# ASSOCIATIONS AMONG FATTY FOOD SENSATIONS, DIET, AND EXPECTORATED EMULSIONS

by

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**A Thesis**

*Submitted to the Faculty of Purdue University*

*In Partial Fulfillment of the Requirements for the degree of*

**Master of Science**


Department of Nutrition Science

West Lafayette, Indiana

August 2021

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*For Mom, Dad, Jeannie, and Wallace*

## ACKNOWLEDGMENTS

Thanks to Dr. Fengqing Maggie Zhu and her lab group at Purdue Video and Image Processing Laboratory (VIPER) for time and resources on the image analysis.

Thanks to all my lab members at SPIT lab for their help to complete the study.

Finally, thank you to Dr. Cordelia Running for her constant patience and guidance.

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# ABSTRACT

Saliva influences chemical and textural sensations, yet details on sources of individual variability for these phenomena are still lacking. In this project, we investigated fatty sensations, dietary habits, and saliva's emulsifying properties. Through a remote tasting and spitting protocol, participants were asked to rate sensory properties of fatty candies with varying concentrations of added linoleic acid (LA) as well as discriminate among fatty candies with/without LA and high/low fat ranch dressings. Additionally, participants swished and expectorated an oil/water mixture, and the expectorated emulsion was visually analyzed. Dietary habits were also assessed by 3-day dietary recalls.

Linear mixed model was used to analyze sensory response, diet, and spit data. Sensory ratings of fatty candies indicate differences based on successful completion of either discrimination tasks. People who passed either discrimination tests (N=26 passed LA; N=22 passed high/low fat tests) rated higher "Fattiness" for the highest LA concentration. In contrast, people who failed the tests (N=36 failed LA; N=40 failed high/low fat tests) rated higher "Bitterness" with the highest LA concentration. Importantly, only 7 individuals overlapped in these two groups who passed the discrimination tasks. Lower total fat intake and larger expectorated fat layer were associated with higher "Bitterness," particularly among those who passed the LA discrimination test and those who failed the high/low fat test. Moreover, lower protein and greater carbohydrate intake seemed to associate with the greater formation and stability of oral emulsions, particularly in individuals who failed the high/low fat discrimination task. Other factors such as total fat intake, medication usage, and BMI were mixed. In conclusion, sensory experience of fatty candies may vary based on the ability of an individual to sense the LA or fat content, and saliva's ability to emulsify fat into water may vary with diet.

# CHAPTER 1. LITERATURE REVIEW

## 1.1 Introduction to Fat Structure, Function and Sensation

Fat has a unique role in the human diet. Dietary fat provides an essential component to maintain normal physiological function in many aspects such as vision, brain development, vital cardiovascular function, and cell growth (Connor et al., 1992; Uauy et al., 2001). On the other hand, overconsumption of fat may lead to negative health outcomes and increase the risk of obesity-related inflammation, diabetes, and cardiovascular diseases (Galgani & García, 2014; Hooper et al., 2015; Imamura et al., 2016; Nettleton et al., 2017). Structures of fatty acids, including degree of saturation, chain length, and position of double bonds, alter physiological outcomes in humans. Overconsumption of trans (TFA) or saturated fatty acid (SFA) is potentially harmful, whereas replacing TFA or SFA with monounsaturated (MUFA) or polyunsaturated fatty acid (PUFA) may reduce obesity and improve cardiovascular health (Ascherio & Willett, 1997; Jakobsen et al., 2009; DiNicolantonio & O'keefe, 2017; Panth et al., 2018). Besides influencing human health, fat also contributes to a variety of oral sensations including aroma, taste, and mouthfeel of the foods (Drewnowski & Almiron-Roig, 2010; Keast & Costanzo, 2015; Relkin et al., 2004; Running & Mattes, 2016; Silva Lannes & Maria, 2013)**.**

Oral perception of fat is a combination of sensations that activate a variety of sensory mechanisms (Mattes, 2009b). Once foods are in the oral cavity, fat stimulates smell (retronasal olfaction), mouthfeel (texture), and taste (gustation) (Mattes, 2005; Schiffman et al., 1998). The chemical and physical structure of fats influence how they behave as tastants, odorants, or texturants, and may influence how fat releases differently from foods upon mastication and saliva interactions (Running et al., 2013; Running & Mattes, 2016; Tucker et al., 2014). Chemical or physical stimuli from fat then interact with specific receptors and trigger a series of sensory signals to the brain (Chandrashekar et al., 2006). The integration of sensory input generates a complex oral experience from foods (Rolls, 2005).

Great variation of fat flavor perception has been observed amongst people (Mattes, 2009a; Running et al., 2013; Stewart et al., 2010; Tucker & Mattes, 2013). The ability of fat to contribute

to multiple aspects of flavor makes it a unique stimulus. Here, we define 'flavor' as a combination of olfactory, tactile, gustatory sensations, which are evoked by foods in the oral cavity (de Roos, 2005). Multifaceted stimulation including texture, aroma, and taste elicited by fat may be perceived and interpreted differently by different people. Consistently, research demonstrates that some people are hypersensitive to fat while others are less sensitive (Asano et al., 2016; Kamphuis et al., 2003; Kindleysides et al., 2017; Mattes, 2009a; Stewart et al., 2010; Tucker & Mattes, 2013). However, which aspects or combinations of fat flavor (taste, aroma, texture) are the primary drivers for individual variability is unclear. Exploring the association behind the sensory experience from fat, and the individual variability in that experience is warranted to better understand human eating habits.

### 1.1.1 Textural Sensation from Fat

Perception of fat is often primarily considered as a textural sensation. In-mouth textural sensation is perceived by oral mechanoreceptors which respond to tactile stimuli from food particles (Engelen & Van Der Bilt, 2008). Generally, the attributes for describing fatty foods such as greasiness, oiliness, or creaminess, are textural properties likely caused by triglycerides (Drewnowski & Almiron-Roig, 2010). Triglyceride structure is formed by a glycerol attached to three fatty acid molecules with ester bonds (Figure 1.1). Most of the fat present in foods, whether animal or plant-based, is predominantly in the form of triglyceride (Lawson, 1995). Depending on the structure of the fatty acids attached to glycerol, including chain length and the degree of unsaturation, the physical properties of triglycerides that contribute to the textural sensation of fat may differ (Frankel, 2012). Short chain length or unsaturated fatty acids have lower melting points. Thus, these fatty acids and their derivatives are more fluid than others (Berg et al., 2002). Upon stimulation, oral fats induce textural signals to the somatosensory cortical areas, which process a wide range of eating experiences from fat (Rolls et al., 1999). The oral sensation of fat texture also changes over time, from mastication, mixing with saliva, swallowing, and even after swallowing.

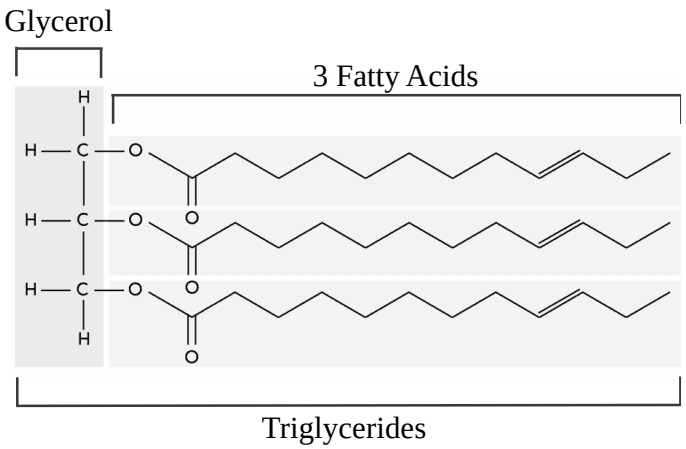


Figure 1.1: Triglycerides consist of three long-chain fatty acids esterified to glycerol

The textural sensation from dietary fat is quite diverse, and influenced by different fat sources, content, and formulation. Studies showed that oil droplet size, as well as droplet concentration, may influence the textural perception of fat (Mela et al., 1994). Smaller particle size with higher droplet numbers generates slightly higher perceived fat content, and the perception of fat tends to linearly increase as concentration increases. In dairy-based fluids, higher fat content is associated with higher creaminess and perceived fat content ratings (Mela, 1988). In addition to how fat incorporates differently in food form, the ever-changing oral environment influences the interaction of fat with other molecules such as shifting its surface electrostatic attraction (Silletti et al., 2007), altering oral shear forces (Dresselhuis et al., 2008), and changing surface retention of oil/fat on the tongue (Dresselhuis et al., 2008). These diverse physical properties of fat during food ingestion alter people's oral experience of fat and preference for fatty foods.

### 1.1.2 Chemical Stimulation from Fat Taste

In addition to the mechanical cues that are generated from fat, the perception of fat is also mediated through chemoreception, likely through the chemistry of fatty acids hydrolyzing from the larger triglyceride molecule. Olfaction is also a known sensation from fat (Boesveldt & Lundström, 2014; Bolton & Halpern, 2010; Chale-Rush et al., 2007), whereas gustatory cues have been demonstrated more recently. Over the past few years, considerable discussions have contemplated whether fat can be perceived solely as a taste when masking other sensory qualities (Chalé-Rush et al., 2007; Fukuwatari et al., 2003; Running et al., 2015; Running & Mattes, 2016; Tsuruta et al., 1999). By

now, considerable evidence indicates that fatty acids can be detected as a taste quality in the oral cavity.

Because identified fat taste receptors in the oral cavity are unlikely to bind with the large triglyceride molecules, triglycerides are unlikely to stimulate taste (Liu et al., 2016). Thus, the gustatory stimulation of fat taste is more likely generated from non-esterified fatty acids (Mattes, 2009b). Non-esterified fatty acids hydrolyze off of triglycerides, either naturally over time or by the action of lipase. These free fatty acids (which are non-esterified) are shown to be an effective taste stimulus and an important target for studying eating behavior from fat (Besnard, 2016; Keast & Costanzo, 2015; Stewart et al., 2011). Furthermore, fatty acids with various chain-length or degrees of saturation also stimulate different qualities of gustation (Mattes, 2009a; Running et al., 2015, 2017; Running & Mattes, 2015). Short- and medium-chain fatty acids may be characterized as sour and irritating, respectively, whereas long-chain fatty acids produce a unique perceptible sensation that is distinctly different from those with shorter carbon chain (Running et al., 2015). The detection threshold for long-chain fatty acids also shown to be lower (more sensitive) in unsaturated fatty acids than saturated fatty acids (Running & Mattes, 2015). However, this taste sensation from long-chain fatty acids, which is generally unpleasant, is very different from the textural sensations from fat.

## 1.2 The Sixth Taste — Oleogustus

Five traditional primary tastes including sweet, salty, sour, bitter, and umami have been globally recognized as gustatory qualities. Evidence that non-esterified fatty acids can also be perceived through chemoreception has been supported by animal anatomical or behavioral studies as well as human trials (Gaillard et al., 2008; Gilbertson, 1998; Keast & Costanzo, 2015; Laugerette et al., 2005; Mattes, 2009; Running & Mattes, 2016). However, lack of consensus on what constitutes primary taste leaves the definition obscured. Six criteria were proposed in order for a chemosensation to qualify as a basic taste quality (Mattes, 2011a): 1) has an adaptive, evolutionary advantage, 2) is stimulated by a defined class of chemicals, 3) is activated by specialized taste receptors and follows unique transduction, 4) is perceived through gustatory nerves and is processed in taste centers, 5) is not overlapping with other primary tastes, and 6) induces functional physiological and/or behavioral responses.

The taste of fatty acids has been tested as an effective stimulus (Chalé-Rush et al., 2007b; Newman & Keast, 2013) and fatty acid taste signal contributes to various complex ingestive behaviors (Chow, 2007; Chow & Chang, 2007; Surai & Fisinin, 2010). Some specialized receptors such as cluster of differentiation (CD) 36 and G protein-coupled receptor (GPCR) 120 have been documented as receptor candidates for fatty acid taste on taste bud cells in human (Galindo et al., 2012; Gilbertson & Khan, 2014; Simons et al., 2011), and an afferent signal is perceived through gustatory nerves and processed in taste centers (De Araujo & Rolls, 2004; Rolls et al., 1999). Fatty acid taste has been confirmed as unique from other traditional primary tastes (Running et al., 2015), and this sensation is responsible for evoking physiological and/or behavioral responses related to lipid metabolism (Mattes, 2011b). Taste perception of non-esterified fatty acids, specifically long-chain fatty acids, has therefore been tested by considerable studies to qualify for all criteria as a sixth basic taste (Running & Mattes, 2016).

Fat "taste" as a concept is often confused with the textual perception of fat, which is mainly attributed to the triglyceride form, not the non-esterified fatty acids. The descriptions such as 'fattiness', 'creaminess,' 'oiliness,' 'thickness,' or 'greasiness' likely contribute more to the mouthfeel characteristics of triglycerides (Drewnowski & Almiron-Roig, 2010; Mattes, 2009b). Given the different sensations from the taste of fatty acids, as well as the unpleasant nature of this taste, a new term was needed to isolate the taste sensation from other sensory attributes commonly characterized as "fattiness." The term "oleogustus" was specifically proposed to describe the taste of long-chain fatty acids (Running et al., 2015).

### 1.2.1 Description of Long-Chain Fatty Acids

Long-chain fatty acids, including saturated (e.g., stearic acid), monounsaturated (e.g., oleic acid), polyunsaturated (e.g., linoleic acid, linolenic acid) likely stimulate fat taste (Chale-Rush et al., 2007; Ebba et al., 2012; Running et al., 2015; Running & Mattes, 2015; Tucker et al., 2017). The chemical structures of several long-chain fatty acids are shown in Figure 1.2. These particular fatty acids have all been tested in taste experiments. The number of double bonds within these fatty acids alters how sensitive humans are to the taste, with linoleic and linolenic acids having a lower detection threshold (i.e., higher sensitivity) than oleic acids (Running & Mattes, 2015). Oleic acid and linoleic acid are more often selected for taste experiments compared to linolenic acid, since

the latter is more expensive, more difficult to purchase pure and at food grade, and more susceptible to oxidation when manipulating in the study (Yun & Surh, 2012). Oleic acid and linoleic acid both contain 18 carbon atoms with one (omega-9) and two (omega-6) cis-form double bonds, respectively. Both fatty acids have been used in many sensory studies as an effective stimulus to elicit oleogustus (Garneau et al., 2017; Running et al., 2015; Running & Mattes, 2015; Stewart et al., 2011; Tucker et al., 2014). Linoleic acid and linolenic acid are two essential fatty acids that humans must consume from diet to maintain optimal health (Smith & Mukhopadhyay, 2012; Spector & Kim, 2015). They are most commonly found in plant oil, nuts, and seeds as part of the plants' stored triglycerides (Mattes, 2009b; Whelan & Fritsche, 2013). As essential fatty acids, they are used to create major components of cell membrane structure, as precursors for various hormones, and serve as components of molecules that modulate signal transduction (Glick & Fischer, 2013).

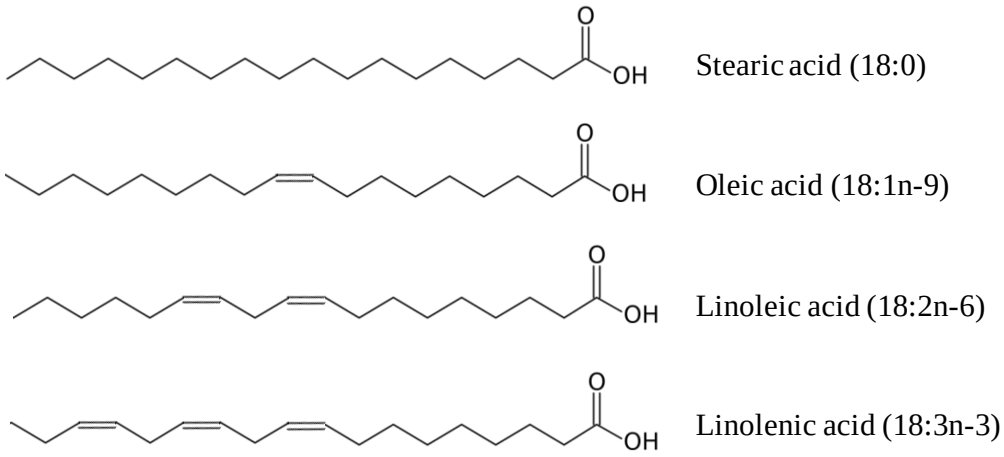


Figure 1.2: Examples of chemical structure of long-chain fatty acids

### 1.2.2 Variability of Oleogustus Sensitivity

Although accumulating psychophysical evidence suggests that humans are able to taste non-esterified fatty acids, oral sensitivity to oleogustus is highly variable among individuals (Chevrot et al., 2014; Mattes, 2009b; Running et al., 2013; Stewart et al., 2010; Tucker & Mattes, 2013). Studies showed that the threshold of fatty acid detection may vary over four orders of magnitude, and some suggested there are hypo- or hyper-tasters of fat (Kamphuis et al., 2003; Mattes, 2009a;

Stewart et al., 2010; Tucker & Mattes, 2013). However, it is worth noting that such a high magnitude of variability might be due to methodological issues rather than the true biological variation (Running, 2015; Tucker & Mattes, 2013). Other factors, including genetics, gender, body mass index (BMI), as well as dietary habits have also been suggested to account for the variability (Running et al., 2013; Stewart et al., 2010, 2011; Tucker, et al., 2014). Still, the magnitude and mechanisms of how these factors influence oleogustus detection thresholds and suprathreshold intensity are still being established.

## 1.3 Dietary Influence on Fat Taste

Taste perception from the same stimuli varies across different individuals. Due to the taste detection and intensity differences among people, the amount of tastants required to reach the same level of satisfaction or pleasantness from food may differ across individuals. Understanding the mechanisms leading to the variation of perceptual experience is particularly important for promoting better food choices. Aside from the genetic variation, dietary fat exposure has been linked with sensitivity to fat perception. Research indicates that higher sensitivity to fatty acid taste is correlated with lower energy and dietary fat consumption (Stewart et al., 2010; Stewart & Newman, et al., 2011). Possibly, a habitual high-fat diet suppresses oleogustus intensity in the mouth, the motility of the gastrointestinal (GI) tract, and the release of GI hormones (Little & Feinle-Bisset, 2011; Stewart & Keast, 2012; Tucker et al., 2014). The impairment of the fat sensing mechanisms in either mouth or GI tract then could predispose individuals to excessive fat intake and obesity. Several studies report this inverse correlation of oleogustus sensitivity with fat consumption or BMI (Stewart et al., 2010; Stewart, et al., 2011), and this demonstrates the potential influence of diet on gustatory detection. Nonetheless, other studies indicate some environmental or behavioral factors may override this physiological regulation of fatty acid taste and diet in the free-living situation (Samra, 2010; Woods, 2004). Detailed mechanisms are being studied that might explain molecular level shifts in the sensory system linked to diet, satiety, and taste perception (Costanzo et al., 2018, 2019; Méjean et al., 2015; Mennella et al., 2014; Mounayar et al., 2013).

## 1.4 The Influence of Diet on Saliva

Saliva has been targeted as a potential flavor mediator, as it interacts with the foods in the oral cavity (Muñoz-González et al., 2018). As the first biological fluid that encounters foods, saliva mediates a variety of functions including preliminary digestion, serving as a solvent to carry flavor compounds to the receptors, and protecting oral surfaces from damage (Dawes et al., 2015). Saliva secretion and composition have been shown to vary within and between individuals (Humphrey & Williamson, 2001). Potentially, diet may be responsible for some of the variation of saliva characteristics (Crawford & Running, 2020; Louro et al., 2021; Méjean et al., 2015; Martine Morzel et al., 2017; Simões et al., 2021a).

### 1.4.1 Saliva Secretion by Different Salivary Glands

Humans have three pairs of major salivary glands (parotid, submandibular, and sublingual) as well as hundreds of minor salivary glands that are distributed around the oral cavity including in the tongue, cheeks, throat, and lips. Salivary glands are composed of clusters of cells called acini which is the basic unit that produces and secretes saliva in the oral cavity. Saliva is a complex fluid comprised mostly of water, as well as various proteins, electrolytes, and enzymes. The parotid, submandibular, and sublingual salivary glands are responsible for 90% of total saliva secretions, whereas minor salivary glands secrete the remaining 10% (Pedersen et al., 2018). Parotid glands produce watery, serous secretions while submandibular and sublingual glands produce slimier, mucus secretions. Minor salivary glands of the lips, cheeks, and throat contribute mostly to mucus quality due to their high protein and glycoprotein content (Iorgulescu, 2009). However, the main minor salivary glands of the tongue, von Ebner's glands, are responsible for a watery, serous secretion of digestive enzymes and proteins with possible taste modulating functions (Gurkan & Bradley, 1988; Kock et al., 1992; Li & Snyder, 1995; Schmale et al., 1990; Spielman et al., 1993). These von Ebner's glands are collocated with taste buds, secreting their saliva directly into clefts of the tongue created by the circumvallate and foliate papillae. Different combinations of saliva from different salivary glands may interact with taste stimuli and further influence the eating experience.

### 1.4.2 Differences in Saliva Characteristics by Stimulation

Salivary composition and flow rate vary markedly by many intrinsic and extrinsic factors such as degrees of hydration, physiological status, or external stimulation (Dawes, 1987; Kubala et al., 2018). Human saliva can be grouped into two types, unstimulated and stimulated saliva. Unstimulated saliva, also known as resting saliva, is when no external stimulation is present, whereas stimulated saliva is when secretion is triggered by psychological, mechanical, or chemical stimuli. In unstimulated saliva, around 60% of the total volume of the whole saliva is produced by submandibular glands. For stimulated saliva, more than half of the total volume of saliva is secreted by the parotid glands. Secretion from sublingual glands only takes up a small percentage in both the unstimulated and stimulated states of the salivary glands (Iorgulescu, 2009). Although minor salivary glands are not the main contributor to whole saliva volume, they produce oral mucus with high protein content for lubrication (Navazesh & Kumar, 2008) and their flow rate is not affected by taste stimulation with acid, which is the strongest stimulant for major salivary glands (Wang et al., 2015). Depends on different type, duration, and intensity of the stimulation, salivary composition and characteristics vary between unstimulated and stimulated saliva (Gomar-Vercher et al., 2018; Jasim et al., 2016; Muddugangadhar et al., 2015).

