ORTHONASAL, RETRONASAL, AND ORAL-CAVITY-ONLY DISCRIMINATION OF VAPOR PHASE FATTY ACIDS

A Thesis

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ABSTRACT

The effectiveness of linoleic, oleic, and stearic acids, per se, as odorants has not been clearly established. Chalè-Rush and others (2007) reported detection thresholds for complex mixtures containing these fatty acids, with the stearic acid mixture at 67-69°C. Tamburrino and Halpern (2007) suggested that these fatty acids can not be easily identified by orthonasal or retronasal smelling. The objectives of the first study were to create an effective modified odorant delivery container (MODC) for orthonasal presentations that would limit unwanted aroma loss while the container is idle, and to validate the use of the MODC for discrimination testing methodology using food extracts that were known to be effective odorants (orange, strawberry, and peppermint) so that the MODC could be used for future studies. The objectives for the second and third studies were to determine if linoleic, oleic, and stearic acid could be discriminated against an odorless sample when presented orthonasally, retronasally, and oral-cavity-only (OCO). With an n = 30, the results from the first study showed an extremely high correct discrimination response of 97%, which demonstrated that the MODC was suitable for orthonasal odorant delivery in discrimination studies. With an n = 30, the results from the second study suggested that the three fatty acids tested were significantly different from an odorless sample when presented orthonasally and retronasally. With an n = 30, linoleic acid when presented OCO appeared to not be discriminable from an odorless sample, but there was not enough evidence to suggest that oleic and stearic acids were or were not significantly different from an odorless sample when presented OCO. With a greater sample size, oleic and stearic acid responses might yield significant results for OCO. These findings suggest that these vapor phase fatty acids can stimulate the receptors in the nasal cavity. However, linoleic acid does not stimulate the trigeminal receptor neurons in the oral cavity. It is inconclusive if oleic acid and stearic acid are oral cavity trigeminal stimulants.

BIOGRAPHICAL SKETCH

Bryson's journey in food science began at the Chicago High School for Agricultural Sciences in Chicago, IL. In high school, Bryson was exposed to various agriculture related fields such as agricultural finance, agricultural mechanics and technology, horticulture, animal science and food science. After completing two USDA sponsored summer apprenticeships during his high school career, one in product development and one in food analysis research, Bryson's decision to pursue a bachelor degree in food science was solidified. In 2002, he enrolled in Alabama A&M University (AAMU) where he majored in food science.

During his undergraduate career at AAMU, Bryson received numerous awards including the most outstanding freshman and sophomore of the year award for the AAMU School of Agriculture and Environmental Sciences, membership to the Alpha Kappa Mu National Honor Society, and an IFT scholarship. Additionally, Bryson held various leadership positions including Treasurer of the Food Science Club and the Keeper of Records for Kappa Alpha Psi Fraternity, Inc. Gamma Phi Chapter. Bryson spent his summers taking part in internship opportunities. He interned at Cargill Foods in Memphis, TN as a Quality Assurance Chemist and spent the summer of 2003 at Cornell University, doing research with Professor Harry Lawless, as part of the Food Science Summer Scholars Program. For three weeks in May 2005, Bryson was awarded an International Travel Award by the Center for Hydrology, Soil Climatology and Remote Sensing (HSCaRS) giving him the opportunity to spend three weeks in Ghana doing soil research and studying the Transatlantic Slave Trade.

After conferring his bachelor degree from AAMU, Bryson worked as a Sensory Intern for 12 months at Kellogg Foods in Battle Creek, MI. The internship not only allowed him to gain a full year of valuable work experience, but gave him an opportunity to increase his interest in pursuing a M.S. in food science, concentrating in

the area of sensory evaluation. This internship also gave him and a better understanding of how his role as a future sensory scientist could make an impact in the food industry.

As a graduate student majoring in food science with a minor in Enology at Cornell University, he was selected to participate in a 16-week leadership course for future life scientists. This course allowed him to further execute his duties as the President of the Black Graduate and Professional Student Association and Vice President of Phi Tau Sigma Honor Society at Cornell University. His research concentration is in sensory science with a particular focus in orthonasal and retronasal olfaction of vapor phase long chain fatty acids. His committee chair is Bruce Halpern who is a member of the Field of Food Technology and is a Professor of Psychology and a Professor of Neurobiology and Behavior at Cornell University. Currently, Bryson is coming to the end of his M.S. degree program and he knows that his advanced degree has enhanced his knowledge of sensory science and has allowed him to be more marketable in the Food Industry. After graduation he is looking forward to starting his career in the Sensory Group at Kraft Foods in Glenview, IL.

This thesis dedicated to my late grandfather (Papa), Henry Chester Bolton.

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CHAPTER 1

LITERATURE REVIEW

1.1 Purpose of Literature Review

There are many different sensory tests that can be used to assess a large variety of chemicals, foods, and beverages. The two categories of products that can be tested are food products and non-food products. The purpose of this review is to give an overview of how sensory tests have been applied to smelling research of fatty acids.

1.2 Sensory Evaluation

Sensory evaluation is defined as being "a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing" (Anonymous 1975). Professional organizations, such as the Institute of Food Technologist (IFT) and the American Society for Testing Materials (ASTM), have accepted and endorsed this definition, along with many other sensory evaluation societies (Lawless and Heymann 1998). The ability to evoke, measure, analyze, and interpret data is a "must have" for any sensory scientist.

1.3 Types of Sensory Tests

There are many sensory tests that can test for many things. However, before a choosing a sensory test the objective must be known. The "central dogma" for all sensory tests is that the test method must match the objectives of the test (Lawless and Heymann 1998). With the objective known, a question of interest can then be formulated. There are three types of sensory testing that use different types of participants and have various goals.

1.3.1 Affective Testing

Affective/ preference sensory tests should be used when one is trying to determine which two or more products are liked or preferred by consumers. Affective

tests can be used to develop, maintain or improve a product (Meilgaard, Civille and Carr 2007). Other uses for these tests include: to assess the market potential, to perform category reviews, and to provide support for advertising claims (Meilgaard, Civille and Carr 2007). Affective tests have a vast variety of tests and scales that are used, such as barter scales, hedonic scales, just-right scales, and paired preference tests (Lawless and Heymann 1998). These tests use participants who are untrained, and in some studies the participants are first screened for product usage.

1.3.2 Descriptive Testing

Descriptive sensory tests should be used when one is trying to determine the sensory profile of a particular product or products or how the products differ in a specific sensory attribute. The descriptive profile of a product can be used to determine underlying ingredient and processing variables that can affect the acceptance of a product. The participants are screened for sensory acuity and trained for three to six months until they are considered ready to be effective "instruments"

1.3.3 Discrimination Testing

The objectives of a discrimination test can be to determine if a difference exists between two samples, if two samples are similar enough to be used interchangeably, or a combination of the two (Meilgaard, Civille and Carr 2007). If a difference is found using this useful analytical tool, the sensory attributes of the products can then be later identified through qualitative and quantitative data analysis methods (Stone and Sidel 2004). The triangle, the duo-trio, and the paired comparison tests are some of the more common discrimination tests that are used by sensory professionals (Stone and Sidel 2004).

1.3.3.1 Triangle Test

In a triangle test, the participant is presented with three samples; two of the samples are the same and one is different. The participant is then asked to pick the odd

sample by properly evaluating each sample using instructions given by an experimenter. The probability of the participant choosing the correct sample is 1/3; p = 1/3, and the probability of the participant choosing an incorrect sample is 2/3; q = 2/3.

There are six possible serving combination orders for a triangle tests, and these orders should be presented randomly to the participants. The six possible orders are AAB, ABA, ABB, BAA, BBA, and BAB. “A” corresponds with sample one and “B” corresponds with sample two. The participants are typically allowed to reevaluate each sample. However, allowing participants to reevaluate samples varies from study to study. One drawback with the triangle test is that it does not convey the direction of difference as the paired comparison test does. See section 1.3.3.3 for the Paired Comparison Test.

1.3.3.2 Duo-Trio

In the duo-trio test the direction of the difference is also not indicated. In this test the participant is presented with a reference sample and two test samples. One of the test samples is the same as the reference sample and the other is not. The participant is then asked to pick the sample that is the same as the reference sample. In a duo-trio test the probability of getting the test correct is 1/2; p = 1/2. The duo-trio test can have a constant reference sample (where the reference sample is the same) and a balanced reference sample (where half of the participants receive one reference and the other half receives a different reference).

1.3.3.3 Paired Comparison

The two types of paired comparison test are the directional paired comparison method, which is also called the 2-alternative forced choice (2-AFC) method, and the difference paired comparison method, which is also called the simple different or the same/ different test. In the 2-AFC the participant is presented with two different samples and asked to determine which sample is higher in a particular attribute. In the

same/ different test the participant is presented with two samples and asked to determine if the samples are the same or if they are different. The same/ different test is best used when the samples have a lingering effect, or if the samples are made up of a complex stimulus (Meilgaard, Civille and Carr 2007). Similar to the duo-trio, the probability of getting either test correct is 1/2; p = 1/2. The 2-AFC method is appropriate when the two samples are known to be different in one sensory dimension (Lawless and Heymann 1998). So not only does this method test for a difference, but it quantifies the degree and the direction of the difference. When the sensory attribute difference is not known, then the simple different test should be used (Lawless and Heymann 1998).

1.4 Statistical Analysis for Triangle Discrimination Tests

The correct and incorrect response data of a discrimination test can be used to determine if two samples were perceived to be different or if they were perceived to be the same. These conclusions are based on a certain level of confidence. Choosing the appropriate statistical test can seem like challenging task but if the one knows the assumptions that are associated with each test then the task becomes much easier. The major assumption is that each participant was forced to make a decision; therefore the data only display correct and incorrect responses. The three statistical methods that can be used interchangeably to test the discrimination response data are the binomial, the chi-square adjusted, and the Z approximation to the normal distribution (Lawless and Heymann 1998).

1.4.1 Binomial Test

The binomial test is based on the binomial expansion of $(p + q)^n$ where p is the probability of a certain event happening, and q is the probability of it not happening, (q = 1 – p). This test can predict what will happen if the test is repeated. The triangle test is a one-tailed binomial test, because the experimenter knows which sample is the

different sample (O'Mahony 1986). Please see Equation 1.1 for the binomial test equation.

$$P(y)=\frac{n!}{y!(n-y)!}p^{y}p^{n-y}$$

Equation 1.1: Shows the binomial test equation used to determine what will happen if a test is repeated. *P(y)* = probability of a specific outcome, *n* = total number of responses, *y* = total number of correct responses, *p* = probability of making the correct judgments by guessing.

Equation 1.1 calculates the probability of any specific outcome. This equation can then be used to calculate the minimum number of correct participants needed to conclude that a difference exist between two samples. In addition, tables were developed by Roessler and others (1978) which allow for a quick estimate of the significance for discrimination tests.

1.4.2 Adjusted Chi-square Test (χ^2)

The chi-square test is another statistical analyses method that is used to determine the significance by comparing frequencies of observed and expected/ hypothesized data sets (O'Mahony 1986). These expected/ hypothesized data sets have been put into a statistical table that has been derived from the chi-square distribution. The chi-square distribution tables can be found in many statistical textbooks, see O'Mahony (1986).

One major advantage that the chi-square test has over the binomial test is that it can be used in one and two-way classifications with multiple categories. In addition, the chi-square test is equipped to analyze three categories of nominal responses rather than just two. One assumption with the chi-square test is that each observation is

independent (O'Mahony 1986). The continuity correction value of -0.5 is inserted into the chi-square equation because the chi-square distribution is continuous, and the observed results from a discrimination test are integers (Lawless and Heymann 1998). Since it is not possible for a half of a participant to have a correct response the statistical approximation can be cut by ½ maximally (Lawless and Heymann 1998). See Equation 1.2 for the adjusted chi-square equation.

$$\chi^2 = \left[\frac{(|O_1 - E_1| - 0.5)^2}{E_1}\right] + \left[\frac{(|O_2 - E_2| - 0.5)^2}{E_2}\right]$$

Equation 1.2: Shows the adjusted chi-square equation. Where O_1 = observed number correct responses, O_2 = observed number of incorrect responses, E_1 = expected number of correct responses: (n) times (p), p = 0.333 for triangle tests, E_2 = expected number of incorrect responses: 1 minus (n) times (p) equals q, q = 0.667 for triangle tests.

1.4.3 Normal Distribution and the Z-Test on Proportion

The normal or the Gaussian distribution is a symmetrical bell-shaped curve, and many statistical theories are based on this curve (O'Mahony 1986). The area under the normal distribution curve can be used to test the probability of an event occurring by chance alone. Statistical z-tables have been calculated, constructed and published in many textbooks, see Lawless (1998). The critical z-score for 95% confidence level for a one-tailed test equates to 1.645. Therefore, if the calculated z-score is greater than 1.645, then there is sufficient evidence to conclude that a difference exists between the two samples in question, and the null hypothesis would then be rejected. On the other hand, if the calculated z-score does not exceed 1.645, then there is insufficient

evidence to conclude that a difference exist between the two samples in question (we would fail to reject the null hypothesis). See Equation 1.3 for the z-score calculation.

$$Z = \frac{X - np - 0.5}{\sqrt{npq}}$$

Equation 1.3: Shows the z-score calculation. X = total number of correct responses, n = total number of correct and incorrect responses, p = probability of correct decision by chance (0.333 for a triangle test), and q = 1 – p.

1.4.4 Type I and Type II Errors

There are two types of statistical errors that can be made in statistical analysis of data. One is a Type I error, which is also called a false positive or an (α) error; the other error is called a Type II error, which is also called a false negative or a (β) error. A Type I error occurs when the experimenter rejects the null hypothesis (H_0) when in fact it was true. The selection of an appropriate (α) controls the chance of a Type I error occurring. A Type II error occurs when the experimenter fails to reject the (H_0) when it was actually false. A Type II error is the risk of not finding a difference when a difference actually exists. Therefore, the power of a test can be defined as being 1 minus β.

1.5 Smelling

The olfactory and the trigeminal systems are the two sensory systems that are used by humans in smelling (Rawson 2000, Halpern 2004). However, which system is used is dependent on the nature of the compound (Savic-Berglund 2004). For example, purely olfactory odorants such as vanillin, coumarin, octanoic acid, and phenylethyl alcohol activate the olfactory system which is located only in the nasal cavity (Chen and Halpern 2008, Cometto-Muñiz, Cain and Abraham 2005, Doty and

others 1978), whereas other odorants such as peppermint (Dragich and Halpern 2008, Stephenson and Halpern 2009), acetone, and butanol activate both the olfactory and trigeminal systems in the nasal cavity (Savic-Berglund 2004). In addition, trigeminal odorants can activate different parts of the brain when compared to olfactory odorants. These activated parts of the brain (brain stem, cerebellum, and the posterior part of the anterior cingulate) are also activated by pain stimuli (Savic-Berglund 2004). In addition to the trigeminal receptor neurons existing in the nasal cavity, they also exist in the oral cavity (Silver and Finger 1991). Therefore, odorants that can stimulate the trigeminal receptor in the nasal cavity could also be effective in the oral cavity (Halpern 2004).

