# INVESTIGATING THE SWEETNESS OF HONEY AND ITS USE IN FOOD SYSTEMS

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**ABSTRACT**

Dietary recommendations for reducing added sugar and increasing the intake of whole grains, fruits, vegetables, and unsaturated fats aim to improve overall health. Consumer liking of foods positively correlates with sweetness, but nutritious foods are often bitter. Knowledge of the sweetness intensity of different sweeteners plays a key role in consumer acceptance. Unlike many commercial sweeteners, the sweetness of honey has yet to be quantified. This research aims to quantify the relative sweetness of honey, compare the sweetness of honey and sucrose in food systems, and measure the impact of added sweeteners on liking nutritious, bitter foods. To quantify the sweetness of honey, semi-trained panelists (n=55) rated sweetness intensities of four honey varieties (Clover, Wildflower, Alfalfa, and Orange) and sucrose on the global sensory intensity scale (GSIS). Sweeteners were diluted in a six-level concentration series that covered the range of sugar content of most commercially sweetened beverages (12.5 – 125g/L). In aqueous solutions, honey is equivalently sweet to sucrose when measured in unit mass. The relative sweetness of honey was then measured in common food and beverage systems; consumers (n≥101 rated their overall liking and the sweetness intensity of honey- or sugar-sweetened conditions. To assess the influence of added sweeteners on the palatability of nutritious foods, consumers rated their overall liking, sweetness intensity, and bitterness intensity of three conditions (unsweetened, honey-or-sugar sweetened) of nutritious foods. The relative sweetness of honey varied by product and temperature of preparation and consumption; however, the results suggest that honey increases acceptance of bitter nutritious foods with less added sugar and kcals than sucrose.

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## Chapter 1: Introduction

### Rationale and Significance

The sweetness potency of many commonly used sweeteners has been compared to sucrose, but the sweetness potency of honey has been understudied. Previous studies that involve sweetness evaluation of honey lacked formal sensory methodologies, leaving a knowledge gap (Raja Nurfatin et al., 2021; Remeňová et al., 2017; Yankova-Nikolova et al., 2024). A factor that makes it difficult to generalize the relative sweetness of honey is its chemical complexity. Honey is comprised of sugar, water, protein and enzymes, vitamins, minerals, and phenolic and volatile compounds; the quantities and composition of individual compounds within these chemical classes vary by floral source, batch of honey, year of harvest, geographical location, processing methods and more (Da Silva et al., 2016). Additionally, honey has intrinsic aroma, and these aromas may be contributing to the sweetness intensity of honey, as there is strong evidence showing that specific aromas can increase sweetness perception (Da Silva et al., 2016; Zhang et al., 2023). Relative sweetness intensities are typically determined through dose-response curves that showcase perceived sweetness intensity as a function of sweetener concentration. However, the relative sweetness intensity of a sweetener in aqueous solutions does not necessarily translate to food and beverage systems because various tastants, aromas, and textures make these mediums much more complex. Typically, added sugars contribute to sweetness and the palatability of food products, including intrinsically bitter foods, because sweetness suppresses bitterness (Davis & Running, 2023). Many foods that health professionals recommend are bitter (e.g. leafy greens, whole grains, and extra virgin olive oil), and we want to investigate the impact of honey and sugar on the acceptance of nutritious foods.

## Objectives

**Objective 1:** Quantify the sweetness of honey in water and the impact honey aromas have on sweetness (chapter 3).

**Objective 2:** Quantify the potency of honey as a sweetener in food systems, compared to sucrose, measuring its impact on overall liking, sweetness intensity, and bitterness intensity (chapter 4).

## Chapter 2: Literature Review

### Sweeteners

#### *Sugars*

Written records of honey can be traced back to 2100 BC, and there are drawings of honey bees from 3100 BC (Bogdanov et al., 2008; Crane, 2004). On average, honey contains sugars (80%), water (17-20%), proteins (0.1-3.3%), organic acids (0.57%), as well as vitamins, minerals, phenolic and volatile organic compounds (VOCs) (Da Silva et al., 2016; Kamal & Klein, 2011). The composition of honey is affected by the species of honeybees that made the honey, the climate, geography, and weather of the region in which the honey was produced, along with processing techniques, including temperature of extraction, packaging, storage conditions, and more (Da Silva et al., 2016). The flavor complexity of honey is primarily attributed to the presence of sugars and VOCs.

Monosaccharides, fructose, and glucose are the dominant sugars in honey and account for 75% of total sugar (Da Silva et al., 2016; Kamal & Klein, 2011). In addition to monosaccharides, honey contains disaccharides and oligosaccharides, including sucrose and many rare sugars such as turanose, trehalose, isomaltose, nigerose, and more (Da Silva et al., 2016; Doner, 1977; Zhu et al., 2024). The botanical origin of honey has a significant impact on the sugar content; floral honey typically contains more fructose and glucose than honeydew honey (i.e., honey derived from a non-flowering plant)(Kamal & Klein, 2011; Manyi-Loh et al., 2011). Floral honey contains 38.19% fructose and 31.28% glucose, while honeydew honey contains 31.80% fructose and 26.08% glucose, on average (Doner, 1977). It has been noted that the sweetness of honey is related to a high fructose content; however,

this statement is not backed by quantitative sensory analysis (Bogdanov et al., 2008).

In addition to the sugar content, the volatile organic compounds present in honey significantly impact its sensory characteristics. The VOCs within honey are most often identified through gas chromatography-mass spectrometry (GC/MS) analysis with headspace solid phase microextraction (HS-SPME) (Kaškoniene & Venskutonis, 2010; Mannaş & Altuğ, 2007; Serra Bonvehí & Ventura Coll, 2003; Siegmund et al., 2018). The VOCs in honey are secondary metabolites from the plants the honey bee derived nectar from, so the plant source of honey has a significant influence on VOCs and the overall flavor of honey (Di Marco et al., 2018). Common chemical classes of the unique VOCs identified in honey include alcohols, aldehydes, esters, hydrocarbons, ketones, and sulfurous compounds (Jerković et al., 2009). Researchers who used gas chromatography-olfactometry (GC-O) to analyze honey samples described many of the VOCs present in honey as floral, fruity, woody, herbal, and sulfuric (Kortesniemi et al., 2018).

Before sucrose production skyrocketed, honey was the primary sweetener available; now, honey accounts for less than 1% of annual sugar production (1.2 million tons per year) (Bogdanov et al., 2008). Today, sucrose is a commonly used sweetener in the food and beverage industry, and it is the universal benchmark to which other sweeteners are compared (Godshall, 2007; Russell et al., 2023). Sucrose is a disaccharide comprised of one glucose and one fructose unit joined together by a 1-2 glycosidic linkage (Clemens et al., 2016). A common form of sucrose is table sugar, a white crystalline product that is extracted from sugarcane or sugar beets (Coulston & Johnson, 2002; Godshall, 2007; Godshall et al., 2021; Pihlsgard, 1997). A major function of sucrose is to provide a sweet

taste and increase the palatability of a food or beverage. Additionally, this sweetener, with a negligible aroma, is widely used in the food and beverage industry to provide bulk, lower water activity, alter boiling and freezing temperatures, and much more (Clemens et al., 2016). There are many well-liked sensory characteristics attributed to sucrose in foods; however, its price is variable due to instability with sugar cane and sugar beet growing regions, and it hydrolyzes in acidic conditions (White, 2008). This fueled the use of high fructose corn syrup (HFCS), an inexpensive and renewable sucrose alternative, made from enzymatic isomerization of glucose (Parker et al., 2010). HFCS is produced and sold in a liquid form that is easy to dispense into liquids, and it is stable in acidic environments; these are valuable characteristics for carbonated beverage production (White, 2008). The two most commonly used HFCS in the food and beverage industry are HFCS-42 (containing 42% fructose) and HFCS-55 (containing 55% fructose) (White, 2008). The top sources of added sugars in the U.S, accounting for more than 50% of total sugar intake when combined, are sugar-sweetened beverages, desserts, sweet snacks, coffee and tea, and candy (U.S Department of Agriculture & U.S Department of Health and Human Services, 2020).

***Sugar alternatives***

In addition to sugars, polyols (sugar alcohols), non-nutritive sweeteners (NNS), and high-potency sweeteners (HPS) are added to foods and beverages to increase sweetness and liking. Sugar alcohols are digestible, but are less calorically dense and less sweet than sucrose, so they are often used to replace sucrose to provide bulk and then combined with another sweetener to reach the desired sweetness intensity (Grembecka, 2015). Sugar

alcohols that are approved for consumer consumption by the FDA and generally recognized as safe (GRAS) are maltitol, mannitol, sorbitol, and erythritol (Saraiva et al., 2020). NNS and HPS provide a sweet taste with minimal calories because these sweeteners are either not metabolized and/or they are highly potent (American Dietetic Association, 2004; Mattes et al., 2023). NNS and HPS that have been given GRAS status include acesulfame potassium (Ace-K), advantame, aspartame, luo han guo fruit extracts, neotame, saccharin, steviol glycosides, sucralose, and thaumatin (Mattes et al., 2023).

Sugar alternatives are most used by consumers in the United States for sugar and caloric reduction as well as weight and blood sugar management (International Food Information Council Foundation, 2019). Although these sweeteners are perceived as sweet, they have different sensory profiles than dietary sugars (e.g., Ace-K and saccharin also elicit bitterness), resulting in some consumer pushback on their use (Chen et al., 2023). Because HPS are used at such low concentrations, sensory properties such as texture and mouthfeel are altered if bulking agents are not added. Sugar alcohols provide bulk and are often used in combination with HPS; however, sugar alcohols have laxative effects and must be used with caution (Kroger et al., 2006; Saraiva et al., 2020). Additionally, concern over the adverse health effects of synthetic sugar alternatives and the preference for naturally derived ingredients have led to changes in consumer sweetener use trends (Mattes et al., 2023; Román et al., 2017).

**Sweetness potency**

The sweetness potency is often reported as a value compared to sucrose. Commercially available high-potency sweeteners range from approximately 200-13,000 times greater

than that of sucrose. Aspartame (i.e. NutraSweet, Equal) is 180-200 times more potent than sucrose; Saccharin (i.e., Sweet'N Low) is 300 times more potent than sucrose; neotame is 7,000-13,000 times more potent than sucrose (Chattopadhyay et al., 2014). However, sweetness intensity does not have a linear relationship with sweetener concentration; rather, the relationship between sweetness intensity and sweetener concentration is exponential, and the parameters of the dose-response function vary by sweetener (Moskowitz, 1970; Wee et al., 2018). So as concentration increases, the relative sweetness compared to sucrose will change (Moskowitz, 1970). The construction of dose-response curves, which model sweetness intensity as a function of sweetener concentration, is a more accurate way to quantify sweetness intensity. One study looked at the sweetness intensity of 16 sweeteners (i.e., saccharides, polyols, and non-nutritive sweeteners) across eight concentrations. It created semi-log dose-response curves for each sweetener (Wee et al., 2018). Many of the tested sweeteners have different growth rates than sucrose and therefore sweetness potency is concentration-dependent; this finding is supported by additional research (Cardello et al., 1999; Fujimaru et al., 2012; Portmann & Birch, 1995; Świąder et al., 2009; Wee et al., 2018; Wiet & Beyts, 1992). Many sweeteners have a recorded plateau in sweetness at high concentrations; one hypothesis for this is that the sweet tastants saturate the tastebuds (Wee et al., 2018). Although honey has been used for thousands of years, there is minimal reported data on its sweetness. It is likely that the chemical complexity of honey is a factor contributing to the lack of sweetness quantification; no two kinds of honey are the same.

## Mechanism of sweetness

Sweet taste receptors are activated by sugar and sugar alternatives through the same mechanism. Within the gustatory system tastebuds, largely located on the dorsal surface of the tongue and soft palate, recognize chemical stimuli as sweet, salty, bitter, sour, or umami (Fernstrom et al., 2012; Yarmolinsky et al., 2009). Tastebuds are comprised of clustered specialized taste cells, each expressing individual taste receptors that bind to specific chemical stimuli (Fernstrom et al., 2012; Yarmolinsky et al., 2009). The majority of taste receptors belong to a family of G-protien coupled receptors; including the type 1 taste receptor (T1R) family (Fernstrom et al., 2012). Sweetness is perceived through the T1R2 and T1R3 receptors and there are several binding sites within each of these receptors for sweet stimuli to bind to (Fernstrom et al., 2012). Different sweeteners bind to different sites of the sweet receptor. For example, some natural and synthetic sweeteners such as sucrose, glucose and sucralose bind to the T1R2 and T1R3 extracellular venus-flytrap (VFT) domains; non-nutritive dipeptide sweeteners like aspartame and neotame bind to the VFT domain of the T1R2 receptor (Nie et al., 2005; Xu et al., 2004). When sweet taste receptors are activated, a neural pathway signals to the brain that the stimulus is sweet (Fernstrom et al., 2012). Sweet taste stimuli have been found to activate the dopamine reward system as well; however, researchers are uncertain about the mechanism (Fernstrom et al., 2012).

## Sugars and Health

The health consequences of added sugar intake have been under investigation in recent years. In 2015, the World Health Organization (WHO) recommended that the intake of free sugars does not exceed 10% of daily caloric intake (World Health Organization, 2015). The

WHO defines free sugars as monosaccharides and disaccharides added to food or beverages by chefs, consumers, and manufacturers, and sugars naturally present in honey, syrup, and fruit juice. The WHO remarks that high sugar consumption increases the risk of developing dental caries (World Health Organization, 2015). A review of the National Health and Nutrition Examination Survey (NHANES) has shown that Americans significantly reduced their added sugar consumption between 2001 and 2018 from 16.2 to 12.7% of daily caloric intake (DiFrancesco et al., 2022). Despite reduction efforts, sugar consumption is higher than what is recommended; however, a publication bias regarding the adverse relationship between free sugar and health must be acknowledged (Joober et al., 2012; Murad et al., 2018; Shields, 2000).

A narrative review of the current WHO sugar recommendation argues that the recommendation should be revised as there are severe limitations to the current body of evidence on sugars and health (Yan et al., 2022). Of the studies reported by the WHO, those that link ill health to sugar intake focused on the sugars of sugar-sweetened beverages (SSBs), while studies that investigated the intake of free sugars from solid sources reported null findings (Yan et al., 2022). Additionally, a review and meta-analysis on the relationship between sugars and obesity did not find a strong correlation between intake of added sugars and obesity; rather, obesity and weight gain are correlated to more energy input than output (Clemens et al., 2016). Additionally, the WHO remarks that free sugars threaten the nutrient quality of diets as they are high in energy and do not provide essential nutrients (World Health Organization, 2015); however, recent studies have showcased that diets void of sugars (<5% daily energy intake) can also be dilute of micronutrients (Fujiwara et al., 2020; Mok et al., 2018; Wong et al., 2019). Some sugar-rich foods (e.g.,

cereals) are a good source of nutrients, and sugars can increase the palatability of nutrient-dense foods such as oatmeal and yogurt (Yan et al., 2022). Regardless of the impact on health, humans have an innate preference for sweet taste, so sweetness is highly correlated with consumer liking of foods and beverages (Clemens et al., 2016).