Stimulated saliva secretion is modulated by various factors, for instance, psychological manipulation (Running & Hayes, 2016), taste (Neyraud et al., 2009), odor (Carreira et al., 2020), or mastication (Polland et al., 2003). Acidic stimulus, such as from citric acid, is one of the strongest stimulants for enhancing salivary flow rate (Bonnans & Noble, 1995). Dietary fatty acids have also been shown to influence fatty acid profiles and salivary flow rate secreted from rat submandibular glands (Escandriolo Nackauzi et al., 2020). In humans, stimulating with various concentrations of fat within milk and cream cheese, no correlation was found between increasing fat concentration and parotid salivary flow (Hodson & Linden, 2004). The milk fat in that study, however, would have been primarily in triglyceride form, mostly stimulating texture. Moreover, the lack of a change in parotid salivary flow rate does not mean that the composition of saliva would be unchanged, as small changes in flow or composition from minor salivary glands would not be reflected in parotid or even whole mouth salivary flow. When stimulated with oleic acid (a long-chain monounsaturated fatty acid), salivary total antioxidant status significantly increased and lipolysis activity decreased, with no change in flow rate, compared with control among people

who were sensitive to the taste of free fatty acid (Mounayar et al., 2013). This indicates that oral sensitivity to tastes may alter salivary characteristics. Moreover, studies have shown that salivary flow rates also vary greatly between individuals, and the functional components within may be diluted in response to changes in the flow (Humphrey & Williamson, 2001). Although the properties and functionalities of the saliva components have been known to be different intra- and inter-individual, the causes of variation and the related influence on flavor remain to be established.

### 1.4.3 Diet and Saliva Composition

The composition and flow rate of saliva are influenced by many factors, such as circadian rhythms, age, gender, several disease states, diet, and medication (Dawes, 1975, 1987; Dodds et al., 2005; Mandel, 1974). Diet has been gaining interest by researchers as a possible source of saliva variation. Dietary exposure to certain types of chemical stimuli in the oral cavity may alter one's sensitivity to flavor perception (Puputti et al., 2019). Detailed causes of the alteration by the different compositions of saliva or other possible mechanism are not fully understood.

Data from animal models indicate that dietary exposure to bitter tastants may change the proteomic profile in saliva and in turn influence bitterness intensity (Martin et al., 2018, 2019; Torregrossa et al., 2014). If diet exposure can also similarly alter salivary proteins in humans, it could potentially be a tool to improve diet quality by incorporating more bitter-tasting foods such as vegetables and polyphenol-rich fruits. Indeed, one study that focused on dietary exposure to polyphenols in chocolate milk showed changes in saliva that could potentially reduce the intensity of unpalatable bitter or astringent sensations (Crawford & Running, 2020). Additionally, higher fat (as well as carbohydrate, protein, and overall energy) diet has been shown to be correlated with higher salivary lipase activity (Mennella et al., 2014). Further research is needed to understand the details of how diets could directly influence saliva, and whether that alters the human experience of flavor from foods in the diet.

## 1.5 The Role of Saliva on Flavor

As the physical and biochemical medium present during the eating process, saliva has also been suggested as a flavor mediator (Canon et al., 2018; Maddu, 2019; Matsuo & Carpenter, 2015;

Muñoz-González et al., 2018; Pedersen et al., 2018; Spielman, 1990). Despite the digestive and mechanical roles of saliva in oral food processing, the role of saliva in flavor perception has not yet been fully understood.

Saliva is the primary liquid component that moisturizes the oral cavity. It protects the taste receptor cells from dryness and infection by covering the external environment of the taste buds. Saliva also serves as a solvent to carry taste compounds, as food particles must dissolve in solution in order to stimulate receptors (Dulac, 2000; Matsuo, 2000). In addition to acting as a transport medium, salivary components have also been shown to be related to the magnitude of taste response. There is mounting evidence showing the detection level of the primary tastes may be modulated by interacting with salivary constituents including water, electrolytes, enzymes, and proteins (Neyraud, 2014; Spielman, 1990).

Different compositions of saliva may modify how individuals perceive the flavor of food. Saliva physically and chemically interacts with the food matrix and alters the perception of stimuli in the matrix. The buffering characteristics of saliva, which is mainly due to bicarbonates, neutralize sour stimuli by lowering the concentration of free hydrogen ions (Christensen et al., 1987; Helm et al., 1982; Norris et al., 1984). Higher concentrations of salivary NaCl decreases the taste sensitivity for saltiness through adaptation, as the level of secreted salivary NaCl surrounding the taste receptors would render the total taste system to be less sensitive to NaCl from foods (Delwiche, 1996; McBurney & Pfaffmann, 1963; O'Mahony & Heintz, 1981). The amount of endogenous glutamate in human saliva is said to perform synergistic effect with disodium ribonucleotide to enhance the umami taste and alter the hedonic responses to monosodium glutamate (MSG) (Scinska-Bienkowska et al., 2006; Yamaguchi, 1991). Sweet taste has also been suggested to be influenced by salivary proteins. For example, some salivary proteins may interfere with glucose transport to taste receptors, and salivary alpha-amylase produces smaller, sweet-tasting sugars from larger starch molecules (Marquezin et al., 2016; Rodrigues et al., 2017). The presence of amylase also influences texture by decreasing the thickness of foods that contain starch (Janssen et al., 2007). Studies of bitter taste acceptance in infants have shown that salivary protein profiles with a higher abundance of cystatin correlate with greater acceptance of bitter solutions (Morzel

et al., 2014). Thus, saliva has an important role in oral perception of tastes and has gained attention in the sensory field.

## 1.6 Changes of Textural Perception when Saliva Interact with Oil/Fat

Food choice is highly driven by flavor and texture (Maarsman, 2016; Steptoe et al., 1995, IFIC, 2020). Fats are often considered to contribute to pleasant oral sensation and food preference (Drewnowski, 1997). Palatability and preference are often associated with foods rich in fat (Drewnowski & Almiron-Roig, 2010). For instance, creamier texture derived from higher fat content in yogurts or dairy products is more favorable to many customers (Folkenberg & Martens, 2003). Yet, some individuals seem to genuinely prefer lower fat products, such as fat-free milk (Bakke et al., 2016). Some individual differences in perception of fat may result from the interaction with saliva.

To detect a fat content difference, oral sensors may perceive textural and taste changes as foods are being orally processed. When exposed to an emulsion, some individuals' saliva destabilizes the emulsion structure, creating larger coalesced or flocculated oil droplets (Silletti et al., 2007; Vingerhoeds et al., 2005). These larger oil droplets feel different in the oral cavity compared to a well-dispersed emulsion of small oil droplets (Dresselhuis et al., 2008). Depending on the properties of fat that saliva is mixing with, saliva may also emulsify the fat into the aqueous saliva/food mixture (Glumac et al., 2019). The function of saliva is quite versatile as it can play opposite roles (stabilize or destabilizing fat in water mixtures) when comes across different structures (Kupirovič et al., 2017; Silletti, 2008). Additionally, different people's saliva has different effects on fat/water mixtures or emulsions (Vingerhoeds et al., 2005).

### 1.6.1 Saliva as an Emulsion De-Stabilizer

Salivary components influence fat emulsions (Vingerhoeds et al., 2005). Studies have shown that saliva may de-stabilize stable fat emulsions into nonhomogeneous mixtures (Sarkar et al., 2017; Silletti et al., 2007), but that different individuals' saliva varies in their ability to destabilize emulsions (Vingerhoeds et al., 2005). Flocculation and coalescence are the main mechanisms if emulsion destabilization occurs (Glumac et al., 2019). Flocculation is a process when oil droplets

in suspension aggregate together into clusters, while coalescence is when oil droplets merge together into larger single droplets. When dispersed lipids flocculate or coalesce, sensory properties deviate from a stable emulsion in respect to viscosity and surface texture (Vingerhoeds et al., 2005). Tactile cues of food emulsions are related to the properties of the particles within the emulsions. As the amount of oil droplets increase, textural qualities such as perceived fattiness, creaminess, and thickness increase (Chen, 2015).

Fat emulsions can be destabilized by salivary proteins interacting with added emulsifiers (Vingerhoeds et al., 2005, 2009). Saliva may disrupt the electrostatic affinity of some emulsifiers, which are located around the surface of the oil droplets (Silletti et al., 2007). However, the influence of saliva on these emulsifiers is quite varied among people. While some people's saliva aggregates oil droplets from homogeneous emulsions, other people's saliva does not have this effect on emulsions (Vingerhoeds et al., 2005).

### 1.6.2 Saliva as an Emulsion Stabilizer

While certainly, saliva can destabilize some emulsions, in other circumstances saliva may improve lipid dispersion in water. Saliva is thought to be an effective emulsifier when mixing with dietary fat such as vegetable oil or animal-based fat in whole foods (Glumac et al., 2019). Thousands of proteins have been identified in whole saliva (Bandhakavi et al., 2009), and many have been demonstrated to interact with lipids. Some salivary proteins, identified as having molecular weight of 27 kDa to 55 kDa, were suggested to be major functional components for saliva-induced emulsion formation (Glumac et al., 2019). In another study, mucins, one of the dominant salivary proteins in saliva, are shown to be surface-active and may serve as a biological surfactant for the stabilization of emulsion systems (Shi et al., 1999). A mixed monolayer is formed, consisting of various salivary proteins around oil droplets. These monolayer molecules act as emulsifying agents to stabilize oil droplets against aggregation in the mouth (McClements, 2016). As food companies trying to enhance the pleasantness of the eating experience by improving the oral sensation of fatty foods, understanding the interaction effect between the oral environment and fat perception becomes crucial.

## 1.7 Saliva's Influence on Oleogustus

Saliva has been shown to play a vital role in affecting textural sensation and interacting with traditional primary tastes. However, as a relatively newly discovered primary taste, the relationship between oleogustus and saliva has not been fully established. Effective stimulation of oleogustus, taste from non-esterified fatty acids, has been speculated to interact with salivary components and alter taste sensation (Neyraud, 2014; Tucker et al., 2014). Whether from binding of fatty acids with salivary proteins, salivary lipase releasing free fatty acids from triglycerides, or saliva altering emulsion structure and access of fatty acids to receptors, saliva may modulate oleogustus.

### 1.7.1 Fatty Acid – Salivary Protein Interaction

Salivary proteins may interact with tastants at a molecular level. Consistent evidence shows salivary proteins bind to bitter compounds correlating with palatability, bitterness, and astringency (Baxter et al., 1997; Dinnella et al., 2009, 2010; Dinnella et al., 2011; Ferruzzi et al., 2012; Lu & Bennick, 1998; Morzel et al., 2017; Shimada, 2006; Torregrossa et al., 2014). Other salivary proteins with a known affinity for taste compounds such as long-chain fatty acids have also been identified as potential taste modifiers (Matsuo, 2000). Lipocalin-1 (also known as von Ebner's gland protein, and secreted by the von Ebner's glands) can bind to small lipophilic compounds (Kock et al., 1992) and has a potential relationship with oleogustus (Mounayar et al., 2013; Neyraud, 2014; Schmale et al., 1990). Its binding affinity to lipophilic compounds such as fatty acids changes the properties of these compounds (Dartt, 2011; Glasgow et al., 1995). It has been speculated that the binding effect that happens between lipocalin-1 and fatty acids may cause the varied sensory detection of fatty acids among humans by carrying the fatty acid stimuli to (Gilbertson, 1998) or protecting the taste receptors from the detergent-effect of fatty acids (Bläker et al., 1993). However, relatively few studies have directly focused on the correlation between saliva properties and the gustatory responses to fat.

### 1.7.2 Antioxidant Capacity of Saliva

Saliva contains several antioxidant molecules to protect against reactive harmful compounds that can damage the oral environment. They help to prevent free radicals by detoxifying reactive oxygen species (ROS) that are naturally present in foods, and also potentially generated during

mastication when food and air are mixed in the mouth. Besides damaging epithelial cells and disturbing oral microorganisms, ROS also participate in the oxidation of some flavor compounds, such as polyunsaturated fatty acids which are especially susceptible to this reaction (Battino et al., 1999; Marcus, 2013). When polyunsaturated fatty acids are oxidized by exposure to light, air, or bacteria, rancidification occurs and generates off-flavors (Kochhar, 1996). In addition, studies have shown that animals can distinguish the difference between oxidized and fresh oils (Kimura et al., 2004). The antioxidant capacity in human saliva has been speculated to associate with the oxidation of fatty acids and lead to the liberation of flavor compounds involved in fat perception (Schwartz et al., 2021). Although other studies found no difference between linoleic acid and oxidized linoleic acid in a human trial, the authors did not rule out the possibility that peroxidase activities may alter the detection thresholds of fatty acid (Chalé-Rush et al., 2007b), which may differ between linoleic acid and its oxidized form. The alteration of antioxidant capacity in response to fatty acid exposure may be due to the protective nature of avoiding oxidation.

### 1.7.3 Fatty Acid – Salivary Protein Interaction

Fat present in foods is mostly in the form of esterified fatty acids such as triglycerides. As the predominant form of fat present in foods, triglycerides can be broken down into smaller parts such as fatty acids by an enzyme called lipase, which is found in small amounts in human saliva. The enzymatic digestion starts with cleaving individual fatty acids from the glycerol backbone by hydrolyzing the ester linkages (Chapus et al., 1988). Likely, only fatty acids can be detected by the receptors that detect oleogustus. Notably, the level of salivary lipolysis within the oral cavity is related to sensitivity to oleogustus (Feron & Poette, 2013; Pepino et al., 2012). In the rat, salivary lipolytic activity seems to correlate with the perception of fat (Kawai & Fushiki, 2003). In humans, studies indicate a possible physiological role of lingual lipase in the regulation of salivary fatty acid concentration (Feron & Poette, 2013; Neyraud et al., 2017). This regulation could help explain the diverse sensitivity that is experienced by different subjects.

However, in other studies, there was only weak evidence that lingual lipase can take a significant role in oral fat taste detection in humans (Kulkarni & Mattes, 2014). Some argue that lingual lipase is hardly present in humans (Gilbertson, 1998; Spielman et al., 1993; Voigt et al., 2014). The activity of lingual lipase in human adults is certainly less than in rats (Schiffman et al., 1998;

Spielman et al., 1993; Stewart et al., 2010). Additionally, the contribution of this enzyme in the oral cavity may not release sufficiently and practically high enough quantities of fatty acid compared to the unesterified fatty acids inherently present in the food matrix (Kulkarni & Mattes, 2014).

## 1.8 Overview of Research

In order to investigate the relation of dietary habits and properties of saliva on the sensory experience from fatty foods, we designed a study including remote tasting sessions, swishing and spitting out an oil/water mixture, and dietary recalls exploring the correlation of perception of fat, habitual diet, and saliva's emulsifying properties. Our hypotheses for the study were:

1. People who are more sensitive to fat and fatty acid taste would have saliva that results in less emulsifying ability.
2. People who consume high amounts of fat in their diet would be less sensitive to fat and fatty acid taste, and thus also have saliva with more emulsifying effects.

# CHAPTER 2. MATERIALS AND METHODS

## 2.1 Introduction

The goal of this work was to remotely assess whether the perception of oleogustus is related to saliva's emulsification capabilities or to dietary habits. The study included a screening survey, consent, demographics surveys, one remote tasting/spitting session, and three 24-hour dietary recalls. Participants who were interested in the study were first screened via an online survey. Those who qualified were given a detailed consent form to review and sign and a demographics survey to fill out. A one-hour remote tasting/spitting session was held via video call with the researcher. Following the remote tasting session, 3-day dietary records were collected. Due to the COVID-19 pandemic, all interactions of subjects with researchers occurred online. Participants and researchers communicated through emails and samples were delivered or picked-up while adhering to social distancing guidelines. The tasting kit contained survey log-in instructions, tasting samples, equipment for spiting protocol (kitchen timer and photo box), nose clip, and rinsing water. Photos of an expectorated oil/water samples were analyzed for the size of the cream layer after the mixture was swished and spat out. All materials and methods were approved by the Purdue University Institutional Review Board, and all subjects provided digital, written informed consent.

## 2.2 Samples

All the materials and corresponding company names are listed in Table 2.1.

Table 2.1: Materials used in Tasting Visit

| Product | Company | Company Location |
|---|---|---|
| Linoleic Acid (LA) | MilliporeSigma | St. Louis, MO |
| White Melting Wafer [a] | Ghirardelli® | San Francisco, CA |
| Original Ranch Salad Dressing [b] | Hidden Valley® | Santa Barbara, CA |
| Original Ranch Light Salad Dressing [c] | Hidden Valley® | Santa Barbara, CA |
| Vegetable Oil [d] | Crisco® | Orrville, OH |
| Tap Water | — | — |
| Electric Purple Soft Gel Paste Food Color [e] | AmeriColor® | Nashville, TN |

[a] Ingredients: Sugar, palm and palm kernel oil, nonfat dry milk, whole milk powder, sorbitan tristearate, soy lecithin, natural flavors, salt.
[b] Ingredients: Vegetable Oil (Soybean and/or Canola), Water, Sugar, Salt, Nonfat Buttermilk, Egg Yolk, Natural Flavors, Less Than 1% of: Spices, Garlic*, Onion*, Vinegar, Phosphoric Acid, Xanthan Gum, Modified Food Starch, Monosodium Glutamate, Artificial Flavors, Disodium Phosphate, Sorbic Acid and Calcium Disodium EDTA Added To Preserve Freshness, Disodium Inosinate & Guanylate. *Dried
[c] Ingredients: Water, Vegetable Oil (Soybean and/or Canola), Maltodextrin, Buttermilk, Sugar, Salt, Modified Corn Starch, Less Than 2% of: Spices, Garlic*, Onion*, Natural Flavors, Egg Yolk, Phosphoric Acid, Vinegar, Artificial Flavor, Disodium Phosphate, Xanthan Gum, Monosodium Glutamate, Disodium Inosinate & Guanylate, Sorbic Acid and Calcium Disodium EDTA Added To Preserve Freshness. *Dried
[d] Soybean Oil
[e] Ingredients: Water, sugar, U.S. certified colors: Red 3 (E127), Blue 1 (E133), modified corn starch, vegetable gum, citric acid, and less than 1/10 of 1% sodium benzoate and potassium sorbate (as preservative)

### 2.2.1 Linoleic Acid (LA) Candies

Linoleic acid was selected due to its liquid state at room temperature and its higher potency as an oleogustus stimulus at lower concentrations (Running et al., 2015, 2017; Running & Mattes, 2015). Linoleic acid was stored under nitrogen gas in the freezer to minimize oxidation. When preparing candies, aliquots of linoleic acid were thawed at room temperature in a water bath covered with an opaque box to avoid direct light before use. Ghirardelli® white melting wafers were used as the base to make fatty candies. Melting wafers were heated while stirring in a bowl on a hot plate (Thermo Scientific Cimarec Hot Plate With Magnetic Stirrer SP131635) around 65-75$^{o}$C. When the wafers were fully melted, linoleic acid was added at 0.1 % (w/w) or 1 % (w/w) concentration

and gently stirred until fully mixed. The warm, melted wafers were then poured into a candy mold (CAKETIME 126 Cavity Square Silicone Mold/Mini Candy Molds, mold size: 11.53"x7.63"x0.47", each cavity: 2.5 ml, 0.6"x0.6"x0.4") and spread out evenly. Candies were refrigerated until solid. Control candies (no linoleic acid) were prepared in the same way. After the fatty candies were cool and solid, they were packaged into small (1 oz, 30 mL) plastic cups with lids and stored until ready for participant pick-up. Each fatty candy weighed around 0.8 g. Samples were stored at room temperature and consumed by the participants within 3-4 days after they were made in the laboratory.

### 2.2.2 High/Low Fat Ranch Dressings

Hidden Valley® Original Ranch Salad Dressing was selected for the high/low-fat discrimination task, as these products are available in single-serving packages and can be stored at room temperature. Ranch salad dressing was served in the original 1.5 Ounce (44 mL) serving size To Go Cups. The product identity was masked by covering the cups with blank shipping labels (Avery® TrueBlock® White Laser Shipping Labels). A comparison of the high and low-fat ranch nutrition labels is listed in Table 2.2.

Table 2.2:High/Low Fat Ranch Comparison

Serving size: 1.5 fl oz (44 mL)

| | Calories | Total Fat | Carbohydrate | Protein | Sodium | Cholesterol |
|---|---|---|---|---|---|---|
| **High Fat** | 200 | 21 g | 2 g | 1 g | 380 mg | 5 mg |
| **Low Fat** | 90 | 8 g | 5 g | 0 g | 450 mg | 5 mg |

### 2.2.3 Oil/Water Mixture

The oil/water mixture that was used to observe the emulsifying properties of saliva was prepared by mixing Crisco® vegetable oil, tap water, and AmeriColor® electric purple soft gel paste food color. Food color was added for the purpose of better visualizing emulsion changes. The color paste was mixed with water to make 0.1% (w/w) coloring in water solution in a blender (*Instant*™

ACE™ NOVA). Total 15ml of oil/water mixture was made by adding 7.5 ml of oil and 7.5 ml of water/color mixture in an amber glass vial. An empty clear glass vial (Fisherbrand™ Class A Clear Glass Threaded Vials) and small funnel were provided for participants to spit the sample into. A control bottle was made with the same oil/water mixture in a glass vial (Fisherbrand™ Class A Clear Glass Threaded Vials) as a reference in the photos.

### 2.3 Participants

All participants were recruited from Saliva, Perception, Ingestion, and Tongues (SPIT) laboratory's participant pool and Purdue University's campus through local advertisements & social media. Eligibility criteria included: be between 18 and 45 years of age, be non-current smokers, have self-reported normal taste function, not have a history of choking or swallowing disorders, and not have any type of severe food allergy or a specific allergy/sensitivity to the study ingredients. Participants were also screened for willingness to comply with all study requirements including self-report demographics, food frequency questionnaire about fat consumption, three-day dietary recalls, and availability for joining an online testing session.

Participants who qualified through the screening survey were provided with a digital consent form to sign via DocuSign (San Francisco, CA). After the consent form was completed, a link was shared with participants to complete a survey on their gender, age, ethnic background, medicine usage, height and weight, and their habitual fat intake using the Block Dietary Fat Screener© (Block et al., 2000). Self-reported demographic data of qualified participants is given in Table 2.3.