1.5.1 Olfaction

The very first olfactory theory is more than 2000 years old. It dates back to a Roman physician named Claudius Galenus who is said to have discovered the olfactory nerves (Bernreuther, Epperlein and Koppenhoefer 1996). Orthonasal and retronasal olfaction are the two types of airflow patterns for olfaction. Orthonasal olfaction is the process in which odorants travel from the external environment inward through the anterior nares towards the olfactory mucosa during nasal inhalation or a sniff (Halpern 2004). Retronasal olfaction occurs when an odorant is in the oral cavity and travels back towards the pharynx and through the nasal cavity towards the olfactory mucosa during exhalation and out through the nose (Halpern 2004). Retronasal olfaction is typically attributed to the release of odorous molecules when food or drink enters the oral cavity and undergo mastication or swallowing (Negoias and others 2008, Halpern 2008) Albeit, orthonasal and retronasal olfaction both use the olfactory system, perceptual difference exists perhaps due to the airflow pattern (Negoias and others 2008, Halpern 2008, Ishikawa and others 2006). In addition, the molecular weight, mass, shape, polarity, resonance structure, type of bond and side

groups has an effect on the smell of an odorant (Rawson 2000). Typically, air-breathing organisms can only detect odorants that are neutral organic compounds, have a strong hydrophobic region and a weaker polar region, and have a molecular weight of about 300 or less (Silver and Walker 1997, Weyerstahl 1994).

Even though many animals have a keener sense of smell than humans do, the human ability to detect odorants is far superior to any olfactometer (Rawson 2000). The olfactometer has a theoretical odor detection limit of about 10^{-19} moles (Reineccius 2006). In the olfactory epithelium, there are about 12 million olfactory receptors in the human nose (Silver and Walker 1997). In sensory evaluation, participants use their sense of smell to detect or describe aromas or flavors. The progressive discovery of odorants is like learning a foreign language and with practice one can become more articulate (Weyerstahl 1994).

1.5.2 Olfaction of Fatty Acids

1.5.2.1 Animal Studies

Prior to the 1990's, most of the sensory research on fats focused on the textural properties of fats/ oils rather than other sensory properties such as olfactory cues (Ramirez 1992). Rodents exhibit an ingestion preference for unsaturated fatty acids under normal biological conditions. However, when the olfactory ability is eliminated in rodents by sinus irrigation with zinc sulfate (Fukuwatari and others 2003), by an olfactory bulbectomy (Ramirez 1993), or by olfactory nerve sectioning (cited after: Kinney and Antill 1996), the preference for fats is eliminated, which suggests that there is an olfactory component in fat perception (Kinney and Antill 1996). The intranasal irrigation with zinc sulfate not only destroys receptor neurons, but it can also cause severe chemical and morphological changes in the olfactory bulb (Margolis and others 1974). The accidental ingestion of zinc sulfate during irrigation has also been found to be poisonous (cited after: Kinney and Antill1996). An advantage that

the olfactory nerve sectioning procedure has over the irrigation with zinc sulfate, and the olfactory bulbectomy procedure, is that it results in complete functional recovery; which is due to the regeneration of olfactory neurons after 31 days (cited after: Kinney and Antill 1995). Rats that had bulbar lesions on the reported area in the olfactory epithelium that was previously reported to be responsive to short-chain fatty acids did not result in a decrease ability to detect fatty acids (Bisulco and Slotnick 2003). However, the use of long-chain fatty acids might have changed the results because longer chain fatty acids may use different transduction mechanisms (Mattes 2005).

1.5.2.2 Human Studies

Schiffman and others (1998) used three different oils (bleached and deodorized soybean oil, medium chain triglyceride (MCT) oil, and light mineral oil) and four different emulsifiers (Tween-80 (Polysorbate 80, CAS Number: 9005-65-6), 0.2%; Emplex (stearoyl lactalate; American Ingredients. Co., Kansas City, MO., USA), 0.5%; sodium caseinate, 2%; and acacia gum, 5%) in solution, and found that the detection thresholds did not differ significantly when nose clips were applied to 12 young participants (Mean age = 23.7, SD = 3.37) and 12 elderly participants (Mean age = 87.3, SD = 4.12). These results suggested that olfaction was not the basis for detection (Schiffman and others 1998). However, it should be noted that in Schiffman and others (1998) Table 2, that there may have been retronasal smelling of the mixture containing Emplex and MCT because the detection thresholds differ by more than one SD with and without nose clips. The absence of inferential statistics in Schiffman and other (1998) makes it difficult to draw firm conclusions. In another study, thickness attribute ratings of dairy products (skim milk, low-fat milk, whole milk, half-and-half, half-and-half plus cream, and heavy cream) did not differ significantly when nose clips were worn to prevent access of potential odorants to the nasal cavity (Schiffman and others 1998).

Chalè-Rush, Burgess and Mattes (2007a) used a sonicator to improve homogeneity of water in oil emulsions, ethylenediaminetetraacetic acid (EDTA) as a chelating agent, and gum acacia and mineral oil in order to reduce viscosity and texture as sensory cues of fatty acid mixtures, because they wanted to ask if the fatty acids were "taste" stimuli. They sought to minimize fatty acid oxidation because they wanted to study taste responses to non-oxidized fatty acids. Their second study determined detection taste thresholds for linoleic, oleic, and stearic acids. However, the addition of multiple solutes can increase the viscosity and decreases the vapor pressure resulting in a decrease in the aroma. This approach was effective in minimizing fatty acid oxidation and homogeneity of the fatty acid sample. In the same series of studies, they measured detection thresholds for mixtures containing linoleic, oleic, or stearic acids. Chalè-Rush, Burgess and Mattes (2007b) found that the retronasal detection thresholds for smelling these mixtures were higher than multimodal (taste, tactile, smell) detection thresholds.

Mattes (2005) stated that thresholds were higher in one study when participants were not able to use their olfactory system. However, previous studies have suggested that the olfactory system does not alter the perception of fats (Schiffman and others 1998, Mela and Christensen 1987). Mela and Christensen (1987) eliminated the ability to see, touch, and smell; and their reports indicated that the chewing and swallowing of a cornmeal based snack foods that varied on vegetable oil content did not alter the perceived oiliness. Mattes (2001) used the postprandial rise of serum triglycerides (TAG) as indicator for fat sensory detection, but it did not support an olfactory contribution (Mattes 2001). It was inconclusive if the olfactory only presentation failed to alter the TAG concentration, or if the stimulus was not detected (Mattes 2001).

Tamburrino and Halpern (2007) found that with a list of identifiers (chalk, silly-putty, linseed oil, glue, soap, and flour), linoleic, oleic, and stearic acid can not be easily identified when presented orthonasally and retronasally to a group of untrained participants. The use of trained participants might offer a possibility for identification (Tamburrino and Halpern 2007).

1.6 General Research Objective

The effectiveness of linoleic, oleic, and stearic acids, per se, as odorants has not been clearly established. Therefore, the goal of this research was to determine if these fatty acids could be discriminated by the olfactory and or the trigeminal systems.

CHAPTER 2

MODC ASSESSMENT FOR ORTHONASAL PRESENTATIONS

2.1 Introduction

To assess the sense of smell in humans many tests have be developed to measure various aspects of smelling. Pierce and Halpern (1996) used the gray covers from Kodak Ektar ® 1000 film canisters for orthonasal and retronasal inhalation. The gray covers were used to hold a specific amount of solid odorant and the participants were asked to identify the sample by normal and diaphragmatic breathing (Pierce and Halpern 1996). Chen and Halpern (2008) developed an odorant delivery container (ODC) for retronasal and oral-cavity only presentations. The ODC is a very effective presentation container for various odorants (Stephenson and Halpern 2009). However, the inhalation straw and the airflow hole may provide an opportunity for aroma loss and oxidation of a sample that has the propensity to oxidize.

2.2 Objective

The goal of this experiment was to develop a modified odorant delivery container (MODC) for orthonasal inhalation procedures that would decrease aroma loss and the potential for oxidation. Once developed, its ability to be an effective ODC was assessed through a series of three different triangle discrimination tests using known odorants. The objective of this study was to determine, using the MODC, if natural food extracts such as orange, peppermint, and strawberry could be discriminated orthonasally against a solvent, which in this case was sunflower oil. See the Table 2.1 for the food extracts that were used.

2.3 Hypothesis

The research hypothesis is that the food extracts (orange, strawberry, and peppermint) would be found to be different orthonasally and retronasally from the

odorless sample. The probability of picking the correct sample by chance is 1/3. Thus the H_o, p = 1/3, q = 2/3; H_a, p >1/3 and it's a one-tailed test.

2.4 Participants

The participants were 30 paid volunteers. The participant's age and gender were not recorded but all participants were at least 18 years of age. The participants were affiliated with Cornell University and were recruited using flyers that were posted around Cornell University's campus. See Figure A.1 for the recruitment poster. All participants that participated in this study had the ability to communicate in written and spoken American English, were non-smokers, non-pregnant, non-lactating, and were asked not to eat or drink anything one hour prior to the study. The participants were not screened for their ability to detect certain odorants prior to the study, nor were there any other chemosensory data collected. The Cornell University Institutional Review Board for Human Participants (IRBHP) first reviewed and approved the protocol. Each participant first read the Informed Consent Form, asked any questions they had, and, if they decided to participate in the study, and signed the informed consent form which was approved by the IRBHP. The participants were informed that this study would test their ability to detect different odorants by orthonasal smelling. For purposes of this study, orthonasal smelling was described inhaling through the nose, in addition to watching a short two minute instructional video that described how to perform the test. See Figure A.2 for the orthonasal ballot instructions.

2.5 Materials

2.5.1 Stimuli

The stimuli used in this study were: organic alcohol free orange, strawberry, and peppermint extracts (Frontier Natural Flavors (FNF) Co-op, Norway, IA 52318, U.S.A.), (www.frontiercoop.com). Strawberry, orange (Halpern 2004, Dragich and Halpern 2008, Sun and Halpern 2005), and peppermint (Dragich and Halpern 2008,

Stephenson and Halpern 2009, Sun and Halpern 2005) extracts have been used in previous odorant sensory studies. These three extracts have been found to be effective odorous stimuli for the olfactory and oral cavity trigeminal systems, even though strawberry has been previously been suspected to be a non trigeminal stimulus (Dragich and Halpern 2008). Expeller pressed organic high heat sunflower oil (Distributed by Spectrum Organic Products, LLC a subsidiary of the Hain Celestial Group, Inc. Millville, NY 11747 USA) was used to dilute the food extracts to the their appropriate concentrations and it was used as a control sample during the discrimination procedure. Sunflower oil was chosen because it was the solvent that was used by FNF.

Table 2.1: Shows a summary of the food extracts that were used and their appropriate concentrations. It also shows the solvent that was used to dilute the food extracts.

Food Extract	Concentration (%v/v)	Solvent
Orange	77	Sunflower Oil
Peppermint	33	Sunflower Oil
Strawberry	50	Sunflower Oil

2.5.2 Ellipso Portion Cups

The method of preparation was designed after Chen and Halpern (2008). The ODC was a new and odorless, homopolymer polypropylene, black tapered elliptical container. (Ellipso Portion Cups, Newspring ® Packaging, Kearny, NJ 07032, U.S.A.), (http://www.instawares.com). The containers were 118ml in volume, 5.1cm high, 0.4 mm in wall thickness, with an upper major axis of 7.8 cm and a minor axis of 4.9 cm. The lower major axis was 5.4 cm with a minor axis of 2.7 cm. Each container had a tight fitting, transparent homopolymer polypropylene elliptical lid.

2.5.3 Modified Odorant Delivery Container (MODC) for Orthonasal Testing

Five ml Eppendorf ® ep disposable pipette tips (http://www.daigerr.com) with a length of 12 cm were first cut to be 8.2 cm. Four cm of the cut pipette tip was then inserted into two 1 cm diameter holes that were made directly into two indentations that were in the lid when it was received from the manufacturer. The indentations in the lid were 3 cm away from the elongated edge of the lid. The holes were made using a #5 cork borer. Plastic caps were then cut from 5ml sample vials (03-338-1C), (Fisherbrand ®, Distributed by Fisher Scientific) and then placed on the pipette tips to decrease aroma loss and the potential for oxidation. Please see Figure 2.1 for a photograph of the MODC's.

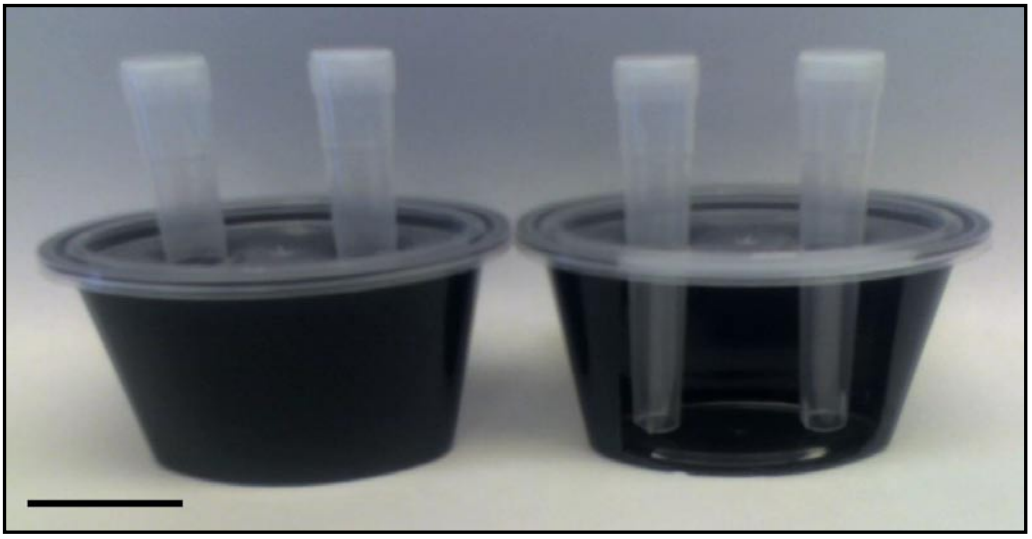

Figure 2.1: Shows a photograph of two MODC's. The MODC on the left shows an exterior view and the MODC on the right shows an interior view. The interior view was made possible by cutting away a portion of the wall of the MODC. The MODC has two cut and capped pipette tips inserted into the holes that were made into the lids. The MODC has a total volume of 118ml. During discrimination testing the MODC contained 5ml of liquid stimuli (orange extract/ strawberry extract/ peppermint extract/ sunflower) which just covered the bottom of the container. The horizontal calibration line represents 3cm.