**Taste modulation**

Taste perception has a large influence on the acceptance and consumption of beverages; thus, reduction of bitterness intensity could increase acceptance of nutritious but bitter foods (Nadathur & Carolan, 2017). One strategy to modulate bitterness intensity is through suppression by other tastants. For example, sucrose, sodium chloride, and citric acid are all able to reduce the bitterness intensity of caffeine (Pangborn, 1960). Conversely, the presence of bitterness can also diminish the intensity of sweet, salt, and sour tastes. For example, the addition of a Tbsp of sugar to a cup of coffee will reduce its bitterness and increase its sweetness, but that coffee will taste less sweet than a Tbsp of sugar in plain water. Sugar is often added to bitter-tasting compounds to balance its intensity and increase acceptability (e.g., coffee and cocoa) (Harwood et al., 2013). Since added sugar reduction is highly recommended, solely relying on sugar to reduce bitterness intensity is not a sustainable method in gaining acceptance of nutritious, bitter foods.

An alternative mode of taste modulation is odor induced taste enhancement. The process of eating stimulates more than the gustatory system, olfactory and somatosensory systems are also involved (Small, 2012). The olfactory epithelium can be stimulated through both orthonasal and retronasal olfaction. Orthonasal olfaction refers to volatile compounds entering the nasal cavity via the nostrils though sniffing and breathing. While retronasal

olfaction refers to volatile compounds entering the nasal cavity via the throat via chewing and swallowing (Wilson, 2021). Numerous studies have demonstrated that retronasal olfaction can influence the intensity of tastes. Observations of sweet taste enhancement through aroma research can be traced back to 1935 (Blakeslee, 1935). Further studies have demonstrated that taste enhancement through aroma is a psychological effect, as there is an overlap between gustatory and olfactory processing in the orbitofrontal cortex (OFC) of the brain (Pan et al., 2023).

Many odors that have been found to enhance sweetness perception are characterized as sweet-smelling (Spence, 2022a). A hallmark aroma that demonstrates this effect is vanillin, the key aroma of vanilla. Many studies have indicated an increase in the perception of sweetness through the presence of vanilla (Spence, 2022b, 2022a). Vanilla is often added to sweet foods and beverages like ice cream, cola, and many baked goods, which has led to consumers associating this smell with sweetness (Sakai, 2001; Spence, 2022b). Moreover, aromas intrinsic to fruits, including 3-methyl butyl acetate, linalool, and benzyl alcohol, also demonstrated sweetness enhancement (Bartoshuk & Klee, 2013; Lim et al., 2014; Xiao et al., 2021; Zhang et al., 2023). Odor-induced taste enhancement can alter taste perception. The insight gained on this topic by sensory scientists may be valuable for food product developers to modify taste attributes in foods with less reliance on sugars and salts.

## Sensory Testing

A well-designed sensory test is critical to producing reliable and reproducible data that measures the impact of stimuli on human perception. Sensory science utilizes humans as measurement tools, so it is necessary to consider that humans have a variety of perceptual

differences and are prone to biases (Meilgaard et al.). It is essential to define the overall goals of the sensory test before making methodological decisions that include testing location and scale use. Two common types of sensory tests are analytical and affective tests. The goal of analytical tests is to understand the sample being tested, while affective tests seek to understand consumers' hedonic opinions of the samples (Drake et al., 2023). Clearly defining the goal of a sensory test is essential, as it lays the groundwork for determining further testing methodology.

After the goal of a sensory test has been determined, a key factor to consider is the testing location. Since analytical tests seek to minimize all variables besides those that are being analyzed, testing is conducted with trained or semi-trained panelists in a controlled environment located on-site of a sensory facility. Within a testing facility, panelists complete testing in booths or individual rooms. This prevents one panelist from influencing the ratings of another. Testing facilities also have controlled lighting, temperature, and noise, minimal outside aromas, and well-controlled sample preparation and presentation (Drake et al., 2023). Affective tests utilize untrained consumers and can be conducted in a sensory laboratory. However, the environment of sensory laboratories strays from everyday consumption environments, so experiences within a laboratory do not directly translate to an experience a consumer will have with a product they have purchased (Meilgaard et al., n.d.; Sosa et al., 2008). Hence, affective tests are typically conducted in a central location or in the home of a consumer. Central location tests (CLT) are conducted in areas where consumers congregate (e.g., shopping malls, food courts, local fairs, etc.) (Meilgaard et al., n.d.). Home usage tests (HUT) are used to gather insight into products used under normal conditions, and they often involve multiple family members (Drake et al., 2023; Meilgaard

et al., n.d.). Whether conducted in a controlled laboratory setting or a location familiar to a consumer, the location of sensory testing plays a crucial role in obtaining accurate and reliable results.

The final aspect of sensory methodology that will be discussed in detail is the determination of scale use. To effectively understand how tested stimuli impact individual perception, scaling methods were developed to transform individual experiences into quantifiable numbers; humans cannot share perceptual experiences with one another, they can only be described (Hayes et al., 2013). Analytical tests often utilize magnitude scales, which provide ratio data, while affective tests tend to utilize scales that are simple to understand, such as category and visual analog scales (VAS).

Scaling methods for analytical tests have continued to evolve. Magnitude estimation, an early scaling method introduced by S.S Stevens in the 1950s, assigns a number to a sensation, producing a scale with ratio data (e.g., assigning a number to the adjective "barely detectable" and twice that value to "weak") (Bartoshuk et al., 2002). Originally, magnitude estimation scales were absolute, meaning the top of the labeled magnitude scale (LMS) was anchored with the most intense sensation possible for the attribute in question (Green et al., 1993). However, this lacked the ability to show sensitivity among individuals. If researchers sought to compare differences in individual sensitivity, an absolute magnitude estimate would lead to invalid assumptions as adjectives assigned to a numerical value do not show an individual perceived intensity (Bartoshuk et al., 2002; Hayes et al., 2013). For example, if the top of the scale were anchored "most intense spice level" the adjective "strong" would reflect a different intensity for an individual who

consumes spicy food daily vs. someone who has had few experiences with spicy food. For this reason, the top anchor was renovated to, “strongest sensation of any kind”; the generalized Labeled Magnitude Scale (gLMS) now considers all senses and is not confined to the sensation being analyzed (Bartoshuk et al., 2002; Hayes et al., 2013). Additionally, training participants in rating sensations that are outside of the study modality (e.g., to train participants in using the scale to rate intensities of sounds in a study looking at sweetness) is recommended by Linda Bartoshuk, a pioneer of the gLMS, and her labratory (Bartoshuk et al., 2002; Green & Hayes, 2003). The gLMS has been widely used in the field for decades; however, researchers have observed that participants treat the anchors as categories (Hayes et al., 2013). Linda Bartoshuk now advocates for the use of the Global Sensory Intensity Scale (GSIS). The GSIS is similar to the GLMS in that it is a generalized scale ranging from “no sensation" to “strongest sensation of any kind,” but it has no additional anchors. This addresses the weakness of participants using other generalized scales as a categorical, instead of continuous, scale (Hayes et al., 2013). Proper use of a generalized scale is not intuitive, and it is difficult to rate one modality while considering all modalities without proper training.

While the scales used in analytical tests are often complex to understand and require training, scale training is rarely employed in affective testing. With that, the scales used on untrained consumers must be quick to use and simple to understand; often, these are category or line scales. Category scales are divided into “numeric and/or semantic” sections (Hayes et al., 2013); a 9-point hedonic scale ranging from “1-Dislike Extremely” to 9-“Like Extremely” has been a historically popular scale used by food scientists (Wichchukit & O’Mahony, 2015). A commonly used line scale that came into use in the 1960s is the Visual

Analog Scale (VAS), which has minimum and maximum anchors and is used to rate the intensity of a particular attribute on a continuous line (Bartoshuk et al., 2002; Hayes et al., 2013). In contrast to the relatively simple scales used in affective testing, analytical test participants are often trained in the use of magnitude estimation scales.

Defining the research question before determining sensory methodologies helps minimize biases. Analytical testing is intended to reveal specific details about a sample so testing methods are designed to eliminate potential biases and are usually carried out in a controlled environment with trained panelists using ratio scales such as the GSIS. On the other hand, affective testing is aimed at understanding consumer opinions about the sample. Testing methods for affective testing are meant to capture unfiltered opinions about the sample and are often done in familiar environments, such as a food court, with untrained panelists using simple scales.

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## Chapter 3: Quantifying the intensity of sweetness and impact of aroma in honey from four floral sources.


### Abstract

Unlike many commercial sweeteners for which sweetness dose-response curves have been constructed, honey's sweetness has yet to be quantified. Honey differs from most commercial sweeteners in that it has a robust aroma; this aroma may impact its perceived sweetness. This study quantified the sweetness intensity and the impact of aroma on the perceived sweetness of four different honey varieties (Clover, Wildflower, Alfalfa, and Orange) compared to sucrose. Each sweetener evaluated was diluted to six concentrations in water ranging from 12.5 g/L to 125 g/L. Panelists ($n$=55) rated the sweetness intensities with and without aroma, in replicate, on the Global Sensory Intensity Scale (GSIS). Additionally, the volatile organic compounds in the honey samples were profiled using gas chromatography-mass spectrometry (GC/MS) analysis. Honey and sugar were equivalently sweet at a given concentration (g/L), with aroma present ($p= 0.251$). Due to the lower caloric density of honey, an equivalent sweetness to sugar can be achieved with 21% fewer kilocalories (kcals). Additionally, aroma significantly increased sweetness intensities for all sweeteners ($p=0.042$), and especially honeys. In a 100 g/L solution, the aromas in honey increased its sweetness by 23-43%, depending on the floral source. Compounds with sweet aroma characteristics were identified at high concentrations in all honey samples using GC/MS analysis, including furfural, benzaldehyde, benzene acetaldehyde, and dimethyl sulfide. Additionally, (S)-limonene and toluene were present in high quantities in the orange and alfalfa samples. This study can inform appropriate honey usage levels and identify major volatiles that may enhance sweetness

## Introduction

Honey is a sweetener with a long history of use, but its sweetness intensity is not well characterized. Past studies looking at the sweetness of honey have not used formal sensory methodologies, and this topic remains understudied (Raja Nurfatin et al., 2021; Remeňová et al., 2017; Yankova-Nikolova et al., 2024). Honey is derived from plant nectar collected by honeybees and is comprised of sugar, water, protein, enzymes, organic acids, vitamins, minerals, pigments, and phenolic compounds. The exact ratio of sugars, as well as the other constituents of honey, varies by the botanical source, climate, processing, and more (Da Silva et al. 2016). Approximately 80% of honey's composition consists of sugars, and of those, approximately 75% are monosaccharides (Da Silva et al., 2016; Escuredo et al., 2013; Kozłowicz et al., 2020). In addition to monosaccharides, researchers have identified more than 25 disaccharides and oligosaccharides in various honeys, such as sucrose, palatinose, trehalose, and melibiose (de la Fuente et al., 2007; Kaškoniene & Venskutonis, 2010; Sanz et al., 2004).

Most simple carbohydrates bind to the VFT sites of the T1R2 and T1R3 taste receptors, triggering the delivery of neurotransmitters to the afferent cranial nerve, which the brain processes as tasting sweet (Fernstrom et al., 2012). Although the processing mechanism is the same, different carbohydrates elicit different sweetness intensities. Some sweeteners can reach a greater maximum intensity, while others are more potent. For example, fructose and sucrose have a similar maximum sweetness intensity, and both sweeteners elicit a much stronger sweetness intensity than glucose (Clemens et al., 2016). On the other hand, aspartame is much more potent than sucrose, and significantly less aspartame is needed than sucrose to achieve a similar sweetness intensity (Wee et al., 2018). The

construction of a dose-response curve, which models sweetness intensity as a function of sweetener concentration, is a common way to represent the sweetness intensity of a sweetener. A known relationship between concentration and sweetness intensity is crucial for food product producers, consumers, and nutritionists to target a specific sweetness intensity in foods and meals (Wee et al.,2018).

An additional factor that impacts sweetness intensity is aroma. A growing body of research has found that certain aromas can influence the perception and intensity of basic tastes (sweet, salt, sour, bitter, and umami) (Bartoshuk & Klee, 2013; Frank & Byram, 1988; Spence, 2022; Wang et al., 2018, 2019; Zhang et al., 2023). Eating and drinking is a multimodal sensory experience that combines gustatory, olfactory, and somatosensory sensory systems (Small, 2012). The cognitive experience of flavor combines inputs from taste and retronasal olfaction (odors emitted from foods in the mouth), and there is often an overlap in the reception of these sensory inputs (Bartoshuk & Klee, 2013; Prescott, 1999; Small, 2012). Many of the aromas that have been reported as sweet taste enhancers are typically paired with sweet taste, such as fruity and floral aromas (Bartoshuk & Klee, 2013; Frank & Byram, 1988; Spence, 2022). Vanillin, the primary odor compound in vanilla, is a hallmark example of an aroma compound with this effect (Ventura & Mennella, 2011; Wang et al., 2018; Yeomans et al., 2008). Using sweet-enhancing volatiles is a promising strategy for reducing added sugars in food (Hopfer et al. 2022).

Unlike most other commercial sweeteners, honey has an intrinsic aroma. Its aroma is commonly described as sweet, floral, citrus, medicinal, and woody (Siegmund et al., 2018). The aroma composition of honey varies by floral source, and researchers have identified over 600 volatile organic molecules in various kinds of honey (Jerković et al., 2009). The

most common chemical classes of volatiles identified in honey include aldehydes, ketones, acids, alcohols, esters, hydrocarbons, and sulfurous compounds (Jerković et al., 2009; Kaškoniene & Venskutonis, 2010). Aldehydes that are commonly identified in honey include benzaldehyde (characteristic of almonds), phenylacetaldehyde (sweet, rose, green, and grassy), heptanal (fatty, pungent odor), hexenal (strong green grass), nonanal (orange, fatty, aldehydic), and furfural (sweet and woody) (Radovic et al.; Piasenzotto et al., 2003; Jerković et al., 2009; Da Silva et al. 2016; Siegmund et al. 2018). Terpenes such as linalool and its derivatives like lilac alcohol and lilac aldehyde have also been identified in many honeys (Piasenzotto et al. 2003; Jerković et al. 2009). Individual compounds have been identified as markers for particular honey varieties, such as methyl anthranilate, a marker of Spanish citrus honey (White & Bryant, 1996; Soria et al., 2003; Piasenzotto et al., 2003). So, how sweet is honey, and how do its aromas impact its sweetness? In this study, we aim to characterize the sweetness of honey in three parts. First, we quantified the relative sweetness of honey from four floral sources: alfalfa, wildflower, orange, and clover. Second, we quantified the impact of total aroma on honey sweetness. Third, we identified key volatile organic compounds present in the honey samples that may impact sweetness.

