or expectorate. Immediately after swallowing or expectorating, panelists rated the perceived flavor intensity. The panelists continued to rate the perceived flavor intensity every ten seconds for a minute after swallowing or expectorating. This process followed ASTM Time-Intensity procedure (ASTM E1909-11). The panelists rinsed their mouths with water three times between samples while taking a three minute break. The same method was used for all twelve samples.

Data Analysis

The data were analyzed using SAS version 9.2 as in previous experiments. The
analysis was conducted on the intensity value at each time point. For each product
data were grouped based on whether the panelists swallowed or expectorated the
sample. First, the means were obtained for each group; the means were calculated
for each concentration at each time point.

Univariate ANOVA were run on the data with the intensity rating at each time
point as a dependent variable. Panelist, swallow, and concentration were the
predictor variables. When the data were analyzed by for one concentration, judge
was a random variable. The analysis of variance was conducted with all
concentrations grouped and with each concentration individually.

Example SAS code for ANOVA of all concentrations:
```
proc glm data=xxx.eb;
class concentration rep judge swallow;
model intensity0 intensity10 intensity20 intensity30
intensity40 intensity50 intensity60
= concentration rep judge swallow;
means concentration rep swallow/snk;
run;
```

Example SAS code for ANOVA of one concentration:
```
proc glm data= ebconc4 outstat=glmstatseb4;
class judge swallow;
model intensity0 intensity10 intensity20 intensity30
intensity40 intensity50 intensity60 = judge swallow;
random judge;
means swallow/snk;
run;
```

All data analysis were carried out on all ethyl butyrate solutions, puddings, and
cookies (Part 2 SAS Code, Appendix E).

Results

Ethyl Butyrate

At 0 seconds, the flavor intensity was perceived to be significantly higher when swallowed than when expectorated for the 1 and 4 ppm concentrations (Table 3.1, Figure 3.1 and 3.4). At 40 and 50 seconds, the flavor intensities were perceived to be significantly higher when expectorated than when swallowed for the 2 ppm concentration (Table 3.1, Figure 3.2). When all concentrations were combined and analyzed only based on swallowing and expectorating, the flavor was perceived to be significantly higher at 0 seconds when swallowed than when expectorated (Table 3.1, Figure 3.5). Overall, when all concentrations were combined, there were no differences in perceived flavor intensity when swallowing or expectorating at any later time point.

Table 3.1: Average perceived intensities, F and p values for each concentration, all time points and all judges (N=11) when the sample was swallowed and when it was expectorated. Small letters indicate a significant difference between swallowing and expectorating within the row (p<.05)

Concentration of Ethyl Butyrate and Time	Swallow	Expectorate	F-value	p-value
1 ppm				
0 seconds	5.5[a]	4.9[b]	6.74	0.01
10 seconds	4.0	4.1	0.51	0.48
20 seconds	2.8	2.8	0.99	0.33
30 seconds	1.8	1.9	0.50	0.49
40 seconds	1.1	1.0	1.55	0.22
50 seconds	0.7	0.6	1.33	0.26
60 seconds	0.5	0.5	0.27	0.61
2 ppm				
0 seconds	4.8	4.5	0.69	0.41
10 seconds	3.4	3.8	0.67	0.42
20 seconds	2.2	2.8	2.81	0.10
30 seconds	1.3	1.7	2.28	0.14
40 seconds	0.6[b]	1.2[a]	4.17	0.05
50 seconds	0.3[b]	0.8[a]	4.37	0.04
60 seconds	0.3	0.5	2.28	0.14
3 ppm				
0 seconds	5.9	5.3	1.91	0.18
10 seconds	4.4	4.5	0.06	0.81
20 seconds	3.1	3.3	0.28	0.60
30 seconds	2.1	2.3	1.13	0.30
40 seconds	1.4	1.4	0.18	0.68
50 seconds	0.8	0.8	0.007	0.94
60 seconds	0.6	0.6	0.02	0.89
4 ppm				
0 seconds	5.9[a]	4.8[b]	6.58	0.02
10 seconds	4.6	4.4	0.14	0.71
20 seconds	3.3	3.4	0.06	0.81
30 seconds	2.3	2.3	0.009	0.92
40 seconds	1.6	1.5	0.19	0.67
50 seconds	1.0	0.9	0.03	0.86
60 seconds	0.7	0.5	0.90	0.35
All Concentrations				
0 seconds	5.5[a]	4.9[b]	11.5	0.0009
10 seconds	4.1	4.2	0.19	0.67
20 seconds	2.8	3.1	1.4	0.25
30 seconds	1.9	2.1	1.8	0.18
40 seconds	1.2	1.2	0.74	0.39
50 seconds	0.72	0.78	0.46	0.50
60 seconds	0.52	0.54	0.09	0.76

Figure 3.1: Shows the mean perceived intensity for all judges (N=11) and all time points for the 1 ppm ethyl butyrate solution.
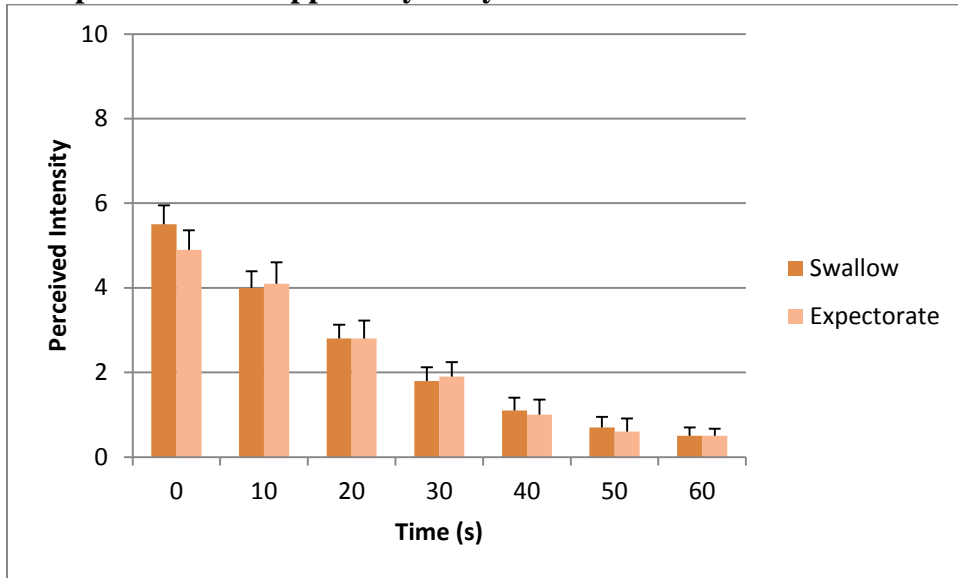
Figure 3.2: Shows the mean perceived intensity for all judges (N=11) and all time points for the 2 ppm ethyl butyrate solution.
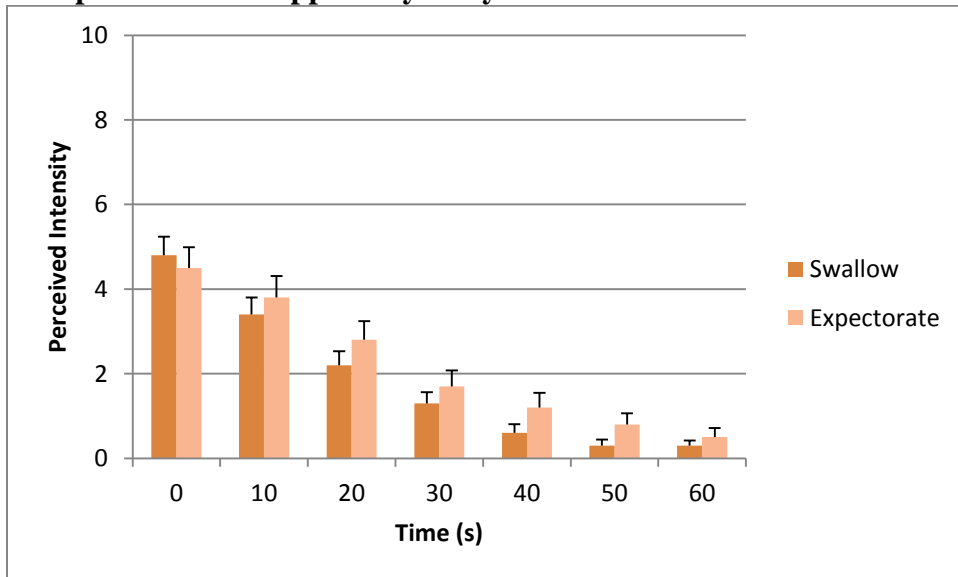
Figure 3.3: Shows the mean perceived intensity for all judges (N=11) and all time points for the 3 ppm ethyl butyrate solution.
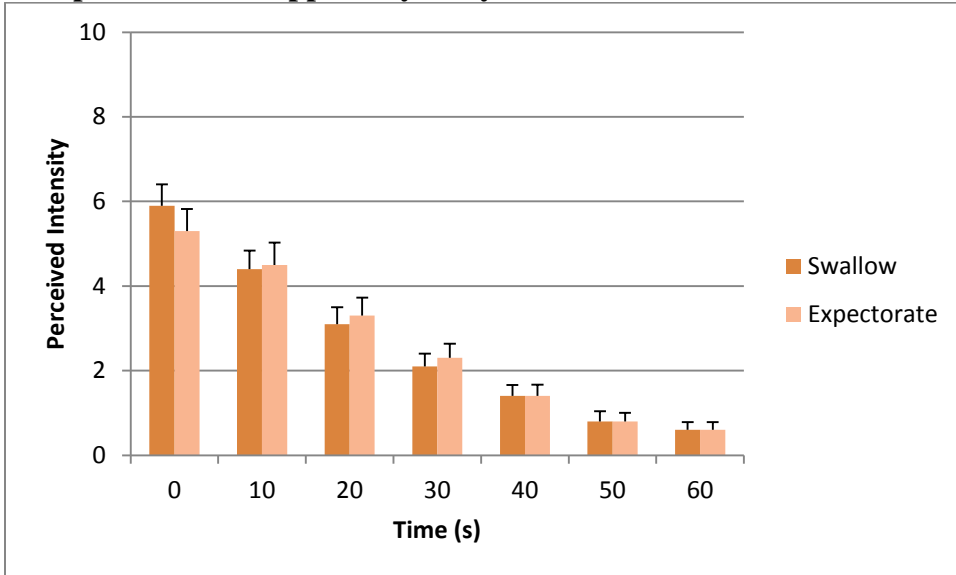
Figure 3.4: Shows the mean perceived intensity for all judges (N=11) and all time points for the 4 ppm ethyl butyrate solution.
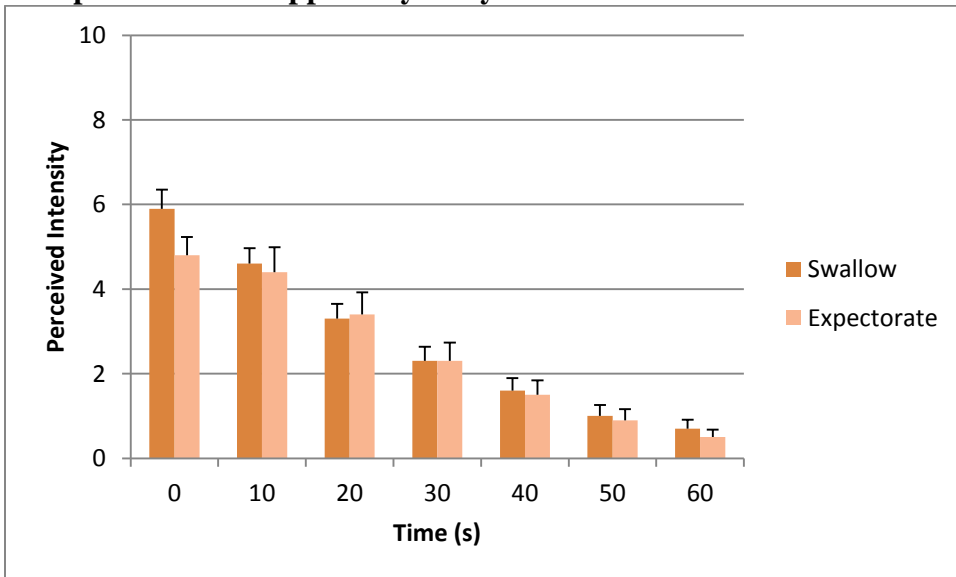
Figure 3.5: Shows the mean perceived intensity for all judges (N=11) and all time points collapsed over all four concentrations of ethyl butyrate solutions.
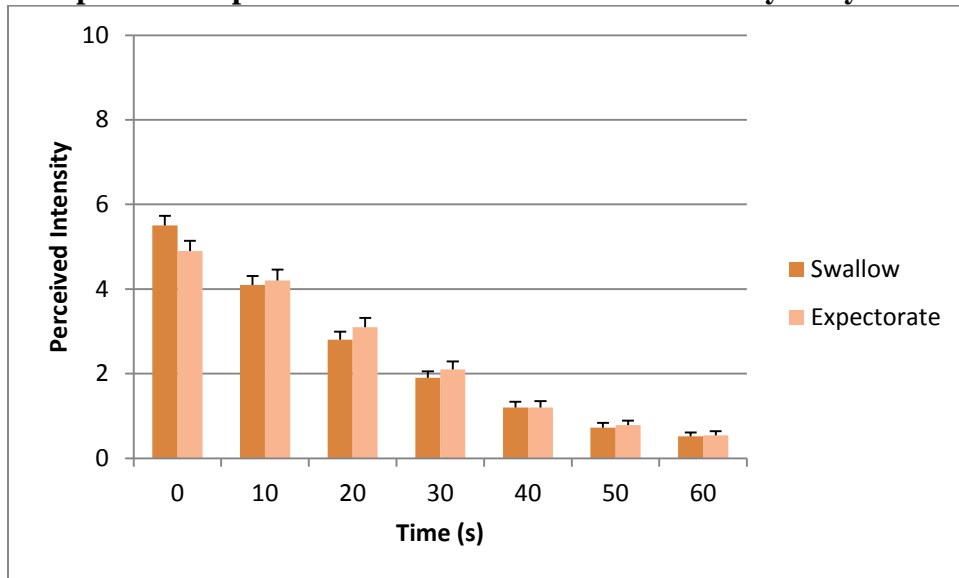
Puddings