2.6 Methods

2.6.1 Training

Each participant watched a 2 minute instructional video on how to perform an orthonasal inhalation triangle discrimination procedure. After the completion of the video each participant was asked to demonstrate this inhalation method before they were allowed to participate in the study. See section 2.6.2 or Figure A.2 for the orthonasal ballot instructions.

2.6.2 Participant's Orthonasal Ballot Instructions

The participants were presented with three, 3-digit coded odorant containers. Two of the samples were the same and one was different. The participants were then asked to pick up one container and remove the two caps from the two plastic pipette tips. They were then asked to keep the containers upright and not to tilt the containers. The pipette tips were then angled from side to side by the participants to make sure that the tips were set at the correct position of the participant's nostrils. Then the participants inhaled moderately one time with the pipette tips angled and placed directly under the nose so that both edges of the tubes were gently grazing the outer rim of their nostrils. A brief intermission for two to three seconds was then taken, and then the inhalation orthonasal procedure was repeated. The inhalation orthonasal procedure could be repeated up to five times for each container. The participants continued this procedure until the odor was committed to memory. The pipette tips were then recapped and the participants were then asked to evaluate the next sample in the same fashion. The participants were advised that once a container was recapped that they would not be allowed to reevaluate the sample again. The objective was to commit the odorant to memory, and then use the computer mouse to click on the sample box that was on the computer screen that was most different. After the participants clicked on the sample (most different) their next set of 3-digit

samples appeared on the computer screen. They repeated this procedure until all discrimination procedures were completed.

2.6.3 Procedure

All participants performed the test on the same day in the last week of July 2008 in Cornell University's Sensory Lab in Stocking Hall. The temperature of the room was not recorded but it was noted to be well above the normal room temperature. All extracts were recently purchased, and the stock solutions were made the day of the experiment. Disposable plastic gloves (USDA approved) were worn by the experimenter throughout the odorant presentations and replaced if they came in contact with an odorant. All samples were prepared and presented within 6 hours at room temperature. The presentation order was a complete randomized block design to eliminate any order effects. It was decided to administer the samples one at a time to eliminate the need to monitor each panelist while they were performing the tests. The discrimination tests were presented using Compusense (Guelph, Ontario, Canada). Once all of the odorant triangle discrimination tests were completed the participants were compensated monetarily. The data were then exported and analyzed for significance. See Table A.1 for food extract orthonasal responses.

2.7 Results

Based on the binomial distribution table at a probability level of 0.05, 15 correct responses out of a total of 30 responses is the minimum number required to establish a statistically significant difference (Roessler and others 1978). See Figure 2.2 for the correct and incorrect orthonasal responses for food extracts vs. odorless stimuli.

2.7.1 Orange Extract vs. Sunflower Oil

The results showed that out of a total of 30 responses, 29 were correct. According to the one-tailed binomial test at an alpha-risk of 0.05, the critical z-score is

1.645. Since the calculated z-score was 7.165 and it is higher than 1.65 there is enough evidence to conclude that there was a difference. The null hypothesis that probability picking the odd sample was due by chance was rejected; therefore, a difference exists.

2.7.2 Peppermint Extract vs. Sunflower Oil

The results showed that out of a total of 30 responses, 30 were correct. According to the one-tailed binomial test at an alpha-risk of 0.05, the critical z-score is 1.65. Since the calculated z-score was 7.552 and it is higher than 1.645 there is enough evidence to conclude that there was a difference. The null hypothesis that probability picking the odd sample was due by chance is rejected; therefore, a difference exists.

2.7.3 Strawberry Extract vs. Sunflower Oil

The results showed that out of a total of 30 responses, 29 were correct. According to the one-tailed binomial test at an alpha-risk of 0.05, the z-score is 1.65. Since the calculated z-score was 7.165 and it is higher than 1.645 there is enough evidence to conclude that there was a difference. The null hypothesis that probability picking the odd sample was due by chance is rejected; therefore, a difference exists.

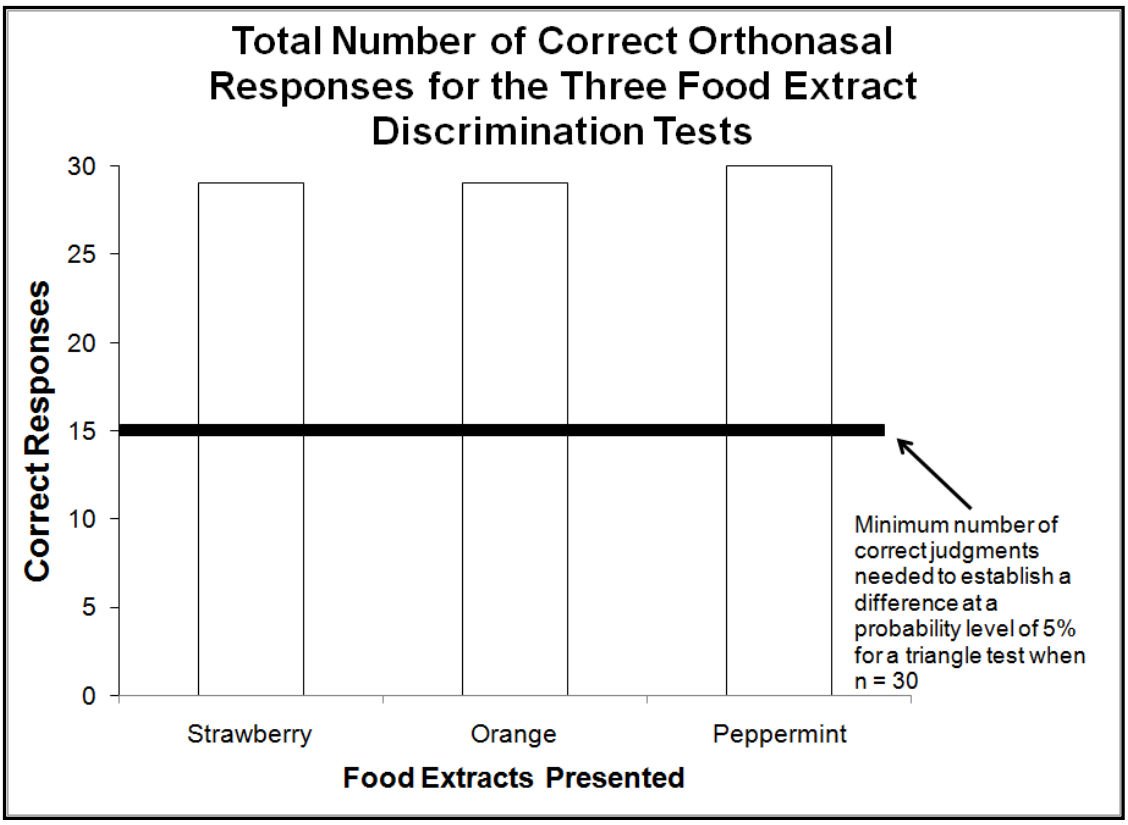

Figure 2.2: This chart shows the total correct responses for the food extract triangle discrimination tests. With regard to the x-axis, strawberry represents strawberry extract vs. sunflower oil; orange represents orange extract vs. sunflower oil; and peppermint represents peppermint extract vs. sunflower oil. The y-axis represents the total number of correct responses for each test. The horizontal black bar represents the minimum number of correct judgments needed to establish a difference at probability level of 5% for a triangle test when n = 30.

2.8 Discussion

In all three discrimination tests, the results were significant which indicates that an aroma difference does exist between the food extracts and the sunflower oil. With an extremely high total of correct responses, there were 29 correct responses for the orange and strawberry extracts vs. sunflower oil. Moreover, there were 30 correct responses for the peppermint vs. solvent discrimination test.

2.9 Conclusion

Since the data has such high discrimination ability it was decided that the MODC's were suitable for orthonasal odorant delivery. In addition, the series of three triangle tests appeared to be sufficient and not overwhelming to the participants.

CHAPTER 3

FATTY ACID DISCRIMINATION BY ORTHONASAL AND RETRONASAL SMELLING

3.1 Introduction

Fats/ lipids make up a large variety of chemical compounds such as monoglycerides, diglycerides, triglycerides, phosphatides, cerebosides, sterols, terpenes, fatty alcohols and fatty acids (Lobb and Chow 2008). Fatty acids are primarily composed of carbon, hydrogen, and oxygen, and are arranged in linear carbon chains of variable lengths. They have a methyl group on one end, and a carboxyl group on the other end. These fatty acids can either be saturated (no double bond), monounsaturated (containing one double bond), or polyunsaturated (containing two or more double bonds).

Linoleic 18:2(n-6), oleic 18:1(n-9), and stearic 18:0 acids all have 18 carbon chains but they differ in their degree of unsaturation. Linoleic acid is an essential omega-6-fatty acid, and it is present in almost every vegetable fat. It has high amounts in corn, safflower, sunflower, and soybean oils and it has low amounts in animal fats and fish oils (Lobb and Chow 2008). Oleic acid is found in high amounts in canola and olive oil (White 2008); it is the major fatty acid in meats, contributing up to 30% of the total fatty acid content (Wood and others 2008). Oleic acid in olive oil has been found to increase high-density-lipoproteins and decrease blood pressure (Ruiz Gutierrez and others 1996). Stearic acid does not raise low-density-lipoproteins concentration levels, unlike other saturated fatty acids (Grundy 1994). Stearic acid has also been used in conjunction with short chain fatty acids to form salatrim (short and long acyl triacylglycerol molecule), which is also known as Benefat ® (Akoh 2008). This molecule has a similar taste, texture and functional properties to conventional fats and oils (Akoh 2008).

Linoleic acid and γ-linoleic acid (GLA) are known to play an important role in the physiology and pathophysiology of the human skin (Lobb and Chow 2008). Linoleic acid is also known to be the most abundant polyunsaturated fatty acids in the human epidermis (Chapkin and Ziboh 1984). In a recent study, based on biophysical techniques, an Efamol ® evening oil primerose capsule taken every day, which contained 345 mg GLA showed significant improvement in skin moisture, elasticity, firmness, fatigue resistance and a decrease in roughness in the skin of adults (Muggli 2005).

Before the mid 1990's there were few if any studies that centered on the gustatory properties of fatty acids (Gilbertson 1998), because the size of the fat/ oil droplets was considered too large to bind to a sensory receptor (Fukuwatari and others 1997). The gustatory system of rats was found to have the ability to detect fatty acids via inhibition of the delayed rectifying K^+ channels, by using a patch clamp recording method in rats (Gilbertson and others 1997). An immunohistochemical staining technique revealed that the CD36 receptor, which is located in the apical membrane of taste cells, transports free fatty acids across a cell membrane (Fukuwatari and others 1997).

Most dietary fats are in triglyceride form and they must first be in the free fatty acid form to be absorbed by the intestine (Abumrad 2005). Kawai and Fushiki (2003) reported that lingual lipases (enzymes in the mouth) are released to perceive triglycerides in the oral cavity.

There has been an increasing interest in the dietary fat sensory response of humans in recent years (Mattes 2005, Schiffman and others 1998, Chalè-Rush, Burgess and Mattes 2007a, Warwick and Schiffman 1990). The sense of taste, smell, and tactile/ touch can be used to detect fats (Drewnowski 1997). The combination of these three senses can make the identification of relevant sensory systems difficult.

The effectiveness of linoleic, oleic, and stearic acids, per se, as odorants has not been clearly established. Chalè-Rush and others (2007b) reported detection thresholds for complex mixtures containing these fatty acids, with the stearic acid mixture at 67-69°C. Oleic acid has been reported to have a fat odor (Acree and Arn 2004), and a lard like odor (Rickman 2009), but it has also been reported to be odorless (Moncrieff 1967, O'Neil 2006). Stearic acid has been reported to be odorless (Moncrieff 1967) or have a slight tallow odor (O'Neil 2006). Tamburrino and Halpern (2007) suggested that these fatty acids can not be easily identified by orthonasal and retronasal smelling.

3.2 Objective

The objective of this study was to determine if the vapor-phase fatty acids (linoleic, stearic and oleic) could be discriminated orthonasally and retronasally when presented against an odorless sample. The use of these three chemicals will make it possible to evaluate the role of smelling via the nostrils (orthonasal smelling) and smelling from the mouth (retronasal smelling) in response to these natural fatty acids. This study will permit an improved understanding of the role of smelling in human responses to foods.

3.3 Hypothesis

The two research hypotheses are that specific vapor-phase fatty acids (linoleic, stearic and oleic) would be found to be different orthonasally and retronasally from the odorless sample. The probability of picking the correct sample by chance is 1/3. Thus the H_o, $p = 1/3$, $q = 2/3$; H_a, $p > 1/3$ and it's a one-tailed test.

3.4 Participants

The participants were 30 paid volunteers (17 Males and 13 Females) with an age range of 19 to 60 and a mean age of 26.6 and a standard deviation of 9.3. The participants were affiliated with Cornell University and were recruited using flyers that were posted around Cornell University's campus. See Figure A.1 for recruitment

poster. All participants that participated in the study were at least 18 years of age, had the ability to communicate in written and spoken American English, were non-smokers, non-pregnant, non-lactating, and did not eat or drink one hour prior to the study. The participants were not screened for their ability to detect certain odorants prior to the study, nor were there any other chemosensory data collected. The Cornell University Institutional Review Board for Human Participants (IRBHP) reviewed and approved the protocol. Each potential participant read the Informed Consent Form, asked any questions they had, and, if they decided to participate in the study, signed the informed consent form which was approved by the (IRBHP). The participants were informed that this study would test their ability to detect different odorants by orthonasal and retronasal smelling. For purposes of this study, orthonasal smelling was described as inhaling through the nose. Retronasal smelling was described as smelling from inside of the mouth while exhaling out the nose. Both procedures were demonstrated by the experimenter. Each participant was asked to demonstrate this procedure correctly to the experimenter before they were allowed to begin the tests.

3.5 Materials

All fatty acid chemicals were purchased from Sigma-Aldrich Incorporated (St. Lousis, MO). The fatty acids were: Linoleic Acid 60% (CAS Number: 60-33-3), Oleic Acid FCC, Kosher FG (CAS Number: 112-80-1), and Stearic Acid 95 % reagent grade (CAS Number: 57-11-4). The presented concentrations were determined from Tamburrino and Halpern (2007) and through bench top testing. See Table 3.1 for the presented linoleic and oleic concentrations that were used in this study and in Chapter four. Mineral oil United States Pharmacopeia (U.S.P.) was used to dilute linoleic and oleic acids and presented at 100% concentration as a control stimuli in this study. Stearic acid which is solid at room temperature (21.5°C) was presented as the test

stimulus and NaCl was presented as the control stimulus for stearic acid during orthonasal and retronasal presentations.