Table 2.3: Subject Demographics

| | **Counts** | **Mean age in years (range)** | **BMI in kg/m$^2$ (range)** |
|---|---|---|---|
| **Total** | 62 | 24.7 (18-38) | 24.1 (14.8-46.8) |
| **Male** | 21 | 26.8 (18-38) | 23.7 (18.1-30.5) |
| **Female** | 41 | 23.7 (18-32) | 24.4 (14.8-46.8) |

An "other" category was listed in the gender question but no participant selected this option.

## 2.4 Study Design

In our study design, we include one remote tasting session to collect sensory responses and spat out sample/saliva images and three diet records. Detailed order of the data collecting process is shown in Figure 2.1.

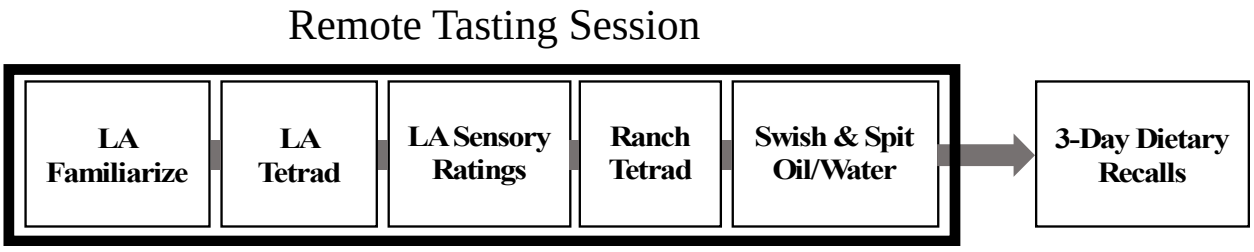


Figure 2.1: Data collecting process

### 2.4.1 Tasting Kit

To maintain social distance during COVID-19 pandemic, studies were conducted remotely by sending a tasting kit to each participant and complete the testing remotely. All the samples were freshly made each week and packaged in the laboratory. Participants received one tasting kit which was delivered to or picked up by participants during the scheduled period. Each tasting kit contained instructions, an exclusive confirmation code, and materials that were needed to complete the study. After receiving the package, participants were asked to store the tasting kit at room temperature and avoid direct sunlight until further notice.

### 2.4.2 Remote Tasting Session

Participants were asked to refrain from eating, drinking, or any oral care activities for 1 hour before the sensory test. Tasting visits were conducted via WebEx video chat (Milpitas, CA) that was hosted by one of our researchers one participant at a time. RedJade® (Redwood City, CA) sensory software was used to display on-screen prompts and collect sensory data. Technology Assisted Dietary Assessment (TADA) program (designed and managed by the Purdue Video and Image Processing Laboratory) was used to take pictures of the expectorated oil/water mixture over time for adjusting environment lighting and the angle of the photo. Participants used their own computer

devices to guide them through study instructions and sensory evaluation, but the researcher was present through the video chat to assist as needed.

### *Check-In*

At the beginning of the online tasting visit, participants were asked to log in to RedJade® sensory software with an exclusive password. After participants successfully logged in, a list of ingredients was displayed on-screen to reaffirm the participant did not have allergies to any study ingredients. Before the actual sensory evaluation, participants followed the instructions to complete a series of warm-up questions for them to familiarize the operating process of the study and verify whether they were paying attention to the instructions.

### *Warm-Up Questions – Intensity Scale*

To familiarize participants with the intensity scale and sensory software, warm-up questions were shown as follows: rate the intensity of "The brightness of the sun in a clear sky," "The brightness of a dark closet," "The loudness of a shout," "The loudness of a whisper," "The sweetness of pure sugar," and "The bitterness of black coffee" (adapted from Hayes, Allen, & Bennett, 2013). Intensity ratings were directly displayed on a modified generalized visual analog scale (gVAS) to collect intensity ratings (Figure 2.2, adapted from Kershaw & Running, 2019). The visual analog scale for intensity ratings corresponded to points on a 110-point scale with internal semantic labels denoted from "None" (0), "Barely detectable" (5), "Weak" (25), "Moderate" (45), "Strong" (65), "Very strong" (85) to "Strongest ever" (105). The numerical values that participants rated were not displayed on-screen (i.e., participants could see the location of their mark on the scale but did not know the numerical value that it represented).

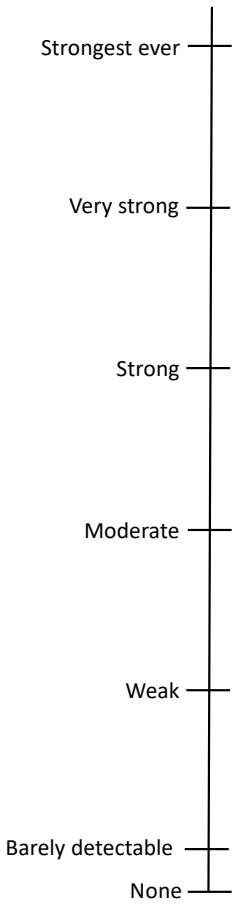


Figure 2.2: Visual analog scale used for intensity rating

***Warm-Up Questions – Intensity Scale***

To familiarize participants with the discrimination tetrad test, participants were asked to select the two identical shapes from the four choices shown on the screen (Figure 2.3). Participants were instructed to "Check the box of two of the selections that are the same (the other two, which should also be the same, will be un-checked)." Participants were required to select two choices before they could move on to the next question.

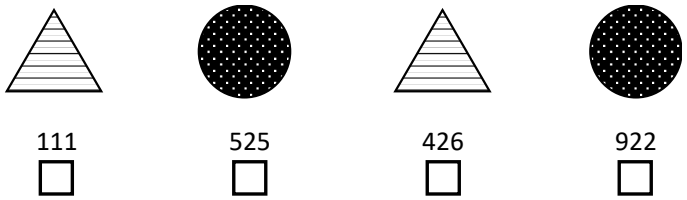


Figure 2.3: Example of discrimination test display

***Sensory Evaluation***

In the actual sensory evaluation tests, participants wore nose clips during the test to minimize olfactory cues. Participants were not allowed to take off their nose clips unless instructed. Each sample was labeled with a three-digit code and served in counterbalanced order controlled by the RedJade survey. Before tasting samples, participants were asked to locate the samples for each question in the tasting kit and place them in front of them. Participants were provided with a bottle of Good & Gather ™ purified drinking water or Kroger® purified drinking water to rinse and clean their palates in between samples.

***LA Familiarization Task***

Since most people have never experienced oleogustus isolated and defined, two fatty candies with no and 1% linoleic acid were given to participants for familiarizing themselves with the taste (wearing nose clips). Each sample was served in a 1-oz plastic cup with a lid. The one that contained added linoleic acid was described to the participants on-screen as having "added flavor." Participants were asked to taste both LA and plain candies following the order shown on-screen and remember the taste differences before the next discrimination test. After both candies had been tasted at least once, re-tasting was allowed.

***LA Discrimination Tetrad Test***

After a one-minute rinsing break, four samples including two plain fatty candies and two fatty candies with 1 % linoleic acids were served to participants. The order of samples was counterbalanced for each participant. Participants were told for these four samples, two of them were identical and then other two also identical. Participants were instructed to taste and select two

candies that taste the same, and reminded the remaining two samples should also taste the same. The display of the test was similar as described for the warm-up questions above (Figure 2.2). Rinsing between samples and re-tasting were allowed.

***LA Sensory Ratings***

After an enforced rinsing break of 60 seconds, participants were prompted to rate the sensory qualities of LA candies with 0, 0.1%, and 1 % linoleic acids (labeled with random 3-digit codes, tasted while wearing nose clips and in counterbalanced order controlled by the software). Participants were asked to eat the whole fatty candy and chew/move it around in their mouth for 30 seconds before swallowing. A 30-second countdown timer was embedded on-screen. After swallowing the sample, participants were asked to rate the intensity of its overall flavor, sweetness, bitterness, and fattiness on the gVAS, as shows in Figure 2.1. A 60 second rinsing break was enforced in between samples.

***High/Low Fat Discrimination Tetrad Test***

After participants completed the sensory ratings for all three fatty candies, a two-minute break was forced before continuing to the next test. Following the forced break, four Hidden Valley® Ranch Salad Dressings including two cups of the original flavor and two cups of reduced fat flavor were presented (labels hidden as described above, labeled instead with randomized 3-digit codes). Samples were tested in counterbalanced order for each participant. Again, participants were informed that among the four samples were two sets of identical pairs. Participants, while wearing nose clips, were asked to taste and select two samples that taste the same from four samples, and reminded that the remaining two sample should also taste the same. Four mini tasting spoons were provided to taste each sample separately. The display of the test was similar to the one described in the warm-up questions above (Figure 2.2). Rinsing between samples and re-tasting were allowed.

***Swish & Spit Oil/Water***

Before continuing the last part of the tasting session, participants were prompted to set up a photo box in a good lighting environment and log into the TADA app on their own devices. The photo box was made with copy paper (Boise Paper® X-9, 8.5”x11”) folded up, with markings to indicate

where the samples should go as well as color markers secured to the background. Detailed instructions regarding the photo box set-up (Figure 2.4) were displayed on-screen, as well as provided by the researcher via video call. The oil/water mixture for swishing/spitting was served in a 30-ml amber vial with a cap. A second oil/water mixture was provided as a control for the image to be taken in the photo box. After setting up the box, participants were instructed to shake the oil/water mixture, pour it into their mouths, and swish for 30 seconds without swallowing. A 30-second countdown timer was embedded on-screen. At the end of 30 seconds, the oil/water mixture along with the accumulated saliva was expectorated into a clear glass vial (a funnel was provided to help participants spit into the vial). Participants then photographed the spat-out sample, in the photo box next to the control sample, at 0 seconds, 30 seconds, 1 minute, and 3 minutes. A timer was provided as part of the photo box to ensure more precise times were recorded automatically in the photos. Participants were asked to start the timer immediately after the mixture was spat out. Images captured by the TADA app were automatically uploaded to the system. For those who had trouble with this app, images were taken with their regular cameras and uploaded to our system through a Qualtrics link.

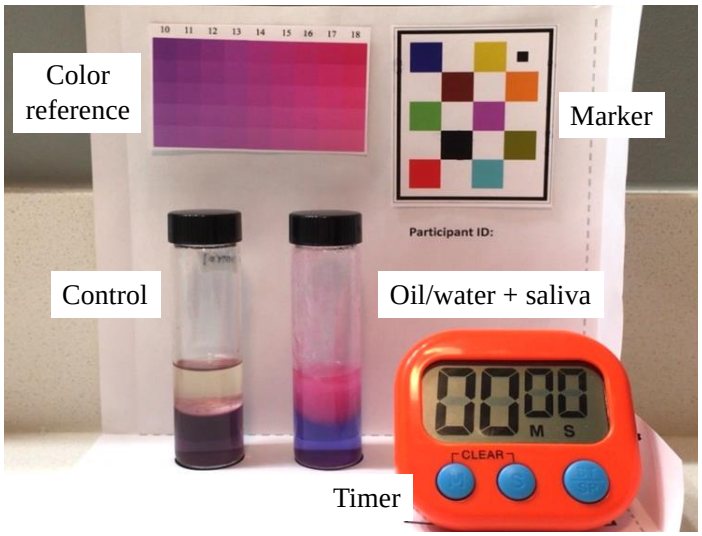


Figure 2.4: Photo box set-up

### 2.4.3 Three-Day Dietary Recalls

Dietary intake data were collected and analyzed using the Automated Self-Administered 24-hour (ASA24®) Dietary Assessment Tool, version (2020), developed by the National Cancer Institute,

Bethesda, MD (Subar et al., 2012) (https://epi.grants.cancer.gov/asa24). Participants sent links to recall their diet for three non-consecutive days including two weekdays and one weekend (days controlled and counterbalanced by the researchers). Special holidays were excluded to avoid major diet changes. Meals, drinks, as well as serving sizes, were collected.

## 2.5 Data Preparation

### 2.5.1 Demographics & Anthropometrics

Demographics and anthropometrics were all self-reported. Age was calculated by 2020 (the year the study was conducted) minus the year of birth for each participant (we collected year of birth instead of precise birthdays as this makes the data less identifiable, offering more protection for our subjects' privacy). BMI was calculated as weight (kilograms)/height (meters) squared.

### 2.5.2 Dietary Recalls

Summary of the nutrition information including macro- and micronutrients from each participants' food record was generated from ASA24®. Individual participant values for each nutrient category were calculated by taking the average across the three separate recalls. Nutrients in the ASA24 U.S. version are provided by USDA's Food and Nutrient Database for Dietary Studies (FNDDS 2015-16), while the food group data is provided by the USDA's Food Patterns Equivalents Database (FPED 2015-16).

### 2.5.3 Spit Image Analysis

#### *Fat Layer Size*

Images of expectorated oil/water samples were taken and uploaded by each participant, as described in section 2.3.2. The ratio of the top and total mixture of oil/water separation was calculated by counting the length of the pixel number from the top line to the layer line ($\boldsymbol{Top\ layer}\ \% = \frac{\boldsymbol{pixel\ count\ of\ the\ top\ layer\ length}}{\boldsymbol{pixel\ count\ of\ the\ total\ sample\ length}}$) using Apple Inc. Preview Version 11.0 (1017). See Figure 2.5 for demonstration.

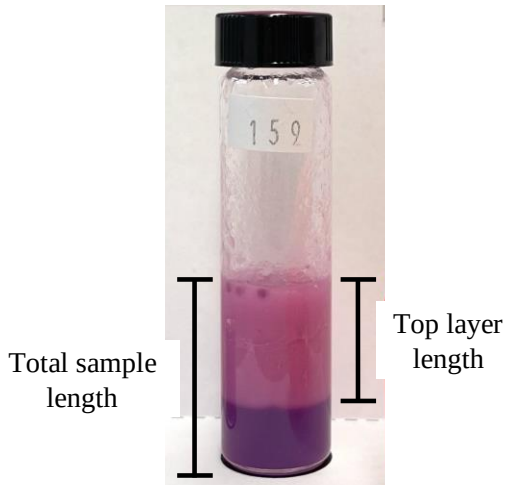


Figure 2.5: Demonstration of the layer measurement

***Fat Layer Characteristics***

Characteristics including a number of distinct layers observed in the spat-out sample images (no separation vs two layers formed), distinctness of the line between layers (no distinct line vs blurry line vs clear separation), the opacity of the top layer (very clear vs intermediate vs very opaque), and homogeneity of the top layer (distinct bubbles/droplets vs intermediate vs homogenous) were rated by three independent research personnel. Rating discrepancies among researchers were resolved through group consensus.

***Fat Layer Color***

The color of the top layer was analyzed by using labelme (Image Polygonal Annotation with Python) to mark the area for the color calculation to avoid shadow or reflection. To define the representative layer color, the color difference among each pixel within the marked area was calculated compared to all other pixels in the marked area. The pixel that had the smallest color differences to the others was considered as the representative (CIE76). The HSV color system was used, which has components for Hue (the color, such as blue or red), Saturation (how strong the color is), and Value (the brightness). Two methods were used to analyze the color of the fat layer. The first method was comparing each representative color pixel HSV value with the standard color blocks on the color strip (Figure 2.6) using General Image Manipulation Program (GIMP-2.10). Because each participant's photos were taken at home, this color strip was attached to the photo

box to serve as a standard to color-correct for the unique lighting conditions in each participant photo. The color of the pixel was selected by using the color picking tool and taking the average of the given square area. The color strip was based on a 5 Hue (H) value increment from 240 to 0 horizontally and 10 Saturation (S) value increment from 60 to 100. Value (V) was not controlled since the color strip was given under the same value (determined by the lighting in the room where the photo was taken).

The second method calibrated each representative pixel by using the fiducial marker that was developed by Purdue Video and Image Processing Laboratory to fix the lighting condition for each image (Fang et al., 2015; Xu et al., 2012). Both the color strip and the fiduciary marker were printed by the Purdue Video and Image Processing Laboratory which has a color corrected printed and paper to ensure all color strips and fiduciary markers were identical in coloring.

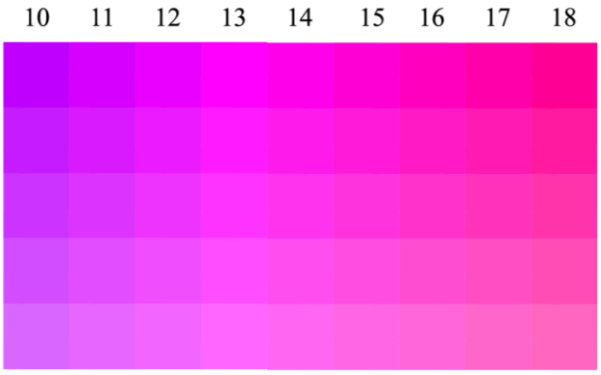


Figure 2.6: Color strip (for color reference)

The final results were presented as mean value and the range of the representative HSV from the two methods described above. When calculating the average of Hue, which has a circular range of values from 0-360 with 0 and 360 being equal, when values span the 360/0 line the lower values should have 360 added to get the correct calculation. For example, to calculate the average of 350 and 10, when simply sum up the value and divided by 2 ($\mathbf{(350 + 10) \div 2 = 180}$), 180 would not be the average between the two (this is a green color, where 350 and 10 are red 0-purple to red-orange). Instead, adding up 360 ($\mathbf{(350 + 10 + 360) \div 2 = 360}$) to the low value will cause the

mean to fall in the correct location to average the two hue measurements. See demonstration in Figure 2.7.

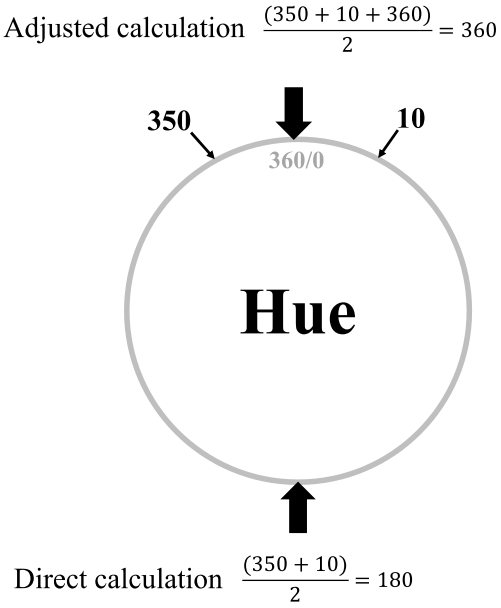


Figure 2.7: Demonstration of Hue average calculation method

### 2.5.4 Statistics

A total 62 individuals completed the testing protocols including remote self-reported demographics, tasting visit, and 3-day dietary recalls. To test whether the true discriminators was observed, Chi-Square goodness of fit tests was tested on R version 3.6.2 (Vienna, Austria) for the distribution of merged tetrads (both correct chance 1/9, just LA correct chance 2/9, just high/low fat correct chance 2/9, both incorrect chance 4/9), LA tetrad (correct chance 1/3, incorrect chance 2/3), and high/low fat tetrad (correct chance 1/3, incorrect chance 2/3). To compare the demographics between individual who pass or fail LA group and fat/low fat group, unpaired two-sample Wilcoxon tests was performed on R version 3.6.2 (Vienna, Austria). To compare the demographics for merged discrimination groups, Kruskal-Wallis test and multiple pairwise-comparison with p-value adjusted by Bonferroni was computed on R version 3.6.2 (Vienna, Austria) as well.

***Sensory Ratings of Fatty Candies***

A quick PCA analysis in OriginPro 2019 was used to reduce the number of dietary factors to analyze in this experiment, as well as to determine which factors related to the photos (ratings of opacity, homogeneity, number of layers, and layer size) were best to use in the analysis. Factors with larger vectors in different directions in the final PCA plot were selected as mostly likely to explain more of the variance in our dataset. From that, factors were reduced to macronutrients from ASA24 data and layer size from the photo analysis. These were analyzed along with linoleic acid concentration, tetrad outcomes, use of medications, BMI, and gender. Linear mixed models (LMM) were performed using SAS 9.4 (Cary, NC) to evaluate the fixed effects on sensory ratings for both tetrad tests. Proc MIXED statements were used to fit models to make statistical inferences about the sensory ratings. Options selected were: Kenward-Roger for the fixed effects standard error and degrees of freedom and restricted maximum likelihood (REML) for estimation. Subjects were included in the model as a repeated measure, with covariance structures set as compound-symmetry. LSMEANS statement was computed for the CLASS variables in the MODEL statement and the denominator degrees of freedom was determined with p-values and confidence limits adjusted for multiple comparisons using the Tukey-Kramer approach.

All sensory models were initially tested with the factors: linoleic acid concentration, emulsion layer size at 3 minutes, ASA macronutrients (fat, protein, carbohydrate), use of medications (yes/no), gender, and BMI. Models were sequentially reduced by removing the factor with the highest, non-significant p-value, and rerunning the analysis until either all factors were significant, or patterns of significance no longer changed with further reductions. Interactions of linoleic acid concentration with emulsion layer size at 3 minutes as well as with macronutrients were also tested, but interactions were not significant and so were removed from final models.

The final sensory model was reduced to:
***Intensity = Lionleic TotalFatASA LayerSize3***

***Intensity*** indicates the ratings of the sensations on the modified gVAS (0-110).

***Linoleic*** corresponds to the concentration of linoleic acids that were added to the fatty candies. This factor was treated as a categorical variable as this improved the model fit.

***TotalFatASA*** indicates the mean of total fat in grams generated from ASA24®.

***LayerSize3*** indicates the measurement of the top layer of the expectorated emulsion, as a ratio of the top layer to total sample volume using the image analysis described above.

For "Bitterness," the intensity was transformed with square root to improve the distribution of the residuals. Data were also analyzed within the groups of participants who pass or failed the tetrad discrimination tasks. Proportions of individuals who passed/failed the tetrad tests were also analyzed using Chi-Square analysis to determine if these proportions were different from chance. Data visualizations were generated using OriginPro2021 for figures of box plots.

***Expectorated Fat Layer***

As with the sensory models, the top layer of the expectorated emulsion was also analyzed with the factors: macronutrients from ASA, medication use, BMI, and gender. As with the sensory models, we sequentially removed factors from the model by removing the factor with the highest p-value, unless all remaining factors were significant or patterns of significance for remaining factors no longer changed. We analyzed the top emulsion layer at 0 min, 3 min, and the change in size of the layer from 0-3 minutes.