At 0 seconds, the flavor intensity was perceived to be significantly higher when swallowed than when expectorated for the 0.5% and 1.5% concentrations (Table 3.2, Figure 3.6 and 3.8).The 1.0% concentration also showed a trend to be more intense in flavor intensity when swallowed than when expectorated. At 10 seconds, the flavor intensity was perceived to be significantly higher when swallowed than when expectorated for the 1.5% concentration (Table 3.2, Figure 3.8). When all concentrations were combined and analyzed only based on swallowing and expectorating, the flavor was perceived to be significantly higher

at 0 seconds and 20 seconds when swallowed than when expectorated. (Table 3.2,

Figure 3.9).

Table 3.2: Shows average perceived intensities, F and p values for each concentration, all time points and all judges (N=11) when the sample was swallowed and when it was expectorated. Small letters indicate a significant difference between swallowing and expectorating at that row's concentration (p<.05)

Concentration of Almond Extract and Measurement Times				
0.5%	Swallow	Expectorate	F-value	p-value
0 seconds	5.6^a	4.4^b	10.75	0.003
10 seconds	4.0	3.7	1.22	0.28
20 seconds	3.1	2.6	2.83	0.10
30 seconds	1.9	1.7	1.29	0.26
40 seconds	1.2	1.2	0.11	0.74
50 seconds	0.8	0.8	0.05	0.82
60 seconds	0.6	0.6	0.07	0.79
1.0%				
0 seconds	5.7	5.1	2.97	0.09
10 seconds	4.3	4.4	0.14	0.72
20 seconds	3.0	3.0	0.06	0.82
30 seconds	2.0	1.9	0.40	0.53
40 seconds	1.5	1.2	3.01	0.09
50 seconds	1.1	0.8	3.81	0.05
60 seconds	0.9	0.7	1.85	0.18
1.5%				
0 seconds	6.4^a	5.0^b	9.53	0.004
10 seconds	4.8^a	4.2^b	4.82	0.04
20 seconds	3.4	2.9	3.01	0.09
30 seconds	2.2	2.0	0.76	0.39
40 seconds	1.5	1.3	0.72	0.40
50 seconds	1.0	0.8	2.26	0.14
60 seconds	0.7	0.6	1.31	0.26
All Concentrations				
0 seconds	5.9^a	4.8^b	22.7	<.0001
10 seconds	4.4	4.1	2.67	0.10
20 seconds	3.3^a	2.8^b	4.43	0.04
30 seconds	2.0	1.8	2.00	0.16
40 seconds	1.3	1.2	1.58	0.21
50 seconds	1.0	0.8	3.91	0.05
60 seconds	0.7	0.6	2.70	0.10

Figure 3.6: Shows the mean perceived intensity for all judges (N=11) and all time points for the 0.5% flavor concentration pudding when swallowed vs. when expectorated.

Figure 3.7: Shows the mean perceived intensity for all judges (N=11) and all time points for the 1.0% flavor concentration pudding when swallowed vs. when expectorated.

Figure 3.8: Shows the mean perceived intensity for all judges (N=11) and all time points for the 1.5% flavor concentration pudding when swallowed vs. when expectorated

Figure 3.9: Shows the mean perceived intensity for all judges (N=11) and all time points for the all pudding samples when swallowed vs. when expectorated

Cookies

There were no significant differences in perceived flavor intensity for any of the

cookies at any of the time points (Table 3.3, Figures 3.10, 3.11, 3.12, and 3.13).

Table 3.3: Shows average perceived intensities, F, and p values for each concentration, all time points and all judges (N=11) when the sample was swallowed and when it was expectorated. Small letters indicate a significant difference between swallowing and expectorating at that row's concentration (p<.05)

Concentration of Orange Extract				
0.5%	Swallow	Expectorate	F-value	p-value
0 seconds	6.0	6.0	0.1	0.76
10 seconds	5.1	5.0	0.3	0.59
20 seconds	4.1	3.8	0.9	0.36
30 seconds	3.0	2.8	0.5	0.49
40 seconds	2.1	2.1	0.0	0.99
50 seconds	1.4	1.5	0.2	0.66
60 seconds	0.9	1.0	0.2	0.66
1.0%				
0 seconds	6.6	6.6	0.0	0.99
10 seconds	5.5	5.6	0.1	0.76
20 seconds	4.2	4.3	0.1	0.76
30 seconds	3.1	3.3	0.6	0.45
40 seconds	2.3	2.5	1.2	0.29
50 seconds	1.5	1.7	1.7	0.21
60 seconds	1.2	1.3	0.8	0.38
1.5%				
0 seconds	6.5	6.2	0.8	0.39
10 seconds	5.4	5.2	0.6	0.45
20 seconds	4.3	3.9	1.9	0.19
30 seconds	3.3	2.9	1.7	0.21
40 seconds	2.6	2.2	2.8	0.12
50 seconds	1.9	1.5	3.1	0.095
60 seconds	1.4	1.0	2.9	0.11
All Concentrations				
0 seconds	6.5	6.2	0.9	0.34
10 seconds	5.4	5.2	2.7	0.52
20 seconds	4.2	4.0	1.4	0.24
30 seconds	3.2	3.0	0.8	0.38
40 seconds	2.4	2.3	0.3	0.57
50 seconds	1.6	1.6	0.2	0.64
60 seconds	1.2	1.1	0.6	0.43