Table 3.1: Shows a summary of the fatty acids (liquids at 21°C) that were used and their appropriate concentrations. It also shows which solvent was used to dilute the fatty acids. Stearic acid stimulus was 95% w/w and was excluded from this table.

Fatty Acid	Concentration (%v/v)	Solvent
Linoleic	66.6	Mineral Oil
Oleic	40	Mineral Oil

3.5.1 Odorant Delivery Container (ODC) for Retronasal Testing

See Chapter 2 for manufacturer information of the Ellipso containers that were used in this study. One 5 mm in diameter hole was made into one of the two indentations that were in the lid when it was received from the manufacturer. The indentation was 3.5 cm away from the elongated edge and the 5mm hole was made using a using a 3/16 spiral drill bit. In the other indentation that was located 1.8 cm away from the former indentation, a 1.3 cm in diameter hole was made with a #6 cork borer. A 6.5 cm long homopolymer polypropylene straw (Jetware Unwrapped Plastic drinking straw, Jet Plastica Industries, Inc., 1100 Schwab Road, Hatfield PA 19440) was inserted 3.25 cm into the 5 mm in diameter hole. Deviating from the previous methodology in Chen and Halpern (2008), the straw was not taped to the lid which was due to a smaller hole size which provided a tighter fitting. A 5 ml Eppendorf ® ep disposable pipette tip (Hamburg, Germany) with a length of 12 cm was then cut to be 4 cm. The cut pipette tip was then inserted into the 1.3 cm in diameter hole, 2 cm. Plastic caps were then cut from 5ml sample vials (03-338-1C), (Fisherbrand ®, Distributed by Fisher Scientific) and then placed on the tubes to decrease aroma loss. To prevent particulate inhalation of stearic acid and sodium chloride (NaCl) crystals

(Polystormor ™ AR® (ACS) Mallinckrodt Baker, Inc., Phillipsburg, NJ 08865), a 1x1 inch Kimwipe ® (Kimberly-Clark ®, Irving, TX 75038) was taped around straw's tip that was located inside the containers. Please see Figure 3.1 for a photograph of the ODC's.

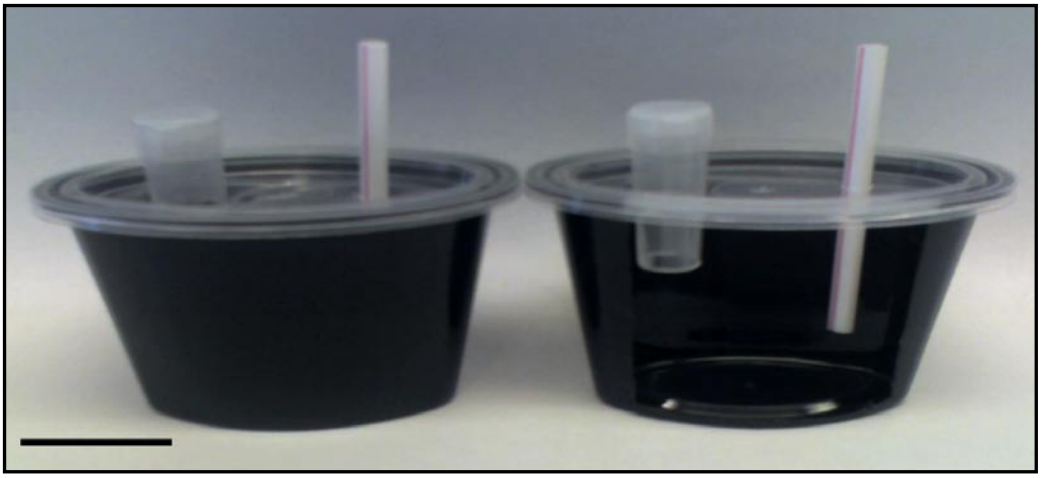

Figure 3.1: Shows a photograph of two ODC's. The ODC on the left shows an exterior view and the ODC on the right shows an interior view. The interior view was made possible by cutting away a portion of the wall of the ODC. The ODC has one cut and capped pipette tip and one straw that were inserted into the holes that were made into the lids. The ODC had a total volume of 118ml. During discrimination testing the ODC contained 5ml of liquid stimuli (linoleic acid/ oleic acid/ mineral oil) or 2 grams of solid stimuli (stearic acid/ NaCl) which just covered the bottom of the container. The horizontal calibration line represents 3 cm. The ODC shown in the interior view in this photo (on the right) does not have a Kimwipe ® taped around the straw.

3.5.2 MODC for Orthonasal Testing

See Chapter 2 for MODC that was used. In addition, to prevent particulate inhalation of stearic acid and NaCl, a 1x1 inch Kimwipe ® (Kimberly-Clark ®, Irving, TX 75038) was taped around the inner rim of the two cut pipette tips that were located inside of the container.

3.5.3 Nose Clips

All retronasal presentations began with the participants properly securing their nose clips (Spirometrics Nose Clip #2104, Spirometrics, Gray, ME; 207-657-6700) around the nostril area of the nose before the plastic cap was removed from the pipette tip. Each nose clip was used once and then discarded. Please see Figure 3.2 for a photograph of a nose clip.

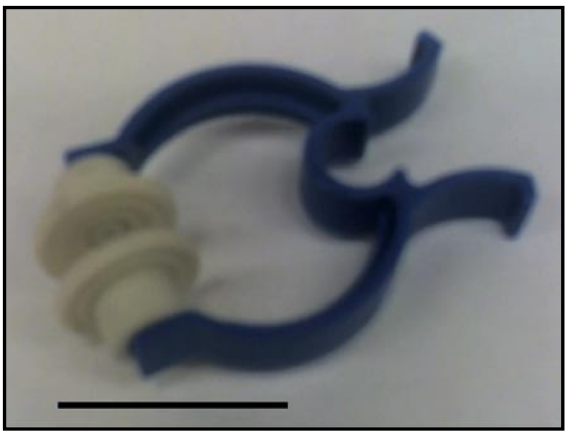

Figure 3.2: Shows a photograph of a nose clip. The horizontal calibration line represents 3 cm.

3.6 Methods

3.6.1 Sample Preparation

3.6.1.1 Linoleic Acid and Oleic Acid

Linoleic and oleic acids stock solutions were first transferred into light resistant glass jars that were wrapped in foil and sealed under compressed pre pure nitrogen to prevent oxidation. According to the Material Safety and Data Sheet for oleic and linoleic acid, it was noted that oleic acid's storage temperature was 2° - 8°C, but linoleic acid's storage temperature was not available. Linoleic and oleic acid was stored in a refrigerator at 4.5°C. It was further noted that this storage temperature solidified linoleic and oleic acid. Each testing day, linoleic and oleic acid was thawed

at room temperature for 2-3 hours before they were diluted in volumetric flasks. Once appropriate concentrations were made, the sample was mixed by turning the flask upside down 10-15 times. With the flask inverted at 180˚, the headspace (air pocket) was allowed to pass through the entire volumetric flask. This allowed for a gradual mixing process for the fatty acids and the mineral oil. Once the concentrations were made, pipettes were used to pipette 5 ml of the diluted fatty acid (test) and 5 ml of the mineral oil (control) into the appropriate odorant containers. Then the appropriate lids and tubes were securely fastened to the containers. The sample concentrations were made daily.

3.6.1.2 Stearic Acid

The oxidation potential of stearic acid is lower than linoleic and oleic acid, so there was not a need to store the stearic acid under nitrogen, but it was stored in a freezer (-18.5°C). However, the stearic acid was transferred into light resistant glass jars that were wrapped in foil to prevent light oxidation. Each testing day, the stearic acid was brought to room temperature (21.5°C) in a closed container for one hour. Then two grams (2.4 ml) of stearic acid and two grams (0.9ml) of NaCl (control sample) were placed into the appropriate odorant containers with a ceramic spatula. The lids with the appropriate tubes were then securely fastened on to the containers. Aluminum foil was then secured/ wrapped on to the lids of the samples that contained stearic acid or NaCl to mask the identity of the sample. To prevent visual identification by looking through the apical portion of the pipette tip, participants were asked to close their eyes during orthonasal and retronasal presentations of stearic acid and NaCl. Eye closure was monitored by the experimenter. These visual masking techniques were avoided for linoleic and oleic acid presentations after bench top testing revealed that identification could not be determined.

3.6.2 Experimental Design

The experimental design was a completely randomized block design and the presentation order was generated using Compusense (Guelph, Canada). Therefore, the order in which the participants performed the tests was randomized. The odd numbered participants received the orthonasal test first, and the even numbered participants received the retronasal test first. A 2-3 minute intermission was given to participants in between orthonasal and retronasal odorant testing procedures.

3.6.3 Participant's Orthonasal Ballot Instructions

The participants were presented with three, 3-digit coded odorant containers. Two of the samples were the same and one was different. The participants were then asked to pick up one container and remove the two caps from the two plastic pipette tips. They were then asked to keep the containers upright and not to tilt the containers. The pipette tips were then angled from side to side by the participants to make sure that the tips were set at the correct position of the participant's nostrils. Then the participants inhaled moderately one time with the pipette tips angled and placed directly under the nose so that both edges of the tubes were gently grazing the outer rim of their nostrils. A brief intermission for two to three seconds was then taken, and then the inhalation orthonasal procedure was repeated. The inhalation orthonasal procedure could be repeated up to five times for each container. The participants continued this procedure until the odor was committed to memory. The pipette tips were then recapped and the participants were then asked to evaluate the next sample in the same fashion. The participants were advised that once a container was recapped that they would not be allowed to reevaluate the sample again. The objective was to commit the odorant to memory, and then circle the sample that was most different.

3.6.4 Participant's Retronasal Ballot Instructions

The participants were presented with three, 3-digit coded odorant containers. Two of the samples were the same and one was different. The participants first secured the nose clip to their nostril. Then they picked up one container and remove the cap from the plastic pipette tip. They were then asked to keep the containers upright and not to tilt the containers. The participant's lips were then placed around the straw and they inhaled moderately one time. The straw and the container were then removed from their mouth area. Then the nose clip was removed and the participants exhaled through their nose while keeping their mouth closed. A brief intermission for two to three seconds was then taken, and then the inhalation/ exhalation retronasal procedure was repeated. The inhalation/ exhalation retronasal procedure could be repeated up to five times for each container. The participants continued this procedure until the odor was committed to memory. The pipette tip was then recapped and the participants were then asked to evaluate the next sample in the same fashion. The participants were advised that once a pipette tip was recapped that they would not be allowed to reevaluate the sample again. The objective was to commit the odorant to memory, and then circle the sample that was most different.

3.6.5 Procedure

Over the course of seven days, samples were prepared in the morning and six participants were tested during the afternoon during December 2008. Two participants were tested at a time in a temperature controlled room (21.5˚C) with fluorescent lighting. The participants received paper ballots that contained sample evaluation instructions. See section 3.8.1 or Figure A.2 for orthonasal instructional ballot and section 3.8.2 or Figure A.3 for retronasal instructional ballot. Once all odorant discrimination tests were completed, the participants were compensated monetarily

and their correct responses were reported to them. See Table A.2 for orthonasal responses and Table A.3 for retronasal responses.

3.7 Results

Standard inferential statistics methods were applied to the data to determine if there were significant differences between the fatty acids and the controls, between orthonasal and retronasal discriminations, and between the male and female participants. See Figure 3.3 for the correct and incorrect orthonasal responses for the fatty acid discrimination test. See Figure 3.4 for the correct and incorrect retronasal responses for the fatty acid discrimination test. Based on the binomial distribution table at a probability level of 0.05, 15 correct responses out of a total of 30 responses is minimum number needed to establish a difference (Roessler and others 1978).

3.7.1 Orthonasal Results

3.7.1.1 Linoleic Acid

The results showed that out of a total of 30 responses, 26 were correct. According to the one-tailed binomial test at an alpha-risk of 0.05, the critical z-score is 1.65. Since the calculated z-score was 6.003 and it is higher than 1.65 there is enough evidence to conclude that there was a difference. The null hypothesis that probability picking the odd sample was due by chance is rejected; therefore, a difference exists.

3.7.1.2 Oleic Acid

The results showed that out of a total of 30 responses, 25 were correct. According to the one-tailed binomial test at an alpha-risk of 0.05, the critical z-score is 1.65. Since the calculated z-score was 5.616 and it is higher than 1.65 there is enough evidence to conclude that there was a difference. The null hypothesis that probability picking the odd sample was due by chance is rejected; therefore, a difference exists.

3.7.1.3 Stearic Acid

The results showed that out of a total of 30 responses, 25 were correct. According to the one-tailed binomial test at an alpha-risk of 0.05, the critical z-score is 1.65. Since the calculated z-score was 5.616 and it is higher than 1.65 there is enough evidence to conclude that there was a difference. The null hypothesis that probability picking the odd sample was due by chance is rejected; therefore, a difference exists.

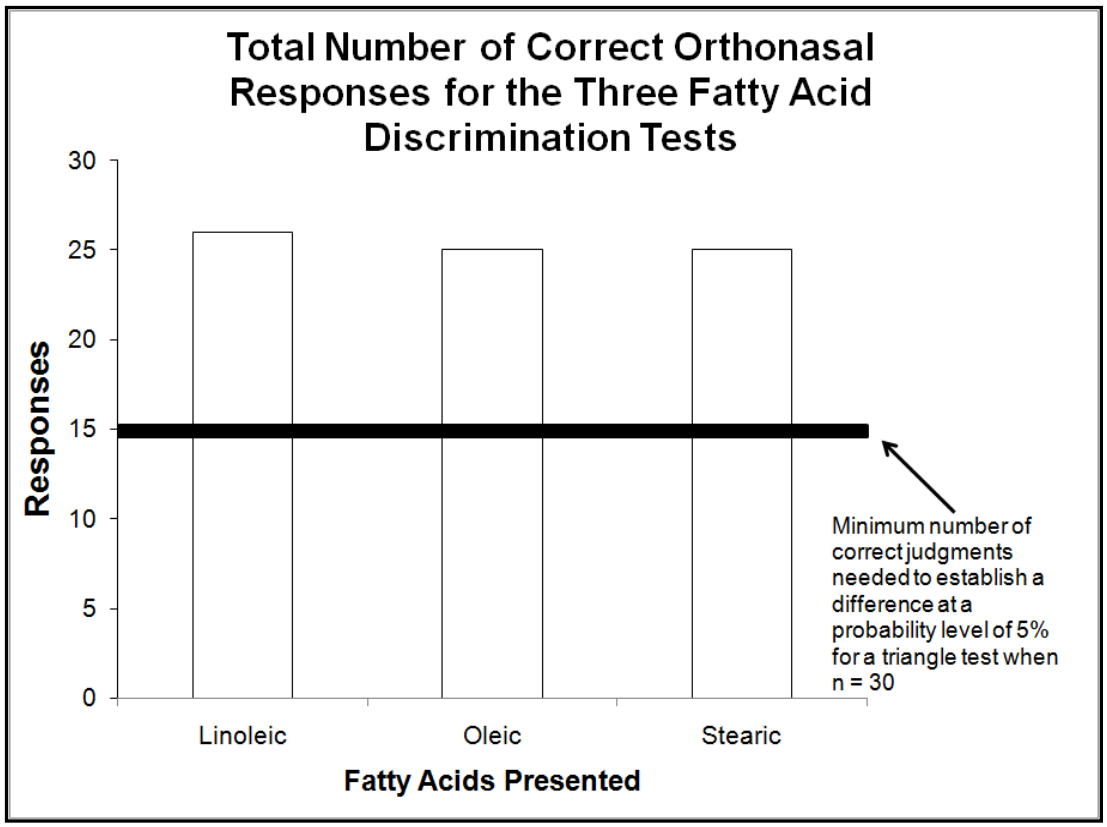

Figure 3.3: This figure shows a bar graph that shows the total correct responses for the orthonasal fatty acid triangle discrimination tests. With regard to the x-axis, linoleic represents linoleic acid vs. mineral oil; oleic represents oleic acid vs. mineral oil; and stearic represents stearic acid vs. mineral oil. The y-axis represents the total number of correct responses for each test. The horizontal black bar represents the minimum number of correct judgments needed to establish a difference at probability level of 5% for a triangle test when n = 30.