## Materials and Methods

### *Quantification of Honey Sweetness and the Impact of Aroma*

#### Sweet Taste Solutions

Sugar (Domino pure cane sugar, West Palm Beach, FL) and four varieties of honey (Clover, Alfalfa, Wildflower, and Orange; Dutch Gold, Lanc Co.) were each diluted to six concentrations in reverse osmosis purified water (Besco Water Treatment, Inc. Battle

Creek, MI) (hereafter referred to as "concentration set"). Each concentration set consisted of 12.5, 25, 50, 75, 100, and 125 g of sweetener/L water. Concentration set doses are displayed by volume (tsp/cup), calories (kcal/cup), and comparable consumer beverages in Table A1. Solutions were prepared in 1 L batches, pumped into 2 oz black cups (15 mL servings), and labeled with random 3-digit codes. Prepared samples were kept refrigerated and brought to room temperature for 30 minutes before serving. Honey samples were stored in 1 lb. bottles and kept frozen (-20ºC) until sample preparation; honey was not used beyond 7 days post thawing.

**Sensory Test Design**

Subjects (n=66) were recruited to participate in a training/screening session to qualify for six sample evaluation sessions to rate sweetness intensities on the Global Sensory Intensity Scale (GSIS)(Hudson et al., 2018). Training on the GSIS took place in small groups and began by asking panelists to think of their strongest remembered sensation of any kind. This sensation was used as their personal top anchor for the scale (numerically 100). Next, they were asked to rate the intensities of 18 remembered sensations, such as "the brightness of a dimly lit room" and "strongest oral pain experienced" and place a dash and numerical value on a hard copy of the scale for each sensation; remembered sensations used for training were adapted from Bartoshuk et al. (2002). Subjects shared their ratings in a facilitated group discussion and were encouraged to adjust their scale usage as needed. Next, they rated the intensities of 12 physical stimuli. Stimuli included various tastes, trigeminal sensations, and sounds; 6 of the physical stimuli were rated for sweet taste intensity (carrot, Ritz cracker (Mondelez International, Chicago, IL), banana, Coke (The

Coca-Cola Company, Atlanta, GA), marshmallow (Kraft Heinz, Chicago, IL), 100g/L sweet taste solution). Finally, they transferred their ratings onto a digital scale, administered through the RedJade sensory software (RedJade Sensory Solutions LLC, Pleasant Hill, CA, USA). Individuals were screened for understanding based on correct rankings of select remembered sensations and physical stimuli (e.g., a carrot is less sweet than Coke, and Coke is less sweet than remembered "strongest sweetness experienced"). Of the 66 individuals who completed the training, 55 met the criteria and completed the full study design.

The remainder of the study consisted of six sessions, during which subjects (n=55) rated the sweetness intensity of solutions; surveys were administered via RedJade. Subjects evaluated 20 concentration sets, rating each sweetener type four times. Subjects evaluated samples in sessions 1-3 without intervention ("with aroma"), while in sessions 4-6, subjects wore nose clips (Frienda, purchased via Amazon.com) to block olfactory perception ("without aroma"). At the beginning of each session, panelists completed a warm-up exercise in which they rated the intensity of three basic taste solutions, namely: sweet (100 g sugar/ 1 L $H_2O$), sour (1 g citric acid/1 L $H_2O$), and salty (3.5 g NaCl/ 1 L $H_2O$). Panelists rated 3-4 concentration sets of solutions per session (three sets in sessions 1, 2, 4, 5; four sets in sessions 3 and 6). To minimize sources of variation and potential biases, the presentation order of concentration sets and samples within a set were randomized via a complete block design; however, each set always began with the 50 g/L concentration of the set. Additionally, sampling protocol was standardized: panelists were instructed to sip the entire 15 mL sample, swish in their mouth for five seconds, expectorate the sample, and then give the intensity rating, with an enforced 30-second break between samples and

three minutes between sets. During that time, they rinsed their mouth with room temperature water and expectorated into a spit cup.

Subjects were compensated with a $15 e-gift card for participating in the training session and a $10 e-gift card/session for the remainder of the study. The Michigan State University IRB approved all sensory testing protocols for this study (STUDY00007723).

**Analysis**

All analyses were performed using R version 4.3.2 (2023-10-31 ucrt). Dose-response curves relating sweetness intensity to sweetener concentration were fit using a Hill equation using the drc package (v3.0.1; Ritz et al, 2015) for sugar and all four honey varieties, and curves were visualized using ggplot2 (v3.4.4; Wickham, 2016). Dose-response curves were used to extract the relative sweetness of honey and iso-sweet concentrations of honey compared to sucrose. We conducted a mixed-effect model ANOVA to analyze the effect of aroma, sweetener, concentration, and subject and their 2-way and 3-way interactions on sweetness intensity. We conducted a second mixed-effect model ANOVA excluding sweetness intensity ratings without aroma, analyzing the effect of sweetener, concentration, and subject, and their 2-way and 3-way interactions (afex v1.3-1). To determine in which cases aroma significantly enhanced sweetness, we conducted paired t-tests on sweetness intensity ratings with and without aroma for individual sweeteners at each concentration tested. ANOVAs and t-tests were performed using the stats package (v4.3.2; R Core Team 2023), and a significance level of $\alpha=0.05$ was used for all comparisons.

### *Determination of volatile organic compounds in honey*

#### Sample preparation and GC/MS protocol

The volatile profiles of each honey were characterized using Headspace-Solid Phase Microextraction (HS-SPME) Gas-Chromatography/Mass-Spectrometry (GC/MS). Four samples were prepared for each honey variety; two commercial honey bottles for each variety were analyzed, with two technical replicates prepared from each bottle. To prepare the honey samples for extraction, eight grams of honey along with equal parts of a saturated NaCl solution were combined in 40 mL vials equipped with a mininert valve (Supelco Inc., Bellefonte PA), and heated in a 40°C hot water bath for 10 minutes. Following heating, vials were vortexed for 10 seconds and equilibrated at room temperature for 5 minutes. Volatiles were extracted from the headspace of prepared samples for 10 minutes using a manual SPME device (Supelco Inc., Bellefonte PA ) equipped with a 65µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (Supelco Inc., Bellefonte PA ). Following extraction, the SPME fiber was inserted into the splitless inlet (200°C) of the gas chromatograph (Agilent 6,890 Hewlett-Packard Co., Wilmington, DE, USA), coupled to a mass spectrometer (Pegasus III TOF MS with Agilent 6890 GC, LECO, USA) and desorbed for 30 seconds (Park et al., 2024). During desorption, the first 20 cm of the column (HP-5 60 m x 0.25mm) were cyrofocused using liquid nitrogen (Song et al. 1998). The oven program began at 40°C and increased to 280°C at a rate of 43°C/min, for a total run time of 7 min. The column carrier gas, helium, had a constant flow rate of 1.50 mL/min. Additionally, the SPME fiber was kept in the injector port for the entirety of the run to desorb all volatiles from the fiber and ensure there was no carryover of volatiles between samples.

### Volatile standards

A standard blend was prepared with 0.4 µL each of the following 19 volatiles: acetone, 1-hexanol, phenylethyl alcohol, benzyl alcohol, decanal, furan, furfural, heptane, linalool, myrcene, octanal, o-xylene, phenylacetaldehyde, S-(-)-limonene (Sigma Aldrich, Milwaukee, WI), hexanal, benzaldehyde (Aldrich Chemical Co., Milwaukee, WI), 2-methyl butanal (Alfa Aesar, Ward Hill, MA), dimethyl disulfide (Tokyo Kasei, Portland, OR), and nonanal (Fluka Chemika Corp., Buchs, CHE). Two-tenths of a microliter of the standard blend was added to a 20mm filter paper (Gelman Instrument Co., Ann Arbor, MI) and dropped into a custom gas-tight 4.4 L volumetric flask with a Mininert valve (Alltech Assoc., Inc., Deerfield, IL); the blend was incubated in the flask for 24 hours to allow complete vaporization of the standards (Song et al. 1997).

Chromatographs were analyzed using LECO deconvolution software (Song et al. 1997). The identification of volatile compounds was determined by comparing retention times of external standard compounds and mass fragmentation spectra patterns from the National Institute for Standard and Technology (NIST) spectral library (version 2.0) for all compounds reported. Only compounds with a high probability match (>5000) in at least two replicates or compounds identified using an external standard are reported.

## Results And Discussion

### *Quantification of honey's relative sweetness*

The average sweetness intensity ratings for each sweetener with aroma are visually represented as sweetness dose-response curves (Figure 1). There was no statistical difference in average sweetness intensity ratings between all five sweeteners rated with

aroma (Figure 1, Table A2). The coefficients of the fitted Hill equation used to define the dose-response curves for each sweetener are given in Table 1. When the maximum response is not constrained, the generated maximum values are unrealistic for sweetness intensities when using a generalized scale (up to 88, with 100 representing the strongest sensation of any kind). Consequently, we constrained the maximum to 53.6, the average rating among our subject pool for "strongest sweetness ever experienced." The sweetness intensity of each sweetener measured, with aroma, can be estimated at any concentration within the experimental range using the dose-response curve equation (Table 1).

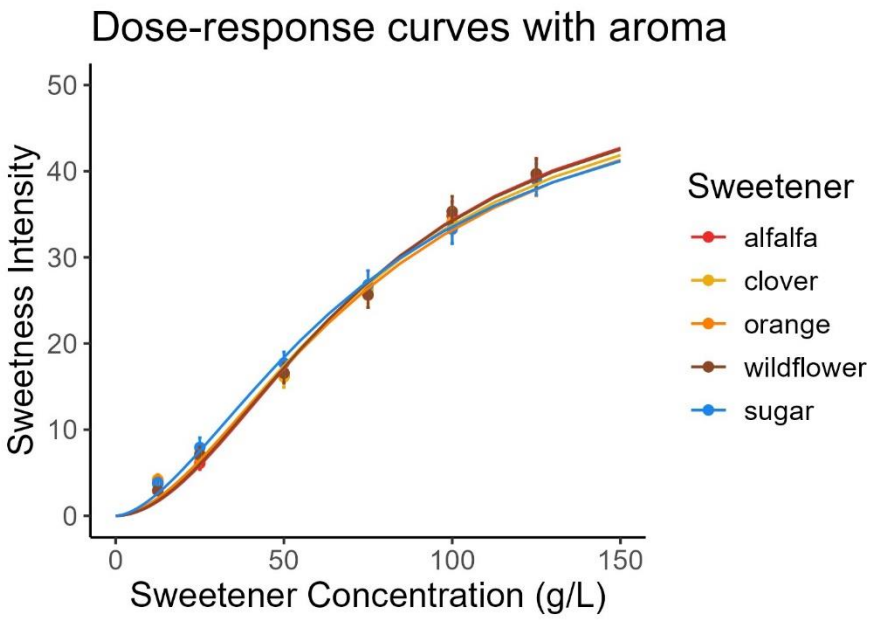


**Figure 1:** Mean sweetness intensity ratings with aroma as a function of sweetener concentration by mass (g/L) for all sweeteners (alfalfa honey, clover honey, orange honey, wildflower honey, and sugar). Error bars represent the standard error of the mean (±1 se).

**Table 1:** Hill equation and its coefficients for sweetness intensity dose-response curves of solutions rated with aroma. *X* represents the concentration of a sweetener (g/L), *n* represents the Hill coefficient, and $EC_{50}$ is the concentration (g/L) that produces a 50% maximum response. Maximum response was constrained to 53.6.

$$\mathbf{f(x)} = \frac{\mathbf{1}}{(\mathbf{1} + \frac{\boldsymbol{EC_{50}}}{\boldsymbol{x}})^{\boldsymbol{n}}}$$

| Sweetener | $EC_{50}$ (g/L) | *n* |
|---|---|---|
| **Alfalfa honey** | 79.2 | 1.77 |
| **Clover honey** | 75.9 | 1.81 |
| **Orange honey** | 77.3 | 1.78 |
| **Wildflower honey** | 75.3 | 1.89 |
| **Sugar** | 74.6 | 1.67 |

Knowing the potency of a sweetener is essential for recipe development and food formulation. In addition, it is common for consumers to measure sweetener content in terms of volume (tsp added to foods at home) or nutritional profile (kcal and g of added sugars in packaged foods). Notably, honey is approximately 70% more dense than sugar (Table A3), meaning 1 tsp of honey contains more sugar than 1 tsp of table sugar. The sugar content of the honey samples used in this study ranged between 79-81 g sugars/100 g honey, with the majority coming from the monosaccharides glucose (32-37%) and fructose (38-41%) (Table A4) (Oroian, 2013; Zhu et al., 2024). On the other hand, ordinary table sugar is at least 99% sucrose, a polysaccharide containing one glucose and one fructose unit. One factor that may affect the differences in sweetness intensity between the sweeteners is the composition of saccharides, as sucrose is less sweet than fructose per unit mass (Wee et al. 2018). In addition to differences in sugar content, honey has a reported caloric density that is 21% lower than that of sucrose (sugar: 3.85 kcal/g; honey 3.04 kcal/g) (FoodData Central 2019b, a). Since honey and sugar are equivalently sweet per unit mass, 21% fewer calories are needed to achieve the same sweetness when using

honey (Table A5).

The implications of the differences in volumetric and caloric densities between the two sweeteners are shown in Table 2. Given the 1.7x higher density of honey, the same sweetness as 1 tsp (or 1 Tbsp or cup) of sugar can be achieved with approximately 40% lower volume of honey. Table 2 can be used as a guide for consumers, dietitians, and product developers who are looking to substitute sugar with honey. However, these equivalencies are based on the sweeteners alone in water and do not account for the functional role of sucrose, loss of aroma volatiles through cooking and baking, and interactions with other aromas and tastants from foods. Dose-response curves that express sweetness intensity as a function of volumetric sweetener concentration (mL/L) and caloric density (kcal/L) are provided (Figure A2).