Gender was not significant in any models, so was removed from all.

This, the model used was:

***Layer = TotalFatASA ProtASA CarbASA MedsYN BMI***

***Layer*** indicates the fat layer size at 0, 3 minutes and changes from 0-3 minutes.

***TotalFatASA, ProtASA****, and* ***CarbASA*** correspond the mean of total fat, protein, and carbohydrate in grams generated from ASA24®, respectively. ***MedsYN*** indicates whether participants indicated they were on any medications (categorical variable). ***BMI*** denotes body mass index in kg/m2 from self-reported height and weight data. All of these factors were significant for some of the layer size at 3 minutes data. However, with this large number of factors, this model is likely overparametrized. Thus, we also tested the model without the medications or BMI factors. As with the sensory models, we also examined the data by groups who passed or failed the LA and high/low fat tetrad discrimination tasks.

# CHAPTER 3. RESULTS

## 3.1 Introduction

From our experiments, we found a wide range of variability in the human perception of fat and fatty acids, which may correlate with dietary behavior and salivary emulsifying capabilities. We found that participants, as a group, could not reliably discriminate the linoleic acid (LA) from plain candies, nor could they discriminate high- and low-fat ranch dressings. However, participants did experience increasing intensity of sensation with increasing LA concentration in the candies, and groups who successfully discriminated the LA candies from plain or high from low fat ranch dressings showed different patterns in their sensory experience of the increasing LA concentrations from groups who failed the discrimination tasks. Additionally, some dietary components, particularly macronutrients, correlated with the effectiveness of saliva at emulsifying the expectorated oil/water mixture. Details are given below.

## 3.2 Summary Data on Participants, Discrimination Tasks, and Diet

Summary data of participants and their performance in the LA tetrad test is shown in Table 3.1, the high/low fat tetrad test in Table 3.2, and the merged results in Table 3.3. Chi-Square goodness of fit tests were used to test for the distribution of merged tetrad tests (both correct chance 1/9, just LA correct chance 2/9, just high/low fat correct chance 2/9, both incorrect chance 4/9), LA tetrad test (correct chance 1/3, incorrect chance 2/3), high/low fat tetrad test (correct chance 1/3, incorrect chance 2/3). The distribution of people in each category was not significantly different from chance for LA tetrad test ($p = 0.1508$), high/low fat tetrad test ($p = 0.7194$), and merged tetrad tests ($p = 0.3019$). Thus, we overall conclude our participants could not, as a group, discriminate the LA or high/low fat samples.

Table 3.1: Summary data of participants and diet by LA tetrad test

| | Fail LA | Pass LA | p-value[1] |
|---|---|---|---|
| | Median (range) | Median (range) | |
| **Counts** | 36 | 26 | - |
| **BMI (kg/m$^2$)** | 23.5 (14.8-46.8) | 22.5 (18.3-38.6) | 0.2538 |
| **Fat_FFQ[2] (g)** | 93.1 (44.7-168.7) | 93.5 (56.7-144.7) | 0.6737 |
| **Fat[3] (g)** | 67.7 (31.8-162.2) | 80.2 (45.3-140.1) | 0.1121 |
| **Protien[3] (g)** | 80.0 (25.8-144.6) | 83.4 (32.7-121.9) | 0.1368 |
| **Carbohydrate[3] (g)** | 217.1 (102.1-442.0) | 200.9 (77.0-312.2) | 0.9154 |
| **Calorie[3] (kcal)** | 1910.1 (777.6-3619.9) | 1867.2 (1311.4-2726.2) | 0.4148 |
| | | Chi-square goodness of fit test: p-value = 0.1508 [4] | |

LA: Linoleic acid; BMI: Body Mass Index
[1] Significant level for two-samples Wilcoxon test between those who failed and passed LA test
[2] Total Fat intake from Food Frequency Questionnaire - Dietary Fat Screener©(NutritionQuest)
[3] Mean of the nutrient information from 3-day dietary recalls (ASA24)
[4] Significant level for Chi-square test with the probabilities of random guessing in LA tetrad

No significant differences of BMI, fat intake from Block Dietary Fat Screener©, total fat, protein, carbohydrate, calorie intake from ASA24 were observed between those who passed or failed the LA tetrad test (See Table 3.1 for details).

Table 3.2: Summary data of participants and diet by high/low fat tetrad test

| | Fail High/Low Fat | Pass High/Low Fat | p-value[1] |
|---|---|---|---|
| | Median (range) | Median (range) | |
| **Counts** | 40 | 22 | - |
| **BMI (kg/m$^2$)** | 23.2 (18.1-38.6) | 23.6 (14.8-46.8) | 0.5513 |
| **Fat_FFQ[2] (g)** | 98.3 (44.7-168.7) | 91.9 (63.9-144.7) | 0.6323 |
| **Fat[3] (g)** | 70.6 (31.8-147.0) | 80.3 (44.6-162.2) | 0.4342 |
| **Protien[3] (g)** | 80.1 (25.8-144.6) | 85.7 (26.0-134.9) | 0.4517 |
| **Carbohydrate[3] (g)** | 201.8 (77.0-442.0) | 218.1 (132.7-349.4) | 0.1109 |
| **Calorie[3] (kcal)** | 1825.9 (777.6-3919.9) | 2015.4 (1232.0-2774.1) | 0.1212 |
| | | Chi-square goodness of fit test: p-value = 0.7194 [4] | |

BMI: Body Mass Index
[1] Significant level for two-samples Wilcoxon test between those who failed and passed high/low fat test
[2] Total Fat intake from Food Frequency Questionnaire - Dietary Fat Screener©(NutritionQuest)
[3] Mean of the nutrient information from 3-day dietary recalls (ASA24)
[4] Significant level for Chi-square test with the probabilities of random guessing in high/low fat tetrad test

No significant differences of BMI, fat intake from Block Dietary Fat Screener©, total fat, protein, carbohydrate, calorie intake from ASA24 were observed between those who passed and failed the high/low fat tetrad test (See Table 3.2 for details).

Table 3.3: Summary data of participants and diet by both tetrad tests

| | Pass Neither | Pass Only LA | Pass Only High/Low Fat | Pass Both | p-value[1] |
|---|---|---|---|---|---|
| | Median (range) | Median (range) | Median (range) | Median (range) | |
| **Counts** | 21 | 19 | 15 | 7 | - |
| **BMI (kg/m$^2$)** | 23.5 (18.1-32.6) | 21.6 (18.3-38.6) | 23.7 (14.8-46.8) | 23.6 (19.7-26.6) | 0.679 |
| **Fat_FFQ[2] (g)** | 101.5 (44.7-168.7) | 92.7 (56.7-127.9) | 83.1 (63.9-127.9) | 101.5 (72.7-144.7) | 0.457 |
| **Fat[3] (g)** | 61.9 (31.8-147.0) | 81.6 (45.3-123.0) | 81.7 (44.6-162.2) | 70.6 (55.3-140.1) | 0.213 |
| **Protien[3] (g)** | 65.4 (25.8-144.6) | 84.7 (43.6-117.0) | 86.6 (26.0-134.9) | 69.8 (32.7-121.9) | 0.084 |
| **Carbohydrate[3] (g)** | 196.9 (102.1-442.0) [a] | 217.3 (77.0-312.2) [a] | 231.7 (184.5-349.4) [a] | 184.8 (132.7-292.0) [a] | 0.041* |
| **Calorie[3]** | 1595.0 (777.6-3619.9) | 1900.8 (1371.3-2648.9) | 2093.2 (1232.0-2774.1) | 1746.0 (1311.4-2726.2) | 0.111 |
| | | | | Chi-square goodness of fit test: p-value = 0.3019 [4] | |

LA: Linoleic acid; BMI: Body Mass Index
[1] Significant level of Kruskal-Wallis test among groups
[2] Total Fat intake from Food Frequency Questionnaire - Dietary Fat Screener©(NutritionQuest)
[3] Mean of the nutrient information from 3-day dietary recalls (ASA24)
[4] Significant level for Chi-square test with the probabilities of random guessing in merged tetrad
[a] No difference was found with multiple pairwise-comparison Wilcoxon rank sum test adjusted by Bonferroni
* $p < 0.05$

No significant difference of BMI, fat intake from Block Dietary Fat Screener©, total fat, protein, calorie intake from ASA24 was observed among merged groups including those who did not pass any tetrad tests (Pass Neither), passed only LA tetrad test (Pass only LA), passed only high/low fat tetrad test (Pass Only High/Low Fat), and passed both tetrad tests (Pass Both). Significant difference was observed in carbohydrate intake ($p = 0.041$). However, after correcting for multiple comparisons post hoc pairwise comparisons did not show differences between groups (See Table 3.3 for details).

## 3.3 Expectorated Emulsion Layer Size Changes

Changes of fat layer size over time within individual's samples were visually observed. Wide variation was found between individual's spat-out samples over time. An example for a stable emulsion in the expectorated sample is shown in Figure 3.1. An example for a less stable emulsion is shown in Figure 3.2. In these images, a larger upper layer size (as analyzed in the statistical models) generally means more emulsified fat—implying that individual's saliva was better at emulsifying the mixture. Less change in layer size over time also indicates better ability of saliva to stabilize the emulsified fat.

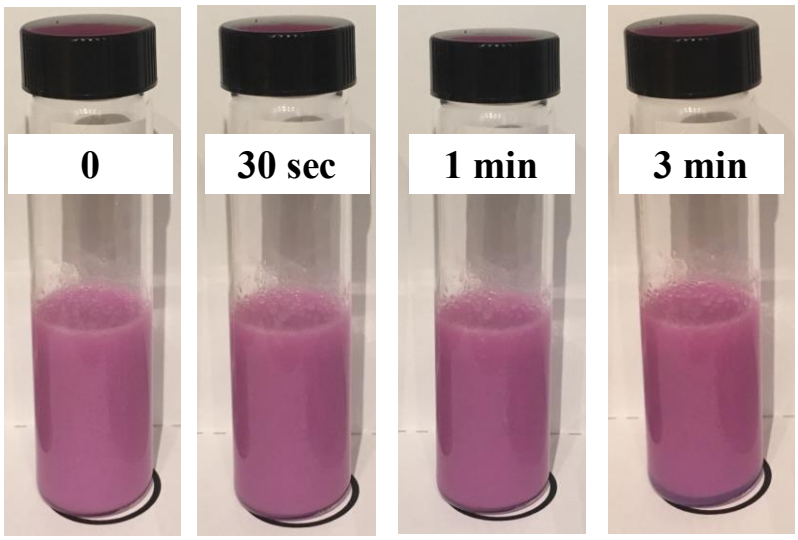


Figure 3.1: Example for high emulsifying capability

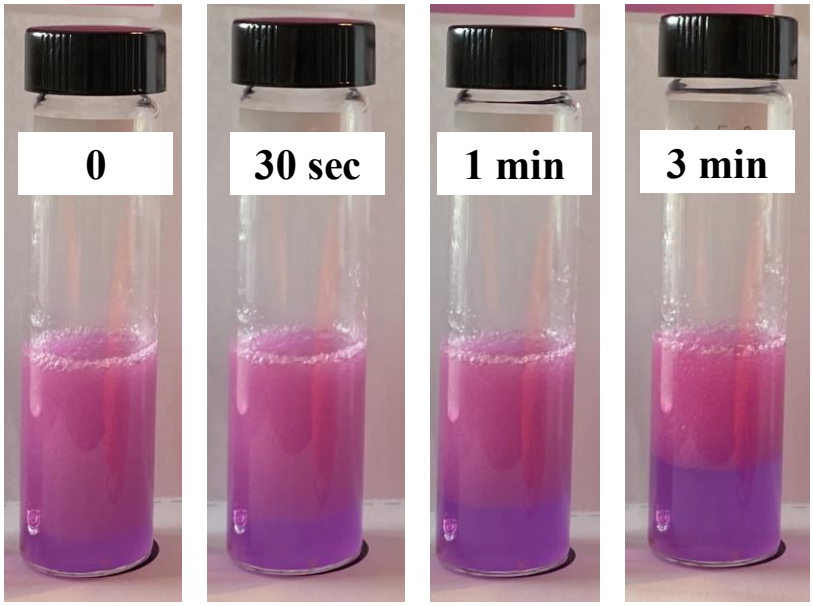


Figure 3.2: Example for less emulsifying capability

### 3.4 Expectorated Emulsion Characteristics Ratings

Expectorated oil/water sample images were collected from each participant. Four characteristics including: “Phase,” “Layer Line,” “Opacity Rating,” and “Homogeneity Rating” for the spat-out

samples were visually analyzed by three personnel in a consensus group meeting. Summary counts for each characteristic at different time point is displayed in Table 3.4.

Table 3.4: Summary counts for expectorated emulsion characteristics ratings

| Characteristics | Rating | 0 (Counts) | 30 sec (Counts) | 1 min (Counts) | 3 min (Counts) |
|---|---|---|---|---|---|
| **Phase**[1] | 1 layer | 17 | 7 | 4 | 0 |
| | 2 layers | 45 | 55 | 58 | 62 |
| **Layer Line**[2] | No distinct layers | 17 | 7 | 4 | 0 |
| | Blurry line | 18 | 10 | 5 | 4 |
| | Clear separation | 27 | 45 | 53 | 58 |
| **Opacity Rating**[3] | Very clear | 0 | 0 | 0 | 0 |
| | Intermediate | 22 | 16 | 19 | 26 |
| | Very opaque | 40 | 46 | 43 | 36 |
| **Homogeneity Rating**[4] | Distinct droplets | 5 | 7 | 5 | 18 |
| | Intermediate | 23 | 24 | 28 | 20 |
| | Homogenous | 34 | 31 | 29 | 24 |

[1] Number of distinct layers observed in spat out sample image
[2] Rating for how distinct the line between the layers is
[3] Rating for the opaqueness of the upper layer (clear to opaque)
[4] Rating for the homogeneity of the upper layer (droplets or not)

For the characteristic of "Phase," counts for one layer gradually decreased as more samples separated into two layers over time. For "Layer Line," the interface between oil/water became clearer over time. For "Opacity Rating," no samples were rated as "very clear," and the opaqueness was shifted but not linearly over time. For "Homogeneity Rating," more samples formed distinct droplets over time. Example images for each characteristic of the spat-out emulsions are shown below. "Phase" indicates the number of layers observed in the spat-out sample (See Figure 3.3). "Layer Line" represents the characteristics of the interface between the oil and water layers (See Figure 3.4). "Opacity Rating" refers to the opaqueness of the upper fat layer (See Figure 3.5). "Homogeneity Rating" is how homogenous the upper fat layer is (See Figure 3.6).

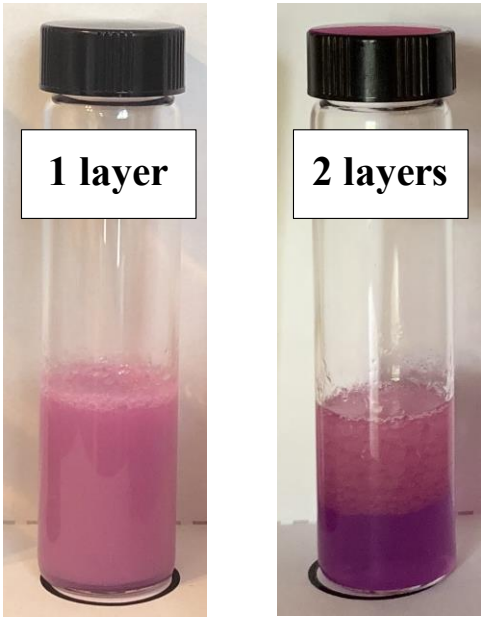


Figure 3.3: Example samples of different phases

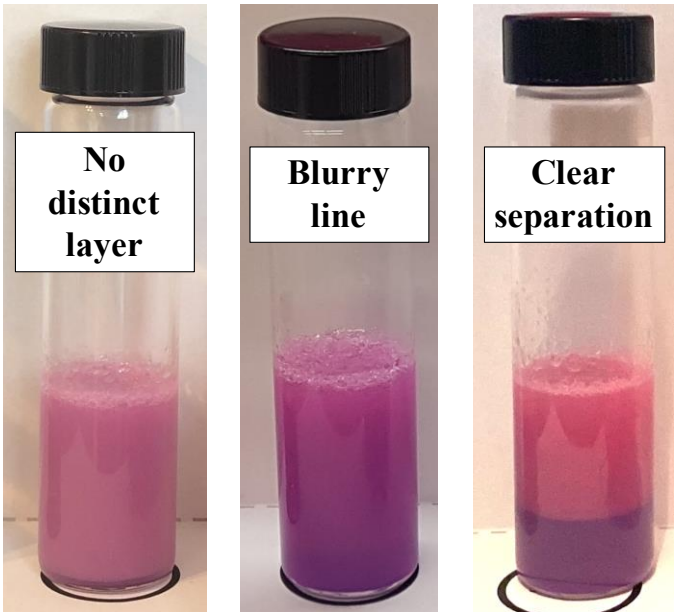


Figure 3.4: Example samples of different layer line

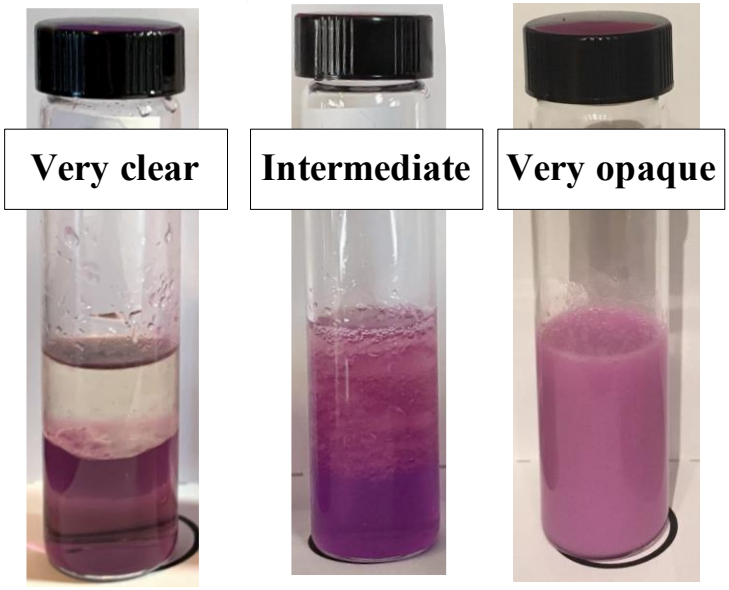


Figure 3.5: Example samples of different opacity rating

(left: this sample with “very clear” layer is the control since we did not observe any expectorated fat layer was characterized in this category)

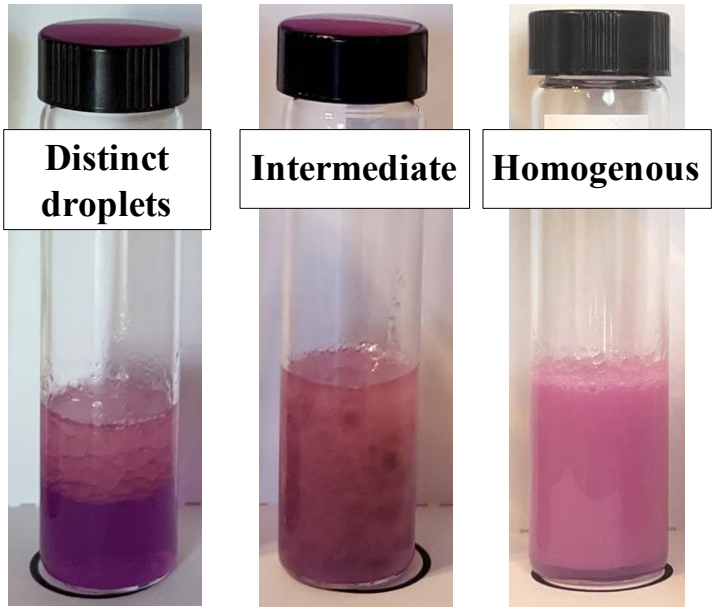


Figure 3.6: Example samples of different homogeneity rating

## 3.5 Color Analysis of Expectorated Fat Layer

The color of the upper fat/cream layer of the expectorated oil/water sample images was analyzed by two methods. In method 1, representative HSV value for each sample were calibrated using the fiduciary marker to correct the lighting environment. In method 2, representative HSV value for each sample were individually compared with the color reference strip (captured together in the same image). Reference color strip we designed did not vary in 'Value' (set consistent as 100). See Table 3.5 for mean color ratings. Note these means must be calculated with the perspective that the 0-360 range of color is circle—so the average of a Hue of 330 and a Hue of 30 would be 0 (see Figure 3.7).

Table 3.5: Summary HSV values by two analytical methods

| | Method 1: Corrected Color[1] | | | Method 2: Reference Color[2] | | |
|---|---|---|---|---|---|---|
| **Time** | **Hue** | **Saturation** | **Value** | **Hue** | **Saturation** | **Value** |
| | **Mean (range)** | **Mean (range)** | **Mean (range)** | **Mean (range)** | **Mean (range)** | **Mean (range)** |
| **0** | 331.5 (293.2-2.5) | 49.1 (28.3-67.1) | 44.6 (15.4-79.2) | 318.5 (235.0-60.0) | 54.8 (10.0-100.0) | 100.0 (-) |
| **30 sec** | 333.5 (296.1-25.1) | 48.7 (7.3-67.5) | 45.3 (14.5-82.8) | 317.2 (265.0-40.0) | 57.6 (10.0-100.0) | 100.0 (-) |
| **1 min** | 334.0 (293.0-23.3) | 48.9 (7.8-68.8) | 45.6 (16.3-81.9) | 316.8 (260.0-45.0) | 56.5 (10.0-100.0) | 100.0 (-) |
| **3 min** | 335.7 (283.5-35.9) | 49.8 (6.5-70.9) | 48.4 (24.2-82.3) | 321.0 (260.0-45.0) | 56.9 (10.0-110.0)[3] | 100.0 (-) |

[1]Corrected HSV value by the fiduciary marker
[2]Reference HSV value by comparing the closest color blocks on the color reference, 'Value' was consistent on the color reference
[3]Due to the rounding issue, the maximum 'Saturation' was beyond limit at 3 minutes ('Saturation' 0-100)

The mean for 'Hue', 'Saturation', and 'Value' in both methods did not substantively change over time, which is not unexpected. However, wide variation was observed among participants. We were unable to use these data in statistical models as the colors and photo quality varied too widely among participants. Additionally, the HSV from both methods did not perfectly match. Nonetheless, the wide range of color observed can be seen through Table 3.5. The HSV color model mapped to a cylinder is displayed in Figure 3.7 as reference.