3.7.2 Retronasal Results

3.7.2.1 Linoleic Acid

The results showed that out of a total of 30 responses, 28 were correct. According to the one-tailed binomial test at an alpha-risk of 0.05, the critical z-score is 1.65. Since the calculated z-score was 6.778 and it is higher than 1.65 there is enough evidence to conclude that there was a difference. The null hypothesis that probability picking the odd sample was due by chance is rejected; therefore, a difference exists.

3.7.2.2 Oleic Acid

The results showed that out of a total of 30 responses, 17 were correct. According to the one-tailed binomial test at an alpha-risk of 0.05, the critical z-score is 1.65. Since the calculated z-score was 2.517 and it is higher than 1.65 there is enough evidence to conclude that there was a difference. The null hypothesis that probability picking the odd sample was due by chance is rejected; therefore, a difference exists.

3.7.2.3 Stearic Acid

The results showed that out of a total of 30 responses, 25 were correct. According to the one-tailed binomial test at an alpha-risk of 0.05, the critical z-score is 1.65. Since the calculated z-score was 5.616 and it is higher than 1.65 there is enough evidence to conclude that there was a difference. The null hypothesis that probability picking the odd sample was due by chance is rejected; therefore, a difference exists.

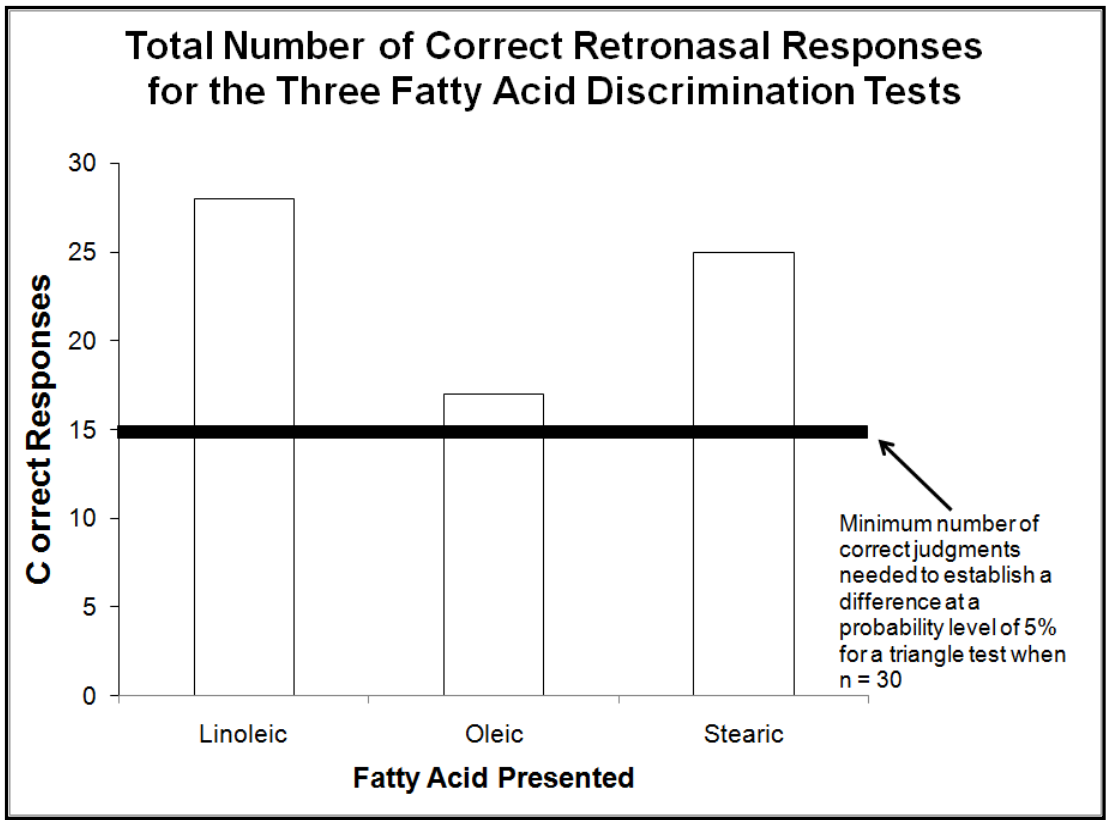

Figure 3.4: This figure shows a bar graph that shows the total correct responses for the retronasal fatty acid triangle discrimination tests. With regard to the x-axis, linoleic represents linoleic acid vs. mineral oil; oleic represents oleic acid vs. mineral oil; and stearic represents stearic acid vs. mineral oil. The y-axis represents the total number of correct responses for each test. The horizontal black bar represents the minimum number of correct judgments needed to establish a difference at probability level of 5% for a triangle test when n = 30.

3.7.3 Independence Test

When testing for independence using the Likelihood-ratio for correct responses vs. gender separately for each type of fatty acid test, we can reject the assumption for independence for the oleic acid test ($p = 0.0267$) but we failed to reject the null hypothesis for the linoleic ($p = 0.5982$) and the stearic acid tests ($p = 0.815$). Therefore, male participants are more likely to respond incorrectly when presented with an oleic acid discrimination test orthonasally and retronasally. See Figure 3.5 for the contingency analysis further details.

When testing for independence using the Likelihood-ratio for correct responses vs. each orthonasal and retronasal fatty acid test, we can reject the assumption for independence for the oleic acid test ($p = 0.0224$) but we failed to reject the null hypothesis for the linoleic ($p = 0.385$) or the stearic acid tests ($p = 1.000$). Therefore, participants are more likely to respond incorrectly when performing an oleic acid discrimination test retronasally. See Figure 3.6 for the contingency analysis for further details.

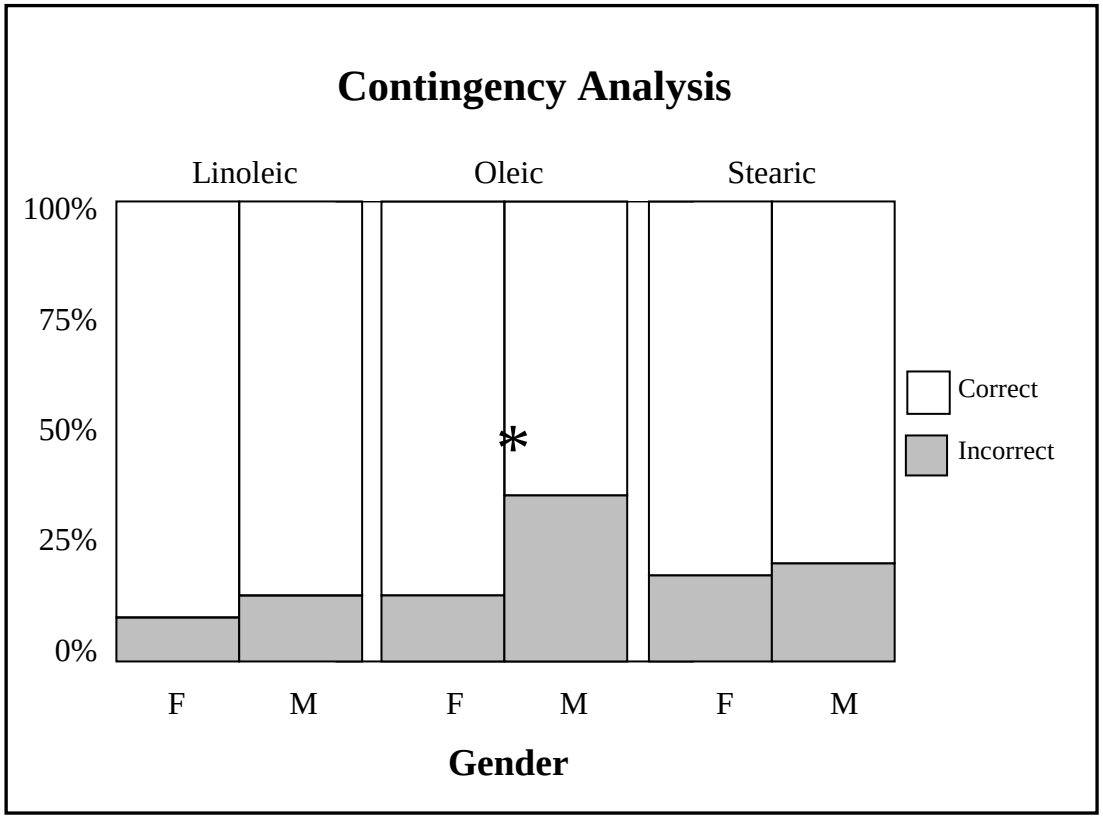

Figure 3.5: This figure shows a contingency analysis for the total percentage of correct and incorrect responses for orthonasal and retronasal presentations - based on gender. With regard to Linoleic, Oleic, and Stearic, represents the three fatty acid discrimination test (linoleic acid vs. solvent, oleic acid vs. solvent, and stearic acid vs. NaCl) respectively. With regard to gender on the x-axis, an F represents Female and an M represents Male. The percentage values on the y-axis, represents the total response percentage. An "*" symbol represents significance.

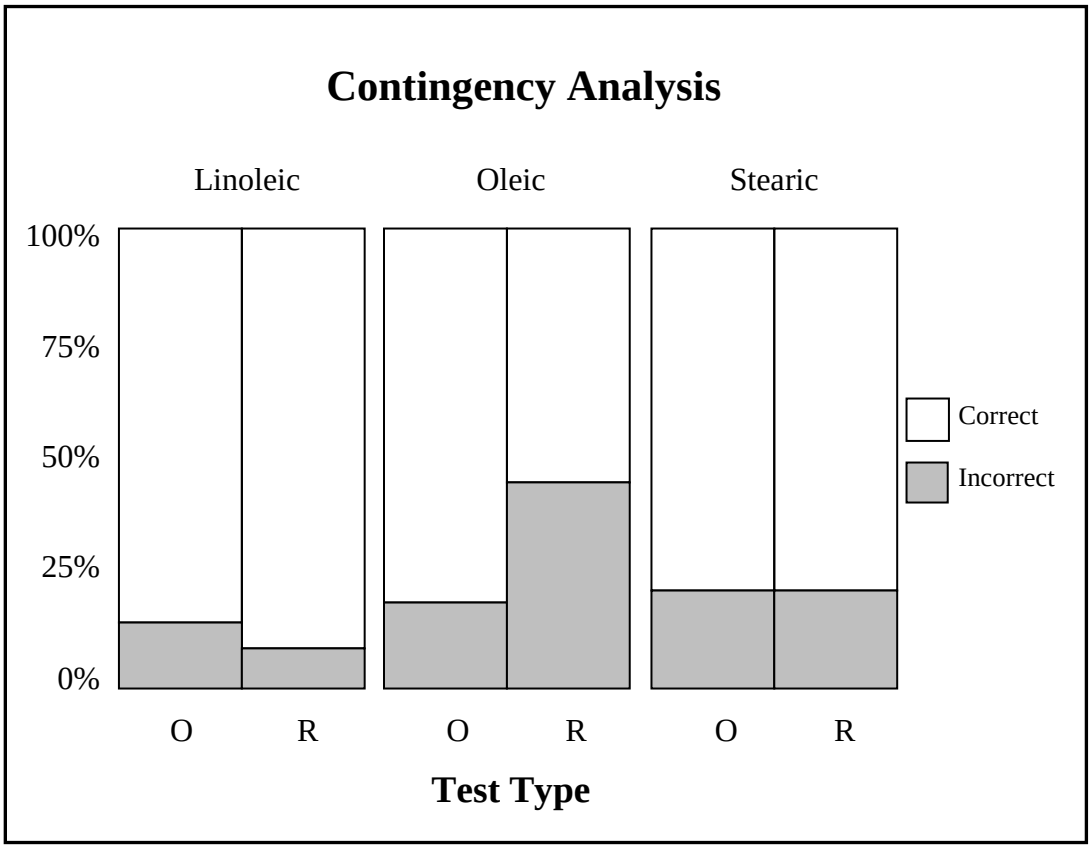

Figure 3.6: This figure shows a contingency analysis for the total percentage of correct and incorrect responses for orthonasal and retronasal presentations - based on the type of test. With regard to Linoleic, Oleic, and Stearic, represents the three fatty acid discrimination test (linoleic acid vs. solvent, oleic acid vs. solvent, and stearic acid vs. NaCl) respectively. With regard to the type of test on the x-axis, an O represents Orthonasal Presentation and an R represents Retronasal Presentation. The percentage values on the y-axis, represents the total response percentage. An "*" symbol represents significance.

3.8 Discussion

See Chapter 5 for General Discussion.

3.9 Conclusion

See Chapter 6 for Conclusion.

CHAPTER 4

ORAL CAVITY ONLY (OCO)

4.1 Introduction

The results from Chapter 3 indicate that linoleic, oleic, and stearic acid can be discriminated from an odorless sample when presented orthonasally and retronasally. These results raise the question if these fatty acids are olfactory only stimuli or if they can stimulate the trigeminal system as well. To test if these fatty acids can stimulate a component of the trigeminal system, an OCO discrimination test was performed. If the results are significant it would suggest that the perception of these fatty acids could depend upon a combination of the olfactory and the trigeminal systems. Because there are separate trigeminal components in the nasal cavities and the oral cavity, it's possible that these fatty acids could stimulate the nasal cavity trigeminal system but not the oral cavity trigeminal system, and not be olfactory stimuli at all.