**Table 2**: Equivalently sweet volumes of honey and sugar; grams of total sugars and kcal of reported volumes. Values were calculated using the average densities (1.43g/mL, 0.83 g/mL), sugar content (0.794g/g, 0.998g/g), USDA kcal values (3.04kcal/1g, 3.85kcal/1g) for honey and sugar, respectively. Our measured densities differ slightly from USDA reported density.

| **Equivalently sweet concentrations** | | **Grams of sugars** | | | **kcal** | | |
|---|---|---|---|---|---|---|---|
| **Sugar** | **Honey** | **Sugar** | **Honey** | **Difference** | **Sugar** | **Honey** | **Difference** |
| 1 tsp | 0.58 tsp | 4.19 | 3.34 | 0.85 | 16.2 | 12.8 | 3.4 |
| 1 Tbsp | 0.58 Tbsp | 11.98 | 9.53 | 2.45 | 46.2 | 36.48 | 9.72 |
| 1 cup | 0.58 cup | 199 | 158 | 41 | 770 | 608 | 162 |

***Quantification of the impact of aroma volatiles on honey's relative sweetness***

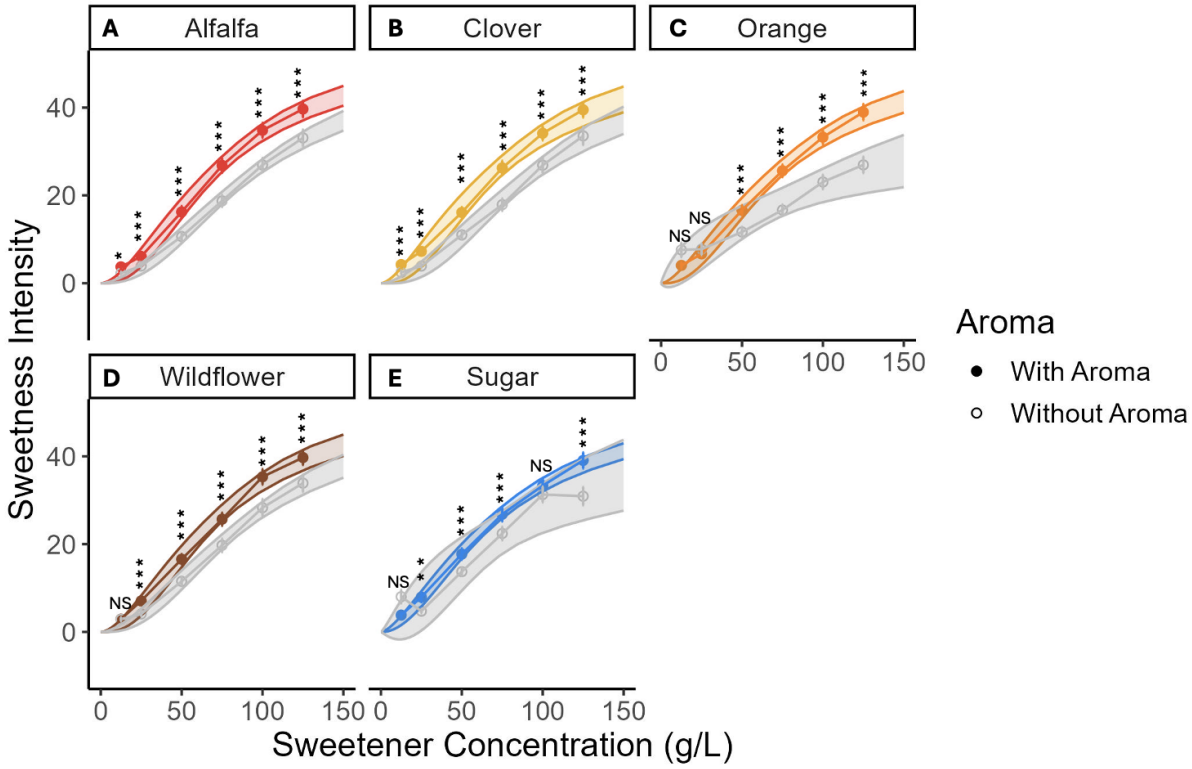


**Figure 2:** Mean sweetness intensity ratings as a function of sweetener concentration (g/L) with and without aroma for each of the sweeteners: **(A)** alfalfa honey, **(B)** clover honey, **(C)** orange honey, **(D)** wildflower honey, and **(E)** sugar. Error bars represent the standard error of the mean (±1 se). Ratings with aroma that are statistically greater than ratings without aroma are defined at p≥0.05 as NS, p<0.05 as *, p<0.01 as **, and p<0.001 as ***.

The differences in saccharide composition between the sweeteners may be responsible for honey and sugar eliciting equivalent sweetness intensities despite a 20% difference in sugar content; however, taste-aroma interactions are an essential factor to consider. Evaluation of changes in sweetness intensity ratings given with and without nose clips provides further evidence for the impact of aroma on perceived sweetness (Figure 2). Aroma significantly affected perceived sweetness across sweeteners (p= 0.042). At 100 g/L, comparable to the sugar concentration of Coke (Table A1), the aromas in alfalfa, clover, orange, and wildflower honey enhanced sweetness by 27.9, 25.5, 42.6, and 23.1%,

respectively; in comparison, the aromas in sugar enhanced sweetness by 5.4% (Table A6). The decrease in sweetness ratings for sugar without aroma, which has a very faint aroma (Urbanus et al., 2014), could be due to an overall muting of sensory input when the nasal cavity and aroma perception were blocked. Without aroma, orange honey was the sweetest at low concentrations but the least sweet at higher concentrations. One potential explanation for this result may be the presence of other tastants, such as acids, that suppress sweetness perception (Spence, 2022).

All the honey varieties are less sweet than sugar without aroma but are equivalently sweet with aroma; this observation indicates that taste-aroma interactions significantly impact perceived sweetness and suggests that the volatiles present in honey enhance sweetness. One hundred grams of honey has 20.6g of sugar and 81 kcal less than one hundred grams of sugar, yet the two sweeteners are equivalently sweet; the aromas in honey are bridging the gap in sugar disparities.

***Determination of volatile organic compounds in honey***

GC/MS results reveal that the orange honey had the most diverse aroma profile (87 compounds identified), while the clover had the fewest distinct aroma compounds profile (64 compounds identified); a complete list of compounds identified in each honey variety is provided in Tables A7-A10. The fifteen most abundant volatile organic compounds (VOCs) found in the headspace of any of the tested honey varieties, along with any additional VOCs confirmed using an external standard, are displayed in Table 3. Furfural, benzaldehyde, dimethyl sulfide, and phenylacetaldehyde comprised a large percentage of the total ion count (TIC) in each of the four honey varieties, and all these compounds have a sweet aroma characteristic. Other sweet-smelling compounds identified at lower concentrations

in each variety include 3-methyl-2-butenal, decanal, and phenylethyl alcohol. Many other aroma characteristics were congruent with sweetness (e.g., caramel, fruity, cherry, apple, etc. ). Other commonly identified aroma families that may or may not be congruent with sweetness include floral, fatty, vegetable, and musty.

(S)-limonene is the most abundant compound in the orange and alfalfa honey (21.11% and 10.26% TIC), and toluene is also present at high levels in both samples (8.04% and 2.80% TIC); however, neither VOC was identified in clover or wildflower honey. Interestingly, (s)-limonene has a "camphoraceous, herbal, and terpenic" aroma, which is not necessarily congruent with sweetness. The aromas in the orange honey contributed to a 40%, or greater, increase in sweetness intensity at a concentration of 50g/L and beyond. Toluene has a sweet aroma characteristic and is the second most abundant compound in the orange honey. This poses the question of whether the concentration of an aroma compound, its aroma characteristic, the complexity of the mixture, or the combined aroma character is a more significant factor contributing to sweet taste enhancement by aroma volatiles. Further work exploring sweetness enhancement with individual compounds present at high and low concentrations (e.g. (s)-limonene and phenylethyl alcohol) will help broaden the understanding of the sweetness-enhancing ability of honey's aromas.

**Table 3:** Average %TIC (total ion chromatogram) of the 15 most abundant compounds in any of the tested honey varieties (alfalfa, orange, clover, wildflower) and any additional compounds that were identified using an external standard

| Compound name | %TIC | | | | |
|---|---|---|---|---|---|
| | alfalfa | orange | clover | wildflower | Aroma characteristic |
| **(s)-Limonene†** | 10.26 | 21.11 | - | - | Camphoraceous; herbal; terpenic |
| **Furfural†** | 4.49 | 5.23 | 8.86 | 8.40 | sweet; caramellic; bready |
| **Benzaldehyde†** | 5.98 | 4.51 | 8.37 | 4.98 | sweet; cherry; maraschino cherry |
| **Toluene** | 2.80 | 8.04 | - | - | sweet |
| **Dimethyl sulfide** | 1.16 | 1.36 | 6.50 | 4.92 | sweet; vegetable; sulfurous |
| **Phenylacetaldehyde†** | 0.94 | 1.47 | 5.74 | 3.90 | sweet; fermented; floral |
| **Pivaloyl acetonitrile** | 4.67 | - | 1.31 | - | - |
| **Ethanol** | 2.08 | 2.88 | 0.36 | 1.61 | alcoholic; medicinal; ethereal |
| **Dimethyl disulfide†** | 0.52 | 0.81 | 3.99 | 1.47 | vegetable; onion; cabbage |
| **3-Penten-2-ol** | 4.13 | 0.07 | 1.97 | 0.52 | vinyl; green |
| **3-Methylbutanal** | 1.05 | 0.96 | 1.77 | 2.57 | aldehydic; fatty; ethereal |
| **(+)-Neoisomenthol** | 1.72 | 1.19 | 1.61 | - | mentholic; musty; woody |
| **2-Butenal, 2-methyl-,(E)-** | 2.60 | 0.06 | 1.63 | - | - |
| **2-Methylbutanal†** | 0.67 | 0.54 | 1.82 | 2.23 | malty; musty; fermented |
| **cis-Linalool Oxide** | 0.68 | 3.94 | 0.18 | 0.36 | floral |
| **Dimethyl silanediol** | 1.12 | 0.53 | 1.45 | 1.33 | - |
| **P-Menth-1-en-9-al** | - | 1.08 | - | - | herbal; spicy |
| **Octane** | 0.97 | 0.86 | 1.49 | 0.82 | - |
| **Acetic acid** | - | 0.33 | - | 1.46 | sour; acidic; vinegar |
| **Hotrienol** | 0.43 | 2.34 | 0.29 | 0.47 | tropical |
| **Nonanal†** | 0.78 | 1.09 | 0.71 | 0.87 | aldehydic; fatty; cucumber |
| **3-Hepten-2-one** | 0.19 | 1.22 | - | - | - |
| **3-methyl-3-Buten-1-ol** | 1.24 | - | 0.63 | 0.15 | sweet; fermented; yeasty |
| **Acetone†** | 0.51 | 0.34 | 0.60 | 0.99 | apple; solvent; pear |
| **3-methyl-2-Butenal** | 1.25 | 0.02 | 0.73 | 0.15 | sweet; cherry; nutty |
| **Decamethylcyclopentasiloxane** | 0.21 | 0.07 | 1.17 | 0.17 | - |
| **Myrcene†** | 0.08 | 0.63 | - | - | spicy; peppery; plastic |
| **2-Butanol** | - | 0.03 | - | 0.60 | sweet; fruity; apricot |
| **Decanal†** | 0.20 | 0.29 | 0.22 | 0.43 | sweet; aldehydic; floral |
| **2-Butanone** | 0.19 | - | 0.14 | 0.46 | camphoraceous; acetone; fruity |
| **Octanal†** | 0.30 | 0.19 | 0.22 | 0.33 | aldehydic; fatty; herbal |
| **Benzyl alcohol†** | - | - | 0.32 | 0.18 | sweet; floral; fruity |
| **Hexanal†** | 0.25 | - | - | 0.25 | vegetable; aldehydic; clean |
| **Phenylethyl Alcohol†** | 0.48 | 0.06 | 0.31 | 0.14 | sweet; dried rose; floral |
| **Heptane†** | 0.10 | - | 0.15 | - | sweet; ethereal |
| **O-xylene†** | 0.07 | 0.05 | 0.03 | 0.10 | geranium |
| **Furan†** | 0.04 | 0.04 | 0.05 | 0.03 | ethereal |
| **1-Hexanol†** | 0.04 | - | - | - | sweet; pungent; herbal |

*† Identification confirmed with an external standard.*

## Conclusion

When measured in units of mass, sugar and all four honey varieties were iso-sweet in water across the concentration range measured, representing the full range of typical commercial sweetened beverages. However, honey is a more potent sweetener per unit of energy, delivering an equivalent sweetness intensity with 21% fewer kcals. Consumers must use a lower volume of honey when substituting it for sugar to reap these nutritional benefits since it is 70% more dense than sugar. On a volumetric (tsp) basis, consumers can use 42% less honey to achieve similar sweetness (i.e. 0.58 tsp of honey per 1 tsp of granulated table sugar).

We found that aromas meaningfully contributed to the total sweetness intensity of honey. Sugar is sweeter than honey when olfactory perception is blocked. However, this difference disappears when honey aroma is present—the aromas in honey bridge the gap in the perceived intensity of the tastants. Generally, honey or aromatic sweeteners could be a valuable nutritional strategy to reduce added sugar intake without sacrificing the sweetness of foods. However, this study only characterized aqueous solutions, and these results do not account for more complex interactions in food systems.

Through GC-MS analysis, ≥64 aroma compounds were identified in the headspace of each honey variety. (S)-limonene, furfural, and benzaldehyde were the most abundant volatiles in the tested honey samples. Knowing that honey aromas enhance sweetness, future studies should investigate the specific contribution of honey volatiles to sweetness intensity.

**Author Contributions: Hannah Mulheron:** conceptualization; methodology; formal analysis; writing – original draft. **Aubrey DuBois**: conceptualization; methodology; formal

analysis; writing – review and editing. **Emily Mayhew:** conceptualization; methodology; supervision; resources; formal analysis; writing – review and editing; funding acquisition.