Figure 3.10: Shows the mean perceived intensity for all judges (N=11) and all time points for the 0.5% flavor concentration cookie when swallowed vs. when expectorated.
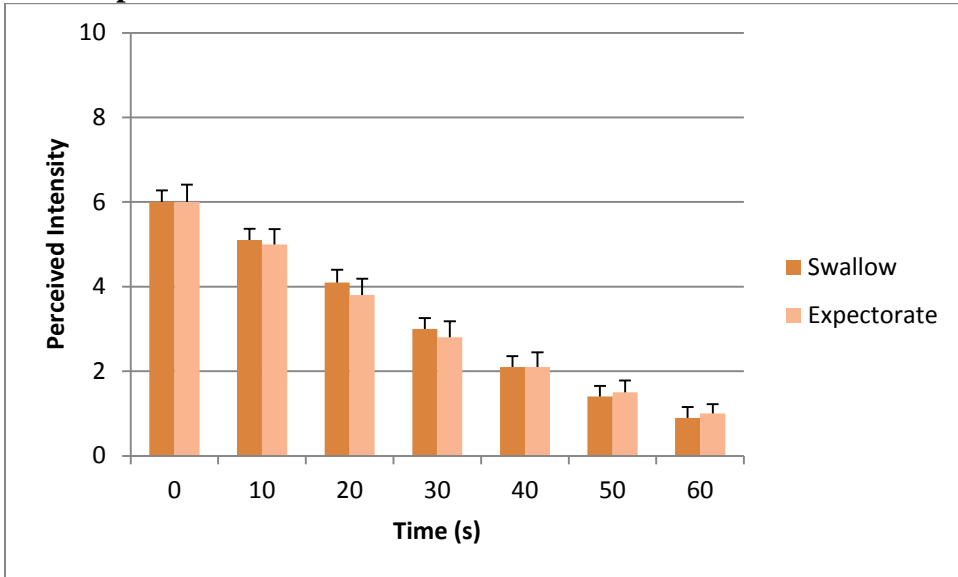
Figure 3.11: Shows the mean perceived intensity for all judges (N=11) and all time points for the 1.0% flavor concentration cookie when swallowed vs. when expectorated.
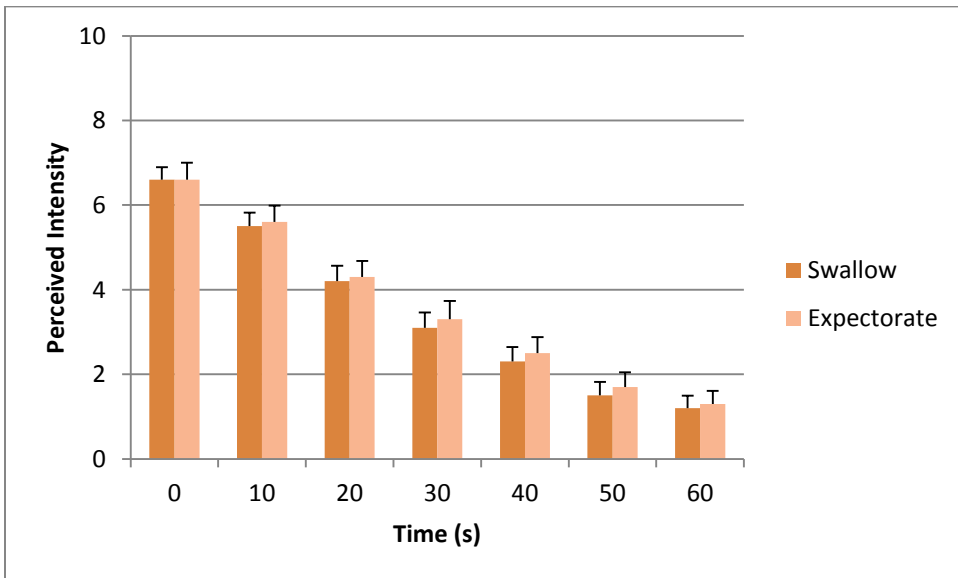
Figure 3.12: Shows the mean perceived intensity for all judges (N=11) and all time points for the 1.5% flavor concentration cookie when swallowed vs. when expectorated.
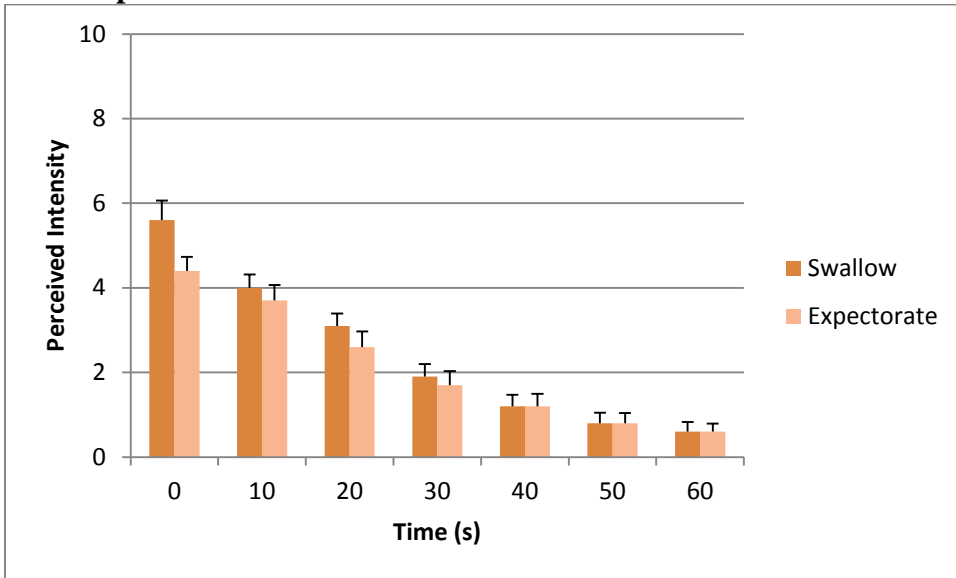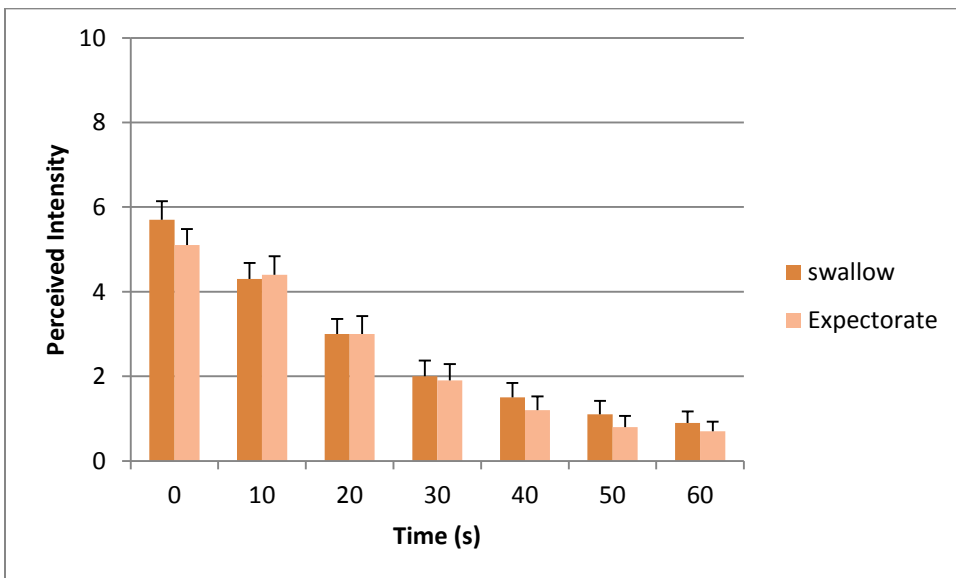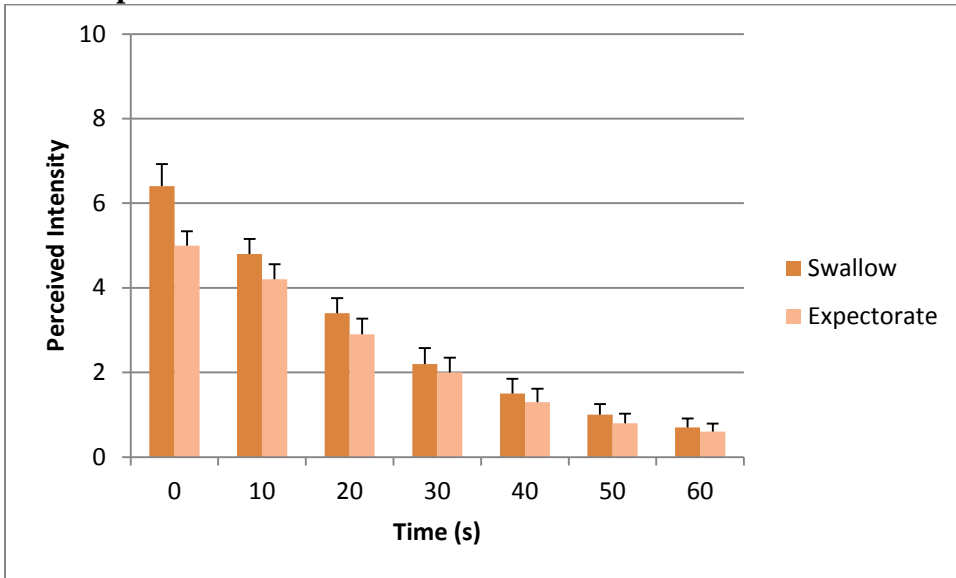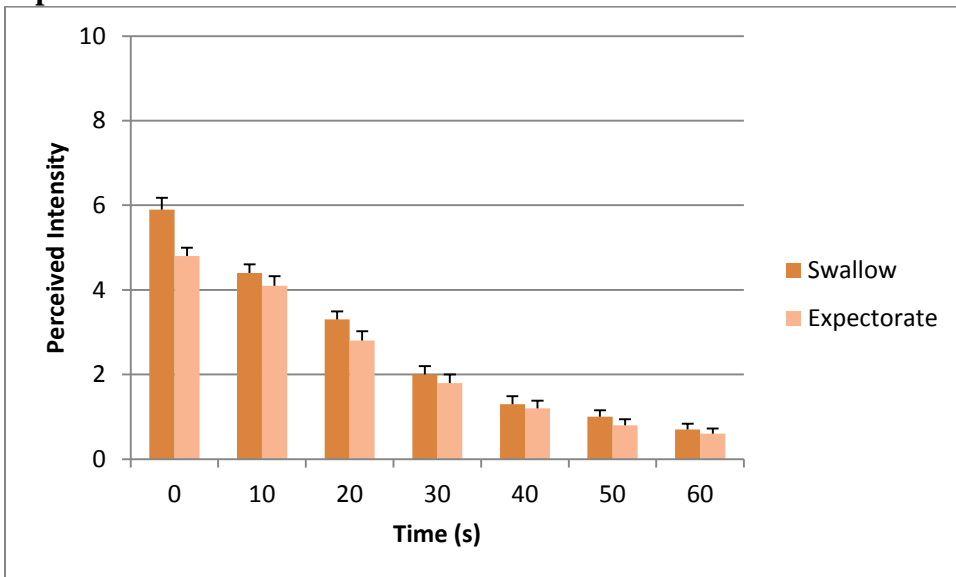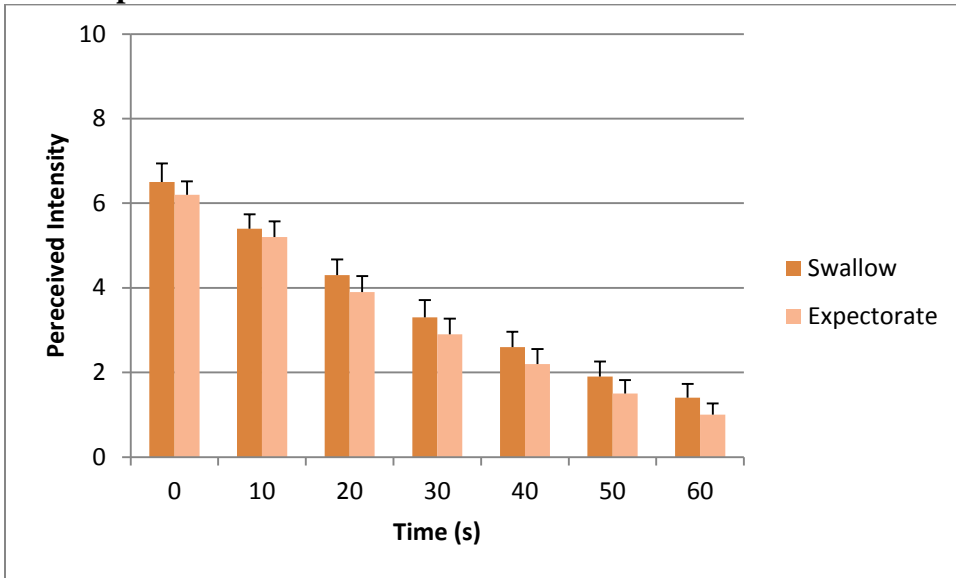