4.2 Objective

This study was performed to determine if these fatty acids could be discriminated by OCO when presented against an odorless sample.

4.3 Hypothesis

The research hypothesis is that vapor-phase fatty acids (linoleic, stearic and oleic) would be discriminated from an odorless liquid when presented OCO. The probability of picking the correct sample by chance is 1/3. Thus the H_o, $p = 1/3$, $q = 2/3$; H_a, $p > 1/3$ and it's a one-tailed test.

4.4 Participants

The participants were 30 paid volunteers (14 Males and 16 Females) with an age range of 20 to 42, and a mean age of 26, and a standard deviation of 4. The participants were affiliated with Cornell University and were recruited using flyers that were posted around Cornell University's campus. Nine out of the total 30

participants were from Chapter 2. See Chapter 2 for further participant details. The use of human participants at this time was with the approval of Cornell University's Institutional Review Board for Human Participants. For the purposes of this study OCO perception was described as inhaling and exhaling through the mouth while keeping the nose clip secured onto the nose (Chen and Halpern 2008).

4.5 Materials

Please see Chapter 3 for Materials.

4.6 Methods

The ODC's were used as the OCO presentation containers. Please see Chapter 3 for further details. The sample preparation was the same as Chapter 3 but the experimental design was from Chapter 2.

4.6.1 Participant's OCO Ballot Instructions

The participants were presented with three, 3-digit coded odorant containers. Two of the samples were the same and one was different. The participants first secured the nose clip to their nose. Then they picked up one container and remove the cap from the plastic pipette tip. They were then asked to keep the containers upright and not to tilt the containers. The participant's lips were then placed around the straw and they inhaled moderately one time. The straw and the container were then removed from their mouth area. While keeping the nose clip on the participants then exhaled through their mouth. A brief intermission for two to three seconds was then taken, and then the inhalation/ exhalation OCO procedure was repeated. The inhalation/ exhalation OCO procedure could be repeated up to five times for each container. The participants continued this procedure until the odor was committed to memory. The pipette tip was then recapped and the participants were then asked to evaluate the next sample in the same fashion. The participants were advised that once a pipette tip was recapped that they would not be allowed to reevaluate the

sample again. The objective was to commit the odorant to memory, and then circle the sample that was most different.

4.6.2 Procedure

Over the course of three days, the samples were prepared in the morning and eight to 12 participants were tested during the afternoon during March 2009. Two to four participants were tested at a time in a temperature controlled room (21.5˚C) with fluorescent lighting. The participants received paper ballots that contained sample evaluation instructions. See section 4.6.1.1 or Figure A.4 for the OCO instructional ballot. Once all odorant discrimination tests were completed by the participants their correct responses were reported to them. See Table A.3 for the OCO responses.

4.7 Results

Standard inferential statistics methods were applied to the data to determine if there were significant differences between the fatty acids and the controls, between OCO discrimination, and between the male and female participants. Please see Figure 4.1 for the correct and incorrect OCO responses for fatty acid discrimination test. Based on the binomial distribution table at a probability level of 0.05, 15 correct responses out of a total of 30 responses is minimum number to establish a difference (Roessler and others 1978).

4.7.1 OCO

4.7.1.1 Linoleic Acid

The results showed that out of a total of 30 responses, nine were correct. According to the one-tailed binomial test at an alpha-risk of 0.05, the critical z-score is 1.65. Since the calculated z-score was -0.5809 and it is lower than 1.65 there is not enough evidence to conclude that there was a difference. We fail to reject the null

hypothesis that the probability picking the odd sample was due by chance; therefore, there is not enough evidence to suggest that a difference exists.

4.7.1.2 Oleic Acid

The results showed that out of a total of 30 responses, 14 were correct. According to the one-tailed binomial test at an alpha-risk of 0.05, the critical z-score is 1.65. Since the calculated z-score was 1.356 and it is lower than 1.65 there is not enough evidence to conclude that there was a difference. We fail to reject the null hypothesis that the probability picking the odd sample was due by chance; therefore, there is not enough evidence to suggest that a difference exists.

4.7.1.3 Stearic Acid

The results showed that out of a total of 30 responses, 14 were correct. According to the one-tailed binomial test at an alpha-risk of 0.05, the critical z-score is 1.65. Since the calculated z-score was 1.3556 and it is lower than 1.65 there is not enough evidence to conclude that there was a difference. We fail to reject the null hypothesis that the probability picking the odd sample was due by chance; therefore, there is not enough evidence to suggest that a difference exists.

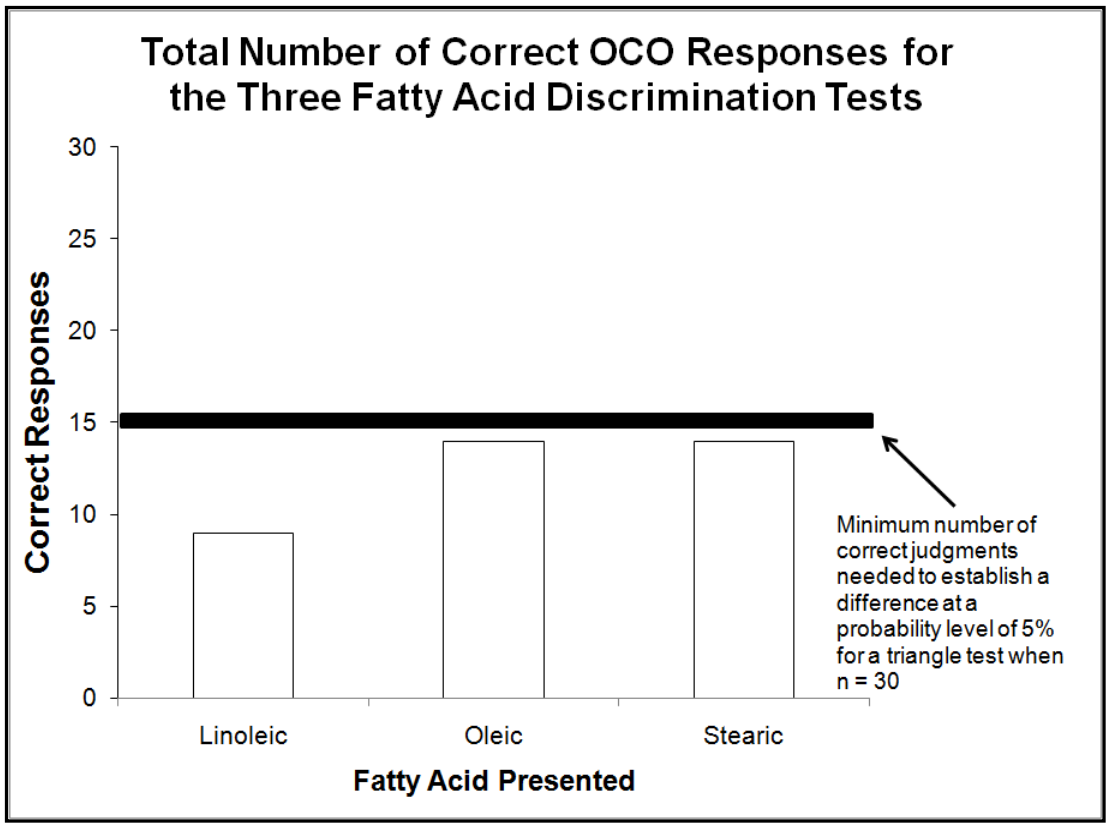

Figure 4.1: This figure shows a bar graph that shows the total correct responses for the OCO fatty acid triangle discrimination tests. With regard to the x-axis, linoleic represents linoleic acid vs. mineral oil; oleic represents oleic acid vs. mineral oil; and stearic represents stearic acid vs. mineral oil. The y-axis represents the total number of correct responses for each test. The horizontal black bar represents the minimum number of correct judgments needed to establish a difference at probability level of 5% for a triangle test when n = 30.

4.7.2 Independence Test

When testing for independence using the Likelihood-ratio for correct responses vs. gender separately for each type of fatty acid test, we fail to reject the assumption for independence for the linoleic acid test ($p = 0.5230$), the oleic acid test ($p = 0.7321$) and the stearic acid test ($p = 0.7321$). Therefore, the gender of a participant cannot be used to predict correct or incorrect responses. See Figure 4.2 for the contingency analysis for further details.

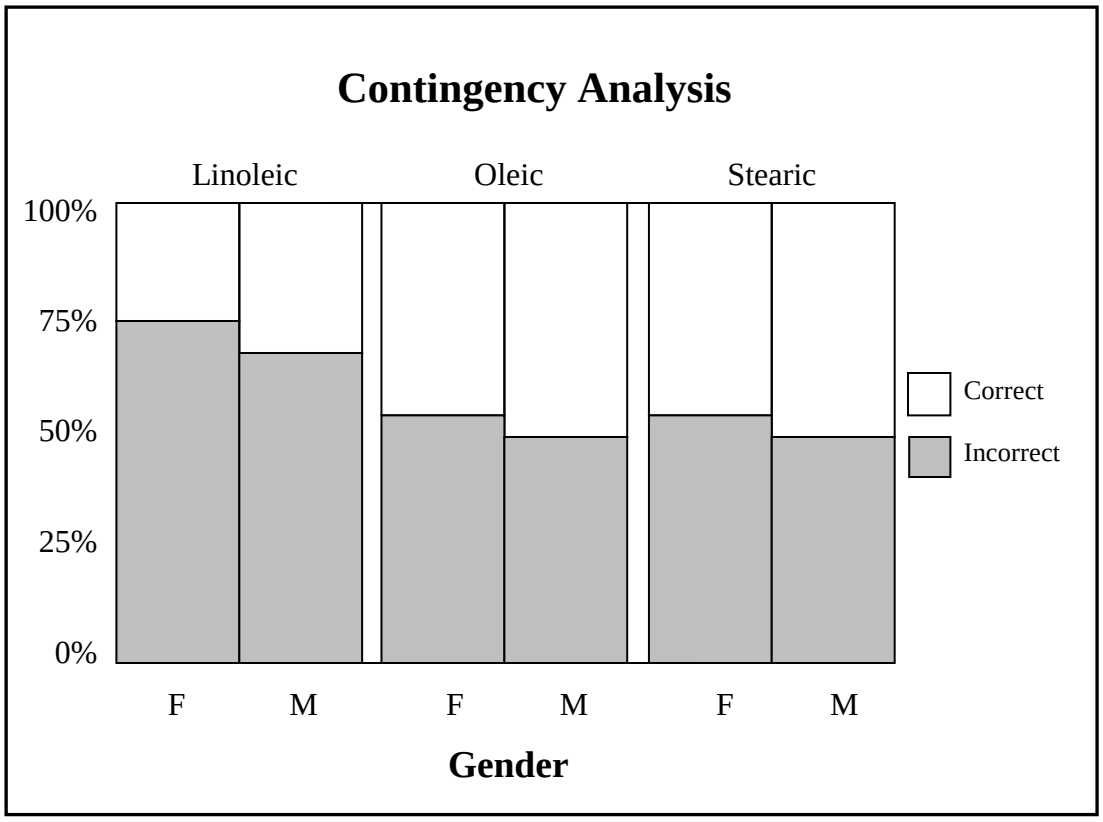

Figure 4.2: This figure shows a contingency analysis for the total percentage of correct and incorrect responses for the OCO presentation which was based on gender. With regard to Linoleic, Oleic, and Stearic, represents the three fatty acid discrimination test (linoleic acid vs. solvent, oleic acid vs. solvent, and stearic acid vs. NaCl) respectively. With regard to gender on the x-axis, an F represents Female and an M represents Male. The percentage values on the y-axis, represents the total response percentage. An "*" symbol represents significance.

4.8 Discussion

See Chapter 5 for General Discussion.

4.9 Conclusion

See Chapter 6 for Conclusion.

CHAPTER 5

GENERAL DISCUSSION

5.1 Evidence

These data indicate that the fatty acids (linoleic, oleic, and stearic) are detectable by the olfactory and/ or the nasal cavity trigeminal system. The OCO linoleic acid response data indicate that the oral cavity trigeminal system does not contribute to retronasal response for linoleic acid. Whether oleic acid or stearic acid can stimulate the oral cavity trigeminal system is inconclusive. Based on a probability level of 0.05, a total of 14 oleic and stearic acid OCO correct responses are not significant.

However, if 15 correct responses were found for oleic or stearic acids, it would reach significance (Roessler and others 1978). With a d-prime of 1.28 for oleic and stearic acid presentations (14/30 correct response), a 3-alternative-forced-choice discrimination test would have rendered significant results (Bi 2006). This phenomenon is also known as the paradox of nondiscriminating discriminators (Byer and Abrams 1953). A larger number of participants for the triangle test might have yielded different results.

Previous data indicated that these fatty acids are detectable if multiple sensory systems, i.e., taste, smell, and tactile systems, were exposed to the fatty acid mixtures (Chalè-Rush, Burgess and Mattes 2007b). Chalè-Rush and others (2007b) report that the lateralization threshold for linoleic acid (nasal irritancy in their figure 1A) is as low as the orthonasal threshold; linoleic acid maybe an effective nasal cavity trigeminal stimulus (Chalè-Rush, Burgess and Mattes 2007b).

After testing for independence, the present data indicate that male participants are more likely to respond incorrectly when presented with an oleic acid discrimination test orthonasally and retronasally. These results were not surprising

since women have been known to perform better in smelling tests (Doty and others 1984). Participants are also more likely to respond incorrectly when performing an oleic acid discrimination test retronasally rather than orthonasally. Finally, the gender of a participant cannot be used to predict correct or incorrect OCO responses, but gender does predict the probability of correct retronasal oleic acid detection.

In the present studies, the fatty acid stimuli for linoleic and stearic acid were less complex fatty acid mixtures when compared to the work of Chalè-Rush and others (2007b). See Table 5.1 for a summary of the total correct responses for the nine discrimination tests. These findings suggest that the vapor phase of linoleic acid does not stimulate the trigeminal receptor neurons in the oral cavity.

Table 5.1: Shows a summary of all participants' correct responses out of the total responses for the three fatty acids discrimination studies. All responses are listed in percentages.

	Fatty Acids Presented		
Test Procedure	Linoleic	Oleic	Stearic
Orthonasal	87a	83a	83a
Retronasal	93a	57a,b	83a
Oral Cavity Only	30b	47b	47b

Within each column, the test procedure's total correct responses that were not significantly different from other test procedures are represented with the same letter after the percentage. If the test procedure correct responses were significantly different from other test procedure's responses they are represented with a different letter after the percentage. Percentages that are followed by two different letters represent a similarity and a difference between different test procedures, e.g., 57a,b means that the numbers of correct responses across participants for orthonasal and retronasal oleic acid did not differ significantly, and that the numbers of correct responses across participants for retronasal versus oral cavity only oleic acid did not differ significantly. Significance was calculated according to the chi-square statistic with one degree of freedom at a probability level of 0.05 under the null hypothesis that the critical chi-square (3.84) is less than or equal to the calculated chi-square.