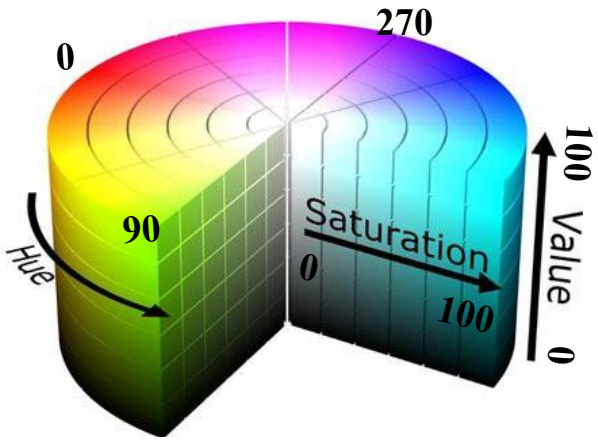


Figure 3.7: HSV color model

(image modified from https://commons.wikimedia.org/wiki/User:SharkD)

## 3.6 Sensory Ratings of Fatty Candies with Varying LA Concentrations

Changes Results are summarized in Table 3.6 for the model:

$$\boldsymbol{Intensity = Linoleic\ TotalfatASA\ LayerSize3}$$

Table 3.6: Summary of the type 3 effects of the full model

| Quality | Effect | Num DF | Den DF | F value | p-value |
|---|---|---|---|---|---|
| **Flavor** | $Linoleic$ | 2 | 122 | 0.44 | 0.6479 |
| | $TotalfatASA$ | 1 | 59 | 0.06 | 0.8019 |
| | $LayerSize3$ | 1 | 59 | 0.09 | 0.7609 |
| **Sweetness** | $Linoleic$ | 2 | 122 | 0.39 | 0.6809 |
| | $TotalfatASA$ | 1 | 59 | 0.21 | 0.6520 |
| | $LayerSize3$ | 1 | 59 | 0.38 | 0.5377 |
| **Bitterness** | $Linoleic$ | 2 | 122 | 14.26 | <.0001* |
| | $TotalfatASA$ | 1 | 59 | 5.92 | 0.0180* |
| | $LayerSize3$ | 1 | 59 | 4.78 | 0.0327* |
| **Fattiness** | $Linoleic$ | 2 | 122 | 4.14 | 0.0182* |
| | $TotalfatASA$ | 1 | 59 | 0.14 | 0.7082 |
| | $LayerSize3$ | 1 | 59 | 0.23 | 0.6315 |

$LMM: Intensity = Linoleic\ TotalfatASA\ LayerSize3$[1]

Num DF: Numerator degree of freedom; Den DF: Denominator degree of freedom

[1] Linear Mixed Model (LMM) was used to evaluate the main effects of linoleic acid concentration ($Linoleic$), total fat intake from ASA24 ($TotalfatASA$), and the fat layer size at 3 minutes ( $LayerSize3$) on each quality ratings ($Intensity$). $Intensity$ for Bitterness was transformed with square root when running the model.

*$p < 0.05$

No effects were significant for "Sweetness" and "Overall flavor." Further reducing the models for "Overall flavor," "Sweetness," and "Fattiness" did not change the patterns of significance. For "Fattiness," LA concentration was significant. For "Bitterness" (square rooted), LA concentration, total fat intake, and layer size at 3 minutes were significant.

To observe the direction of the significance, the effect of LA concentration is shown in Table 3.7, total fat intake in Table 3.8, and layer size at 3 minutes in Table 3.9.

Table 3.7: The effect of LA concentration on sensory ratings

| Quality | 0% Mean (SD) | 0.1% Mean (SD) | 1% Mean (SD) | p-value[1] |
|---|---|---|---|---|
| **Flavor** | 55.5 (18.0) [a] | 53.5 (19.4) [a] | 55.7 (19.8) [a] | 0.6479 |
| **Sweetness** | 57.2 (18.8) [a] | 55.9 (21.7) [a] | 54.9 (23.5) [a] | 0.6809 |
| **Bitterness** | 9.4 (12.0) [a] | 10.1 (11.8) [a] | 21.1 (21.1) [b] | <.0001* |
| **Fattiness** | 50.9 (20.9) [ab] | 46.8 (21.7) [a] | 54.0 (22.2) [ab] | 0.0182* |

$LMM$: $\boldsymbol{Intensity} = \boldsymbol{Linoleic}\ Totalfat ASA\ LayerSize3$ [2]

SD: Standard deviation
[1] Significant level for the fixed effects of linoleic acid concentration contribute to the model on quality rating.
[2] Linear Mixed Model (LMM) was used to evaluate the main effects of linoleic acid concentration ($Linoleic$), total fat intake from ASA24 ($TotalfatASA$), and the fat layer size at 3 minutes ( $LayerSize3$) on each quality ratings ($Intensity$). $Intensity$ for Bitterness was transformed with square root when running the model
[ab] Within the same row of discrimination group, means without shared superscript differ ($p < 0.05$), adjusted by Tukey-Kramer
*$p < 0.05$

LA concentration had a significant effect on "Bitterness" ($p < 0.0001$) and "Fattiness" ($p = 0.0182$) ratings. Higher "Bitterness" and "Fattiness" ratings were associated with the highest LA concentration as shown in the mean ratings for each concentration. For "Bitterness," 1 % LA concentration was rated significantly higher than 0% and 0.1%. No difference for "Bitterness" was found between 0 and 0.1 % LA concentration. "Fattiness" was rated higher for 1% than 0.1% LA concentration. No difference for "Fattiness" was found between 1 and 1% as well as 0% and 0.1 % LA concentration. No significance of LA concentration on "Overall Flavor" and "Sweetness" ratings were found. Box chart displaying "Overall flavor," "Sweetness," "Bitterness" and "Fattiness" rating are shown in Figure 3.8.

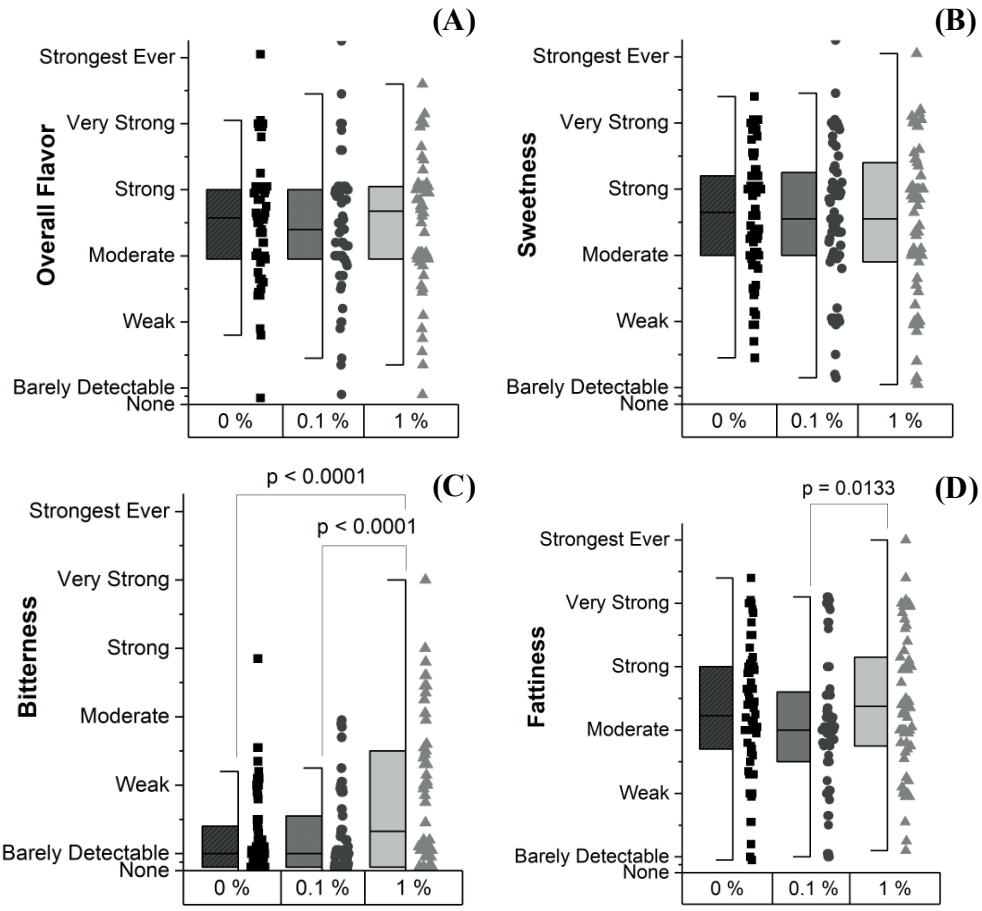


Figure 3.8: Box chart of quality rating

(Boxes indicate 25-75%, whiskers indicate 5-95%, and horizontal line indicates median)

No significant effect was found on “Overall flavor” and “Sweetness” ratings (Figure 3.8 A, B). “Bitterness” rating of 1% LA candy was significantly higher than 0% ($p < 0.0001$) and 0.1% ($p < 0.0001$) LA candies, but no difference was found between 0% and 0.1% (Figure 3.8 C). “Fattiness” rating of 1% LA candy was significantly higher than 0.1% ($p = 0.0133$)., but no difference was found between 1% and 0% as well as 0% and 0.1% (Figure 3.8 D).

Table 3.8: The effect of total fat intake on sensory ratings

| Quality | Estimate | StdErr | DF | t-value | p-value[1] |
|---|---|---|---|---|---|
| **Flavor** | 0.0188 | 0.0746 | 59 | 0.25 | 0.8019 |
| **Sweetness** | 0.0397 | 0.0875 | 59 | 0.45 | 0.6520 |
| **Bitterness** | -0.0194 | 0.0080 | 59 | -2.43 | 0.0180* |
| **Fattiness** | 0.0034 | 0.0902 | 59 | 0.38 | 0.7082 |

$LMM$: $\boldsymbol{Intensity}$ $=$ $Linoleic$ $\boldsymbol{TotalFatASA}$ $LayerSize3$[2]

StdErr: Standard Error; DF: Degrees of Freedom
[1] Significant level for the fixed effects contribute to the model on quality rating.
[2] Linear Mixed Model (LMM) was used to evaluate the main effects of linoleic acid concentration ($Linoleic$), total fat intake from ASA24 ($TotalFatASA$), and the fat layer size at 3 minutes ( $LayerSize3$) on each quality ratings ($Intensity$). $Intensity$ for Bitterness was transformed with square root when running the model.
$*p < 0.05$

Total fat intake had a significant effect on "Bitterness" (p = 0.0180). A negative association was found between "Bitterness" rating and total fat intake. Higher "Bitterness" rating was associated with lower total fat intake. No significant effects of total fat intake on "Overall Flavor," "Sweetness" and "Fattiness" ratings were found).

Table 3.9: The effect of layer size at 3 minutes on sensory ratings

| Quality | Estimate | StdErr | DF | t-value | p-value[1] |
|---|---|---|---|---|---|
| **Flavor** | 4.9131 | 16.073 | 59 | 0.31 | 0.7609 |
| **Sweetness** | -11.697 | 18.8668 | 59 | -0.62 | 0.5377 |
| **Bitterness** | 3.7629 | 1.7205 | 59 | 2.19 | 0.0327* |
| **Fattiness** | -9.3718 | 19.4298 | 59 | -0.48 | 0.6315 |

$LMM$: $\boldsymbol{Intensity}$ $=$ $Linoleic$ $TotalFatASA$ $\boldsymbol{LayerSize3}$[2]

StdErr: Standard Error; DF: Degrees of Freedom
[1] Significant level for the fixed effects contribute to the model on quality rating.
[2] Linear Mixed Model (LMM) was used to evaluate the main effects of linoleic acid concentration ($Linoleic$), total fat intake from ASA24 ($TotalFatASA$), and the fat layer size at 3 minutes ( $LayerSize3$) on each quality ratings ($Intensity$). $Intensity$ for Bitterness was transformed with square root when running the model.
$*p < 0.05$

Layer size at 3 minutes had a significant effect on "Bitterness" (p = 0.0327). A positive association was found between "Bitterness" rating and layer size at 3 minutes. No significant effects of layer size at 3 minutes on "Overall Flavor," "Sweetness" and "Fattiness" ratings were found.

### 3.6.1 Sensory Ratings Analyzed by Discrimination Tetrad Performance

Although the Chi-square test indicated we did not have true discriminators for LA tetrad test, the patterns we observed in the sensory ratings indicate people did experience some sort of sensation from LA candies. Thus, we also analyzed the sensory ratings by whether the participants passed or failed the tetrad tests. Results separated out by LA or high/low fat tetrad tests are shown in Tables 3.10 & 3.11. Notable patterns are present in both the LA and high/low fat tetrad pass/fail groups. Those who passed the test in both groups show increase ratings for "Fattiness" with increasing concentrations of LA. Those who failed the test, on the other hand, show increase ratings for "Bitterness" with increasing concentrations of LA. Importantly, these are not the same groups of people. As shown in Table 3.3, only 7 people overlapped for passing both LA and high/low fat tetrad tests.

#### *By LA Discrimination Tetrad Test*

Differences in sensory ratings of fatty candies were found based on whether people passed the discrimination test. Based on LA tetrad test, 36 participants failed while 26 participants passed the test. The effect of LA concentration (***Linoleic***) on quality ratings of fatty candies when sorted by LA discrimination test is shown in Table 3.10.

Table 3.10: The effect of LA concentration on sensory ratings by LA tetrad test

| **Quality** | **Fail LA ($n = 36$)** | | | | **Pass LA ($n = 26$)** | | | |
|---|---|---|---|---|---|---|---|---|
| | **0%** Mean (SD) | **0.1%** Mean (SD) | **1%** Mean (SD) | **p-value**[1] | **0%** Mean (SD) | **0.1%** Mean (SD) | **1%** Mean (SD) | **p-value**[1] |
| **Flavor** | 56.1 [a] (17.4) | 52.9 [a] (14.3) | 56.6 [a] (19.4) | 0.3953 | 54.7 [a] (19.2) | 54.5 [a] (25.1) | 54.4 [a] (20.6) | 0.9970 |
| **Sweetness** | 55.6 [a] (18.6) | 55.0 [a] (18.7) | 52.3 [a] (24.3) | 0.6048 | 59.5 [a] (19.1) | 57.0 [a] (25.6) | 58.6 [a] (22.3) | 0.8260 |
| **Bitterness** | 10.1 [a] (13.5) | 10.1 [a] (11.4) | 24.0 [b] (19.0) | <.0001* | 8.5 [a] (9.6) | 10.2 [a] (12.7) | 17.1 [a] (24.1) | 0.2541 |
| **Fattiness** | 53.4 [a] (21.8) | 48.9 [a] (22.9) | 55.2 [a] (23.3) | 0.1968 | 47.3 [ab] (19.3) | 44.0 [a] (20.0) | 52.3 [b] (20.9) | 0.0553 |

$LMM$: $\boldsymbol{Intensity} = \boldsymbol{Linoleic}\ Totalfat ASA\ LayerSize3$ [2]

LA: Linoleic acid; SD: Standard deviation

[1] Significant level for the fixed effects of linoleic acid concentration contribute to the model on quality rating

[2] Linear Mixed Model (LMM) was used to evaluate the main effects of linoleic acid concentration ($Linoleic$), total fat intake from ASA24 ($TotalfatASA$), and the fat layer size at 3 minutes ( $LayerSize3$) on each quality ratings ($Intensity$). $Intensity$ for Bitterness was transformed with square root when running the model

[ab] Within the same row of discrimination group, means without shared superscript differ (p < 0.05), adjusted by Tukey-Kramer

*$p < 0.05$

Among those who failed the LA tetrad tests, LA concentration had a significant effect on "Bitterness" rating ($p < 0.0001$). Higher "Bitterness" rating was associated with the highest LA concentration as shown in the mean ratings for each concentration. Whereas among those who passed the LA tetrad test, LA concentration had a trend on "Fattiness" rating ($p = 0.0553$). "Fattiness" was higher for 1% than 0.1% LA concentration. No significant effects of LA concentration on "Overall Flavor" and "Sweetness" ratings were found in either LA pass/fail groups. Box plots displaying "Overall flavor," "Sweetness," "Bitterness" and "Fattiness" rating are shown in Figure 3.9.

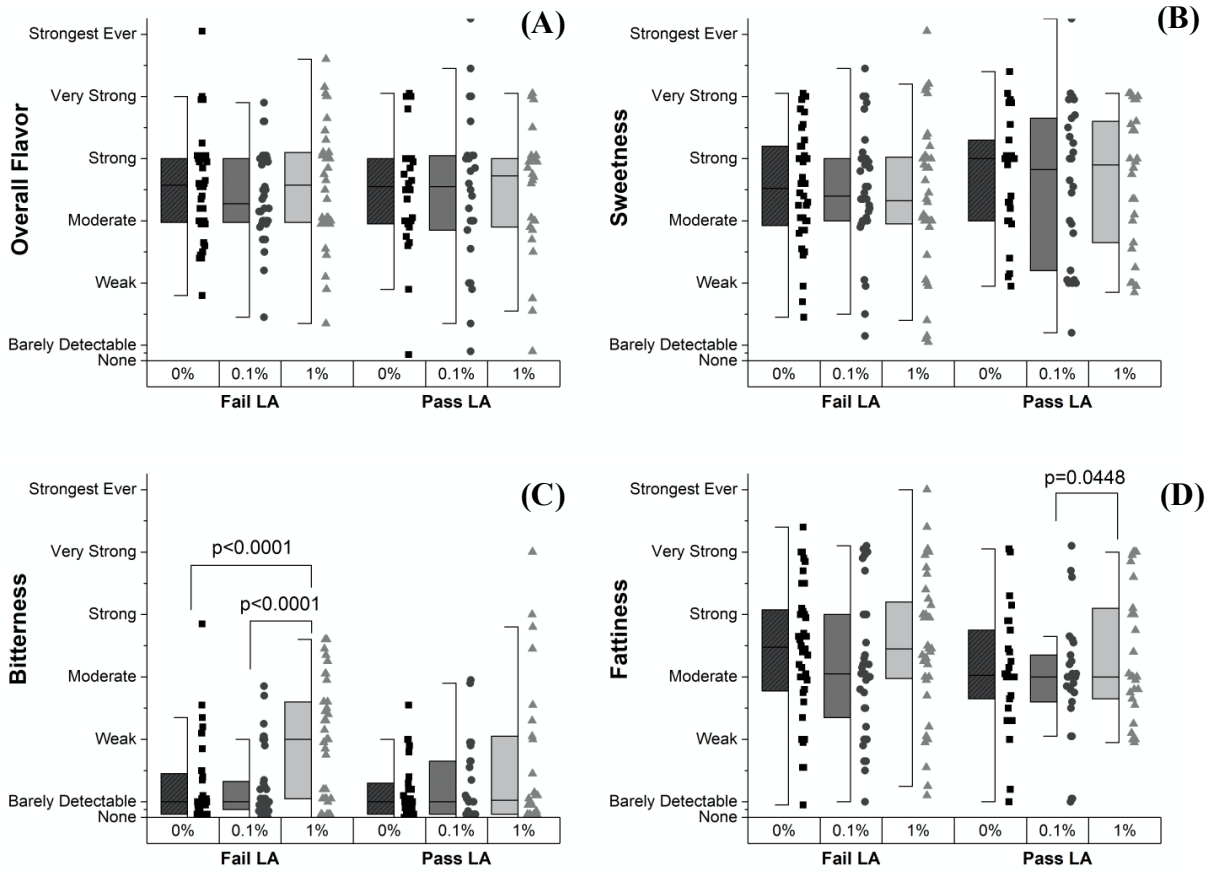


Figure 3.9: Box chart of quality rating sorted by LA tetrad test
(Boxes indicate 25-75%, whiskers indicate 5-95%, and horizontal line indicates median)

No significant effect was found on "Overall flavor" and "Sweetness" ratings (Figure 3.9 A, B). In unsuccessful LA discrimination group, "Bitterness" rating of 1% LA candy was significantly

higher than 0% ($p < 0.0001$) and 0.1% ($p < 0.0001$) LA candies, but no difference was found between 0% and 0.1% (Figure 3.9 C). Although only a trend ($p = 0.0553$) was observed for the overall effect of LA concentration on "Fattiness" in those who passed the LA tetrad test, post hoc comparisons show "Fattiness" rating of 1% LA candy was significantly higher than 0.1% ($p = 0.0448$)., but no difference was found between 1% and 0% as well as 0% and 0.1% (Figure 3.9 D).

### *By High/low Fat Discrimination Tetrad Test*

Based on the high/low fat discrimination test, 40 participants failed while 22 participants passed the test. Similar to looking at the data by whether participants passed or failed the LA discrimination test, the ability to detect fat content was associated with the quality experienced from fatty acids. The effect of LA concentration (***Linoleic***) on sensory ratings when sorted by high/low fat discrimination test is shown in Table 3.11.