Figure 3.13: Shows the mean perceived intensity for all judges (N=11) and all time points for all cookie samples when swallowed vs. when expectorated
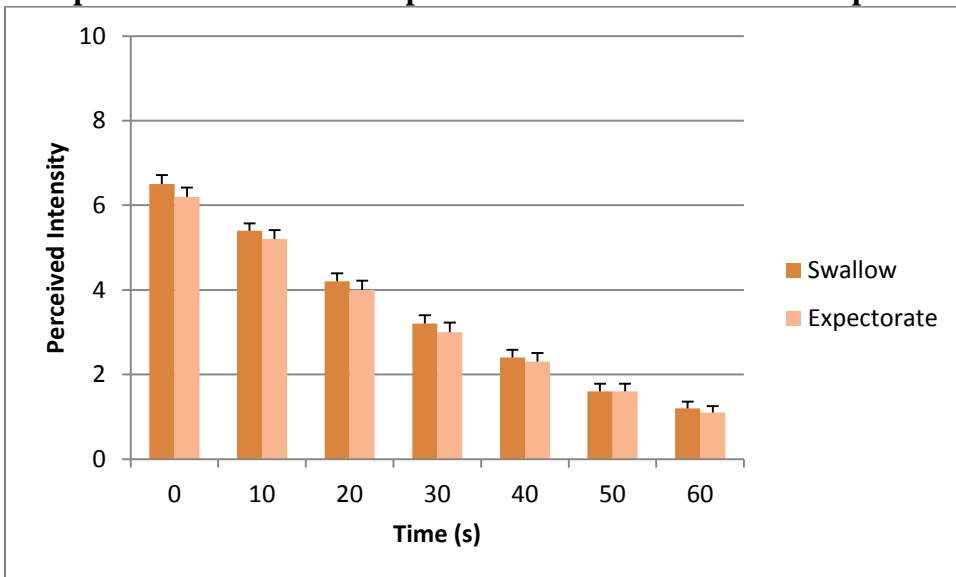
Chapter 4

Part III: Determination of Aroma Compound Concentration In Vivo Using an Atmospheric Pressure Chemical Ionization-Mass Spectrometer

Overview

The objectives of Part III were to (1) determine the concentration of ethyl butyrate in the nasal headspace when a panelist swallowed a solution versus when a panelist expectorated a solution and (2) determine whether more ethyl butyrate is present in the nose space for an extended amount of time when the panelist swallows versus when the panelist expectorates. I hypothesized that the concentration of ethyl butyrate in the nose space would be higher when the panelist swallowed than when the panelist expectorated and that the ethyl butyrate would be present in the nose space for a longer amount of time when the panelist swallowed versus when the panelist expectorated.

Experiment 4

Methods

Panelists

The panelists who participated in experiment 4 also participated in experiment 2. Of the twenty-nine who participated in experiment 2, twenty-five returned to take part in experiment 4. Panelists were paid $5 for participation in experiment 4. All

research and procedures were approved by the Institutional Review Board at the
University of Minnesota (Consent Form, Appendix B, Figure B.1).

Solutions

The solutions in Experiment 4 were prepared the same as the solutions in
Experiment 2. Panelists were presented four samples of each of the 1 ppm, 2 ppm,
3 ppm and 4 ppm solutions. All solutions were served at room temperature in a
volume of 10 mL per sample in a 1-oz plastic soufflé cup.

Instrument Specifications

The atmospheric pressure chemical ionization- mass spectrometer used was a
ZMD 4000 Micromass. It had a deactivated capillary column of .53 mm internal
diameter. The transfer gas was nitrogen at a flow rate of 184 mL/min.

Test Session

This study was conducted using an Atmospheric Pressure Chemical Ionization-
Mass Spectrometer. During the testing session, the panelists were presented with
one sample at a time, handed to them by the moderator. The solutions were
presented in 1-oz. cups and taken into the mouth in a normal drinking motion. All
panelists were given the same solutions in the same order to make data analysis
less complex. Before receiving the sample, the panelists were instructed to inhale

and hold their breath. Upon receiving the sample, the panelists were instructed to put the entire volume into their mouth and hold it without breathing. The panelists were then instructed to reposition themselves on the glass nosepiece and continue to hold their breath for a count of two seconds. At the end of the two seconds, the panelists were instructed to either swallow or expectorate. The panelists swallowed the first four samples as well as a four sample replicate, took a short break and expectorated the last eight samples. Although the term expectorate is used, the panelist allowed the liquid to run out of the mouth into a Styrofoam cup to avoid breaking the nose piece and column. The panelist was instructed to breathe at a normal rate into the nosepiece for the next 60 seconds. At the end of the 60 second collection period, the panelist was asked to move away from the nose piece in order to take a five second rest period. This process was followed for all sixteen samples. Data were collected in Mass Lynx 4.0.

Data Analysis

The initial step of data analysis was to create a calibration curve. This was done by fitting a calibration port to the instrument and injecting dilutions of ethyl butyrate into the instrument. The dilutions of ethyl butyrate were made with pentane. With the calibration port set at a stirring time of 4, standard samples of 0.48 uL/mL were injected at the volumes of 0.4 uL, 1.6 uL, 3.9 uL, 11.0 uL, and

50.0 uL to obtain the calibration curve relating intensity of ethyl butyrate to the concentration (ng/L) in the nasal headspace (Appendix G).

After the calibration curve was completed, data analysis began on the panelist data. The equation of the calibration curve was $y = 0.00039x - 32.45946$. Data were collected from the highest peak, known as the swallow or expectoration breath (Figure 4.1). This breath was the first one exhaled by the panelist after either swallowing or expectorating the sample. To collect this data, the entire peak was chosen in Mass Lynx 4.0 and the ion intensities were copied and pasted into Microsoft Excel. The intensities obtained from the peak were then averaged to give one value per peak which also represented one value per swallowed or expectorated solution. Using the calibration curve equation, the averaged ion intensity was converted to the concentration of ethyl butyrate present in the nose space during this swallow/expectoration breath. This was done by plugging the ion intensity in for "x" in the equation and solving for the ethyl butyrate concentration (y).