5.2 Limitations and Constraints

It is impossible to determine from the present data if these fatty acids can stimulate the trigeminal receptor neurons that are located in the nasal cavity. Through orthonasal lateralization testing methods, one could determine if oleic acid and stearic acid are an olfactory only stimuli or if they can activate both the olfactory and nasal cavity trigeminal systems (Brand, Millot and Henquell 2001). Chalè-Rush and others (2007b) presented their stearic acid containing mixture at 67-69°C, which produced a vapor pressure much higher than that of the present study, which was presented at 21°C.

The present data not only suggest that fatty acids can be sensed by the human olfactory system and/ or the nasal trigeminal system, but that the concentrations used were at a detectable level. Future studies OCO using a higher concentration for linoleic and oleic acids are possible, but since stearic acid was presented at such a high purity (95% w/w) in the present study; further studies might render the same results. Whether the concentrations were below threshold for OCO presentations is unable to be determined from the results. The delivery containers that were used in these studies for retronasal and OCO have been used in Stephenson and Halpern (2009), Parikh and others (2009), Chen and Halpern (2008), and Tamburrino and Halpern (2007) (Chen and Halpern (2008), with minor modifications.

5.3 Applicability to Food Science

Consumers are becoming more knowledgeable about the fats that they are consuming. Fats have been given a negative connotation due to their over consumption in today's society. Since fats are responsible for many of the dietary decisions that consumers make every day; the study of their sensory properties would be of importance. It is suggested that the sensation of fat in foods relies on the combination of taste, smell and texture (Drewnowski 1997, Chalè-Rush, Burgess and

Mattes 2007b, Mattes 2009a). The sensation of fat comes from the aroma of fat-soluble flavor molecules and the texture of foods in the oral cavity during the mastication process (cited after: Drewnowski 1997).

The concentrations of linoleic, oleic, and stearic acid in foods varies from product to product. These fatty acids are usually attached to a glycerol molecule with two other fatty acids to form a triglyceride. However, free fatty acids are still present (in low amounts) in foods. The present findings have indicated that these fatty acids can be smelled (orthonasal and retronasal); however, in foods these fatty acids odorant potential might be suppressed by other compounds that are more volatile and are present in the foods.

5.4 Importance of Research

The detection of dietary fats is a combination of the trigeminal system, the olfactory system, post-ingestive cues (Laugerette and others 2007) and the gustatory system (Mattes 2009b). Further sensory research in these areas will allow for a better understanding of how humans perceive such a necessary nutrient that participates in many metabolic processes (Rebouche and Yao 2008) and serves as a source of energy for the human body. The discovery of new and improved fat replacement products brings with it a desire to have a product that has the same sensory properties of the fat but with fewer calories.

CHAPTER 6

CONCLUSION

The current data have demonstrated that humans can smell fatty acids orthonasally and retronasally. In agreement with Chalè-Rush and others (2007b), these fatty acids (linoleic, oleic, and stearic) are effective stimuli for nasal cavity smelling. The data have also demonstrated that linoleic acid cannot be smelled in the oral cavity, thus eliminating the OCO trigeminal array as a source of the smelling. It is inconclusive if oleic acid and stearic acid are oral cavity trigeminal stimulants. One previous study was unable to demonstrate that linoleic, oleic, or stearic acids could be identified either orthonasally or retronasally (Tamburrino and Halpern 2007). However, further studies should be conducted to determine if these fatty acids are perceptually different when presented against themselves, i.e. (linoleic vs. oleic; linoleic vs. stearic; oleic vs. stearic). In addition, threshold, and descriptive studies using the olfactory, trigeminal, or the gustatory system for that matter would not only determine what concentration can be perceived, but what attributes could be used to describe these fatty acids.

The present data, which avoided the confounding issue of complex, multicomponent stimulus mixtures, confirm the previous report of retronasal smelling of linoleic, oleic, and stearic fatty acids (Chalè-Rush, Burgess and Mattes 2007b). This resolves a long-standing dispute (see Mattes 2009) concerning the ability of humans to smell these fatty acids.

At the same time, the observed absence of discrimination between linoleic acid and mineral oil when the odorants were restricted to the oral cavity (the OCO condition) indicates that the nasal cavity should be the focus of future studies regarding the smelling of linoleic acid. Another OCO discrimination study for oleic and stearic acid with a greater sample size should be pursued before these two fatty

acids are determined not to be OCO trigeminal stimulants. However, to what degree there are differences in retronasal versus orthonasal smelling of these fatty acids remains unresolved. The present finding that participants were less able to discriminate oleic acid when it was smelled retronasally is in accordance with prior observations that retronasal thresholds for odorants are generally higher than orthonasal thresholds (Halpern 2008). Future studies with lower concentrations of fatty acids may extend this generalization to linoleic and stearic fatty acids. This will be important to resolve because smelling of fatty acids released from foods would normally employ a retronasal route.

APPENDIX

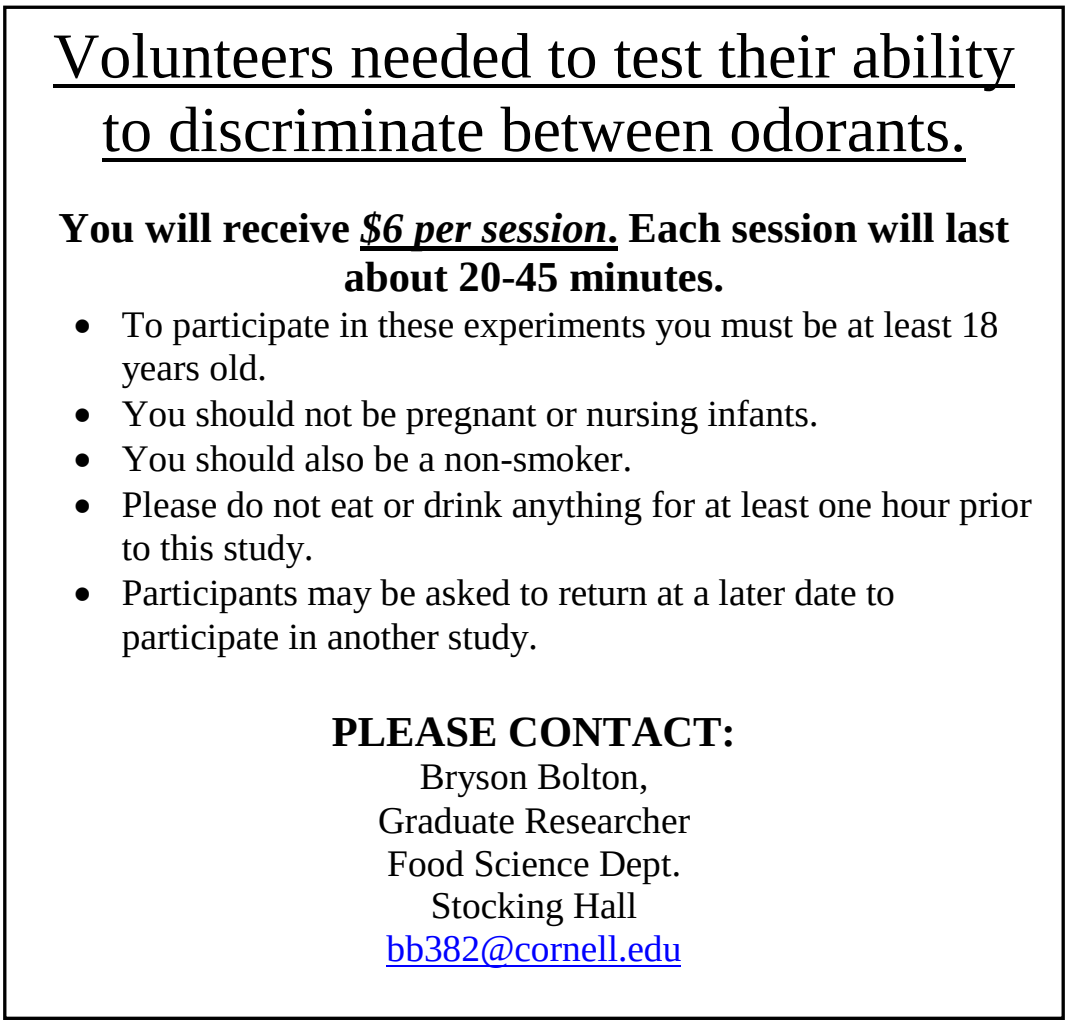

Figure A.1: Shows the participant recruitment poster used to recruit participants for multiple studies.

Table A.1: Summary of the responses from the three orthonasal food extracts discrimination tests.

Participant Number	Gender	Orange	Peppermint	Strawberry
1	F	1	1	1
2	M	1	1	1
3	M	1	1	1
4	M	1	1	1
5	F	1	1	1
6	F	1	1	1
7	F	1	1	1
8	M	1	1	1
9	F	0	1	1
10	F	1	1	1
11	F	1	1	1
12	F	1	1	1
13	F	1	1	1
14	F	1	1	1
15	F	1	1	1
16	M	1	1	1
17	M	1	1	0
18	M	1	1	1
19	M	1	1	1
20	M	1	1	1
21	M	1	1	1
22	M	1	1	1
23	F	1	1	1
24	F	1	1	1
25	F	1	1	1
26	M	1	1	1
27	M	1	1	1
28	F	1	1	1
29	M	1	1	1
30	F	1	1	1
Total Correct Responses		29	30	29

With regard to Gender, an M represents Male, and an F represents Female. In regards to the columns, a 1 indicates a correct response, and a 0 represents an incorrect response.

Table A.2: Summary of the responses from the three orthonasal fatty acid triangle discrimination tests.

Participant Number	Gender	Age	OCO: Linoleic	OCO: Oleic	OCO: Stearic
1	F	23	1	1	1
2	M	29	1	1	1
3	M	28	1	1	1
4	M	30	1	0	0
5	M	28	1	1	1
6	F	25	1	1	1
7	M	23	1	1	1
8	F	28	1	1	1
9	F	28	1	1	1
10	M	23	0	1	0
11	M	23	0	0	1
12	F	21	1	1	1
13	M	21	1	1	1
14	F	21	1	1	1
15	M	19	1	1	1
16	M	19	1	1	1
17	M	28	1	1	1
18	F	29	1	1	1
19	M	20	0	1	0
20	M	28	1	1	1
21	F	58	1	1	1
22	F	60	1	1	0
23	M	25	1	0	1
24	M	23	1	0	1
25	F	19	1	1	1
26	F	24	1	0	1
27	M	22	1	1	1
28	F	21	1	1	0
29	F	28	1	1	1
30	M	25	0	1	1
Total Correct Responses			9	14	14

With regard to Gender, an M represents Male, and an F represents Female. In regards to the columns, a 1 indicates a correct response, and a 0 represents an incorrect response.

Table A.3: Summary of the responses from the three retronasal fatty acid triangle discrimination tests.

Participant Number	Gender	Age	OCO: Linoleic	OCO: Oleic	OCO: Stearic
1	F	23	1	1	1
2	M	29	1	0	0
3	M	28	1	0	1
4	M	30	1	0	1
5	M	28	1	1	1
6	F	25	0	1	0
7	M	23	1	1	1
8	F	28	1	1	1
9	F	28	1	0	1
10	M	23	1	0	1
11	M	23	1	0	1
12	F	21	1	1	0
13	M	21	1	0	1
14	F	21	1	1	1
15	M	19	1	1	1
16	M	19	1	0	1
17	M	28	1	0	0
18	F	29	1	1	1
19	M	20	1	0	1
20	M	28	1	1	1
21	F	58	1	1	1
22	F	60	0	0	1
23	M	25	1	0	0
24	M	23	1	1	1
25	F	19	1	1	1
26	F	24	1	1	1
27	M	22	1	1	1
28	F	21	1	0	1
29	F	28	1	1	1
30	M	25	1	1	1
Total Correct Responses			9	14	14

With regard to Gender, an M represents Male, and an F represents Female. In regards to the columns, a 1 indicates a correct response, and a 0 represents an incorrect response.

Table A.4: Summary of the responses from the three oral cavity only fatty acid triangle discrimination tests.

Participant Number	Gender	Age	OCO: Linoleic	OCO: Oleic	OCO: Stearic
1*	M	28	0	1	0
2*	F	28	1	0	0
3*	M	23	1	0	0
4	F	30	1	0	1
5	F	26	0	0	1
6	M	24	0	0	0
7	F	28	0	1	1
8*	M	25	0	1	1
9	F	28	0	1	1
10	M	24	0	0	0
11*	F	28	0	0	0
12	M	21	0	0	1
13	M	42	0	1	0
14	F	26	1	1	0
15	F	27	0	1	0
16	F	26	0	0	0
17	M	23	1	0	0
18*	M	23	0	0	1
19*	F	25	0	0	1
20	F	25	0	1	0
21	M	26	1	1	0
22	F	25	0	0	1
23	F	24	0	0	0
24	M	32	0	1	1
25*	M	28	1	1	1
26*	M	29	1	0	1
27	F	20	0	1	1
28	M	28	0	1	1
29	F	25	0	0	0
30	F	21	1	1	0
Total Correct Responses			9	14	14

With regard to Gender, an M represents Male, and an F represents Female. In regards to the columns, a 1 indicates a correct response, and a 0 represents an incorrect response. An * represents a participant that participated in the orthonasal and retronasal fatty acid discrimination studies.

Panelist Number:_____ Date:_____

Name:_____ Age:_____ Gender:_____

In front of you there are three coded samples. Write the 3 digit number that is on the container on the three lines below from left to right in the order in which the containers are presented. Once completed, **READ THE DIRECTIONS BELOW CAREFULLY BEFORE STARTING THE TEST.**

Two of these samples are the same and one is different. Please pick up one container and remove the two caps from the two plastic tubes. Try to keep the containers upright. **DO NOT TILT THE CONTAINERS.** Then angle the tubes from side to side to make sure that the tubes are angled to the correct position of your nostrils. Inhale moderately **ONE TIME** with the tubes angled and placed directly under the nose so that both edges of the tubes are gently grazing the outer rim of your nostrils. Take a brief intermission for two to three seconds, and then repeat the inhalation procedure through the plastic tubes. **BUT DO NOT REPEAT THE ORTHONASAL INHALING PROCEDURE MORE THAN FIVE TIMES FOR EACH CONTAINER.** Continue this procedure until the odor of the container is committed to memory. Recap both tubes and evaluate the next sample. Once an odorant inhaling procedure is complete, recap both tubes and go on to the next sample. Once a container is recapped you will not be allowed to go back to evaluate the sample again; so try to commit the odorant to memory because you are trying to pick the odd sample. Once you have evaluated all three samples, circle the number of the sample that is most different. Please feel free to also answer the question below.