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## APPENDIX

**Table A1:** Concentrations of experimental concentration set by volume (tsp/cup), calories (kcal/cup), and comparable consumer products.

| Experimental concentrations (g/L) | Concentration by Volume | | Concentration by Calories | | Comparable consumer product |
|---|---|---|---|---|---|
| | Sucrose (tsp/cup) | Honey (tsp/cup) | Sucrose (kcal/cup) | Honey (kcal/cup) | |
| 12.5 | 0.70 | 0.41 | 11.38 | 8.98 | Pure Leaf Subtly Sweet Iced Tea (9g/L) |
| 25 | 1.40 | 0.81 | 22.75 | 17.97 | C20 Non-GMO Coconut Water The Original (29g/L) |
| 50 | 2.79 | 1.62 | 45.51 | 35.93 | Lipton Iced Tea with Lemon (50g/L) |
| 75 | 4.21 | 2.43 | 68.26 | 53.90 | IZZE Clementine Sparkling Juice (77g/L) |
| 100 | 5.60 | 3.24 | 91.02 | 71.87 | Coke (106g/L) |
| 125 | 7.00 | 4.07 | 113.77 | 89.83 | Simply Lemonade (117g/L) |

**Table A2:** Mixed-effects model ANOVA results showing the effect of subjects, sweetener concentration (g/L), and their 2-way and 3-way interactions for sweetness ratings with aroma.

| | DF | F-VALUE | P-VALUE |
|---|---|---|---|
| Subject | 54 | 91.4 | ~0.00* |
| Concentration | 5 | 231 | 2.7e-95 |
| Sweetener | 4 | 0.25 | 0.91 |
| Subject:Concentration | 270 | 7.20 | 3.1e-148 |
| Subject:Sweetener | 216 | 1.67 | 3.5e-8 |
| Concentration:Sweetener | 20 | 1.19 | 0.85 |
| Subject:Concentration:Sweetener | 1080 | 0.57 | 0.25 |

**p-value generated in R is 0.00 because it is lower than the minimum value computed in R, exact p-value is unknown.*

**Table A3.** Measured density in g/mL for each of the five sweeteners (mean ± standard error).

| Sweetener | Sugar | Average of all 4 honeys | Alfalfa Blossom | Wildflower Blossom | Orange Blossom | Clover |
|---|---|---|---|---|---|---|
| **Density (g/mL)** | 0.83 ± 0.038 | 1.43± 0.013 | 1.42 ± 0.140 | 1.42 ± 0.159 | 1.43 ± 0.126 | 1.44 ± 0.084 |

**Table A4:** Average fructose, glucose, sucrose, and total carbohydrate content ± standard deviation of three samples tested in triplicate. The data presented are summarized from Zhu et al. (2024) Table S2h for Alfalfa1, Clover3, Wildflower2, and Orange1.

| | Alfalfa | Clover | Orange | Wildflower |
|---|---|---|---|---|
| **Fructose** | 36.60 ± 1.28 | 40.36 ± 1.89 | 38.25 ± 0.41 | 41.35 ± 1.90 |
| **Glucose** | 32.49 ± 0.90 | 32.03 ± 0.95 | 31.78 ± 0.34 | 36.98 ± 0.82 |
| **Sucrose** | 0.42 ± 0.04 | 0.55 ± 0.08 | 0.68 ± 0.02 | 0.11 ± 0.01 |
| **Total carbohydrate content** | 79.30 ± 5.55 | 78.52 ± 2.28 | 78.34 ± 1.97 | 80.67 ± 1.66 |

**Table A5**: Volume and caloric comparison of equivalently sweet sugar and honey doses. Honey and table sugar are equivalently sweet by mass. The honeys used in this study have an average sugar content of 0.794g/g; the density of honey is 1.72 times that of sugar (1.43g/mL vs 0.83g/mL); the caloric density of sugar is 1.27 times that of honey (3.85kcal/g vs 3.04kcal/g).

| Equivalently sweet concentrations | | Table sugar (sucrose) | | Honey | | |
|---|---|---|---|---|---|---|
| **Sugar (tsp/ 8 oz. cup)** | **Honey (tsp/ 8 oz. cup)** | **Grams of sugars** | **Kcal** | **Grams** | **Grams of sugars** | **Kcal** |
| **1** | 0.58 | 4.2 | 16.2 | 4.2 | 3.3 | 12.8 |
| **2** | 1.16 | 8.4 | 32.4 | 8.4 | 6.7 | 25.5 |
| **3** | 1.74 | 12.6 | 48.5 | 12.6 | 10.0 | 38.3 |

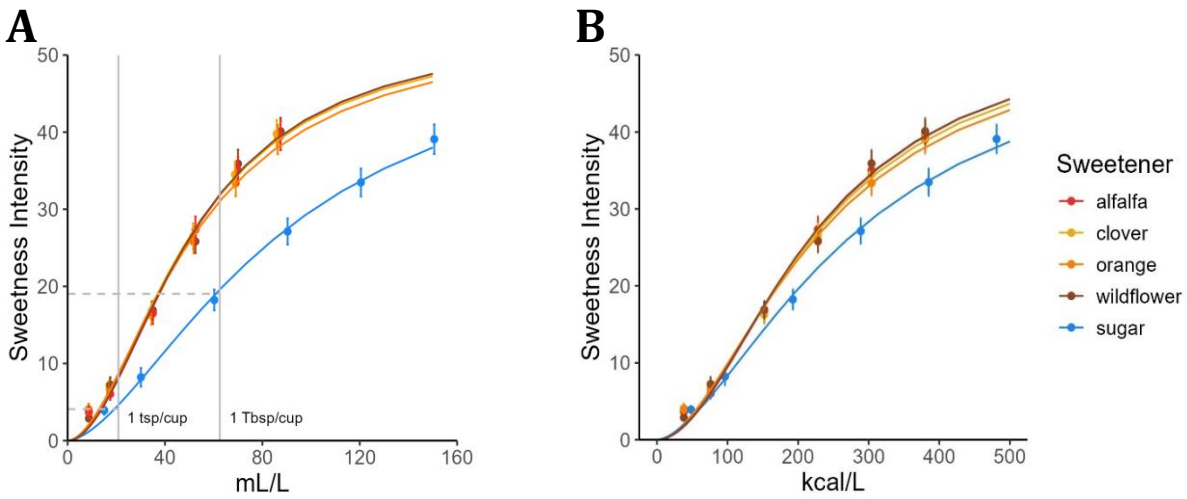



**Figure A2:** Mean sweetness intensity ratings with aroma as a function of sweetener concentration by **(A)** Volume (mL/L) (vertical lines represent the concentrations that are equivalent to 1 tsp/8 oz. cup and 1 Tbsp/8 oz. cup of each sweetener/ cup of water) and **(B)** Calories (kcal/L) for all 5 sweeteners (Alfalfa Honey, Clover Honey, Orange Honey, Sucrose, and Wildflower Honey). Error bars represent the standard error of the mean (±1 se).

**Table A6:** Percent increase in sweetness intensity at each concentration set level for all 5 sweeteners (Alfalfa Honey, Clover Honey, Orange Honey, Sucrose, and Wildflower Honey).

| Sweetener Concentration (g/L) | Sweetener | | | | |
|---|---|---|---|---|---|
| | Alfalfa | Clover | Orange | Sucrose | Wildflower |
| **12.5** | 60.6 | 68.6 | -47.4 | -53.0 | 0.07 |
| **25** | 53.1 | 82.3 | -14.9 | 65.3 | 70.2 |
| **50** | 50.3 | 44.6 | 40.3 | 27.3 | 41.7 |
| **75** | 40.9 | 44.7 | 51.3 | 18.1 | 28.5 |
| **100** | 27.9 | 25.5 | 42.6 | 5.4 | 23.1 |
| **125** | 18.6 | 16.3 | 43.4 | 25.1 | 15.8 |

**Table A7:** Percent total ion count (TIC) of compounds identified in the headspace of alfalfa honey.

| No. | Compound name | avg %TIC |
|---|---|---|
| **1** | Acetaldehyde | 0.06 |
| **2** | Ethanol | 2.08 |
| **3** | Trimethylborane | 0.02 |
| **4** | Acetone† | 0.51 |
| **5** | Furan† | 0.04 |
| **6** | 1,4-Pentadiene | 0.38 |
| **7** | Dimethyl sulfide | 1.16 |
| **8** | 2-methyl propanal | 0.31 |
| **9** | 2,3-Butanedione | 0.77 |
| **10** | 2-Butanone | 0.19 |
| **11** | 3-Penten-2-ol | 4.13 |
| **12** | Trichloromethane | 0.11 |
| **13** | Isobutyronitrile | 0.82 |
| **14** | 2-methyl-2-Propen-1-ol | 0.06 |
| **15** | 3-methylbutanal | 1.05 |
| **16** | 1-Butanol | 0.03 |
| **17** | 2-methylbutanal† | 0.67 |
| **18** | 1-hydroxy-2-propanone | 0.07 |
| **19** | Dimethyl silanediol | 1.12 |
| **20** | 2,3-Pentanedione | 0.13 |
| **21** | Pentanal | 0.08 |
| **22** | Heptane† | 0.10 |
| **23** | Bromodichloromethane | 0.16 |
| **24** | 3-hydroxy-2-butanone | 0.34 |
| **25** | 2-methylbutanenitrile | 0.68 |
| **26** | 3-methyl-3-buten-1-ol | 1.24 |
| **27** | 3-methyl- 1-butanol | 0.69 |
| **28** | Hydroxymethyl 2-hydroxy-2-methylpropionate | 0.02 |
| **29** | 2,5-dimethylfuran | 0.01 |
| **30** | (E)-2-methyl-2-butenal | 2.60 |
| **31** | Dimethyl disulfide† | 0.52 |
| **32** | Methyl 1-methylcyclopropyl ketone | 0.14 |
| **33** | Methallyl cyanide | 0.05 |
| **34** | 3-methylpentanal | 0.15 |
| **35** | Toluene | 2.80 |
| **36** | Butanoic acid | 0.16 |
| **37** | 3-methyl-2-butenal | 1.25 |
| **38** | 3-Hepten-2-one | 0.19 |
| **39** | Octane | 0.97 |

*Spectral match to NIST, † identification confirmed with external standard, ** correct isomer not identified.*

**Table A7 (cont'd).**

| | | |
|---|---|---|
| **40** | **Hexanal†** | **0.25** |
| **41** | Dibromochloromethane | 0.08 |
| **42** | Hexamethylcyclotrisiloxane | 0.16 |
| **43** | Furfural† | 4.49 |
| **44** | 4,4-Dimethyl-3-oxopentanenitrile | 4.67 |
| **45** | (Z)-3-Hexen-1-ol | 0.55 |
| **46** | 1-Hexanol† | 0.04 |
| **47** | Ethylbenzene | 0.14 |
| **48** | o-Xylene† | 0.07 |
| **49** | Oxime-, methoxy-phenyl-_ | 0.04 |
| **50** | 2-Cyclopentene-1,4-dione | 0.03 |
| **51** | 2-Acetylfuran | 0.32 |
| **52** | Heptanal | 0.08 |
| **53** | 2-Heptanone | 0.14 |
| **54** | Benzocyclobutene | 0.04 |
| **55** | 5-methylfurfural | 0.05 |
| **56** | Benzaldehyde† | 5.98 |
| **57** | 1-Octen-3-ol | 0.15 |
| **58** | Dimethyl trisulfide | 0.07 |
| **59** | Octamethylcyclotetrasiloxane | 0.04 |
| **60** | Myrcene† | 0.08 |
| **61** | Octanal† | 0.30 |
| **62** | (S)-Limonene† | 10.26 |
| **63** | Phenylacetaldehyde† | 0.94 |
| **64** | cis-Linalool oxide | 0.68 |
| **65** | trans- Linalool oxide | 0.24 |
| **66** | Nonanal† | 0.78 |
| **67** | Hotrienol | 0.43 |
| **68** | Phenylethyl Alcohol† | 0.48 |
| **69** | Lilac aldehyde** | 0.02 |
| **70** | Decamethylcyclopentasiloxane | 0.21 |
| **71** | Benzyl nitrile | 0.05 |
| **72** | (+)-neoisomenthol | 1.72 |
| **73** | Decanal† | 0.20 |
| **74** | Methyl Salicylate | 0.09 |
| **75** | 3-Phenylfuran | 0.03 |
| **76** | Damascenone | 0.03 |

*Spectral match to NIST, † identification confirmed with external standard, ** correct isomer not identified.*

**Table A8:** Percent total ion count (TIC) of compounds identified in the headspace of clover honey.

| No. | Compound Name | avg %TIC |
|---|---|---|
| **1** | Acetaldehyde | 0.03 |
| **2** | Methanethiol | 0.03 |
| **3** | Ethanol | 0.36 |
| **4** | Acetone† | 0.60 |
| **5** | Furan† | 0.05 |
| **6** | Dimethyl Sulfide | 6.50 |
| **7** | 2-methylpropanal | 0.28 |
| **8** | 2,3-Butanedione | 0.41 |
| **9** | 2-Butanone | 0.14 |
| **10** | 3-Penten-2-ol | 1.97 |
| **11** | Trichloromethane | 0.10 |
| **12** | Isobutyronitrile | 0.27 |
| **13** | 3-methylbutanal | 1.77 |
| **14** | 2-methylbutanal† | 1.82 |
| **15** | 1-hydroxy-2-propanone | 0.06 |
| **16** | Dimethyl silanediol | 1.45 |
| **17** | 2,3-Pentanedione | 0.10 |
| **18** | Heptane† | 0.15 |
| **19** | Bromodichloromethane | 0.16 |
| **20** | 2-methylbutanenitrile | 0.38 |
| **21** | 3-Buten-1-ol, 3-methyl- | 0.63 |
| **22** | 3-methyl-1-butanol | 0.51 |
| **23** | 1-Butanol, 2-methyl-, (S) | 0.11 |
| **24** | 2-Butenal, 2-methyl-,(E)- | 1.63 |
| **25** | Dimethyl disulfide† | 3.99 |
| **26** | Methallyl cyanide | 0.05 |
| **27** | 3-methylpentanal | 0.12 |
| **28** | Spiro[2.4]hepta-4,6-diene | 0.17 |
| **29** | 3-Penten-2-ol | 0.66 |
| **30** | Butanoic acid | 0.67 |
| **31** | 3-methyl-2-butenal | 0.73 |
| **32** | Octane | 1.49 |
| **33** | Dibromochloromethane | 0.08 |
| **34** | Hexamethyl cyclotrisiloxane | 0.14 |
| **35** | 3-Methylbutanoic acid | 0.15 |
| **36** | Furfural† | 8.86 |
| **37** | 4,4-Dimethyl-3-oxopentanenitrile | 1.31 |
| **38** | (z)-3-Hexen-1-ol | 0.31 |
| **39** | o-xylene† | 0.03 |

*Spectral match to NIST, † identification confirmed with external standard, ** correct isomer not identified*

**Table A8 (cont'd).**

| | | |
|---|---|---|
| **40** | **Oxime-, methoxy-phenyl-_** | **0.05** |
| **41** | 2-Cyclopentene-1,4-dione | 0.03 |
| **42** | Nonane | 0.50 |
| **43** | Heptanal | 0.04 |
| **44** | 2-Acetylfuran | 0.28 |
| **45** | 5-methylfurfural | 0.05 |
| **46** | Benzaldehyde† | 8.37 |
| **47** | 1-Octen-3-ol | 0.24 |
| **48** | Dimethyl trisulfide | 0.74 |
| **49** | Octamethylcyclotetrasiloxane | 0.04 |
| **50** | Octanal† | 0.22 |
| **51** | Benzyl Alcohol | 0.32 |
| **52** | Phenylacetaldehyde† | 5.74 |
| **53** | Dihydromyrcenol | 0.15 |
| **54** | trans-Linalool oxide | 0.18 |
| **55** | 1-methyl-4-(1-methylethenyl)-benzene | 0.71 |
| **56** | Nonanal† | 0.71 |
| **57** | Hotrienol | 0.29 |
| **58** | Phenylethyl Alcohol† | 0.31 |
| **59** | Decamethylcyclopentasiloxane | 1.17 |
| **60** | (+)-neoisomenthol | 1.61 |
| **61** | Decanal† | 0.22 |
| **62** | Methyl Salicylate | 0.03 |
| **63** | 3-phenyl furan | 0.03 |
| **64** | Damascenone | 0.03 |

*Spectral match to NIST, † identification confirmed with external standard, ** correct isomer not identified*