Figure 4.1: Example of raw data chromatogram from panelist breath collection using the APCI-MS. The time in minutes is on the X-axis and the ion intensity is on the Y-axis. The peaks before the red line (or 9 minute mark) represent the swallowed solutions while the peaks after the red line (or 9 minute mark) represent the expectorated solutions.
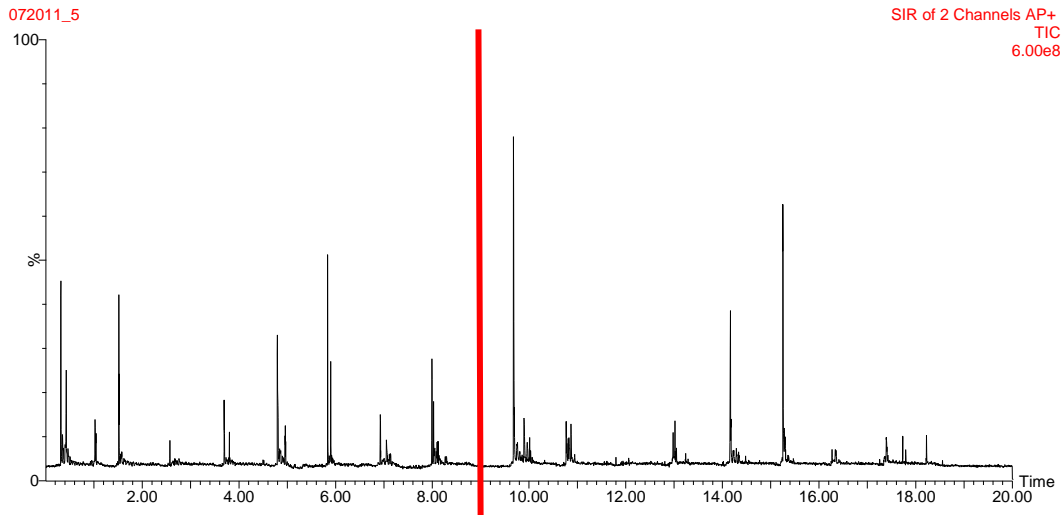
As in the other experiments, SAS version 9.2 was used to analyze the data. The data were analyzed using a univariate analysis of variance model with the concentration present in the nose space as a dependent variable. Judge, swallow, and concentration were the predictor variables. This determined if ethyl butyrate concentrations in the nasal headspace differed between swallowing and expectorating. Similar to the earlier experiments, data were analyzed at all concentration levels together and at each concentration level separately to compare the effects of swallowing and expectorating with the intensity rating at

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each time point as a dependent variable. Judge, swallow, and concentration were

the predictor variables.

Example of SAS code for all concentrations:

```
data xxx.instrument;
proc glm data=xxx.instrument outstat=glmstats;
class judge rep swallow sample;
model concentration= judge rep sample swallow sample*swallow;
random judge;
means rep swallow sample/snk;
run;
```

This analysis was conducted on the data for all concentrations (Part 3 SAS Code,
Appendix F).

Example of SAS code for one concentration:
```
data xxx.concentration2;
set concentration2;
run;
proc sort data=xxx.concentration2;
by swallow;
run;
proc glm data=xxx.concentration2 outstat = glmstats2;
class rep judge swallow;
model concentration
= rep judge swallow;
random judge;
means swallow/snk;
run;
```

Results

The concentration of ethyl butyrate was significantly higher when expectorated

for the ethyl butyrate solutions overall (Table 4.1, Figure 4.3). The concentration

of ethyl butyrate was significantly higher in the nasal headspace when

expectorated for the 2 ppm ethyl butyrate solution (Table 4.1, Figure 4.2).

However, the concentration of ethyl butyrate was not significantly different in the

nasal headspace when swallowed versus expectorated for the 1, 3, and 4 ppm

ethyl butyrate solutions (Table 4.1, Figure 4.2)

Table 4.1: Shows average concentrations of ethyl butyrate (ng/L) present in the nasal headspace, F and p values for all judges (N=25) when the sample was swallowed and when it was expectorated. Small letters indicate a significant difference between swallowing and expectorating at that row's concentration (p<.05)

Concentration	Swallow	Expectorate	F-value	p-value
1 ppm	3123	3834	2.61	0.11
2 ppm	3363	3794	0.39	0.53
3 ppm	5884^b	7729^a	4.42	0.04
4 ppm	6523	8099	2.50	0.12
All	4882^b	5706^a	4.55	0.03

Figure 4.2: Shows the mean concentration of ethyl butyrate present in the nasal head space for all judges (N=25) at all concentrations of ethyl butyrate solutions for swallowing and expectorating. The dotted line represents swallowing, the solid line represents expectorating.
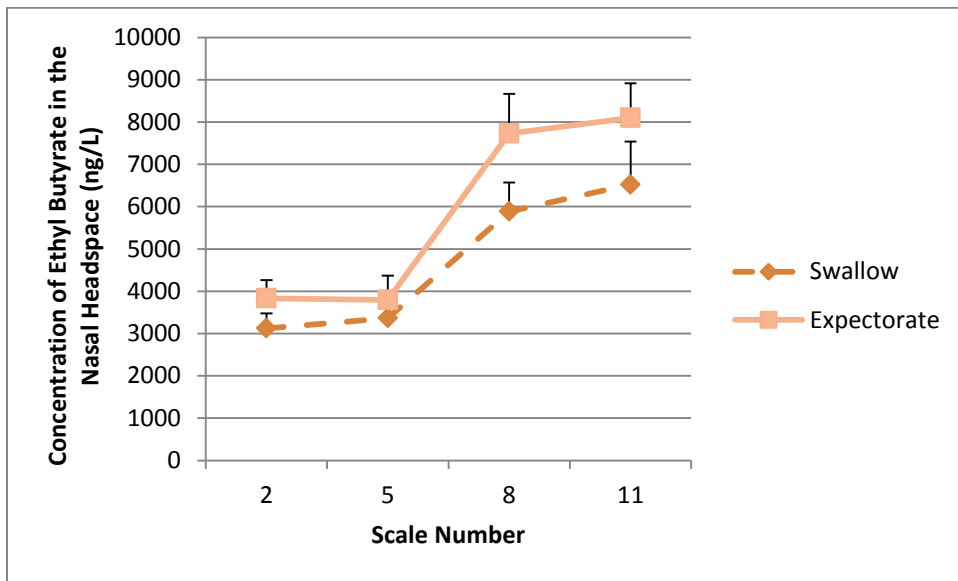
Figure 4.3: Shows the mean concentration of ethyl butyrate present in the nasal headspace for all judges (N=25) overall when solutions are swallowed and   expectorated.
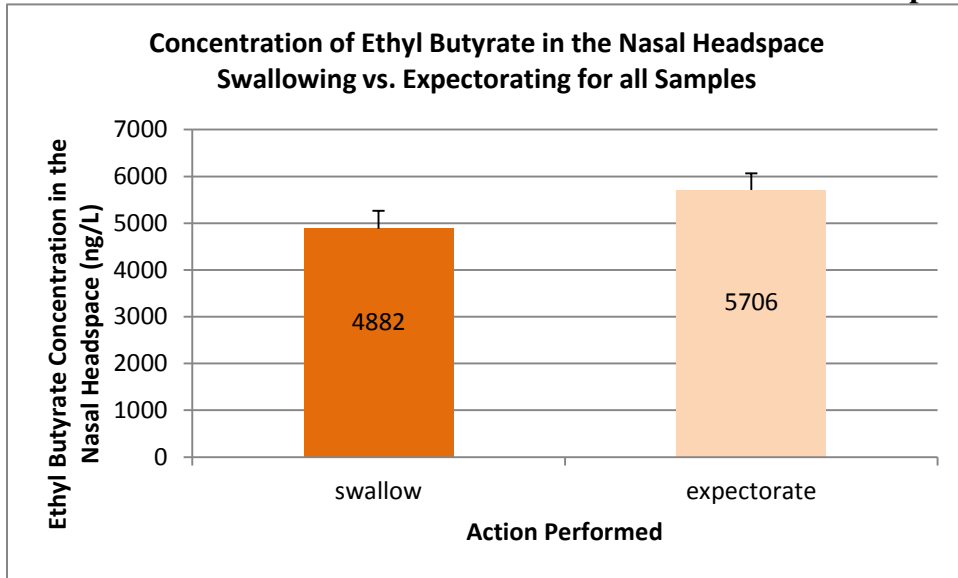
Chapter 5

Discussion

Overall

In experiment 1, we observed that the caffeine solutions were perceived to be more intense when swallowed than expectorated for the 5, 8, and 11 scale number solutions. However, this did not agree with our hypothesis that swallowing and expectorating would have no effect on taste intensity perception for the basic tastes. Ott and Palmer (1990) observed that 0.027% caffeine solutions were not perceived differently when swallowed and expectorated. Interestingly, the only caffeine solution in our study that was not perceived to be different when swallowed and expectorated was a 0.019% caffeine solution. This was a lower concentration than Ott and Palmer served their panelists. The three solutions that were perceived to be bitterer when swallowed than expectorated in our study were 0.076%, 0.15%, and 0.27% caffeine solutions. This could suggest that when serving panelists 10 mL samples of caffeine solution, bitterness is more intense when swallowed than expectorated at levels greater than 0.027%. Bitterness has been defined as "being perceived in the back of the mouth and characterized by solutions of caffeine, quinine, and other alkaloids," (Curic and others 2008). Therefore, if most of the perception is occurring in the back of the mouth and the taste is building, a more intense sensation could have resulted from swallowing.

To avoid this, panelists could have taken a longer break in between caffeine samples or evaluated fewer than sixteen samples in one session.

In experiment 3, we observed that the cookies did not follow the hypothesis that swallowing would yield a more intense flavor than expectorating. Mouth movements and texture play a very complex role in sensory perception. It was noted by Burdach and Doty (1987) that mouth movements resulted in a higher perceived intensity than no mouth movements in the case of rum and orange extracts. Our panelists had to perform mouth movements in the form of chewing in order to process the cookie in both the swallowing and expectorating procedure. As Burdach and Doty noted, the mouth movements lead to a greater perceived intensity. In our study mouth movements occurred whether the panelist swallowed or expectorated the cookie. Because of the mouth movements used to process the cookie, the panelists may have fully perceived the maximum possible intensity which would lead to no difference between swallowing and expectorating.

The Atmospheric Pressure Chemical Ionization Mass Spectrometer was never designed for use when a panelist would expectorate. Even the smallest movement could break the column of the instrument or the glass nose piece leading to hours of repair. In Experiment 4 of this study, panelists were instructed to expectorate solutions while a glass nose piece connected to the APCI-MS was in their nose.