Table 3.11: The effect of LA concentration on sensory ratings by high/low fat tetrad test

| **Quality** | **Fail High/Low Fat ($n = 40$)** | | | | **Pass High/Low Fat ($n = 22$)** | | | |
|---|---|---|---|---|---|---|---|---|
| | **0%** Mean (SD) | **0.1%** Mean (SD) | **1%** Mean (SD) | **p-value[1]** | **0%** Mean (SD) | **0.1%** Mean (SD) | **1%** Mean (SD) | **p-value[1]** |
| **Flavor** | 55.1 [a] (18.6) | 53.7 [a] (19.5) | 56.5 [a] (20.6) | 0.7026 | 56.2 [a] (17.3) | 53.3 [a] (19.7) | 54.2 [a] (18.6) | 0.7534 |
| **Sweetness** | 55.1 [a] (18.6) | 57.4 [a] (22.3) | 53.2 [a] (25.3) | 0.4200 | 59.3 [a] (19.3) | 53.0 [a] (20.8) | 58.1 [a] (20.0) | 0.3095 |
| **Bitterness** | 9.5 [a] (12.8) | 10.8 [a] (12.5) | 24.5 [b] (23.4) | <.0001* | 9.4 [a] (10.7) | 9.0 [a] (10.8) | 14.9 [a] (15.8) | 0.1921 |
| **Fattiness** | 48.6 [a] (22.0) | 46.0 [a] (20.0) | 50.1 [a] (23.6) | 0.4272 | 55.0 [ab] (18.4) | 48.3 [a] (24.9) | 61.0 [b] (17.7) | 0.0111* |

$LMM: \boldsymbol{Intensity} = \boldsymbol{Linoleic}\ \ TotalFatASA\ LayerSize3$ [2]

SD: Standard deviation

[1] Significant level for the fixed effects of linoleic acid concentration contribute to the model on quality rating

[2] Linear Mixed Model (LMM) was used to evaluate the main effects of linoleic acid concentration ($Linoleic$), total fat intake from ASA24 ($TotalfatASA$), and the fat layer size at 3 minutes ( $LayerSize3$) on each quality ratings ($Intensity$). $Intensity$ for Bitterness was transformed with square root when running the model

[ab] Within the same row of discrimination group, means without shared superscript differ ($p < 0.05$), adjusted by Tukey-Kramer

$*p < 0.05$

Among those who failed the high/low fat tetrad test, LA concentration had significant effect on "Bitterness" ratings. Higher "Bitterness" was associated with the highest LA concentration. Whereas among those who passed the high/low fat tetrad test, LA concentration had a significant

impact on “Fattiness” ratings. No significant impact of LA concentration on “Overall flavor” and “Sweetness” ratings was found in either high/low fat discrimination groups. Box plots displaying “Overall flavor,” “Sweetness,” “Bitterness” and “Fattiness” rating are shown in Figure 3.10.

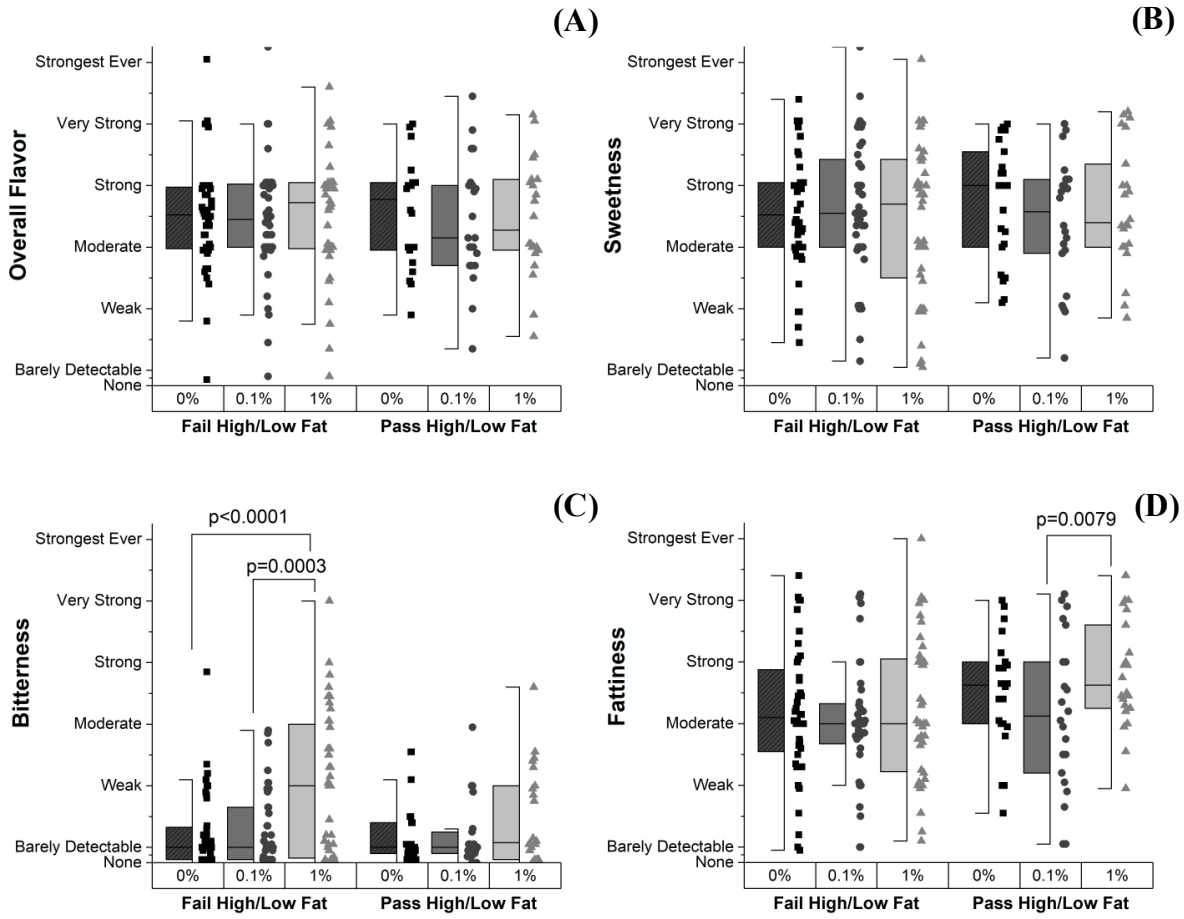


Figure 3.10: Box chart of quality rating sorted by high/low fat tetrad test
(Boxes indicate 25-75%, whiskers indicate 5-95%, and horizontal line indicates median)

No significant effect was found on “Overall flavor” and “Sweetness” ratings (Figure 3.10 A, B). For the group who failed the high/low fat tetrad test, “Bitterness” ratings of 1% LA candy were significantly higher than 0% ($p < 0.0001$) and 0.1% ($p = 0.0003$) LA candies, but no difference for “Bitterness” ratings were found between 0% and 0.1% (Figure 3.10 C). For the group who passed the high/low fat tetrad tests, “Fattiness” ratings of 1% LA candies were significantly higher than

0.1% (p = 0.0079) LA candies, but no difference was found between 0% and 1% as well as 0% and 0.1% (Figure 3.10 D).

### 3.6.2 Sensory Response and Diet

In the overall model (***Intensity = Linoleic TotalfatASA LayerSize3***), we found no effects of total fat intake (***TotalfatASA***) for "Overall flavor" and "Sweetness," and "Fattiness." However, for "Bitterness," total fat intake showed to be a significant effect (p = 0.0180, see Table 3.6). When we looked at individuals who passed or failed the LA or high/low fat tetrad discrimination tasks, these patterns changed slightly.

#### *By LA Discrimination Tetrad Test*

The effect of total fat intake on sensory ratings when sorted by LA discrimination test is shown in Table 3.12. Higher "Bitterness" rating was associated with lower total fat intake only among those who passed the LA tetrad test (p = 0.0032). No association was found among those who failed the test.

Table 3.12: The effect of total fat intake on quality ratings by LA tetrad test

| Quality | Fail LA ($n = 36$) | | | | | Pass LA ($n = 26$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Estimate | StdErr | DF | t-value | p-value[1] | Estimate | StdErr | DF | t-value | p-value[1] |
| **Flavor** | -0.0913 | 0.08784 | 33 | -1.04 | 0.3061 | 0.1659 | 0.1359 | 23 | 1.22 | 0.2344 |
| **Sweetness** | -0.0256 | 0.1106 | 33 | -0.23 | 0.8187 | 0.0885 | 0.1529 | 23 | 0.58 | 0.5684 |
| **Bitterness** | -0.0055 | 0.0106 | 33 | -0.52 | 0.6053 | -0.0393 | 0.0119 | 23 | -3.29 | 0.0032* |
| **Fattiness** | -0.0579 | 0.124 | 33 | -0.46 | 0.6453 | 0.1905 | 0.1358 | 23 | 1.40 | 0.1738 |

$LMM$: $\boldsymbol{Intensity} = Linoleic\ \boldsymbol{TotalfatASA}\ LayerSize3$ [2]

LA: Linoleic acid; StdErr: Standard Error; DF: Degrees of Freedom

[1] Significant level for the fixed effects of total fat intake contribute to the model on quality rating.

[2] Linear Mixed Model (LMM) was used to evaluate the main effects of linoleic acid concentration ($Linoleic$), total fat intake from ASA24 ($TotalfatASA$), and the fat layer size at 3 minutes ( $LayerSize3$) on each quality ratings ($Intensity$). $Intensity$ for Bitterness was transformed with square root when running the model.

*$p < 0.05$

***By High/low Fat Discrimination Tetrad Test***

The effect of total fat intake on sensory ratings when sorted by high/low fat discrimination test is shown in Table 3.13. Higher "Bitterness" ratings were associated with lower total fat intake only among those who failed the high/low fat tetrad test (p = 0.0226).

Table 3.13:The effect of total fat intake on quality rating by high/low fat tetrad test

| Quality | Fail High/Low Fat ($n = 40$) | | | | | Pass High/Low Fat ($n = 22$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Estimate | StdErr | DF | t-value | p-value[1] | Estimate | StdErr | DF | t-value | p-value[1] |
| **Flavor** | 0.00435 | 0.1017 | 37 | 0.04 | 0.9661 | 0.0427 | 0.1153 | 19 | 0.37 | 0.7152 |
| **Sweetness** | 0.0432 | 0.1240 | 37 | 0.35 | 0.7298 | 0.0287 | 0.1250 | 19 | 0.23 | 0.8208 |
| **Bitterness** | -0.0268 | 0.0113 | 37 | -2.38 | 0.0226* | -0.0090 | 0.0111 | 19 | -0.82 | 0.4238 |
| **Fattiness** | 0.0390 | 0.1220 | 37 | 0.32 | 0.7509 | 0.0120 | 0.1351 | 19 | 0.09 | 0.9300 |

$LMM$: $\boldsymbol{Intensity} = Linoleic\ \boldsymbol{TotalFatASA}\ LayerSize3$ [2]

LA: Linoleic acid; StdErr: Standard Error; DF: Degrees of Freedom

[1] Significant level for the fixed effects of total fat intake contribute to the model on quality rating.

[2] Linear Mixed Model (LMM) was used to evaluate the main effects of linoleic acid concentration ($Linoleic$), total fat intake from ASA24 ($TotalFatASA$), and the fat layer size at 3 minutes ( $LayerSize3$) on each quality ratings ($Intensity$). $Intensity$ for Bitterness was transformed with square root when running the model.

*$p < 0.05$

### 3.6.3 Sensory Response and Spit Layer Size

In the overall model ($\boldsymbol{Intensity} = \boldsymbol{Linoleic\ TotalfatASA\ LayerSize3}$), we found no effects of fat layer size at 3 minutes ($\boldsymbol{LayerSize3}$) for "Overall flavor" and "Sweetness," and "Fattiness." However, for "Bitterness," fat layer size at 3 minutes showed to be a significant effect (p = 0.0327, see Table 3.6). When we looked at individuals who passed or failed the LA or high/low fat tetrad discrimination tasks, these patterns changed slightly.

***By LA Discrimination Tetrad Test***

The effect of layer size on sensory ratings when sorted by LA discrimination test is shown in Table 3.14. A trend was found between bitterness ratings and fat layer size at 3 minutes for those who passed the LA test (p = 0.0568). Higher "Bitterness" rating was associated with larger emulsion

fat layer for this group. No association was found for other sensory ratings among other discrimination groups.

Table 3.14: The effect of fat layer size on quality ratings by LA tetrad test

| Quality | Fail LA ($n = 36$) | | | | | Pass LA ($n = 26$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Estimate | StdErr | DF | t-value | p-value[1] | Estimate | StdErr | DF | t-value | p-value[1] |
| **Flavor** | 29.8306 | 22.3121 | 33 | 1.34 | 0.1904 | -11.447 | 23.8778 | 23 | -0.48 | 0.6362 |
| **Sweetness** | 6.6409 | 28.1039 | 33 | 0.24 | 0.8147 | -26.979 | 26.8806 | 23 | -1.00 | 0.3260 |
| **Bitterness** | 3.3120 | 2.6951 | 33 | 1.23 | 0.2278 | 4.2142 | 2.1011 | 23 | 2.01 | 0.0568 |
| **Fattiness** | 6.8267 | 31.6733 | 33 | 0.22 | 0.8307 | -17.291 | 23.8595 | 23 | -0.72 | 0.4759 |

$LMM: \boldsymbol{Intensity} = Linoleic\ TotalFatASA\ \boldsymbol{LayerSize3}$ [2]

LA: Linoleic acid; StdErr: Standard Error; DF: Degrees of Freedom

[1] Significant level for the fixed effects of fat layer size at 3 minutes contribute to the model on quality rating.

[2] Linear Mixed Model (LMM) was used to evaluate the main effects of linoleic acid concentration ($Linoleic$), total fat intake from ASA24 ($TotalFatASA$), and the fat layer size at 3 minutes ( $LayerSize3$) on each quality ratings ($Intensity$). $Intensity$ for Bitterness was transformed with square root when running the model.

### *By High/low Fat Discrimination Tetrad Test*

The effect of layer size on sensory ratings when sorted by high/low fat discrimination test is shown in Table 3.15. For those who failed the high/low fat test, higher "Bitterness" rating was associated with larger emulsion fat layer at 3 minutes (p = 0.0417). No association was found for other sensory ratings among other discrimination groups.

Table 3.15: The effect of fat layer size on quality ratings by high/low fat tetrad test

| Quality | Fail High/Low Fat ($n = 40$) | | | | | Pass High/Low Fat ($n = 22$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Estimate | StdErr | DF | t-value | p-value[1] | Estimate | StdErr | DF | t-value | p-value[1] |
| **Flavor** | 0.03885 | 20.1692 | 37 | 0.00 | 0.9985 | 19.9863 | 29.6424 | 19 | 0.67 | 0.5083 |
| **Sweetness** | -6.8279 | 24.5993 | 37 | -0.28 | 0.7829 | -26.738 | 32.1281 | 19 | -0.83 | 0.4156 |
| **Bitterness** | 4.7156 | 2.2350 | 37 | 2.11 | 0.0417* | 2.8062 | 2.84260 | 19 | 0.99 | 0.3360 |
| **Fattiness** | -22.075 | 24.1991 | 37 | -0.91 | 0.3676 | 7.6037 | 34.7334 | 19 | 0.22 | 0.8291 |

$LMM: \boldsymbol{Intensity} = Linoleic\ TotalFatASA\ \boldsymbol{LayerSize3}$ [2]

LA: Linoleic acid; StdErr: Standard Error; DF: Degrees of Freedom
[1] Significant level for the fixed effects of fat layer size at 3 minutes contribute to the model on quality rating.
[2] Linear Mixed Model (LMM) was used to evaluate the main effects of linoleic acid concentration ($Linoleic$), total fat intake from ASA24 ($TotalFatASA$), and the fat layer size at 3 minutes ( $LayerSize3$) on each quality ratings ($Intensity$). $Intensity$ for Bitterness was transformed with square root when running the model.
*$p < 0.05$

## 3.7 Emulsifying Properties and Diet

Results of the type 3 test are summarized in Table 3.16 for the model:

$$\boldsymbol{Layer = TotalfatASA\ ProtASA\ CarbASA}$$

Table 3.16: Summary of the type 3 effects of the model

| Layer | Effect | Num DF | Den DF | F value | p-value |
|---|---|---|---|---|---|
| **At 0** | $TotalfatASA$ | 1 | 58 | 0.79 | 0.3778 |
| | $ProtASA$ | 1 | 58 | 3.96 | 0.0512 |
| | $CarbASA$ | 1 | 58 | 0.05 | 0.8306 |
| **At 3 min** | $TotalfatASA$ | 1 | 58 | 2.80 | 0.0997 |
| | $ProtASA$ | 1 | 58 | 0.40 | 0.5278 |
| | $CarbASA$ | 1 | 58 | 7.80 | 0.0071* |
| **Change 0-3 min** | $TotalfatASA$ | 1 | 58 | 14.26 | 0.6271 |
| | $ProtASA$ | 1 | 58 | 5.92 | 0.0092* |
| | $CarbASA$ | 1 | 58 | 4.78 | 0.0237* |

$LMM: Layer = TotalfatASA\ ProtASA\ CarbASA$[1]

Num DF: Numerator degree of freedom; Den DF: Denominator degree of freedom
[1] Linear Mixed Model (LMM) was used to evaluate the main effects of total fat intake ($TotalfatASA$), and protein intake ($ProtASA$), carbohydrate ($CarbASA$) from ASA24 on layer size at 0, 3 minutes or layer change from 0-3 minutes ($Layer$).
*$p < 0.05$

We found that protein intake had a trend for an effect on the fat layer size at time 0 (p = 0.0512). For layer size at 3 minutes, a trend was found for total fat intake (p = 0.0997), and a significant for carbohydrate intake (p = 0.0071). For the layer changes from 0 to 3 minutes, protein intake (p = 0.0092), and carbohydrate intake (p = 0.0237) were significant. See Table 3.12 for test statistics, degrees of freedom, F-value, and p-values for these models as reference.

To observe the direction of the significance, solution for fixed effects is shown in Table 3.17.

Table 3.17: Solution for fixed effects of the full model

| Layer | Effect | Estimate | StdErr | DF | t-value | p-value[1] |
|---|---|---|---|---|---|---|
| **At 0** | $TotalfatASA$ | 0.000768 | 0.000864 | 58 | 0.89 | 0.3778 |
| | $ProtASA$ | 0.001983 | 0.000996 | 58 | 1.99 | 0.0512 |
| | $CarbASA$ | 0.000076 | 0.000355 | 58 | 0.21 | 0.8306 |
| **At 3 min** | $TotalfatASA$ | 0.001205 | 0.00072 | 58 | 1.67 | 0.0997 |
| | $ProtASA$ | -0.00053 | 0.00083 | 58 | -0.64 | 0.5278 |
| | $CarbASA$ | 0.000826 | 0.000296 | 58 | 2.79 | 0.0071* |
| **Change 0-3 min** | $TotalfatASA$ | -0.00039 | 0.000793 | 58 | -0.49 | 0.6271 |
| | $ProtASA$ | 0.002465 | 0.000914 | 58 | 2.70 | 0.0092* |
| | $CarbASA$ | -0.00076 | 0.000326 | 58 | -2.32 | 0.0237* |

$LMM: Layer = TotalfatASA\ ProtASA\ CarbASA$[1]

StdErr: Standard Error; DF: Degrees of Freedom

[1] Significant level for the fixed effects contribute to the model on layer.

[2] Linear Mixed Model (LMM) was used to evaluate the main effects of total fat intake from ASA24 ($TotalfatASA$), total protein intake from ASA ( $ProtASA$) and total carbohydrate intake from ASA ( $CarbASA$) on layer size at 0, 3 minutes, or layer change from 0-3 minutes ($Layer$).

$*p < 0.05$

Significance was found only in carbohydrate intake for layer size at 3 minutes. Protein intake and carbohydrate intake were significant for layer changes from 0-3 minutes. At 3 minutes, carbohydrate intake (Estimate = 0.000826) was positively associated with larger fat layer size. To observe the changes from 0-3 minutes, protein intake (Estimate = 0.002465) was positively associated, whereas carbohydrate intake (Estimate = -0.00076) was negatively with fat layer size changes.

### 3.7.1 Emulsifying Properties Analyzed by Discrimination Tetrad Performance

In the overall model ($\boldsymbol{Layer = TotalfatASA\ ProtASA\ CarbASA}$), we found that the effects of macronutrient intake were associated with saliva's emulsifying properties at different time points. When we looked at individuals who passed or failed the LA or high/low fat tetrad discrimination tasks, these patterns changed slightly.

#### *By LA Discrimination Tetrad Test*

The effects of macronutrient intake including total fat, protein, and carbohydrate as well as medication and BMI on the expectorated fat layer size at 0, 3 minutes, and changes from 0-3 minutes when sorted by LA discrimination test are shown in Table 3.18.

Table 3.18: The effects of diet, medication, and BMI on layer size at 0, 3 minutes and overtime change from 0 to 3 minutes by LA tetrad test

| Layer | | Fail LA ($n = 36$) | | | | | Pass LA ($n = 26$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Effect | Estimate | StdErr | DF | t-value | p-value[1] | Estimate | StdErr | DF | t-value | p-value[1] |
| At 0 | $TotalfatASA$ | -0.00004 | 0.00124 | 32 | -0.03 | 0.9751 | 0.00171 | 0.00109 | 22 | 1.57 | 0.1305 |
| | $ProtASA$ | 0.00297 | 0.00163 | 32 | 1.82 | 0.0780 | 0.00039 | 0.00114 | 22 | 0.35 | 0.7326 |
| | $CarbASA$ | -0.00003 | 0.00057 | 32 | -0.05 | 0.9585 | 0.00029 | 0.00043 | 22 | 0.68 | 0.5011 |
| At 3 min | $TotalfatASA$ | 0.00003 | 0.00078 | 30 | 0.03 | 0.9724 | 0.00315 | 0.00124 | 20 | 2.53 | 0.0197* |
| | $ProtASA$ | 0.00167 | 0.00109 | 30 | 1.54 | 0.1342 | -0.00432 | 0.00149 | 20 | -2.89 | 0.009* |
| | $CarbASA$ | 0.00053 | 0.00035 | 30 | 1.49 | 0.1465 | 0.00076 | 0.00047 | 20 | 1.61 | 0.1234 |
| | $MedsYN$ | -0.05345 | 0.03491 | 30 | -1.53 | 0.1362 | 0.1686 | 0.06004 | 20 | 2.81 | 0.0109* |
| | $BMI$ | 0.00345 | 0.00299 | 30 | 1.15 | 0.2574 | 0.01239 | 0.00576 | 20 | 2.15 | 0.0437* |
| Change 0-3 min | $TotalfatASA$ | -0.00045 | 0.00108 | 32 | -0.42 | 0.6775 | -0.0004 | 0.00126 | 22 | -0.32 | 0.7529 |
| | $ProtASA$ | 0.00206 | 0.00141 | 32 | 1.46 | 0.1544 | 0.00269 | 0.00123 | 22 | 2.04 | 0.0537 |
| | $CarbASA$ | -0.00066 | 0.00050 | 32 | -1.33 | 0.1931 | -0.0007 | 0.00049 | 22 | -1.39 | 0.1793 |

$LMM: Layer = TotalfatASA\ ProtASA\ CarbASA$ [2] $(MedsYN\ BMI)$ [3]

LA: Linoleic acid; StdErr: Standard Error; DF: Degrees of Freedom

[1] Significant level for the fixed effects contribute to the model on fat layer.