**Table A9:** Percent total ion count (TIC) of compounds identified in the headspace of orange honey.

| No. | Compound Name | avg %TIC |
|---|---|---|
| **1** | Acetaldehyde | 0.04 |
| **2** | Ethanol | 2.88 |
| **3** | Trimethylborane | 0.07 |
| **4** | Acetone† | 0.34 |
| **5** | Furan† | 0.04 |
| **6** | Dimethyl sulfide | 1.36 |
| **7** | Methane sulfonyl chloride | 0.01 |
| **8** | 2-methylpropanal | 0.18 |
| **9** | Formic acid | 0.03 |
| **10** | 2,3-Butanedione | 0.30 |
| **11** | 3-Methyl-2-pentanone | 0.11 |
| **12** | 2-Butanol | 0.03 |
| **13** | Acetic acid | 0.33 |
| **14** | Trichloromethane | 0.04 |
| **15** | Isobutyronitrile | 0.32 |
| **16** | 2-Methyl-1-propanol | 0.06 |
| **17** | 3-Penten-2-ol | 0.07 |
| **18** | 3-methylbutanal | 0.96 |
| **19** | 1-Butanol | 0.04 |
| **20** | 2-methylbutanal† | 0.54 |
| **21** | 1-hydroxy-2-propanone | 0.03 |
| **22** | 2,3-Pentanedione | 0.10 |
| **23** | Dimethyl silanediol | 0.53 |
| **24** | Pentanal | 0.02 |
| **25** | Bromodichloromethane | 0.07 |
| **26** | 3-Hydroxy-2-butanone | 0.14 |
| **27** | 2-Methylbutanenitrile | 0.23 |
| **28** | 3-Methylbutanenitrile | 1.04 |
| **29** | 2-Methyl-1-butanol | 0.16 |
| **30** | 2-Methyl-2-butenal | 0.06 |
| **31** | Dimethyl disulfide† | 0.81 |
| **32** | 2-Methyl-3-pentanone | 0.03 |
| **33** | 3-Methylpentanal | 0.02 |
| **34** | Toluene | 8.04 |
| **35** | 3-methyl-2-butenal | 0.02 |
| **36** | 3-Hepten-2-one | 1.22 |
| **37** | Octane | 0.86 |
| **38** | 2-Ethylcyclobutanol | 0.34 |
| **39** | Dibromochloromethane | 0.04 |

*Spectral match to NIST, † identification confirmed with external standard, ** correct isomer not identified*

**Table A9 (cont'd).**

| | | |
|---|---|---|
| **40** | **3(2H)-Furanone, dihydro-2-methyl-** | **0.10** |
| **41** | Hexamethyl cyclotrisiloxane | 0.09 |
| **42** | 3-Methyl-butanoic acid | 0.04 |
| **43** | Acetyl valeryl | 0.07 |
| **44** | Furfural† | 5.23 |
| **45** | Ethylbenzene | 0.37 |
| **46** | o-Xylene† | 0.05 |
| **47** | Oxime-, methoxy-phenyl-_ | 0.04 |
| **48** | 2-Methylpentanoic acid | 0.04 |
| **49** | Cyclopropane, propyl- | 0.06 |
| **50** | 3-Hydroxy-2-butanone | 0.01 |
| **51** | 2-Furanmethanol | 0.01 |
| **52** | Heptanal | 0.09 |
| **53** | 2-Acetylfuran | 0.34 |
| **54** | 2-methyl-3-Octyne- | 0.11 |
| **55** | Pentanoic acid, 2-hydroxy-4-methyl-, methyl ester | 0.06 |
| **56** | 2-Ethylhexanal | 0.01 |
| **57** | Lilac alcohol** | 0.02 |
| **58** | 5-Methylfurfural | 0.15 |
| **59** | Benzaldehyde† | 4.51 |
| **60** | 1-Octen-3-ol | 0.07 |
| **61** | Dimethyl trisulfide | 0.03 |
| **62** | Octamethylcyclotetrasiloxane | 0.03 |
| **63** | Myrcene† | 0.63 |
| **64** | Octanal† | 0.19 |
| **65** | (S)-Limonene† | 21.11 |
| **66** | Phenylacetaldehyde† | 1.47 |
| **67** | Cis-Linalool oxide | 3.94 |
| **68** | Nonanal† | 1.09 |
| **69** | Hotrienol | 2.34 |
| **70** | Phenylethyl Alcohol† | 0.06 |
| **71** | Decamethylcyclopentasiloxane | 0.07 |
| **72** | Benzyl nitrile | 0.17 |
| **73** | Lilac aldehyde** | 0.39 |
| **74** | 2,6,6-Trimethyl-2-cyclohexene-1,4-dione | 0.06 |
| **75** | 2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl- | 0.17 |
| **76** | (+)-neoisomenthol | 1.19 |
| **77** | Terpinen-4-ol | 0.01 |
| **78** | Anethofuran | 0.27 |
| **79** | P-Menth-1-en-8-ol | 0.32 |

*Spectral match to NIST, † identification confirmed with external standard, ** correct isomer not identified*

**Table A9 (cont'd).**

| | | |
|---|---|---|
| **80** | **Decanal†** | **0.29** |
| **81** | 2-Thiophenecarboxylic acid, 3-methyl-, methyl ester | 0.02 |
| **82** | Safranal | 0.07 |
| **83** | P-Menth-1-en-9-al | 1.08 |
| **84** | 3-Phenylfuran | 0.01 |
| **85** | Nonanoic acid | 0.07 |
| **86** | Damascenone | 0.04 |

*Spectral match to NIST, † identification confirmed with external standard, ** correct isomer not identified*

**Table A10:** Percent total ion count (TIC) of compounds identified in the headspace of wildflower honey.

| No. | Compound Name | avg %TIC |
|---|---|---|
| **1** | Acetaldehyde | 0.07 |
| **2** | Methanethiol | 0.03 |
| **3** | Ethanol | 1.61 |
| **4** | Acetone† | 0.99 |
| **5** | Furan† | 0.03 |
| **6** | Dimethyl sulfide | 4.92 |
| **7** | 2-Methylpropanal | 0.45 |
| **8** | Formic acid | 0.12 |
| **9** | 2,3-Butanedione | 0.53 |
| **10** | 2-Butanone | 0.46 |
| **11** | (R)-2-Butanol | 0.60 |
| **12** | 3-Penten-2-ol | 0.52 |
| **13** | Acetic acid | 1.46 |
| **14** | Trichloromethane | 0.13 |
| **15** | 2-Methyl-1-propanol | 0.21 |
| **16** | Isobutyronitrile | 0.04 |
| **17** | 3-Methylbutanal | 2.57 |
| **18** | 2-Methylbutanal† | 2.23 |
| **19** | 1-Hydroxy-2-propanone | 0.04 |
| **20** | 2,3-Pentanedione | 0.06 |
| **21** | Dimethyl silanediol | 1.33 |
| **22** | 2,5-Dimethylfuran | 0.01 |
| **23** | Bromodichloromethane | 0.20 |
| **24** | 3-Hydroxy-2-butanone | 0.20 |
| **25** | 2-Methylbutanenitrile | 0.22 |
| **26** | 3-Methyl-3-buten-1-ol | 0.15 |
| **27** | 3-Methyl-1-butanol | 0.50 |
| **28** | Dimethyl disulfide† | 1.47 |
| **29** | 2-Methyl-1-butanol | 0.29 |
| **30** | Methallyl cyanide | 0.07 |
| **31** | 3-Methyl-pentanal | 0.05 |
| **32** | Butanoic acid | 0.07 |
| **33** | 3-Methyl-2-butenal | 0.15 |
| **34** | Octane | 0.82 |
| **35** | Hexanal† | 0.25 |
| **36** | Dibromochloromethane | 0.10 |
| **37** | 3(2H)-Furanone, dihydro-2-methyl- | 0.07 |
| **38** | Hexamethylcyclotrisiloxane | 0.17 |
| **39** | 3-Methylbutanoic acid | 0.10 |

*Spectral match to NIST, † identification confirmed with external standard, ** correct isomer not identified*

**Table A10 (cont'd).**

| | | |
|---|---|---|
| **40** | **Furfural†** | **8.40** |
| **41** | (Z)-3-Hexen-1-ol | 0.32 |
| **42** | Hexanenitrile | 0.13 |
| **43** | Oxime-, methoxy-phenyl-_ | 0.06 |
| **44** | o-Xylene† | 0.10 |
| **45** | Heptanal | 0.11 |
| **46** | 2-Acetylfuran | 0.45 |
| **47** | 2-Ethylhexanal | 0.02 |
| **48** | 5-Methylfurfural | 0.09 |
| **49** | Benzaldehyde† | 4.98 |
| **50** | 1-Octen-3-ol | 0.27 |
| **51** | Dimethyl trisulfide | 0.08 |
| **52** | Octamethylcyclotetrasiloxane | 0.05 |
| **53** | Octanal† | 0.33 |
| **54** | Benzyl Alcohol | 0.18 |
| **55** | Phenylacetaldehyde† | 3.90 |
| **56** | Dihydromyrcenol | 0.17 |
| **57** | cis-Linalool oxide | 0.36 |
| **58** | Nonanal† | 0.87 |
| **59** | Hotrienol | 0.47 |
| **60** | Phenylethyl Alcohol† | 0.14 |
| **61** | Isophorone | 0.15 |
| **62** | Decamethylcyclopentasiloxane | 0.17 |
| **63** | Lilac aldehyde** | 0.01 |
| **64** | Octanoic Acid | 0.18 |
| **65** | Ethanone, 1-(1,4-dimethyl-3-cyclohexen-1-yl)- | 0.07 |
| **66** | Decanal† | 0.43 |
| **67** | Methyl salicylate | 0.02 |
| **68** | Myrtenal | 0.07 |
| **69** | 3-phenylfuran | 0.02 |
| **70** | Nonanoic acid | 0.12 |
| **71** | Damascenone | 0.04 |

*Spectral match to NIST, † identification confirmed with external standard, ** correct isomer not identified*

## Chapter 4: A comparison of honey and sugar used to sweeten foods, and their efficacy in increasing acceptance of nutritious but bitter foods


### Abstract

The standard American diet does not meet the recommended levels of many micro-nutrients but exceeds the recommended sugar intake. A Mediterranean dietary pattern is recommended for improved health; however, key foods in the diet are bitter. Adding sugar can reduce bitterness and improve acceptance but goes against nutritional recommendations. We previously found that honey is equally sweet by mass and 1.27x sweeter than sugar per kcal in aqueous solutions, but this relative sweetness has not been validated in foods. First, to measure the relative sweetness of honey in diverse products, consumers rated overall liking and the sweetness intensity for honey- or sugar-sweetened Greek yogurt, oatmeal, vinaigrette, dressed leafy greens, and three varieties of teas. Results indicated honey was only equivalently sweet by mass in the salad and significantly less sweet in other products (t-test; $p<0.05$). A second experiment compared the impact of added honey and sugar on acceptance of nutritious, bitter foods; consumers rated overall liking, sweetness intensity, and bitterness intensity of foods (Greek yogurt with walnuts, vinaigrette, dressed leafy greens, dressed farro salad) prepared in three sweetener conditions (unsweetened, honey, or sugar). Except for the bitterness of the salad with sugar, sweetened conditions were more liked, sweeter, and less bitter than the unsweetened condition (Two-factor ANOVA; $p<0.05$). Honey suppressed bitterness to an equal or greater degree than sugar for all foods tested. While the relative sweetness of honey is product-dependent, our results suggest it can be used to reduce bitterness and increase liking with fewer kcals than sucrose.

## Introduction

Current Dietary Guidelines for Americans suggest restricting added sugars to less than 10% of daily caloric intake (U.S Department of Agriculture and U.S Department of Health and Human Services 2020). Despite these recommendations, Americans over-consume added sugars (DiFrancesco et al., 2022). The standard American diet (SAD) is characterized by a high consumption of ultrarefined carbohydrates and saturated fats and low consumption of fiber and other micronutrients that come from whole grains, fruits, and vegetables (Grotto & Zied, 2010; Totsch et al., 2018). The underconsumption of dietary components such as calcium, potassium, vitamin D, and dietary fiber is a public health concern for Americans (U.S Department of Agriculture & U.S Department of Health and Human Services, 2020). A Mediterranean Diet (MD) contrasts with the SAD; this dietary style is recognized for high consumption of extra virgin olive oil, legumes and nuts, unrefined cereals, fruits and vegetables, and fish, with moderate to low consumption of dairy products, meats, and wine (Kris-Etherton et al., 2001; Serra-Majem et al., 2019). Evidence suggests that the Mediterranean diet can improve overall health and increase life expectancy by lowering the risks of various cardiovascular diseases, diabetes, cognitive decline, obesity, cancer, and more (Keys et al. 1986; Serra-Majem et al. 2019; Mentella et al. 2019).

Humans have an innate preference for sweetness, and consumer preference is a significant barrier against increasing healthy food consumption (Clemens et al., 2016). Many nutrient-dense foods associated with MD, such as extra virgin olive oil, whole grains, and leafy greens, are quite bitter; however, this taste is innately undesirable (Isabelle Lesschaeve & Ann C Noble, 2005). One way to reduce the bitterness of a food is by adding sweeteners

(e.g., adding sugar to coffee to reduce its bitterness); however, added sugars should be minimized (Beck et al., 2014).

Non-nutritive sweeteners, sweet substances that provide little to no energy, such as stevia, aspartame, and monk fruit are commonly used in today's food and beverage industry (Chattopadhyay et al., 2014; Russell et al., 2023; Wee et al., 2018). However, many sugar alternatives have sensory properties that differ from sugar (Coulston and Johnson 2002; Chen et al. 2023). Additionally, there is a growing consumer avoidance of non-nutritive sweeteners (Chen et al., 2023). The drawbacks of non-nutritive sweeteners have motivated food producers and sensory scientists to pursue alternative methods of sugar reduction.

Odor-induced sweet taste enhancement is a potential avenue for sugar reduction. In a companion study, we found that the naturally occurring aromas in honey significantly contribute to its sweetness intensity (Mulheron et. al, in preparation). Honey has about 20% less total sugar than an equivalent mass of sucrose, yet aqueous solutions dosed with equivalent masses of honey and sugar were equivalently sweet (Mulheron et al., in preparation). Additionally, honey is less calorically dense than sucrose (3.04kcal/g vs. 3.85kcal/g); so, using honey to sweeten foods instead of sucrose may be a potential way to lower total sugar and caloric consumption while still maintaining sweetness and consumer acceptability of the product (FoodData Central, 2019a, 2019b).

In the present study we conducted a consumer test to validate that honey and sugar are equivalently sweet by mass in foods that are commonly sweetened. In a second experiment, we investigated the efficacy of honey and sugar in increasing the acceptability of bitter, nutrient-dense foods that are associated with a Mediterranean diet.