This forced most panelists to expectorate without leaning their heads forward due to the fragile nature of this nose piece (the expectorating process observed when panelists were performing experiments 1-3 included the panelist leaning forward to expectorate, with seemingly less difficulty). The extra movements and force needed to expectorate with the nose piece in the nose could have allowed the volatile ethyl butyrate to be pushed into the nasal headspace more readily than during a normal expectorating motion, leading to a higher ion concentration that disagreed with our hypothesis. By looking at Figure 4.1, you can see that the peaks for the expectoration breath were higher in this panelist than for the swallowing breath. This could be a result of the extra force needed to expectorate or from the volatile compound escaping into the surrounding air. Also, you can see that the area under the curve was greater when the solutions were swallowed than when the solutions were expectorated, however this was not observed in the time intensity data collected in Part 2 of my research. More work should be done on time intensity perception and area under the curve using APCI-MS collection to determine if there is a correlation. Deleris and others (2011) solved this problem by attaching the nose piece to glasses to make the expectorating process more comfortable. The results obtained from their study supported the hypothesis that expectorating lead to a smaller ion concentration in the nasal head space than swallowing.

In order to draw conclusions about the concentration of ethyl butyrate in the nasal headspace, a better method for expectorating should be developed. Because of the volatility of the compound, ethyl butyrate solutions cannot be expectorated prior to adjusting the nose piece because expectoration would release the compound into the nose and into the air adjacent to the nose. The use of a long tube for expectoration may ease the difficulty for panelists expectorating with the nose piece in their mouths. The long tube would also reduce the amount of volatile ethyl butyrate that escapes into the air during the expectorating process. Taylor and others (2000) noted that the air in the room was sampled before and after a panelist placed the nose piece in the nose to ensure the volatile compound had not escaped into the surrounding air. In my study, the process of expectorating into an open cup may have caused the air surrounding the nose piece to become contaminated with ethyl butyrate from the expectorated sample yielding higher concentrations present in the instrument reading, but not necessarily in the nasal headspace. In order to relate the sensory data to the instrument data, a more precise and natural process is needed. Until then, it is not possible for us to conclude whether swallowing or expectorating leads to a higher concentration of ethyl butyrate in the nasal headspace.

Chapter 6

Conclusions

Flavor intensity was perceived to be more intense for caffeine solutions, ethyl butyrate solutions, and almond extract flavored puddings when panelists swallowed than when they expectorated. Such differences were not observed for sucrose solutions, MSG solutions, and orange extract flavored cookies. As others have concluded (Deleris and others 2011, Burdach and Doty 1987, Buettner and others 2002) expectorating may lead to flavor intensity perception that is less intense than when normally consuming the food. Because of this, as well as my researchers should be cautious when prescribing expectoration to their panelists as it may lead to results different from those obtained from normal eating.

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Appendices

Appendix A: Screener

Figure A.1: Example of screener used to recruit panelists.

Hello!

We are recruiting people to participate in a study on basic taste intensity related to swallowing. The study will be held in room 132 McNeal Hall on the St. Paul Campus. Your attendance is required on [date] for the session in McNeal Hall.

Each session should take no more than 30 minutes. During the session you will be asked to swallow or expectorate solutions and make intensity ratings.

You will receive a total of $5 for participating in the study.

If you are interested in taking part in this study, please answer the questions below and reply to this e-mail. Your information will be evaluated to see if you qualify to be part of the study. If you qualify, you will be contacted and assigned a participation time for two of the four test dates and a time slot on the final test date. We will contact you in the next few days to schedule you for the study. **You may choose not to participate, even if you have qualified.**

Please provide the following information about yourself. All information you provide is strictly confidential.

Are you interested in participating this study?

___ Yes

___ No

What is your age? _____

Do you have any food allergies?

___ Yes

___ No

Availability on XXX

Please indicate the times on <u>date</u> that you would be available attend **session**. (Please mark all applicable times)

Date

_____ 8:00 – 8:30 am

_____ 8:30 – 9:00 am

_____ 9:00 – 9:30 am

_____ 9:30 – 10:00 am

_____ 10:00 – 10:30 am

_____ 10:30 – 11:00 am

_____ 11:00 – 11:30 am

_____ 11:30 – 12:00 pm

_____ 12:00 – 12:30 pm

_____ 12:30 – 1:00 pm

_____ 1:00 – 1:30 pm

_____ 1:30 – 2:00 pm

_____ 2:00 – 2:30 pm

_____ 2:30 – 3:00 pm

_____ 3:00 – 3:30 pm

_____ 3:30 – 4:00 pm

_____ 4:00 – 4:30 pm

_____ 4:30 – 5:00 pm
_____ 5:00 – 5:30 pm
_____ 5:30 – 6:00 pm
If you qualify for the study, we will assign you a time to meet for the sessions.
If you have any questions about the study, please respond to this e-mail
Thank you!

Appendix B: Consent Form

Figure B.1: Consent form used to show Institutional Review Board Approval in all experiments.

CONSENT FORM
Swallowing Study

You are invited to participate in a research study of the relation of swallowing to flavor intensity. You were selected as a possible participant because of your interest, availability, lack of any food allergies, and qualifications. We ask that you read this form and ask any questions you may have before participating in this study.

This study is being conducted by Amanda Peck as her Masters research with her advisor Zata Vickers through the Sensory Center at the University of Minnesota.

Background Information:

The purpose of this study is to measure differences of intensity in flavor solutions based on the subjects swallowing or expectorating of the sample. In addition, this study aims to determine whether the compounds are more present in the retronasal cavity when a panelist swallows or expectorates. All ingredients will be GRAS or FDA approved food additives.

Procedures:

If you agree to be in this study, we would ask you attend one test session that requires you to taste and potentially consume different solutions. The session will take no more than 30 minutes.

Risks and Benefits of being in the Study:

The study has no risks beyond those of normally consuming beverages. The study has no benefits for you other than the compensation.

Compensation:

You will receive a total $5 for participating. You will be compensated at the end of the session.

Confidentiality:

The records of this study will be kept private. In any published material, we will not include information that will make it possible to identify a subject. Research records will be stored securely and only researchers will have access to the records.

Voluntary Nature of the Study:

Participation in this study is voluntary. Your decision whether or not to participate will not affect your current or future relations with the University of Minnesota. If you decide to participate, you are free to not answer any question or withdraw at any time without affecting those relationships.

Contacts and Questions:

The researchers conducting this study are: Amanda Peck and Zata Vickers. You may ask any questions you have now. If you have questions later, **you are encouraged** to contact them at(612) 625-3712, peckx137@umn.edu and (612) 624-2257, zvickers@umn.edu .

If you have any questions or concerns regarding this study and would like to talk to someone other than the researcher(s), **you are encouraged** to contact the Research Subjects' Advocate Line, D528 Mayo, 420 Delaware St. Southeast, Minneapolis, Minnesota 55455; (612) 625-1650.

You may take a copy of this information to keep for your records

Appendix C: Ballot

Figure C.1 : Example of ballot used for all experiments (1-4). The first is an example of the ballot that panelists saw when they swallowed a sample; the second is an example of the ballot that panelists saw when they swallowed the sample.

Appendix D: Part 1 SAS Code

Figure D.1: SAS Code from Part I (Experiment 1 and 2)

Experiment 1: Sweet

SAS code for merging sweet solution data:
Data was merged by swallow, judge, and rep.

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Data';
proc sort data= xxx.sweetswallow;
by swallow judge rep;
run;
proc sort data= xxx.sweetspit;
by swallow judge rep;
run;
data xxx.sweet;
merge xxx.sweetswallow xxx.sweetspit;
by swallow judge rep;
run;
```

SAS code for computing means by concentration, rep and swallow:

```
proc freq data= xxx.sweet;
run;
proc sort data=  xxx.sweet;
by concentration rep swallow;
run;
proc means data= xxx.sweet;
by concentration rep swallow;
var overallaftertaste;
output out =meanssweet mean= overallaftertaste;
run;
```

SAS code for PROC GLM sweet solution data by concentration to determine differences in swallowing and expectorating at each concentration:

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Data';
proc glm data=xxx.sweet outstat=glmsweet;
class judge rep swallow concentration;
model overallaftertaste= judge rep concentration swallow
concentration*swallow;
random judge;
means rep swallow concentration/snk;
run;
data xxx.concentrationsweettwo;
set xxx.sweet;
if concentration=2;
```

```
run;
proc glm data=xxx.concentrationsweettwo outstat=conctwosweet;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
proc freq data= xxx.concentrationsweettwo;
run;
data xxx.concentrationsweetfive;
set xxx.sweet;
if concentration=5;
run;
proc glm data=xxx.concentrationsweetfive
outstat=concfivesweet;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
proc freq data= xxx.concentrationsweetfive;
run;
data xxx.concentrationsweeteight;
set xxx.sweet;
if concentration=8;
run;
proc glm data=xxx.concentrationsweeteight
outstat=conceightsweet;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
proc freq data= xxx.concentrationsweeteight;
run;
data xxx.concentrationsweeteleven;
set xxx.sweet;
if concentration=11;
run;
proc glm data=xxx.concentrationsweeteleven
outstat=concelevensweet;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk
run;
```

Experiment 1: Sour

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Data';
proc sort data= xxx.sourswallow;
by swallow judge rep;
run;
proc sort data= xxx.sourspit;
by swallow judge rep;
run;
data xxx.sour;
merge xxx.sourswallow xxx.sourspit;
by swallow judge rep;
run;
proc freq data= xxx.sour;
run;
proc sort data=  xxx.sour;
by concentration rep swallow;
run;
proc means data= xxx.sour;
by concentration rep swallow;
var overallaftertaste;
output out =meanssour mean= overallaftertaste;
run;
```

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Data';

proc glm data=xxx.sour outstat=glmsour;
class judge rep swallow concentration;
model overallaftertaste= judge rep concentration swallow
concentration*swallow;
random judge;
means rep swallow concentration/snk;
run;
data xxx.concentrationsourtwo;
set xxx.sour;
if concentration=2;
run;

proc glm data=xxx.concentrationsourtwo outstat=conctwosour;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
```

```
run;
proc freq data= xxx.concentrationsourtwo;
run;
data xxx.concentrationsourfive;
set xxx.sour;
if concentration=5;
run;
proc glm data=xxx.concentrationsourfive outstat=concfivesour;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
proc freq data= xxx.concentrationsourfive;
run;
data xxx.concentrationsoureight;
set xxx.sour;
if concentration=8;
run;
proc glm data=xxx.concentrationsoureight
outstat=conceightsour;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
proc freq data= xxx.concentrationsoureight;
run;
data xxx.concentrationsoureleven;
set xxx.sour;
if concentration=11;
run;
proc glm data=xxx.concentrationsoureleven
outstat=concelevensour;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
```