[2] Linear Mixed Model (LMM) was used to evaluate the main effects of total fat intake from ASA24 ($TotalfatASA$), total protein intake from ASA ( $ProtASA$) and total carbohydrate intake from ASA ( $CarbASA$) on layer size at 0, 3 minutes or layer change from 0-3 minutes ($Layer$).

[3] Medications yes or no ($MedsYN$) and BMI kg/m$^2$ ($BMI$) were added to the model for layer size at 3 minutes.

*$p < 0.05$

At 0 seconds (immediately after the oil/water mixture was spat out), a positive effect of protein intake was found on the size of the fat layer (p = 0.078) among those who failed LA tetrad test. At

3 minutes, a positive effect of total fat (p = 0.0197), medication (p = 0.0109), and BMI (p = 0.0437), as well as a negative effect of protein intake (p = 0.009) was found on the size of the fat layer among those who passed LA tetrad test. For the fat layer size changes from 0 to 3 minutes, a positive trend of protein intake was found on the change of the fat layer (p = 0.0537) among those who passed LA tetrad test.

### *By High/low Fat Discrimination Tetrad Test*

The effects of macronutrient intake including total fat, protein, and carbohydrate consumption on the expectorated fat layer size at 0, 3 minutes, and changes from 0-3 minutes when sorted by high/low fat discrimination test are shown in Table 3.19 (BMI and medication use were tested but not significant, and so were removed from the model).

Table 3.19: The effects of diet on layer size at 0, 3 minutes and overtime change from 0 to 3 minutes by high/low fat tetrad test

| Layer | | Fail High/Low Fat ($n = 40$) | | | | | Pass High/Low Fat ($n = 22$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Effect | Estimate | StdErr | DF | t-value | p-value[1] | Estimate | StdErr | DF | t-value | p-value[1] |
| At 0 | $TotalfatASA$ | 0.00065 | 0.00125 | 36 | 0.52 | 0.6048 | 0.00009 | 0.00140 | 18 | 0.06 | 0.9508 |
| | $ProtASA$ | 0.00146 | 0.00124 | 36 | 1.18 | 0.2472 | 0.00346 | 0.00183 | 18 | 1.90 | 0.0742 |
| | $CarbASA$ | 0.00039 | 0.00045 | 36 | 0.87 | 0.3875 | -0.0008 | 0.00076 | 18 | -1.07 | 0.2995 |
| At 3 min | $TotalfatASA$ | 0.00079 | 0.00096 | 36 | 0.82 | 0.4149 | 0.00007 | 0.00098 | 18 | 0.07 | 0.9453 |
| | $ProtASA$ | -0.00172 | 0.00095 | 36 | -1.81 | 0.0781 | 0.00251 | 0.00128 | 18 | 1.96 | 0.0654 |
| | $CarbASA$ | 0.00157 | 0.00034 | 36 | 4.62 | <.0001* | -0.00093 | 0.00053 | 18 | -1.75 | 0.0969 |
| Change 0-3 min | $TotalfatASA$ | -0.00007 | 0.00114 | 36 | -0.07 | 0.9482 | 0.00006 | 0.00129 | 18 | 0.05 | 0.9634 |
| | $ProtASA$ | 0.00308 | 0.00113 | 36 | 2.74 | 0.0096* | 0.00097 | 0.00168 | 18 | 0.58 | 0.5717 |
| | $CarbASA$ | -0.00118 | 0.00004 | 36 | -2.92 | 0.0060* | 0.00011 | 0.00070 | 18 | 0.15 | 0.8793 |

$LMM: Layer = TotalFatASA\ ProtASA\ CarbASA$ [2]

LA: Linoleic acid; StdErr: Standard Error; DF: Degrees of Freedom
[1] Significant level for the fixed effects contribute to the model on layer.
[2] Linear Mixed Model (LMM) was used to evaluate the main effects of total fat intake from ASA24 ($TotalFatASA$), total protein intake from ASA ( $ProtASA$) and total carbohydrate intake from ASA ( $CarbASA$) on layer size at 0, 3 minutes, or layer change from 0-3 minutes ($Layer$).
$*p < 0.05$

At 0 seconds, a positive trend for an effect of protein intake (p = 0.0742) was found on the size of the fat layer among those who passed high/low fat tetrad test. At 3 minutes, a negative trend for protein intake (p = 0.0781) and a positive significance for carbohydrate (p < 0.0001) on the size of

the fat layer were observed among those who failed high/low fat tetrad test; a positive trend of protein intake ($p = 0.0654$) and a negative trend of carbohydrate intake ($p = 0.0969$) were found for the size of the fat layer among those who passed high/low fat tetrad test. For the fat layer size changes from 0 to 3 minutes, a positive association of protein intake ($p = 0.0096$) and a negative association of carbohydrate intake ($p = 0.006$) were found on the change of the fat layer among those who failed high/low fat tetrad test.

# CHAPTER 4. DISCUSSIONS

## 4.1 Introduction

The present study aimed to investigate the perception of fat and fatty acid and its association with habitual diet and saliva's emulsifying properties. To compare people with or without the ability to sense the fat or fatty acids, data were also analyzed separately based on the results of both LA and high/low fat discrimination tetrad tests. Analyses indicate that as a group, participants were unable to discriminate LA from plain candies or high- from low-fat ranch dressing. However, interesting patterns still emerged when analyzing sensory ratings for linoleic acid (LA) candies, as well as when analyzing the stability of emulsions created when swishing and spitting out an oil and water mixture. Some of these patterns were different among the groups of individuals who passed or failed the LA and high/low fat discrimination tasks, indicating that these individuals may sense or interpret the sensations differently. Moreover, some effects also emerged from the habitual diet.

Putting together all of the results, we found that:

- Linoleic acid concentration increased the perception of fattiness and bitterness in linoleic acid candies, especially at the highest concentration.
    - Those who passed the LA and high/low fat discrimination tests experienced more fattiness from the high LA candy.
    - Those who failed the LA and high/low fat discrimination tests experienced more bitterness from the high LA candy.
- Higher dietary fat intake may be associated with reduced bitterness from LA candies (particularly for those who passed the LA discrimination test or failed the high/low fat discrimination test).
- More stable emulsions in the expectorate may associate with higher bitterness (again, particularly for those who passed the LA or failed the high/low fat discrimination tests).
- More dietary protein was associated with less stable expectorated emulsions (smaller layers at time 3 minutes and larger changes over 3 minutes). This was particularly apparent for those who failed the high/low fat discrimination test.
- More dietary carbohydrate was associated with more stable expectorated emulsions (larger

layers at time 3 minutes, smaller changes over 3 minutes). This was particularly apparent for those who failed the high/low fat discrimination test.

- For those who passed the LA discrimination test, greater BMI, medication use, higher dietary fat intake, and lower protein intake were all associated with more stable expectorated emulsions. The reason for different effects in this specific group is unclear.

## 4.2 Sensory Ratings of LA Candies

Higher "Bitterness" and "Fattiness" were found to correlate with high LA concentration within the fatty candies, but not for "Overall flavor" and "Sweetness." These sensory ratings of LA candies were further characterized differently based on the ability to sense the fat based on the results of the discrimination tests (LA and high/low tetrad tests). Similar patterns were observed for those who passed either LA or high/low fat discrimination tests. People who passed the discrimination tests rated the sensation of LA candies more as "fatty"; whereas people who failed the discrimination tests rated them as more "bitter" rather than "fatty." Although we technically did not find true discriminators from the tetrad tests (based on the chi-square distribution tests), these consistent patterns among those who passed or failed the discrimination tests are intriguing. Note that people who passed the LA discrimination test were not the same group of people who passed the high/low fat discrimination. Only 7 participants overlapped among those who passed the tests. Parallel results in both groups indicate the experience from fatty acids may differ based on participants' oral sensitivity to fat content and fatty acids.

### *LA Concentration Influence on Sensory Ratings*

LA concentration was shown to influence "Bitterness" and "Fattiness" ratings of the fatty candies. Non-discrimination groups rated increasing "Bitterness" with higher LA concentration whereas discrimination groups rated increasing "Fattiness" with higher LA concentration instead. For those who passed the discrimination tests, perhaps the ability to better identify the fat (as either a higher fat dressing or as LA in the candies) might allow them to better characterize the LA sensation as "Fattiness." The fact that individuals who failed the discrimination tests rated increasing "Bitterness" with higher LA concentration is also interesting, as it indicates they certainly experience some sensation from LA, despite failing the LA discrimination task. It appeared that

the differences in "Bitterness" rating we observed were not substantial enough to be used as a judgment to pass the discrimination tests. This may be further explained by the mean ratings of "Bitterness" among those who failed the tests, whose ratings were near the tick labels, "Weak" or "Barely detectable." Additionally, the characterization of the sensation of LA as "Bitter" is interesting. Prior work has shown people, as a group, differentiate oleogustus from bitter sensations (Running et al., 2015). Yet, even in that early work, there was substantial overlap among some of the bitter tastants and the long chain fatty acids. As fatty acids are generally considered unpleasant (Running et al., 2015) and people tend to label unpleasant taste they experienced into negative descriptors such as "bitterness" (Grosch & Laskawy, 1984). Perhaps people who were less sensitive to fat still sensed some unpleasantness in the LA candies and labeled it as bitter. People who passed the discrimination tests rated the taste of the LA higher for "Fattiness," but we do not know whether the descriptor is experienced or defined the same among all our individuals. "Fattiness" is generally considered as more of a textural sensation (de Wijk et al., 2011; Malone et al., 2003; Stokes et al., 2013). Participants in our study were not trained on how to define "Fattiness," but this was intentional, so that we could observe how participants would rate the samples when unbiased. It is unlikely that most participants had any experience with the sensation of oleogustus in isolation, so though a select few participants may have been involved in prior experiments at Purdue University studying this taste (Cheon & Mattes, 2020, 2021; Running et al., 2015; Running & Mattes, 2014, 2015). Nonetheless, given the remote testing environment of our work, and the disruptions due to the pandemic, we doubt our participants had enough experience or training with oleogustus to drive the difference in labeling the sensation as "fatty" vs "bitter." A few participants raised questions about the definition of "Fattiness" during the tasting session, but were instructed to use their own judgement. Potentially, those who passed versus failed the tests may be "dumping" the sensation of the oleogustus into different descriptors. "Dumping effects" occur when a salient attribute is experienced, but no descriptor is offered by the sensory survey into which the participant can rate that sensation; so, the participant ends up raising their rating for a different attribute (Clark & Lawless, 1994; Lawless & Heymann, 2010). It appeared that people who failed the tetrads were dumping the unpleasant oleogustus sensation into the descriptor "Bitterness," while those who passed the tetrads placed the sensation in "Fattiness."

While the effect of LA concentration was shown to influence "Bitterness" and "Fattiness," we did not see a significant difference with the lower LA concentration (0.1%) but only with the highest

LA concentration (1%) in our results. We expected to see a linear relationship between quality ratings and all the LA concentrations we tested. However, 0.1% LA was not significantly different from 0% LA in fatty candies either "Bitterness" or "Fattiness." The 0.1% LA candies used in our study should have been above the detection threshold according to the comparable concentration of linoleic acid in other studies (Chalé-Rush et al., 2007b; Chevrot et al., 2014; Mattes, 2009a; Running & Mattes, 2015). Possibly, due to the methodological differences, such as sample matrix (our sample stimulation was made in solid form while others in liquid), the detectable level may be higher in our solid candies. Note that we did not train participants to rate the fatty acid taste before the remote tasting session. Even though we asked participants to familiarize taste differences they experienced from fatty acids, this is very minimal "training" for an unfamiliar sensation. Failure to observe a linear relationship in the "Fattiness" rating might be attributed to the fact that participants are generally naive to fatty acid taste, and untrained with how to use the descriptor of "fattiness." Looking at the data, error bars of 0% "Fattiness" among those who passed or failed the LA test large, indicating inconsistency across participants in how to characterize the plain candy (See Figure 3.9 as reference). Thus, it is critical to keep in mind that the term "Fattiness" measured here and in other studies may differ in its definition and not refer to the exact same percept. More studies are required to confirm whether the patterns we observed for difference in rating LA candies as bitter versus fatty are due to oral biochemical or psychological differences. Our results support the concept that descriptors like "Fattiness" do not entirely refer to the fatty acid taste; thus, a specific term, such as oleogustus, is needed to evaluate the fatty acid taste (Running et al., 2015) and training would help make these ratings more consistent (Running & Mattes, 2014). It is also possible that some participants used "Fattiness" to refer to whether they liked the samples as large variability seen in the 0% (plain candies). More studies would be needed to confirm the fatty sensation experienced among people may refer to different attributes.

### *Dietary Fat Intake Influence on Sensory Ratings*

The ability to sense the fatty acids or the fat content was shown, for some participants, to be negatively associated with the consumption of dietary fat. When sorted by whether they passed or failed the LA discrimination test, a higher "Bitterness" rating was correlated with lower total fat intake only among those who passed the test. It seemed that lower exposure to dietary fat may influence participants' experience from LA candies as more bitter. Although LA concentration did

not have a significant effect on “Bitterness” rating for the group of people who passed the LA test, within this group those with a lower total fat intake tended to rate “Bitterness” higher. However, the effect of total fat consumption only appeared to be significant for those who passed the LA discrimination test, as the same pattern in not apparent among those who failed the LA discrimination test. When grouping participants based on the high/low fat discrimination test, a higher “Bitterness” rating was correlated with lower total fat intake only among those who failed the high/low fat test. This is backwards from the observations for those the LA discrimination test. Notably, in the high/low fat non-discriminators group, a higher “Bitterness” rating was also associated with higher LA concentration. No effect of total fat intake was found among those who passed the high/low fat test. Additionally, no overall different in fat intake was observed between those who passed or failed either discrimination test, so this is unlikely to be due to general differences in diet among discriminator and non-discriminators. This implies that the dietary effect of lower fat intake increasing sensation of fatty acids may not be universal.

A negative association observed in our study between total fat intake and “Bitterness” rating indicated dietary exposure to fat may influence the bitter sensation from the fatty candies for some participants. This is in partial agreement with other studies showing that that higher fat intake may attenuate the taste sensitivity of fatty acid (Stewart et al., 2010; Stewart, Newman, et al., 2011) although for that study the phenomenon was only observed in the lean population. In our study, dietary influence on sensory ratings was shown to be different when analyzing by whether participants passed or failed the LA or high/low fat discrimination test. The ability to sense the fat content (high/low fat discrimination test) may not completely be correlated with the ability to taste the fatty acid (LA discrimination test). In another study, individuals who were more sensitive to fatty acid were better at differentiating fat content within custards, and those individuals also consumed less dietary fat. (Stewart et al., 2010). In our current study, the ability to detect the fat content from ranch dressings and LA from fatty candies did not overlap. A negative effect of dietary fat intake was only associated with the LA discriminator group and high/low fat non-discriminator group. Perhaps the influence of dietary fat on the sensory response from fatty candies may occur in certain groups of people. This has been observed in some prior work, as fat tase sensitivity was only modulated by dietary change in lean but not overweight/obese population (Stewart & Keast, 2012). The detection of oleogustus is influenced by various contributing factors

such as genetics, sex, BMI and diet (Running et al., 2013). Indeed, our study shows that saliva may play a role in changes the structure of fat in water emulsions, and that diet may influence the stability of those structures (more below). This in turn could modulate how individuals detect oleogustus, but those effects could be small and thus more subjects would be required to fully uncover the relationships.

### *Saliva's Emulsifying Effect on Sensory Ratings*

Fat layer size at 3 minutes was selected in the model since its distribution was more spread out than other time points (0, 30 seconds, 1 minute). Fat layer size at 3 minutes may be interpreted two different ways regarding saliva's emulsifying properties. Larger fat layer size at this time implies that individual's saliva was able to form an emulsion within the mouth, but also that the emulsion was fairly stable. The effect of fat layer size at 3 minutes was found to be correlated with only the "Bitterness" rating. The impact was again group-specific when analyzed by the LA or high/low fat discrimination tasks. A positive trend was observed among those who passed LA discrimination test and a positive effect was found among those who failed the high/low fat discrimination test. In both these groups, a larger fat layer size at 3 minutes was shown to be associated with higher "Bitterness" ratings. This implies that better emulsifying properties of saliva (larger fat layer) may be associated with the ability to sense the "Bitterness" from fatty candies. This could be because emulsification increases the surface area of oil droplets, as one large layer of a few large droplets have less surface than thousands of small droplets. The increased interface aids in the efficiency of chemical reactions, such as lipolysis (Bodewes et al., 2015). This effect is seen later in the gastrointestinal tract as well, as digestion is more efficient for finer emulsions in the intestines (Dhillon et al., 2016).The presence of lingual lipase in the oral cavity may be related to the oleogutus by regulating the concentration of salivary fatty acid (Feron & Poette, 2013; Neyraud et al., 2017) and increases the chance of fatty acid to be detected by the taste receptors. Thus, the smaller droplets that would be maintained in the better emulsion at 3 minutes could allow for more lipase to act on fatty foods. While we only observed the emulsion in the fat/water mixture that was expectorated, we hypothesize the same emulsifying properties of saliva would be present while eating the fatty candies. Thus, individuals whose saliva formed better and more stable emulsions would likely disperse the fat in the candies better as well, which could increase the ability of lipase

to act on fat or the access of transporters for fatty acids to access these molecules and deliver them to taste cells.

Thus, some individuals who were unfamiliar with fatty acid taste may describe their unpleasant experience from oleogustus as bitter. Bitterness ratings of plain fatty candies in our study was very low, so it is likely that bitterness in 0.1% and 1% LA candies is due to the fatty acids, not some other bitter compound. Assuming the "Bitterness" may be associated with the oleogustus people experienced, a better emulsion (smaller fat droplets with higher total surface area) would lead to a higher chance for fat to interact with the oral environment. This could also increase the chance for fat to be detected. The tongue surface is hydrophilic once covered with saliva (Ranc et al., 2006). Since lipids are hydrophobic, in order to carry oil droplets to interact with salivary components as well as perceived by the receptors, a higher emulsifying ability creates more accessibility for oil droplets to be sensed. Per our results, a more stable emulsion (likely with more salivary proteins) may generate a stronger sensation from fatty acids due to the increased surface area of the dispersed lipid phase.

However, one study revealed that protein-poor (unstable) emulsions would cause more oil droplets retained on the tongue compared with protein-rich (stable) emulsions (Dresselhuis et al., 2008). Thicker coatings of lipid deposited on the tongue are suggested to lead to a stronger fatty or lubricating mouthfeel sensation (Pivk et al., 2008). However, mouthfeel is a textural, not a chemical, sensation. Potentially, unstable emulsions lead to greater textural contributions and stable emulsions to more taste sensations. Additionally, a longer exposure time due to lipid retention on the surface of the oral environment could potentially enhance fat perception through texture but suppress taste. If the fat forms as large layer (less surface area, more textural sensory contribution), this creates a hydrophobic barrier that could block hydrophilic activities or access to receptors, whereas small oil droplets increase surface area for interaction and do not block access to receptors. On the other hand, fat retention on the tongue could also lead to adaptation, reducing fat sensitivity. Continuous stimulation by a fat layer on the taste and mechanoreceptors on the tongue may result in less sensitivity to detect oral fat alteration (Camacho et al., 2015). Overall, our findings are more consistent with the concepts that either: 1) better dispersed/more stable

emulsions increase fatty taste sensations through increasing surface area, and/or 2) unstable emulsions induce fat layers on the tongue which cause adaptation and reduced fatty taste sensations.

It is unclear why the impact of saliva's emulsifying properties is group-specific, to those who passed or failed our LA or high/low fat discrimination tasks. For LA discriminators, a trend of larger fat layer size at 3 minutes was associated with a higher "Bitterness" rating. As LA tetrad test was conducted to evaluate whether people were sensitive to fatty acid taste, emulsifying properties could influence the ability to sense the fatty acids. On the other hand, a positive association with bitterness was also found among non-discriminators of high/low fat. People who were less sensitive to the fat content within ranch dressings were able to experience some "Bitterness" from fatty candies, and this phenomenon may be explained by saliva's emulsifying properties. Perhaps the emulsion being more stable masked the fat content differences (mostly textural) but allowed these individuals to experience the taste sensation differences. However, why they characterized the taste as bitter rather than fatty is unknown. Perhaps, rather than these effects of more stable emulsion associating with greater bitterness truly being group-specific, this may be a more general trend that we only had the power to observe in certain subgroups. Additionally, the statistical analysis indicates we may not have had true discriminators of high/low fat or of LA to plain candies; this may suggest that the patterns we observed for saliva emulsifying effects on taste sensations may be due to a subgroup we have not yet fully characterized.

Notably, we do not know how well our testing protocols for saliva's emulsifying properties mimicked the actual in-mouth emulsifying actions of saliva, since the layer sizes and changes were observed outside the oral cavity. Emulsification can be accomplished by biochemical as well as mechanical actions (Bodewes et al., 2015). Biochemical emulsification can be achieved by interacting with intrinsic components, such as salivary proteins, while mechanical emulsification occurs through mastication and shearing forces that force larger fat droplets to disperse into smaller ones. In our study, the wide variability of emulsification we observed may have been caused by different swishing patterns and thus different shear forces by each participant. In addition, compared with the duration of oral processing, which usually takes only a few seconds before swallowing, the emulsifying properties observed at 3 minutes in our study may not be in accordance with real-life oral processes. Although we recorded the layer size at additional 30

seconds and 1 minute as well, the distribution of the layer size across individuals was more widely distributed at 3 minutes, which allowed us to observe greater variability and thus be more likely to find distinct patterns in the data. More controlled methods should be performed to better understand the real-time effect of emulsification and the perceptual experience from fat, such as mimicking the oral process of mastication by using a chewed stimulus to collect the saliva. By having participants to chew, the shear force might be more controlled and more similar to the real-live eating experience compare to swishing. Additionally, saliva could be collected separately and added to an oil/water mixture. This would allow us to control the emulsification shearing forces. However, it would also stimulate different saliva than swishing, as the oil/water mixture would not actually interact with oral surfaces. A variety of these techniques could be pursued in future studies to establish more details on how saliva emulsifies oil/water mixtures.