## Materials and Methods

### *Central location test*

Two experiments using central location testing (CLT) in which consumers were recruited on the spot were conducted outside of a dining hall on the campus of Michigan State University. In the first experiment, seven foods and beverages (Greek yogurt, oatmeal, a simple vinegarette, leafy greens salad, hot black tea, hot herbal tea, and iced black tea) were prepared in two conditions, differing by sweetener (honey or sugar). Prepared samples were tested in four product sets across six days: set 1: hot teas, set 2: iced tea and yogurt, and set 3: vinaigrette and leafy greens salad (participants were given the vinaigrette twice, to rate alone and to dress the greens), and set 4: oatmeal. In the second experiment, four foods (Greek yogurt with walnuts, vinaigrette, leafy greens salad, and farro salad) were prepared in three conditions differing by sweetener (unsweetened, honey, or sugar). Prepared samples were tested in three product sets across four days: set 1: Greek yogurt with walnuts, 2: vinaigrette and leafy greens salad, and 3: vinaigrette and farro salad. Product descriptions for both experiments are described in section 2.2.

In both experiments, consumers ($n \geq 101$) rated the overall liking and sweetness intensity of each food application; in Experiment 2, consumers additionally rated bitterness intensity. To reduce potential biases, samples were blinded with 3-digit codes, the presentation order of sweetener conditions was randomized following a complete block design, and there was a mandatory 30-second break between samples during which subjects rinsed with water. Consumers were instructed to rate their overall liking of their first bite/sip, then taste the sample again and rate the sweetness intensity (experiment 1 and 2) and the bitterness

intensity (experiment 2). Overall liking was rated using a Labeled Affective Magnitude Scale (LAM); this hedonic scale was labeled 0= Greatest Imaginable Dislike for Products Like This, 12= Dislike Extremely, 22= Dislike Very Much, 34= Dislike Moderately, 45= Dislike Slightly, 50= Neither Like Nor Dislike, 56= Like Slightly, 68= Like Moderately, 78= Like very Much, 87= Like Extremely, 100= Greatest Imaginable Like for Products Like This. Sweetness and bitterness intensity ratings were collected using a Visual Analog Scale (VAS), labeled 0=No Sweetness or No Bitterness, 100= Extremely Sweet or Extremely Bitter. Additionally, in experiment 2, consumers were screened for PROP (6-*n*-Propylthiouracil) taster status after sample evaluation to determine their sensitivity to bitterness. Consumers rated two warmup sensations on the Global Sensory Intensity Scale (GSIS) to aid in familiarization with a scale that considers all senses. Then, consumers rated the bitterness intensity of a filter paper with saturated PROP on the GSIS to determine taster status (Bartoshuk et al. 1994).

At the end of the test, consumers were asked a series of demographic questions, followed by a sweetener consumption survey including questions on sweetened product consumption, sweetener use, and how much sugar and honey they would add to an 8-ounce cup of tea (Table A1). Sweetened food product categories were adapted from Guthrie and Morton (2000). On the questions of how they would sweeten tea, consumers were provided with visual aids of sweetener and tea volumes. In experiment 2, following the demographic and sweetener consumption questionnaires, consumers completed a Mediterranean diet adherence survey; the survey was adapted from Mattavelli et al. (2023) and included six questions from the original survey (Table A2). Panelists received a $5 electronic gift card for each completed survey. They were allowed to participate in multiple

sessions but not to rate the same product set more than once. These protocols were reviewed and approved by the Michigan State University Institutional Review Board (STUDY00007723).

### *Sample Preparation*

#### Experiment 1:

Foods that are typically prepared with the addition of a sweetener (commercially and at home by consumers) were dosed in equivalent masses of honey (Dutch Gold, Lancaster Co., PA) or sugar (Domino pure cane sugar, West Palm Beach, FL), namely: oatmeal, 0% milkfat Greek yogurt, a simple vinaigrette (rated alone and on a leafy greens salad), and teas (hot black, iced black, hot herbal). To validate the relative sweetness intensity of honey, the same batch of clover honey used in the companion study was the only honey used in this paper; stored at -20ºC and used within seven days of thawing (Mulheron et al., in preparation). This floral source was chosen because clover honey is a commonly sold variety in supermarkets. The added sweetener mass, total sugar, and caloric differences between the two sweeteners for each food application are displayed in Table 1. Detailed recipes for each food application are provided in tables A3-10.

**Table 1:** Amount of sugar and clover honey added to one serving of each food product. The difference in added sugars (Δ sugars) represents the total g added sugars in the sucrose condition - minus the total g added sugars in the honey condition. The difference in added kcal (Δ kcal) represents the disparities in calories: added sugar kcal minus added honey kcal. The vinaigrette was used to dress one-half cup of leafy greens salad (experiment 1&2) and one-half cup of farro salad (experiment 2). In experiment 2, the Greek yogurt was topped with two 2 tablespoons of tbsp chopped walnuts.

| Product | Serving Size | Mass of added sweetener (g) | Δ sugars (g) | Δ kcal |
|---|---|---|---|---|
| **Oatmeal** | ¼ cup | 3.75 | 0.73 | 3.04 |
| **Greek yogurt** | ¼ cup | 4.00 | 0.78 | 3.24 |
| **Vinaigrette** | 2 tbs | 3.15 | 0.62 | 2.55 |
| **Tea** | 4oz | 5.73 | 1.12 | 4.64 |

In addition to an equivalent mass of honey to sugar, hot black tea was tested with two additional concentrations of honey: an equivalent volume of honey and equivalent calories of honey to sugar (Table 2). Detailed recipes for additional teas are provided in tables A11-12.

**Table 2**: Quantities of added honey and sugar tested in hot black tea per four-ounce, 4-oz serving size.

| Sugar-sweetened control | | | Honey-sweetened samples | | | |
|---|---|---|---|---|---|---|
| Mass (g) | Energy (kcal) | Volume (tsp) | Unit of equivalence | Mass (g) | Energy (kcal) | Volume (tsp) |
| **5.73** | **22.1** | **1.36** | **Mass** | **5.73** | 17.4 | 0.79 |
| | | | **Kcal** | 7.27 | **22.1** | 1.00 |
| | | | **Volume** | 9.88 | 30.0 | **1.36** |

**Experiment 2:**

Bitter foods commonly associated with the Mediterranean diet were prepared in three conditions: unsweetened, sugar-sweetened, and honey-sweetened. The sweetened conditions were prepared using an equivalent mass of sweetener and at the same concentrations as experiment 1 (Table 1). The tested foods were 0% milkfat Greek yogurt

(with walnuts) and a simple vinaigrette rated alone and used to top both a leafy greens salad and a farro salad.

### *Analysis*

All analyses were performed using R version 4.3.2 (2023-10-31 ucrt). Data was visualized using ggplot2 (v3.4.4) and GGally (v2.2.1). Welch's 2 sample t-test was conducted to determine any significant differences in overall liking and perceived sweetness of food products sweetened with the predicted iso-sweet concentration of sugar and clover honey. Differences in overall liking and sweetness intensity ratings were also examined within groups of consumers that use either sugar or natural sweeteners most often. Additionally, a 2-way mixed ANOVA followed by Least Significant Difference (LSD) post hoc means separation tests were performed to determine differences in overall liking, sweetness intensity, and bitterness intensity of experiment 2 products. PROP tasters are determined by consumer ratings of PROP strips on the GSIS, and cut-offs of taster status are determined according to Catanzaro et al. (2013). A Pearson's correlation test was used to determine if a person's PROP sensitivity is related to their liking of bitter foods. Consumers were additionally grouped by their most used sweetener and reported quantities of honey and sugar added to tea were compared using paired t-tests.

## Results and discussion

### *Subject demographics*

**Table 3:** General demographics (gender, age, race, ethnicity), sweetener usage, sweet food consumption averages, Mediterranean food consumption averages, and PROP taster status for all consumers. Hot tea (honey EM) represents the first hot teas, black and herbal, tested with an equal mass (EM) of honey to the sugar condition. Hot tea (honey EV, EC) represents the additional hot tea test; black tea is sweetened with equal calories and an equal volume of honey compared to sugar.

| | | Product category | | | | | |
|---|---|---|---|---|---|---|---|
| | **Demographic and dietary characteristics** | **Oatmeal** | **Greek Yogurt** | **Dressed salad** | **Hot Tea (honey EM)** | **Iced Black Tea** | **Hot Tea (honey EV, EC)** |
| **Experiment 1 cohort** | total n | 118 | 188 | 101 | 104 | 104 | 79 |
| | M | 79 | 116 | 72 | 63 | 69 | 46 |
| | F | 37 | 67 | 27 | 37 | 31 | 33 |
| | other/non-binary | 0 | 3 | 1 | 2 | 3 | 0 |
| | age (mean ± sd) | 19.7 ± 5.78 | 20.4 ± 6.21 | 19.9 ± 4.34 | 19.9 ± 5.01 | 21.3 ±7.67 | 19.1 ± 1.81 |
| | Sweetened product consumption (mean servings per day±sd) | 5.36 ± 3.80 | 5.43 ± 3.69 | 5.85 ± 4.43 | 5.19 ± 3.71 | 5.38 ± 3.94 | 5.37 ± 3.17 |
| | ***Sweeteners used*** | | | | | | |
| | sugar | 71 | 108 | 57 | 58 | 55 | 55 |
| | natural sweeteners | 35 | 63 | 32 | 36 | 31 | 30 |
| | natural low calorie sweeteners | 13 | 24 | 11 | 12 | 12 | 10 |
| | articificial sweeteners | 21 | 37 | 18 | 19 | 14 | 25 |
| | do not sweeten | 29 | 47 | 31 | 30 | 36 | 10 |
| | other | 0 | 1 | 1 | 2 | 1 | 0 |
| | | **Greek Yogurt** | | **Dressed Greens Salad** | | **Dressed Farro Salad** | |
| **Experiment 2 cohort** | total n | 103 | | 101 | | 104 | |
| | M | 57 | | 57 | | 59 | |
| | F | 45 | | 42 | | 42 | |
| | other/non-binary | 0 | | 0 | | 1 | |
| | age (mean +/- sd) | 19.8 ± 3.61 | | 19.3 ± 3.38 | | 19.5 ± 3.52 | |
| | ***PROP taster status*** | | | | | | |
| | non-taster | 19 | | 23 | | 28 | |
| | taster | 57 | | 34 | | 40 | |
| | super-taster | 25 | | 42 | | 32 | |
| | Mediterranean diet adherence (mean servings per day +/- sd) | 6.62 ± 3.10 | | 7.10 ± 4.44 | | 8.85 ± 4.74 | |
| | ***Sweeteners used*** | | | | | | |
| | sugar | 74 | | 66 | | 65 | |
| | natural sweeteners | 38 | | 38 | | 35 | |
| | natural low calorie sweeteners | 19 | | 21 | | 14 | |
| | articificial sweeteners | 25 | | 24 | | 19 | |
| | do not sweeten | 18 | | 23 | | 26 | |
| | other | 0 | | 0 | | 0 | |

Demographic and dietary consumption data characterizing consumer test cohorts are presented in Table 3. The majority of participants were college students, with cohort average ages falling between 19.1 and 21.3 years; gender composition was balanced on most test days, though male participants generally outnumbered females in these experiments. In experiment 1, where validating the relative sweetness of honey was the focus, we surveyed consumers on their consumption of sweetened products. On average, consumers reported consuming just over five servings of sweetened foods and beverages daily. In experiment 2, where the impact of sweeteners on bitterness and acceptance of bitter and nutritious foods was the focus, we surveyed consumers on their consumption of foods associated with the Mediterranean diet. On average, consumers report consuming between six and nine servings of foods that adhere to the Mediterranean diet daily. Additionally, in experiment 2, subjects rated the bitterness of PROP strips and were classified as PROP non-tasters, tasters, or super tasters; the majority of consumers were sensitive to PROP. In both experiments, we surveyed consumers about which sweeteners they used; sugar was the sweetener most commonly used by consumers, followed by natural sweeteners (e.g. honey, maple syrup).

***Experiment 1: Relative sweetness of honey in common food and beverage applications***

In experiment 1, consumers gave higher liking and sweetness ratings for sugar-sweetened oatmeal, Greek yogurt, hot herbal tea, and iced black tea than for the honey-sweetened counterpart (Figure 1). Additionally, oatmeal, Greek yogurt, salad dressing, hot black tea, hot herbal tea, and iced black tea sweetened with sugar are perceived as sweeter than the same product sweetened with an equivalent mass of honey (Figure 1).

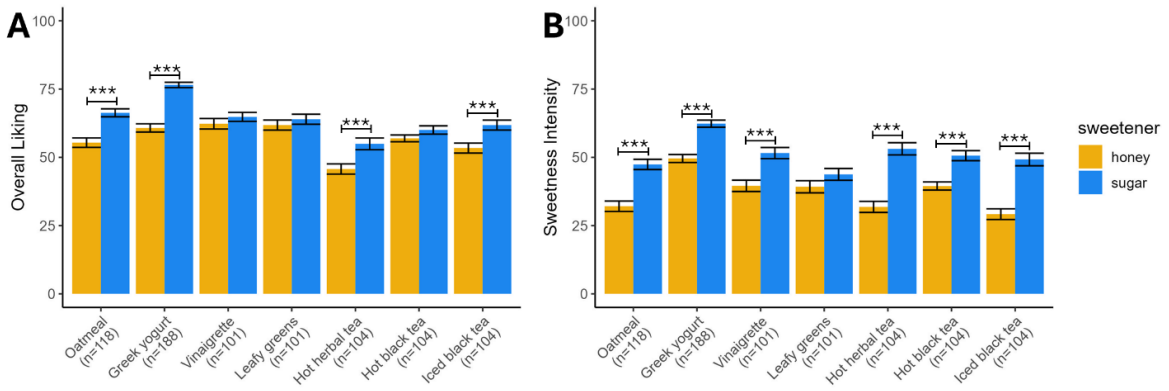



**Figure 1:** Experiment 1 mean **(A)** overall liking and **(B)** sweetness intensity ratings for all tested foods and beverages (oatmeal n=118, Greek yogurt n=188, vinaigrette n=101, hot herbal tea n=104, hot black tea n=104, iced black tea n= 104. Error bars represent the standard error of the mean (±1 se). Significant differences in liking and sweetness intensity ratings for sugar- or honey-sweetened conditions are defined as *** ($p<0.001$).

***Experiment 2: Impact of sweeteners on the acceptance of bitter foods***

In experiment 2, both sweetened conditions were more liked, and rated sweeter and less bitter than the unsweetened control, with one exception - the leafy greens dressed with the sugar-sweetened vinaigrette were not significantly less bitter than the unsweetened condition (Figure 2). The sugar-sweetened Greek yogurt with walnuts was liked more than the honey condition, and there was no significant difference in liking between honey- or sugar-sweetened conditions for the remaining products (vinaigrette, leafy greens, and farro salads) (Figure 2a). There was no significant difference in sweetness rating between the honey and sugar conditions of the yogurt and both salads. Still, when the vinaigrettes were rated independently, the honey-sweetened vinaigrette was the sweetest (Figure 2b). Sweetness intensity did not directly correlate to liking, as the honey-sweetened vinaigrette was sweeter than the sugar-sweetened counterpart, but both vinaigrettes were liked equally. Additionally, the honey- or sugar-sweetened Greek yogurt were equivalently sweet,

but the honey-sweetened condition was less liked (Figure 2). Using honey as a sweetener to reduce bitterness worked as well as or better than sugar for all conditions (Figure 2c).