Experiment 1: Savory (Umami)

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Data';
proc sort data= xxx.savoryswallow;
by swallow judge rep;
run;
proc sort data= xxx.savoryspit;
by swallow judge rep;
run;
data xxx.savory;
merge xxx.savoryswallow xxx.savoryspit;
by swallow judge rep;
run;
proc freq data= xxx.savory;
run;
proc sort data=  xxx.savory;
by concentration rep swallow;
run;
proc means data= xxx.savory;
by concentration rep swallow;
var overallaftertaste;
output out =meanssavory mean= overallaftertaste;
run;
```

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Data';

proc glm data=xxx.savory outstat=glmsavory;
class judge rep swallow concentration;
model overallaftertaste= judge rep concentration swallow
concentration*swallow;
random judge;
means rep swallow concentration/snk;
run;
data xxx.concentrationsavorytwo;
set xxx.savory;
if concentration=2;
run;

proc glm data=xxx.concentrationsavorytwo
outstat=conctwosavory;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
```

```
run;
proc freq data= xxx.concentrationsavorytwo;
run;
data xxx.concentrationsavoryfive;
set xxx.savory;
if concentration=5;
run;
proc glm data=xxx.concentrationsavoryfive
outstat=concfivesavory;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
proc freq data= xxx.concentrationsavoryfive;
run;
data xxx.concentrationsavoryeight;
set xxx.savory;
if concentration=8;
run;
proc glm data=xxx.concentrationsavoryeight
outstat=conceightsavory;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
proc freq data= xxx.concentrationsavoryeight;
run;
data xxx.concentrationsavoryeleven;
set xxx.savory;
if concentration=11;
run;
proc glm data=xxx.concentrationsavoryeleven
outstat=concelevensavory;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
proc freq data= xxx.concentrationsavoryeleven;
run;
```

Experiment 1: Bitter

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Data';
proc sort data= xxx.bitterswallow;
by swallow judge rep;
run;
proc sort data= xxx.bitterspit;
by swallow judge rep;
run;
data xxx.bitter;
merge xxx.bitterswallow xxx.bitterspit;
by swallow judge rep;
run;
proc freq data= xxx.bitter;
run;
proc sort data=  xxx.bitter;
by concentration rep swallow;
run;
proc means data= xxx.bitter;
by concentration rep swallow;
var overallaftertaste;
output out =meansbitter mean= overallaftertaste;
run;
```

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Data';
proc sort data=xxx.bitter;
by swallow concentration;
run;
proc glm data=xxx.bitter outstat=glmbitter;
class judge rep swallow concentration;
model overallaftertaste= judge rep concentration swallow
concentration*swallow;
random judge;
means rep swallow concentration/snk;
run;
data xxx.concentrationbittertwo;
set xxx.bitter;
if concentration=2;
run;

proc glm data=xxx.concentrationbittertwo
outstat=conctwobitter;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
```

```
random judge;
means swallow/snk;
run;
proc freq data= xxx.concentrationbittertwo;
run;
data xxx.concentrationbitterfive;
set xxx.bitter;
if concentration=5;
run;
proc glm data=xxx.concentrationbitterfive
outstat=concfivebitter;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
proc freq data= xxx.concentrationbitterfive;
run;
data xxx.concentrationbittereight;
set xxx.bitter;
if concentration=8;
run;
proc glm data=xxx.concentrationbittereight
outstat=conceightbitter;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
proc freq data= xxx.concentrationbittereight;
run;
data xxx.concentrationbittereleven;
set xxx.bitter;
if concentration=11;
run;
proc glm data=xxx.concentrationbittereleven
outstat=concelevenbitter;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
```

Experiment 2: Ethyl Butyrate
SAS code for merging ethyl butyrate solution data
Data were merged by swallow, judge, and rep:

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Data';
proc sort data= xxx.fruityswallow;
by swallow judge rep;
run;
proc sort data= xxx.fruityspit;
by swallow judge rep;
run;
data xxx.fruity;
merge xxx.fruityswallow xxx.fruityspit;
by swallow judge rep;
run;
proc freq data= xxx.fruity;
run;
proc sort data=  xxx.fruity;
by concentration rep swallow;
run;
proc means data= xxx.fruity;
by concentration rep swallow;
var overallaftertaste;
output out =meansfruity mean= overallaftertaste;
run;
```

SAS code for PROC GLM ethyl butyrate solutions by concentration to determine differences between swallowing and expectorating at each concentration:

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Data';

proc glm data=xxx.fruity outstat=glmstats;
class judge rep swallow concentration;
model overallaftertaste= judge rep concentration swallow
concentration*swallow;
random judge;
means rep swallow concentration/snk;
run;
proc glm data=xxx.concentrationone outstat=conconestats;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
data xxx.concentrationone;
set xxx.fruity;
if concentration=1;
run;
```

```
proc freq data= xxx.concentrationone;
run;


proc glm data=xxx.fruity outstat=glmstats;
class judge rep swallow concentration;
model overallaftertaste= judge rep concentration swallow
concentration*swallow;
random judge;
means rep swallow concentration/snk;
run;
data xxx.concentrationtwo;
set xxx.fruity;
if concentration=2;
run;
proc glm data=xxx.concentrationtwo outstat=conctwostats;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
proc freq data= xxx.concentrationtwo;
run;


proc glm data=xxx.fruity outstat=glmstats;
class judge rep swallow concentration;
model overallaftertaste= judge rep concentration swallow
concentration*swallow;
random judge;
means rep swallow concentration/snk;
run;
data xxx.concentrationthree;
set xxx.fruity;
if concentration=3;
run;
proc glm data=xxx.concentrationthree outstat=concthreestats;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
proc freq data= xxx.concentrationthree;
run;
```

```
proc glm data=xxx.fruity outstat=glmstats;
class judge rep swallow concentration;
model overallaftertaste= judge rep concentration swallow
concentration*swallow;
random judge;
means rep swallow concentration/snk;
run;
data xxx.concentrationfour;
set xxx.fruity;
if concentration=4;
run;
proc glm data=xxx.concentrationfour outstat=concfourstats;
class swallow concentration rep judge;
model overallaftertaste= rep swallow judge;
random judge;
means swallow/snk;
run;
proc freq data= xxx.concentrationfour;
run;
```

Appendix E: Part 2 SAS Code

Figure E.1: SAS Code for Part 2 (Experiment 3)

Experiment 3: Ethyl Butyrate Solution Time Intensity

SAS Code for merging Ethyl Butyrate Solution Time Intensity Data
Data were merged by judge, concentration, rep, and swallow

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Descriptive
Work';
proc sort data = xxx.ebswallow;
by judge concentration rep swallow;
run;
proc sort data = xxx.ebspit;
by judge concentration rep swallow;
run;
data xxx.eb;
merge xxx.ebswallow xxx.ebspit;
by judge concentration rep swallow;
run;
proc freq data=xxx.eb;
run;
proc sort data=xxx.eb;
by concentration rep swallow;
proc means data=xxx.eb;
by concentration swallow;
var intensity0 intensity10 intensity20 intensity30 intensity40
intensity50 intensity60;
output out =meanseb mean=  intensity0 intensity10 intensity20
intensity30 intensity40 intensity50 intensity60;;
run;
```

SAS Code for PROC GLM Ethyl Butyrate Solution Time Intensity Data by
Concentration to determine differences between swallowing and expectorating at
each time point for each concentration
```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Descriptive
Work\Data';
data xxx.ebconc1;
set ebconc1;
run;
proc sort data =xxx.ebconc1;
by swallow rep;
run;
proc glm data= ebconc1 outstat=glmstatseb1;
```

```
class judge swallow rep;
model intensity0 intensity10 intensity20 intensity30
intensity40 intensity50 intensity60
= judge swallow rep;
random judge;
means swallow rep/snk;
run;
proc means data=xxx.ebconc1;
by swallow;
var intensity0 intensity10 intensity20 intensity30 intensity40
intensity50 intensity60;
output out =meansebconc1 mean=ebconc1;
run;


libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Descriptive
Work\Data';
data xxx.ebconc2;
set ebconc2;
run;
proc sort data =xxx.ebconc2;
by swallow rep;
run;
proc glm data= ebconc2 outstat=glmstatseb2;
class judge swallow rep;
model intensity0 intensity10 intensity20 intensity30
intensity40 intensity50 intensity60
= judge swallow rep;
means swallow rep/snk;
random judge;
run;
proc means data=xxx.ebconc2;
by swallow;
var intensity0 intensity10 intensity20 intensity30 intensity40
intensity50 intensity60;
output out =meansebconc2 mean=ebconc2;
run;


libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Descriptive
Work\Data';
data xxx.ebconc3;
set ebconc3;
run;
proc sort data =xxx.ebconc3;
by swallow rep;
run;
proc glm data= ebconc3 outstat=glmstatseb3;
class judge swallow rep;
```