### 4.3 Factors Associated with Saliva's Emulsifying Properties

To evaluate factors that may influence or associate with saliva's emulsifying properties, we analyzed the fat layer size at 0 minutes, 3 minutes, and the changes over this period of time. Fat layer size at 0 minutes we would expect to reflect the initial ability of saliva to emulsify, whereas layer size change over time may be interpreted as the stability of the emulsion. As mentioned above, layer size at 3 minutes may be the combination of both initial ability and stability over time. A positive trend association was found between protein intake and layer size at 0 minutes. When sorted by performance on the LA discrimination task, a positive trend of protein intake was only found among LA non-discriminators. In contrast, when sorted by high/low fat tetrad, a trend was shown among those who passed the test. We suspect these effects by groups may not be genuinely attributed to the ability to discriminate, and instead may simply reflect less power to find effects within all subgroups (patterns of effects are in the same direction in other groups, but the effect is not significant). In other words, we hypothesize a general effect of higher protein intake on saliva's initial emulsifying ability is more likely than an effect specific to the subgroups. Our work does not identify salivary proteins responsible for the emulsification, nor how those proteins may specifically reflect dietary intake of protein. In rats, high protein consumption significantly reduced the total salivary protein content (Kołodziej et al., 2017). As more salivary protein is expected to create better emulsions in a food bolus, this pattern in rats may be opposite what we would expect to find in salivary protein content for our study observed in humans. Yet no research

has directly looked at salivary protein content, dietary protein intake, and saliva's emulsifying ability all at once in a human trial. More studies would be needed to confirm the relationship between dietary protein, salivary proteins, and saliva's emulsifying properties.

For the layer size change from 0 to 3 minutes, lower protein and higher carbohydrate intake significantly associated with increased stability of the emulsions (less change over 3 minutes). When sorted by LA tetrad, only a trend was found between higher protein intake and greater layer size change (less stability) among those who passed the LA tetrad. Higher protein and lower carbohydrate intake were also associated with larger layer size change (less stability) among those who failed the high/low fat tetrad. Again, we hypothesize these group effects are not truly unique to the subgroups, and that the pattern might be observed in other groups with larger and more diverse samples of subjects (patterns of effects in other groups are generally in the same direction but not significant). Studies have shown that mucin and amylase may be responsible for emulsion destabilization (Dresselhuis et al., 2008; Hu et al., 2019; Sarkar et al., 2009; Silletti et al., 2007; Vingerhoeds et al., 2005) and the secretion of mucin and amylase may be influenced by nutritional changes. Salivary amylase enzymatic activity was positively associated with intake of starchy foods and plant-based fatty foods (Louro et al., 2021). This would imply that carbohydrate in the diet may associate with greater amylase secretion, which could destabilize emulsions in the mouth. This would be reversed from our observations, where greater carbohydrate intake was associated with more emulsion stability (larger layer at 3 minutes and less change over time). However, the published studies on amylase and mucin effects on emulsions use mixtures that are already stabilized with commercial emulsifiers. Our study had no emulsifier present—all emulsifications had to come from the saliva and oral shearing forces. Potentially, salivary components may complete with or bind the commercial stabilizers, leading to different effects on pre-stabilized emulsions versus unstabilized mixtures of oil and water. This should be investigated in future research.

When we looked at emulsion layer size at 3 minutes, higher fat, lower protein intake, no medication, as well as higher BMI were significantly associated with larger layer size (more stable over time). Medication tends to reduce salivary flow, which will also change the saliva's composition (Aliko et al., 2015; Bardow et al., 2001; Baum, 1981). Our study did not have the power to investigate

specific medications' effects, but we hypothesize that the driver of medications influence on salivary emulsions is due to changes in salivary flow due to changes in hydration/dryness induced by medications (Aliko et al., 2015; Bardow et al., 2001; Baum, 1981). Higher BMI has also been shown to correlate with lower salivary flow rates (Modéer et al., 2010; Rabiei et al., 2016). This lower salivary flow would lead to higher protein concentration in secreted saliva, which could correlate with our finding of higher BMI associating with greater emulsion stability at 3 minutes. Importantly, this model with medication use and BMI was likely over-fitted (too many factors included, leading to artificial significance). However, the effects of BMI and medication use should be investigated further in studies that can properly balance and control for these variables. Medication and BMI were only included in the LA model when analyzing the layer size at 3 minutes since they were only significant in that model. Removing those two factors from the LA models above does not change patterns of significance in the macronutrient factors.

Overall, dietary fat, protein, and carbohydrate were all associated with saliva's emulsifying properties, just at different time points or time changes. Medication usage and BMI may also take part in influencing oral emulsification. The stronger and more consistent patterns, however, indicate that less dietary protein and more carbohydrate likely correspond to better emulsion stability. In addition, the associations among diet and saliva's emulsifying capabilities may differ in respect to people's sensitivity to fatty acids (LA tetrad) and fat content (high/low fat tetrad); however, more work should test this further, as the lack of effect in some groups may be due to lack of power to observe associations rather than true lack of effects.

### 4.4 Conclusion

Details on associations among fat perception, habitual diet, and saliva's emulsifying properties have not been fully elucidated. In this current study, we provide some new evidence on the potential relationships among these three factors. Discriminators of high and low fat, as well as LA from plain candy, showed higher ratings for "Fattiness" with increasing concentrations of linoleic acid. In contrast, non-discriminators showed higher ratings for "Bitterness" with increasing concentrations of linoleic acid. Future work will need to confirm whether this pattern is an oral biochemical or psychological difference. This current study also offers a preliminary look at both total fat intake and saliva's emulsifying effect on the perception of fat. The effect of

lower habitual fat intake and higher oral emulsification was shown to correlate with higher "Bitterness" ratings from the fatty candies, but this was only observed for LA-discriminators and high/low fat non-discriminators. Moreover, lower protein and greater carbohydrate intake seemed to associate with the greater formation and stability of oral emulsions, particularly in individuals who failed the high/low fat discrimination task. Other factors such as total fat intake, medication usage, and BMI were mixed. Future studies will need to evaluate the causative relationships to better understand the perceptual variation of fat due to these factors. While some connections on the oral sensation of fat, habitual diet, and saliva's characteristics were observed in the current research, clearer association will be validated as more data becomes available and methods improve to unveil the variability of fat perception.

# CHAPTER 5. LIMITATION & FUTURE WORK

## 5.1 Limitations

### 5.1.1 Remote Testing Session

The biggest limitation of this study was conducting a sensory experiment during the COVID-19 pandemic. All study protocols needed to maintain social distance and methods were adjusted to meet regulations and promote safety. Since the sensory data were collected remotely through video meetings, results may differ from traditional, tightly controlled sensory environments. Moreover, to minimize issues with the stability of samples in our take-home kits, we used commercially available products where possible. These commercial products were more stable and did not require refrigeration, however, they had more complex formulations than samples we would have generated in the laboratory kitchen. Thus, the extra ingredients, particularly for the ranch dressings, may have masked some effects that would be more apparent with less tightly controlled emulsions.

Remote testing sessions were held between 8 AM to 6 PM every Thursday and Friday during the data collecting period. Since salivary flow and composition are influenced by circadian rhythms (Dawes, 1975), it is important to note that we did not specifically control this factor in our testing protocol. Although some associations were discerned in respect to the perception of fat, habitual diet, and the characteristics of saliva, future studies should investigate whether effects are consistent, especially within-subjects, across different times of the day.

#### *Fatty Acid Stimulation Matrix*

Oleogustus stimulus was made into solid candies to allow easier storage and improve sample stability for the remote tasting session. For most studies of oleogustus, samples are liquid emulsions, which can result in stronger flavor release compared to solids (Ammari & Schroen, 2018). Due to the different forms of the samples from other studies, the detectable concentration for the fatty acid may not be perfectly comparable to other studies. This may explain why the 0.1 % linoleic acid (LA) candy sensory ratings were not significantly different from the no LA candy. In addition, the base matrix we used was white melting wafers which already contain their own flavor

and fat. This non-neutral background flavor is currently unique to our study, and without additional research, we cannot be certain the background flavor did not shift perception of oleogustus in some way. Potentially, fatty acids may sensitize taste receptors cells to other tastes, or other tastes could sensitize receptors to fatty acids. Current research does not show strong evidence that fatty acids shift perception of other tastes (Mattes, 2007), but whether other tastes shift fatty acids sensation, especially at supra-threshold concentrations, is not well studied.

Additionally, the solid fats of our candies may have been more difficult to clear from the mouth. This could have contributed to variability in sensory ratings, as well as to the difficulty in successfully completing the tetrad discrimination task.

### 5.1.2 Spit Image Analysis

#### *Layer Size Evaluation*

Our strategy to evaluate the emulsifying properties of saliva by measuring the emulsion layer size from images is a novel method. While we designed a photo box to control the position of each sample and equipment as consistently as possible among participants, we learned through this study that we need to provide a light in order to better standardize lighting and shadows. The quality of the images varied across individuals since the procedure was completed by participants without in-person guidance. We did set up a specific angle of photo requirements in the photo-taking app (TADA). However, the images submitted by participants still had some issues with consistency, especially for lighting and color. For analysis, we used the relative length of the fat layer and the total sample to avoid the angle of the photo causing bias in the sample measurement. Some practice photos in advance and more detailed instructions may help improve the image quality in future studies.

Overall, the method we used to observe the emulsification properties of saliva successfully showed variability among subjects. Future studies would benefit from additional efforts to: i) try to avoid backlight or single light sources in photos, ii) put all unnecessary items away from the photo box to avoid any possible reflections, iii) make sure color strip and fiduciary marker are not blocked or cropped in the image, iv) ensure camera view is parallel with the photo box.

### *Emulsifying Characteristics Consensus*

To evaluate the emulsion characteristics of the fat layer, three research personnel rated the images for the numbers of distinct layers observed in the spat-out sample images (no separation vs two layers formed), distinctness of the line between layers (no distinct line vs blurry line vs clear separation), the opacity of the top layer (very clear vs intermediate vs very opaque), and homogeneity of the top layer (distinct bubbles/droplets vs intermediate vs homogenous). Once again, due to the quality of the images and the screen monitors used, it increased the challenges in arriving at consensus for these measurements. In addition, classifying the photos into the categories of each rating was a subjective task. Ratings like layer line distinctness, opacity, and homogeneity were not always rated the same by each member of the lab, and so consensus had to be reached through discussion. Some example photos that caused more disagreement are shown in Figure 5.1. Image quality may be the main issue when rating the characteristics of the samples. Future work can explore more analytical-based methods for assessing features of the expectorated emulsions.

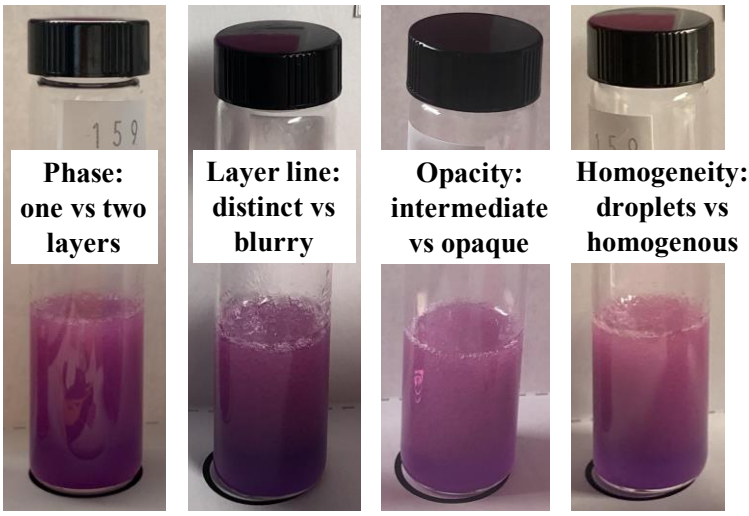


Figure 5.1: Example photos that caused disagreement

### *Color Analysis from the Fat Layer*

We used HSV value to quantify the color of the upper layer of the emulsion. The first step was to select the area for later calculation of the HSV mean. To avoid any reflection or shadow that appeared on the target area, we manually selected the area for analysis. Since the total number of selected pixels was different between images, it caused inconsistency for the later calculation. Each

pixel within the selected area was included to calculate the mean HSV as a representative color of the fat layer. The color range of our fat layer (pinkish, purplish) was around Hue (H from HSV) 270 – 30). However, we still had a wide range of colors observed in the experiment, and these colors did not match our color map (provided in the background of the photo setup) as well as we had hoped. It is still inconclusive regarding which methods would be more reliable in our color analysis. In future studies, controlling lighting better may help in improving the color analysis. Additionally, in-person research could analyze the color more directly with spectrophotometric methods.

The color analysis of the top layer of the emulsion layer was measured in two ways. The first method was using the color strips with various color scales that we attached to the background of the photo box as a reference for the color of the fat layer. Although we made our best guess of how varied the colors would be, we still did not fully cover all the color range we observed which makes the comparison more challenging. The second method was to correct the lighting conditions which were varied among all participants. By using the fiduciary marker attached to the back of the photo box, images were adjusted to a similar lighting condition. However, the adjustment may deviate from its original color which causes some challenges when comparing one color to another since the correction method, the von-Kries model, is a linear transformation. In the real-world situation, the lighting condition cannot simply transform by one model and fix all confounding factors. In this case, the correction may not be accurate. An example of comparing corrected Hue and its visual color is shown in Figure 5.2. By looking at the image, although the lighting condition was calibrated using the methods as mentioned, the condition was still visually different (The left image was more under warm light; the right image was under white or natural light). In addition, Hue 2.5 (indicate mid red) and Hue 293.2 (indicate mid magenta) were widely varied. Importantly, it may not be reliable to pick only one representative value to account for the whole fat layer color since some of the fat layers were non-homogenous. Future work could address how to assess the color of non-homogenous samples, as these were relatively common in our images.

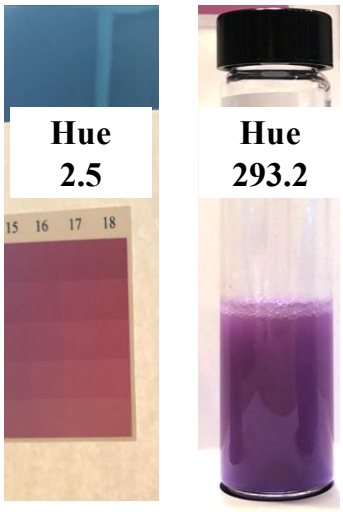


Figure 5.2: Example of corrected Hue and visual color

## 5.2 Future Works

### 5.2.1 Identify Salivary Proteins Responsible for Oral Emulsification

From our current study, saliva can aid in the emulsification of oil/water mixtures. A preliminary study from Glumac et al. revealed that proteins with molecular weights around 27 and 55 kDa were depleted after oral emulsification occurs, and these authors suggested that salivary proteins within this range may contribute to emulsion formation (Glumac et al., 2019). Lipocalin-1 was found in saliva and can bind to small hydrophobic molecules such as fatty acids to aid transportation in the hydrophilic environment (Neyraud, 2014). The molecular weight of the lipocalin family is around 18–40 kDa (Dartt, 2011) which partly overlaps with the range of proteins that were speculated in Glumac et al.'s study. Furthermore, it is also possible that depends on the type of fat (solid vs liquid, for example) that was used in the sample, different salivary proteins be involved in oral emulsification. Proteomics or targeting protein analysis with assays may prove us with more details on the possible functionality of different salivary proteins in the oral emulsification process. Studies could observe which proteins are depleted from the whole saliva when mixing with the fat/water mixture, as most protein analysis methods work better for aqueous samples. The proteins that are most involved in emulsification would partition into the fat layer, depleting the amount left in the aqueous phase.

### 5.2.2 The Association Between Saliva's Destabilizing Effect and Fat Perception

Perception of fat emulsions may change along with emulsion structure alteration during oral processing. Dispersed oil droplets in the stable emulsion may aggregate in the oral environment when saliva is introduced. A study has shown that saliva-induced emulsion destabilization was associated with sensory perception including creaminess, fattiness, and thickness (Vingerhoeds et al., 2009). However, whether saliva's destabilizing effect may influence oleogustus as well has not been tested. Moreover, different emulsifiers behave differently when interacting with saliva (E. Silletti et al., 2007). To study the emulsion destabilization and its association with oleogustus, emulsion samples with different emulsifiers could be used to study the effect. By observing the size of oil droplets change in the spat-out emulsions and the detection threshold of the oleogustus stimuli, it may provide us more details on the sensations experienced from fatty acids. Knowing how oral mechanisms alter the physical and chemical structure of the lipids and the properties of each material contained in the fat emulsions may improve our understanding of the human sensory experience from fat.

### 5.2.3 Dietary Exposure and Saliva's Emulsifying Properties

In our current study, we found that diet is associated with some of the emulsifying properties at different time points. To better confirm whether any of our observed factors are causative, dietary intervention studies may be conducted to observe if specific dietary exposure alters the effectiveness of oral emulsification. In our current study, we observed that dietary fat, protein, and carbohydrate were somehow associated with saliva's emulsifying properties at different individual time points or over time. Protein intake in particular was shown to be negatively associated with emulsion stability. We hypothesize that higher protein intake may lead to worse saliva emulsifying properties (smaller, less stable fat layer). It is also important to verify: i) whether the type of dietary protein may influence the effectiveness of oral emulsification, ii) whether the impact is caused by acute or chronic diet. The findings will improve our understanding of controlling the mechanisms of textural and chemical perception of fat.

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# APPENDIX A LINOLEIC ACID GUMMY

### *Introduction*

In early 2020, we had created a linoleic acid (LA) gummy for use in our original study regarding dietary fatty acid exposure's influence on oleogustus perception and salivary profile. Due to the COVID-19 pandemic, the project was disrupted. However, we report here the formulation of the gummy for potential use in future work. The linoleic acid gummy was preliminarily evaluated by lab members for its stability during storage, texture, flavor release, and optimal stimuli concentration in pilot testing. All the materials and corresponding company names were listed in Table A1.

### *Sample Preparation*

The overall formulation is as shown in Table A2. LA was used for oleogustus stimulation. Locust bean gum (LBG) and xanthan gum, which are both commonly used as a thickener, were mixed together to create the firm texture of the gummy sample. Either LBG or xanthan gum alone would only thicken the solution, but combined they synergize to form a gel. Granulated sugar was added for the purpose of aiding flavor release, and it also aids in dissolution and texture of the gums. Ethylenediaminetetraacetic acid (EDTA) and Tert-Butylhydroquinone (TBHQ) were used as antioxidants to protect linoleic acid from oxidation. White icing color was added because the LA gummies were white due to the emulsion. To make the appearance of the control consistent with the LA gummies, the white food color was used. Ethanol served as a solvent for TBHQ. Powdered ingredients such as granulated sugar, LBG, and xanthan gum were weighed in a plastic container separately. Total deionized (DI) water was weighed and divided the amount into two separate containers equally. This was because LGB and xanthan gum must be initially dissolved separately in order to avoid immediate formation of the synergistic gel. To prepare the antioxidants, EDTA was made into a 2% (w/w) stock solution by mixing with DI water; TBHQ was also made into a 10% (w/w) stock solution by dissolving in ethanol.

LA was stored in a freezer and fully thawed before being weighed in a glass beaker. TBHQ solution was then added to the linoleic acid in the same container. The EDTA solution was weighed in

another beaker along with the white icing color. During preliminary formulations, we observed on average 10% loss of water due to heating. To account for this, 10% extra water was added to the formulation to keep the final concentrations more accurate. Water can be added to the same beaker as EDTA and white food color solution.

In the gummy-making process, two blenders (*Instant*™ ACE™ NOVA) were used to mix the ingredients. Blender A and B were used to indicate two different blenders. The divided amounts of DI water (as mentioned above) were placed in blender A and blender B separately. DI water in blender A and B was pre-heated to around 90-95$^{o}$C (blender had built-in heater). LBG powder was first mixed with granulated sugar in a container to help break apart any powder clumps. LBG /sugar was added in blender A. "Pulse mode" was set as a speed setting, and the sides of the blender were scraped down periodically if needed. While waiting for blender A to fully mix the ingredients, xanthan gum powder was added into blender B with the same process to mix the ingredients. When all the ingredients in blender A and blender B were fully mixed, mixture in blender B was poured into blender A for further blending. Once the combined mixture was fully mixed, and the mixture was cooled to a temperature of 75-80 $^{o}$C. At this temperature, LA/TBHQ and EDTA/dye were added to the combined mixture. When all the ingredients were fully mixed, final mixture was poured into a baking pan and covered with plastic wrap to avoid water evaporation. Sample was cool in a refrigerator.

Once the sample was cool and the gel was set, a multi-square cutter (Gobel 845200 Stainless Steel Brownie and Caramel Cutter) was used to cut the sample into multiple 1-inch x 1-inch pieces. Each small piece of sample was packaged in a vacuum sealer food bag and stored in a fridge or insulated bag to avoid oxidation.

Table 2 0 : List of ingredients and reagents

| Product | Company | Company Location |
|---|---|---|
| Deionized (DI) water | — | — |
| Linoleic acid (LA) | MilliporeSigma | St. Louis, MO |
| Locust bean gum (LBG) | Modernist® Pantry | Portsmouth, NH |
| Keltrol® Xanthan gum | CP Kelco | Atlanta, GA |
| Granulated sugar | Domino® | Yonkers, NY |
| EDTA | Spectrum® Chemical | New Brunswick, NJ |
| Tenox™ TBHQ | Eastman | Kingsport, TN |
| White Icing Color | Wilton® | Woodridge, IL |
| Ethanol (200 proof) | Decon Labs, Inc. | King of Prussia, PA |

EDTA: Ethylenediaminetetraacetic acid; TBHQ: Tert-Butylhydroquinone

Table 2 1 : Concentration of ingredients in final sample

| DI water | LA | LBG | Xanthan | Sugar | TBHQ | EDTA | White Icing Color | Ethanol |
|---|---|---|---|---|---|---|---|---|
| 93% (w/w) | 1.5% (w/w) | 1% (w/w) | 1% (w/w) | 3.5% (w/w) | 0.01% (w/w) | 0.01% (w/w) | 0.1% (w/w) | 0.1% (w/w) |

DI water: Deionized water; LA: Linoleic acid; LBG: Locust bean gum; TBHQ: Tert-Butylhydroquinone
EDTA: Ethylenediaminetetraacetic acid