_____ _____ _____

Optional: What was it about that the sample that you chose that made it seemed to be different? _____
_____
_____

Figure A.2: Shows the instructions and ballot used for orthonasal triangle discrimination tests.

Panelist Number:_____ Date:_____

Name:_____ Age:_____ Gender:_____

In front of you there are three coded samples. Write the 3 digit number that is on the container on the three lines below from left to right in the order in which the containers are presented. Once completed, **READ THE DIRECTIONS BELOW CAREFULLY BEFORE STARTING THE TEST.**

Two of these samples are the same and one is different. First, secure the nose clip on your nostrils. Secondly, pick up the container and remove the cap from the shorter plastic tube. Try to keep the containers upright. **DO NOT TILT THE CONTAINERS.** Place your lips around the red and white straw and inhale moderately **ONE TIME.** Remove the straw from your mouth, close your mouth, move the container away from your mouth, then remove your nose clip and exhale while keeping your mouth closed. Take a brief intermission for two to three seconds, and then repeat the retronasal inhalation exhalation procedure. Continue until the odor of one container is committed to memory. **BUT DO NOT REPEAT THE RETRONASAL INHALING AND EXHALING PROCEDURE MORE THAN FIVE TIMES FOR EACH CONTAINER.** Once an odorant retronasal inhaling procedure is complete, recap the tube and evaluate the next sample. Once a container is recapped you will not be allowed to reevaluate the sample again; so try to commit the odorant to memory because you are trying to pick the odd sample. Once you have evaluated all three samples, circle the number of the sample that is most different. Please feel free to also answer the question below.

_____ _____ _____

Optional: What was it about that the sample that you chose that made it seemed to be different? _____
_____
_____

Figure A.3: Shows the instructions and ballot used for retronasal triangle discrimination tests.

Panelist Number:_____ Date:_____

Name:_____ Age:_____ Gender:_____

In front of you there are three coded samples. Write the 3 digit number that is on the container on the three lines below from left to right in the order in which the containers are presented. Once completed, **READ THE DIRECTIONS BELOW CAREFULLY BEFORE STARTING THE TEST.**

Two of these samples are the same and one is different. First, secure the nose clip on your nostrils. Second, pick up the container and remove the cap from the shorter plastic tube. Try to keep the containers upright. **DO NOT TILT THE CONTAINERS.** Place your lips around the red/ pink and white straw securely and inhale deeply **ONE TIME.** Remove the straw from your mouth, and exhale moderately out of your mouth with the nose clip still on. Take a brief intermission for two to three seconds, and then repeat the oral cavity inhale, exhale procedure. Continue until the odor/ sensation from the container is committed to memory. Then evaluate the next container. **BUT DO NOT REPEAT THE ORAL CAVITY INHALE, EXHALE PROCEDURE MORE THAN FIVE TIMES FOR EACH CONTAINER.** Once an oral cavity inhale procedure is complete, recap the tube and evaluate the next sample. Once a tube is recapped you will not be allowed to reevaluate the sample again. So try to commit the odor/ sensation to memory because you are trying to pick the odd sample. Once you have evaluated all three samples, circle the number of the sample that is most different. Please feel free to also answer the question below.

PLEASE KEEP THE NOSE CLIP ON DURING THE ENTIRE TEST.

_____ _____ _____

Optional: What was it about that the sample that you chose that made it seemed to be different? _____
_____
_____

Figure A.4: Shows the instructions and ballot used for oral-cavity-only triangle discrimination tests.

REFERENCES

Abumrad NA. 2005. CD36 may determine our desire for dietary fats. Journal of Clinical Investigation 115(11):2965-2967.

Acree T, Arn H. 2004. http://www.flavornet.org/flavornet.html. (cited: May 13, 2009):

Akoh CC. 2008. Fat-based fat substitutes. In: C. K. Chow, editor. Fatty Acids in Foods and Their Health Implications. 3rd ed. ed. Boca Raton: CRC Press. P461-471.

Anonymous. 1975. Minutes of Division Business Meeting. Institute of Food Technologist - Sensory Evaluation Division. IFT, Chicago, IL.

Bernreuther A, Epperlein U, Koppenhoefer B. 1996. Enantiomers: why are they important and how to resolve them. In: R. Marsili, editor. Techniques for Analyzing Food Aroma. CRC Press. P142.

Bi J. 2006. Sensory Discrimination Tests and Measurements: Statistical Principles, Procedures and Tables. Ames, Iowa, USA: Blackwell Publishing. 298 p.

Bisulco S, Slotnick B. 2003. Olfactory discrimination of short chain fatty acids in rats with large bilateral lesions of the olfactory bulbs. Chemical Senses 28(5):361-70.

Brand G, Millot JL, Henquell D. 2001. Complexity of olfactory lateralization processes revealed by functional imaging: a review. Neuroscience & Biobehavioral Reviews 25(2):159-66.

Byer AJ, Abrams D. 1953. A comparison of the triangular and two-sample taste-test methods. Food Technology 7(3):185-187.

Chalè-Rush A, Burgess JR, Mattes RD. 2007a. Evidence for human orosensory (taste?) sensitivity to free fatty acids. Chemical Senses 32(5):423-31.

Chalè-Rush A, Burgess JR, Mattes RD. 2007b. Multiple routes of chemosensitivity to free fatty acids in humans. American Journal of Physiology - Gastrointestinal and Liver Physiology 292(5):G1206-12.

Chapkin RS, Ziboh VA. 1984. Inability of skin enzyme preparations to biosynthesize arachidonic acid from linoleic acid. Biochemical and Biophysical Research Communications 124(3):784-792.

Chen V, Halpern BP. 2008. Retronasal but not oral-cavity-only identification of "purely olfactory" odorants. Chemical Senses 33(2):107-18.

Cometto-Muñiz JE, Cain WS, Abraham MH. 2005. Determinants for nasal trigeminal detection of volatile organic compounds. Chemical Senses 30(8):627-42.

Doty RL, Brugger WE, Jurs PC, Orndorff MA, Snyder PJ, Lowry LD. 1978. Intranasal trigeminal stimulation from odorous volatiles: psychometric responses from anosmic and normal humans. Physiology & Behavior 20175-185.

Doty RL, Shaman P, Applebaum SL, Giberson R, Siksorski L, Rosenberg L. 1984. Smell identification ability: changes with age. Science 226(4681):1441-1443.

Dragich AM, Halpern BP. 2008. An oral-cavity component in retronasal smelling of natural extracts. Physiology & Behavior 93(3):521-8.

Drewnowski A. 1997. Why do we like fat? Journal of the American Dietetic Association 97(7):S58-62.

Fukuwatari T, Kawada T, Tsuruta M, Hiraoka T, Iwanaga T, Sugimoto E, Fushiki T. 1997. Expression of the putative membrane fatty acid transporter (FAT) in taste buds of the circumvallate papillae in rats. FEBS Letters 414(2):461-4.

Fukuwatari T, Shibata K, Iguchi K, Saeki T, Iwata A, Tani K, Sugimoto E, Fushiki T. 2003. Role of gustation in the recognition of oleate and triolein in anosmic rats. Physiology & Behavior 78579-583.

Gilbertson TA. 1998. Gustatory mechanisms for the detection of fat. Current Opinion in Neurobiology 8(4):447-52.

Gilbertson TA, Fontenot DT, Liu L, Zhang H, Monroe WT. 1997. Fatty acid modulation of K+ channels in taste receptor cells: gustatory cues for dietary fat. American Journal of Physiology 272(4):C1203-10.

Grundy SM. 1994. Influence of stearic acid on cholesterol metabolism relative to other long-chain fatty acids. American Journal of Clinical Nutrition 60(Suppl):986S-90S.

Halpern BP. 2008. Retronasal olfaction. In: L. R. Squire, Editor-in-Chief. Sensory Systems. Chemical Senses. Encyclopedia of Neuroscience. Amsterdam: Elsevier Online. P297-304.

Halpern BP. 2004. Retronasal and orthonasal smelling. ChemoSense 6(3):1-7.

Ishikawa S, Nakayama T, Watanabe M, Matsuzawa T. 2006. Visualization of flow resistance in physiological nasal respiration: analysis of velocity and vorticities using numerical simulation. Archives of Otolaryngology - Head & Neck Surgery 132(11):1203-9.

Kinney NE, Antill RW. 1996. Role of olfaction in the formation of preference for high-fat foods in mice. Physiology & Behavior 59(3):475-8.

Laugerette F, Gaillard D, Passilly-Degrace P, Niot I, Besnard P. 2007. Do we taste fat? Biochimie 89(2):265-9.

Lawless HT, Heymann H. 1998. Sensory evaluation of food: principles and practices. New York: Chapman & Hall. 819 p.

Lobb K, Chow CK. 2008. Fatty Acid Classification and Nomenclature. In: C. K. Chow, editor. Fatty Acids in Foods and Their Health Implications. 3rd ed. Boca Raton: CRC Press. P1-15.

Margolis FL, Roberts N, Ferriero D, Feldman J. 1974. Denervation in the primary olfactory pathway of mice: biochemical and morphological effects. Brain Research 81(3):469-83.

Mattes RD. 2009a. Is there a fatty acid taste? Annual Review of Nutrition. 29 (Volume publication date August 2009) (doi:10.1146/annurev-nutr-080508-141108). Expected final online publication date for the Annual Review of Nutrition Volume 29 is July 17, 2009. Available from http://arjournals.annualreviews.org/doi/pdf/10.1146/annurev-nutr-080508-141108.

Mattes RD. 2009b. Oral detection of short-, medium-, and long-chain free fatty acids in humans. Chemical Senses 34145-150.

Mattes RD. 2005. Fat taste and lipid metabolism in humans. Physiology & Behavior 86(5):691-7.

Mattes RD. 2001. The taste of fat elevates postprandial triacylglycerol. Physiology & Behavior 74(3):343-8.

Meilgaard M, Civille GV, Carr BT. 2007. Sensory Evaluation Techniques. 4th ed. Boca Raton: Taylor & Francis. 448 p.

Mela DJ, Christensen CM. 1987. Sensory assesment of oiliness in a low moisture food. Journal of Sensory Studies 2273-281.

Moncrieff RW. 1967. The Chemical Senses. Cleveland, OH, USA: CRC Press.

Muggli R. 2005. Systemic evening primrose oil improves the biophysical skin parameters of healthy adults. International Journal of Cosmetic Science 27(4):243-9.

Negoias S, Visschers R, Boelrijk A, Hummel T. 2008. New ways to understand aroma perception. Food Chemistry 108(4):1247-1254.

O'Mahony M. 1986. Sensory Evaluation of Food: Statistical Methods and Procedures. New York: M. Dekker. 487 p.

O'Neil MJ. 2006. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 14th ed. Whitehouse Station, NJ., USA: Merck Research Laboratories: Merck & Co., Inc. 1756 p.

Pierce J, Halpern BP. 1996. Orthonasal and retronasal odorant identification based upon vapor phase input from common substances. 21(5):529-543.

Ramirez I. 1993. Role of olfaction in starch and oil preference. American Journal of Physiology 265(6):R1404-9.

Ramirez I. 1992. Chemoreception for fat: do rats sense triglycerides directly? Appetite 18(3):193-206.

Rawson NE. 2000. The Neurobiology of Taste and Smell. 2nd ed. New York: Wiley-Liss. 479 p.

Rebouche CJ, Yao JK. 2008. Fatty Acid Metabolism in Skelatal Muscle and Nerve, and Neuromuscular Disorders. In: C. K. Chow, editor. Fatty Acids in Foods and Their Health Implications. 3rd ed. Boca Raton: CRC Press. P1197.

Reineccius G. 2006. Flavor chemistry and technology. 2nd ed. / Gary Reineccius. ed. Boca Raton : Taylor & Francis/CRC Press, 2006. 489 p.

Rickman E. 2009. Kitchen Chemistry. Oleic Acid. http://chemistryandphysics.astate.edu/draganjac/oleicacid.html.

Roessler EB, Pangborn RM, Sidel JL, Stone H. 1978. Expanded statistical tables for estimating significance in paired—preference, paired–difference, duo–trio and triangle Tests. Journal of Food Science 43(3):940-3.

Ruiz Gutierrez V, Muriana FJ, Guerrero A, Cert AM, Villar J. 1996. Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources. 14(12):1483-90.

Savic-Berglund I. 2004. Imaging of olfaction and gustation. Nutrition Reviews 62(s3):S205-7.

Schiffman SS, Graham BG, Sattely-Miller EA, Warwick ZS. 1998. Orosensory perception of dietary fat. American Psychological Society Current Directions in Psychological Science(5):137-143.

Silver WL, Finger TE. 1991. The Trigeminal System. In: T. V. Getchell, R. L. Doty, L. M. Bartoshuk, J. B. Snow Jr, editors. Smell and Taste in Health and Disease. New York: Raven. P97-108.

Silver WL, Walker JC. 1997. Odors. In: J. J. Lagowski, editor. Macmillan Encyclopedia of Chemistry. New York: Macmillan Publishing Co. P1-8.

Stephenson D, Halpern BP. 2009. No oral-cavity-only discrimination of purely olfactory odorants. Chemical Senses 34(2):121-126.

Stone H, Sidel JL. 2004. Sensory Evaluation Practices. 3rd ed. ed. Amsterdam ; Boston: Elsevier Academic Press. 377 p.

Sun BC, Halpern BP. 2005. Identification of air phase retronasal and orthonasal odorant pairs. Chemical Senses 30693-706.

Tamburrino R, Halpern BP. 2007. Identification of air-phase fatty acids: both retronasal and orthonasal failure. Chemical Senses 32(6):(abstract page A15). http://chemse.oxfordjournals.org.proxy.library.cornell.edu/cgi/reprint/32/6/A1?etoc.

Warwick ZS, Schiffman SS. 1990. Sensory evaluations of fat-sucrose and fat-salt mixtures: relationship to age and weight status. Physiology & Behavior 48(5):633-6.

Weyerstahl P. 1994. Odor and structure. Journal für Praktische Chemie/Chemiker-Zeitung 336(2):95-109.

White PJ. 2008. Fatty acids in oilseeds (vegetable oils). In: C. K. Chow, editor. Fatty Acids in Foods and Their Health Implications. 3rd ed. Boca Raton: CRC Press. P227-262.

Wood JD, Enser M, Richardson RI, Whittington FM. 2008. Fatty acids in meat and meat products. In: C. K. Chow, editor. Fatty Acids in Foods and Their Health Implications. 3rd ed. Boca Raton: CRC Press. P87-107.