```
model intensity0 intensity10 intensity20 intensity30
intensity40 intensity50 intensity60
= judge swallow rep;
random judge;
means swallow rep/snk;
run;
proc means data=xxx.ebconc3;
by swallow;
var intensity0 intensity10 intensity20 intensity30 intensity40
intensity50 intensity60;
output out =meansebconce3 mean=ebconce3;
run;


libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Descriptive
Work\Data';
data xxx.ebconc4;
set ebconc4;
run;
proc sort data =xxx.ebconc4;
by swallow rep;
run;
proc glm data= ebconc4 outstat=glmstatseb4;
class judge swallow rep;
model intensity0 intensity10 intensity20 intensity30
intensity40 intensity50 intensity60 = judge swallow rep;
random judge rep;
means swallow rep/snk;
run;
proc means data=xxx.ebconc4;
by swallow;
var intensity0 intensity10 intensity20 intensity30 intensity40
intensity50 intensity60;
output out =meansebconce4 mean=intensity0 intensity10
intensity20 intensity30 intensity40 intensity50 intensity60;
run;
```

Experiment 3: Pudding

SAS Code for merging pudding time intensity data
Data were merged by judge, concentration, rep, and swallow:

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Descriptive
Work';
proc sort data= xxx.puddingswallow;
by judge concentration rep swallow;
run;
proc sort data= xxx.puddingspit;
by judge concentration rep swallow;
run;
data xxx.pudding;
merge xxx.puddingswallow xxx.puddingspit;
by judge concentration rep swallow;
run;
proc freq data=xxx.pudding;
run;
proc sort data=xxx.pudding;
by concentration rep swallow;
proc means data=xxx.pudding;
by concentration swallow;
var intensity0 intensity10 intensity20 intensity30 intensity40
intensity50 intensity60;
output out =meanspudding mean=  intensity0 intensity10
intensity20 intensity30 intensity40 intensity50 intensity60;
run;
```


SAS Code for PROC GLM for pudding time intensity data by concentration to
determine differences between swallowing and expectorating at each time point
for each concenctration:

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Descriptive
Work\Data';
data xxx.puddingconc1;
set puddingconc1;
run;
proc sort data =xxx.puddingconc1;
by swallow rep;
run;
proc glm data= puddingconc1 outstat=glmstats;
class judge swallow rep;
model intensity0 intensity10 intensity20 intensity30
intensity40 intensity50 intensity60
```

```
= judge swallow rep;
random judge;
means swallow rep/snk;
run;
proc means data=xxx.puddingconc1;
by swallow;
var intensity0 intensity10 intensity20 intensity30 intensity40
intensity50 intensity60;
output out =meanspuddingconc1new mean=intensity0 intensity10
intensity20 intensity30 intensity40 intensity50 intensity60;
run;


libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Descriptive
Work\Data';
data xxx.puddingconc2;
set puddingconc2;
run;
proc sort data =xxx.puddingconc2;
by swallow rep;
run;
proc glm data= puddingconc2 outstat=glmstatsp2;
class judge swallow rep;
model intensity0 intensity10 intensity20 intensity30
intensity40 intensity50 intensity60
= judge swallow rep;
random judge;
means swallow rep/snk;
run;
proc means data=xxx.puddingconc1;
by swallow;
var intensity0 intensity10 intensity20 intensity30 intensity40
intensity50 intensity60;
output out =meanspuddingconc2new2 mean=intensity0 intensity10
intensity20 intensity30 intensity40 intensity50 intensity60;
run;



libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Descriptive
Work\Data';
data xxx.puddingconc3;
set puddingconc3;
run;
proc sort data =xxx.puddingconc3;
by swallow rep;
run;
proc glm data= puddingconc3 outstat=glmstatsp3;
class judge swallow rep;
```

```
model intensity0 intensity10 intensity20 intensity30
intensity40 intensity50 intensity60
= judge swallow rep;
random judge;
means swallow rep/snk;
run;
proc means data=xxx.puddingconc3;
by swallow;
var intensity0 intensity10 intensity20 intensity30 intensity40
intensity50 intensity60;
output out =meanspuddingconc3new3 mean=intensity0 intensity10
intensity20 intensity30 intensity40 intensity50 intensity60;
run;
```

Experiment 3: Cookie

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Descriptive
Work';
proc sort data= xxx.cookieswallow;
by swallow judge rep;
run;
proc sort data= xxx.cookiespit;
by swallow judge rep;
run;
data xxx.cookie;
merge xxx.cookieswallow xxx.cookiespit;
by swallow judge rep;
run;
proc freq data= xxx.cookie;
run;
proc sort data=  xxx.cookie;
by concentration rep swallow;
run;
proc glm data=xxx.cookie outstat =glmstats;
class judge rep swallow concentration;
model intensity0 intensity10 intensity20 intensity30
intensity40 intensity50 intensity60= judge swallow
concentration*swallow;
random judge;
means swallow*concentration/snk;
run;
```

SAS Code for cookie time intensity data by concentration to determine differences between swallowing and expectorating at each time point for each concentration:

```
libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Descriptive
Work\Data';
data xxx.cookieconc1;
set cookieconc1;
run;
proc sort data =xxx.cookieconc1;
by swallow rep;
run;
proc glm data= cookieconc1 outstat=glmstatsc1;
class judge swallow rep;
model intensity0 intensity10 intensity20 intensity30
intensity40 intensity50 intensity60
= judge swallow rep;
random judge;
means swallow/snk;
run;
proc means data=xxx.cookieconc1;
by swallow;
var intensity0 intensity10 intensity20 intensity30 intensity40
intensity50 intensity60;
output out =meanscookieconc1new mean=intensity0 intensity10
intensity20 intensity30 intensity40 intensity50 intensity60;
run;


libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Descriptive
Work\Data';
data xxx.cookieconc2;
set cookieconc2;
run;
proc sort data =xxx.cookieconc2;
by swallow rep;
run;
proc glm data= cookieconc2 outstat=glmstatsc2;
class judge swallow rep;
model intensity0 intensity10 intensity20 intensity30
intensity40 intensity50 intensity60
= judge swallow rep;
random judge;
means swallow rep/snk;
run;
proc means data=xxx.cookieconc2;
by swallow;
```

```
var intensity0 intensity10 intensity20 intensity30 intensity40
intensity50 intensity60;
output out =meanscookieconc2new mean=intensity0 intensity10
intensity20 intensity30 intensity40 intensity50 intensity60;
run;


libname xxx 'G:\FSCN\Vickers_Lab\Peck\Swallowing\Descriptive
Work\Data';
data xxx.cookieconc3;
set cookieconc3;
run;
proc sort data =xxx.cookieconc3;
by swallow rep;
run;
proc glm data= cookieconc3 outstat=glmstatsc3;
class judge swallow rep;
model intensity0 intensity10 intensity20 intensity30
intensity40 intensity50 intensity60
= judge swallow rep;
random judge;
means swallow rep/snk;
run;
proc means data=xxx.cookieconc3;
by swallow;
var intensity0 intensity10 intensity20 intensity30 intensity40
intensity50 intensity60;
output out =meanscookieconc3 mean=intensity0 intensity10
intensity20 intensity30 intensity40 intensity50 intensity60;
run;
```

Appendix F: Part 3 SAS Code

Figure F.1: SAS Code for Part 3 (Experiment 4)

Experiment 4: Instrument

PROC GLM for ethyl butyrate concentration in nasal headspace (instrument data) to determine differences in concentration in the nasal headspace for swallowing and expectorating at each solution concentration:

```
libname xxx
'\\cfans.ad.umn.edu\cfans$\FSCN\Vickers_Lab\Peck\Swallowing\Da
ta\Instrument Data For SAS';
proc freq data= xxx.instrument;
run;
proc sort data=  xxx.instrument;
by sample swallow rep concentration;
run;
proc means data= xxx.instrument;
by sample swallow;
var concentration;
output out = meansinstrument mean= instrument;
run;
proc glm data=xxx.instrument outstat=glmstats;
class judge rep swallow sample;
model concentration= judge rep sample swallow sample*swallow;
random judge;
means rep swallow sample sample*swallow/snk;
run;
```

PROC GLM for instrument data by ethyl butyrate solution concentration to determine differences in concentration in the nasal head space between swallowing and expectorating for each solution concentration

```
libname
xxx'G:\FSCN\Vickers_Lab\Peck\Swallowing\Data\Instrument Data
For SAS';
data xxx.concentration2;
set concentration2;
run;
proc sort data=xxx.concentration2;
by swallow rep;
run;
proc glm data=xxx.concentration2 outstat = glmstats2;
class rep judge swallow;
model concentration
= rep judge swallow;
```

```
random judge;
means swallow rep/snk;
run;
proc means data=xxx.concentration2;
by swallow;
var concentration;
output out = meansconcentration2 mean=concentration2;
run;

libname
xxx'G:\FSCN\Vickers_Lab\Peck\Swallowing\Data\Instrument Data
For SAS';
data xxx.concentration5;
set concentration5;
run;
proc sort data=xxx.concentration5;
by swallow rep;
run;
proc glm data=xxx.concentration5 outstat = glmstats5;
class rep judge swallow;
model concentration
= rep judge swallow;
random judge;
means swallow rep/snk;
run;
proc means data=xxx.concentration5;
by swallow;
var concentration;
output out = meansconcentration5 mean=concentration5;
run;


libname
xxx'G:\FSCN\Vickers_Lab\Peck\Swallowing\Data\Instrument Data
For SAS';
data xxx.concentration8;
set concentration8;
run;
proc sort data=xxx.concentration8;
by swallow;
run;
proc glm data=xxx.concentration8 outstat = glmstats8;
class rep judge swallow;
model concentration
= rep judge swallow;
random judge;
means swallow/snk;
```

```
run;
proc means data=xxx.concentration8;
by swallow;
var concentration;
output out = meansconcentration8 mean=concentration8;
run;

libname
xxx'G:\FSCN\Vickers_Lab\Peck\Swallowing\Data\Instrument Data
For SAS';
data xxx.concentration11;
set concentration11;
run;
proc sort data=xxx.concentration11;
by swallow;
run;
proc glm data=xxx.concentration11 outstat = glmstats11;
class rep judge swallow;
model concentration
= rep judge swallow;
random judge;
means swallow/snk;
run;
proc means data=xxx.concentration11;
by swallow;
var concentration;
output out = meansconcentration11 mean=concentration11;
run;
```

Appendix G: Calibration Curve

Calibration curve used to calculate ion concentration from ion intensity

Ethyl Butyrate

$y = 0.00039x - 32.45946$
$R^2 = 0.99982$