***Factors contributing to differences in perceived taste intensities***

There is a weak, positive correlation (r=0.08) between the perceived bitterness intensity of the PROP strip ratings and bitterness intensity (p=0.002). A weak negative correlation existed between PROP bitterness intensity and overall liking and sweetness intensity. However, the correlation was insignificant for both attributes (p=0.08 and p=0.34, respectively).

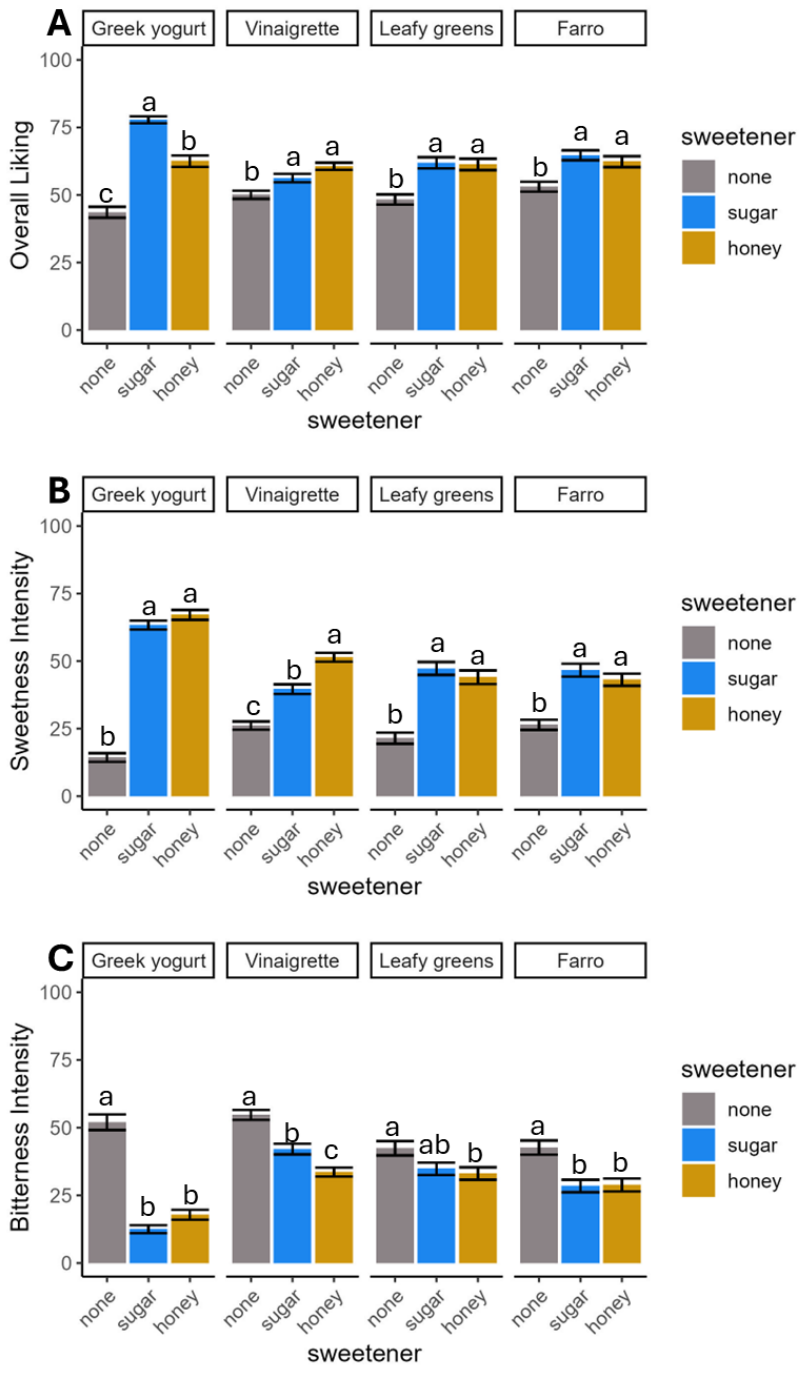



**Figure 2:** Mean **(A)** overall liking, **(B)** sweetness intensity, and **(C)** bitterness intensity of an unsweetened, sweetened with sugar, or sweetened with honey condition of a Greek yogurt with walnuts (n= 103), a simple vinaigrette (n= 205), farro salad (n= 104), and leafy greens salad (n= 101).

The relative sweetness of honey differs between food products and sessions; therefore, many potential causes were considered when interpreting these findings. Variations in tastants (sugars, acids, salts, fats, phenols, etc.) and aromas between food products may have affected the degree to which the aromas in honey influenced sweetness and overall acceptability of the food it was added to. Additionally, the texture of the food product should be acknowledged. One study looked at the effect of yogurt protein content and viscosity and found that as protein content and viscosity increased, the intensity of aroma decreased (Saint-Eve et al., 2006). In other mediums, increased viscosities were inversely correlated with aroma intensity; both Greek yogurt and oatmeal have high viscosities, which may have decreased the degree to which the aromas in honey impacted sweetness intensity (Pangborn and Szczesniak 1974).

In addition to physical differences between the products, oral residency is an essential factor to consider. The sugar-sweetened yogurt was significantly sweeter than the honey-sweetened yogurt in experiment 1 ($p= 5.4 e -7$); there was no significant difference between the sugar- or honey-sweetened samples in experiment 2 ($p= 0.17$) (Figure 1B, 2B). The only difference between the honey- or sugar-sweetened yogurt samples in both experiments was the presence of walnuts in experiment 2. Adding walnuts introduced the need to chew the yogurt, which likely increased the oral residency of the sample in the mouth. A longer oral residency allows the aromas in the honey samples more time to warm up and volatilize. Additionally, chewing breaks down the food structure. It likely contributes to a greater release of aroma compounds, suggesting the aromas in honey had a greater impact on sweetness in the honey-sweetened yogurt samples with walnuts (Liu et al., 2017).

We hypothesize that the temperature at which the food is consumed and prepared is another factor contributing to the sweetness enhancement potential of the aromas in honey. In the companion study, clover honey aromas contributed to a 68.2% sweetness intensity increase at a concentration of 12.5 g/L, and the most abundant volatile was furfural (Mulheron et al., in preparation). It is likely that the concentration of furfural, an aldehyde with a flash point of 140°F, and other heat-sensitive volatiles was reduced due to exposure to high temperatures when the teas and oatmeal were prepared with boiling water (212°F) (Mulheron et al. in preparation, Gong et al. 2023). Greek yogurt was served cold and requires little oral processing, so it is possible that the aromas in the honey did not fully volatilize in the consumer's mouth, reducing the sweetness-enhancing potential of those aromas. On the other hand, the honey had the highest relative sweetness in the applications that involved the vinaigrette, which was never heated in its preparation and served close to room temperature. We theorize that honey would be more effective in oatmeal and tea if it is added to the product closer to the time of consumption once it has cooled off sufficiently.

Additionally, contextual differences in samples served on a particular day/session are another factor to consider. The products in the first experiment had two conditions (honey- and sugar-sweetened), while those in the second experiment had an additional unsweetened condition. The addition of the unsweetened condition in a sample set may have closed the perceptual gap between the honey- and sugar-sweetened conditions. Consumers were able to rate multiple product sets during some days of the study (e.g., on the day of iced tea testing, Greek yogurt (from experiment 1) was also tested to meet the target n≥ 100 consumers). Tasting multiple products in one day may have influenced

consumer perception. Individual differences in consumer perceptions are another factor; the consumer pool for each product varies, and individuals have different preferences and sensitivities to sweetness.

***Consumer sweetener use and impact on product liking***

**Table 4:** Average amounts (tsp) of added sugar and added honey amounts as a function of most-used sweetener for all consumers.

| Sweetener category used the most often | n | Mean added sugar (tsp) ± SD | Mean added honey (tsp) ± SD | Difference in use | p-value |
|---|---|---|---|---|---|
| **Sugar (white table sugar, brown sugar, cane sugar, etc.)** | 492 | 1.56 ± 1.06 | 1.74 ± 1.07 | 0.19 | 1.5 e -5 |
| **Naturally derived sweeteners (honey, agave, maple syrup, etc.)** | 122 | 0.97 ± 0.74 | 1.58 ± 0.75 | 0.61 | 1.4 e -11 |
| **Artificial sweeteners (Equal, Sweet'N Low, Splenda)** | 104 | 1.54 ± 0.96 | 1.89 ± 0.91 | 0.35 | 1.1 e -4 |
| **Naturally derived low-calorie sweeteners (stevia, monk fruit, etc.)** | 43 | 1.43 ± 0.84 | 1.68 ± 0.89 | 0.25 | 0.06 |
| **Do not sweeten** | 120 | 1.14 ± 1.22 | 1.38 ± 1.11 | 0.24 | 1.0 e -03 |
| **Other** | 5 | 0.72 ± 0.41 | 0.84 ±0.57 | 0.12 | 0.65 |

All consumer groups report, on average, that they would add a larger volume of honey than sugar to sweeten one 8oz. serving of tea (Table 4). The average added volume of honey was significantly greater ($p \leq 0.001$) than the average added volume of sugar for cohorts of consumers who most commonly sweeten their food with sugar, naturally derived sweeteners, or artificial sweeteners and for those who do not sweeten their food and beverages. Interestingly, users of natural sweeteners would use more honey than sugar by the largest margin. This finding could be due to many factors. Consumers of natural sweeteners may have a strong liking for honey and want a more pronounced flavor. Additionally, consumers may attribute the term 'natural' to added health benefits and are

therefore less restrictive in added natural sweetener use (Saraiva et al., 2020).

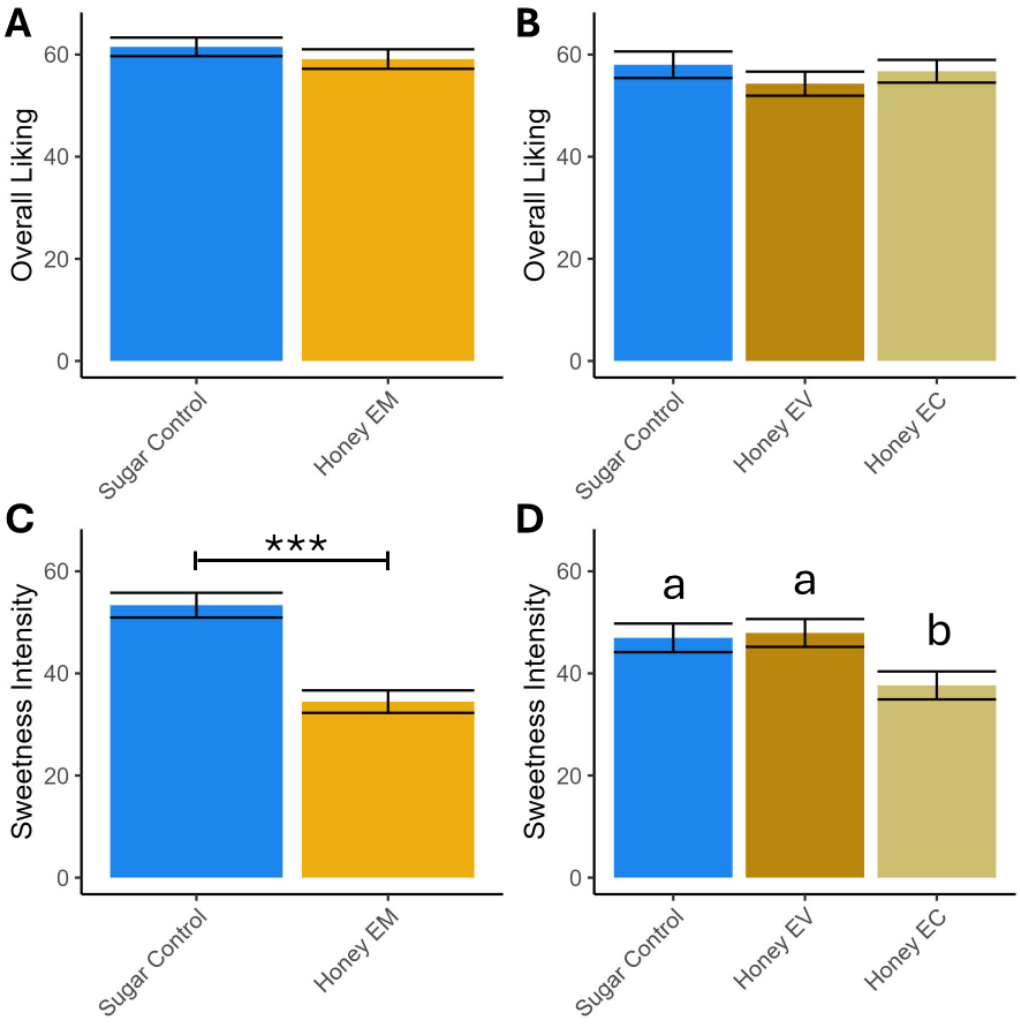


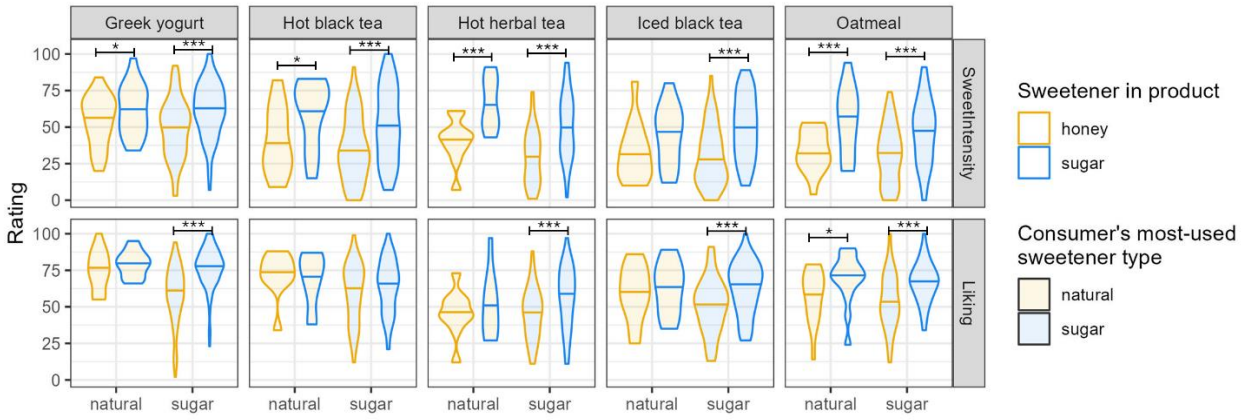

**Figure 3**: Mean overall liking **(A, B)** and sweetness intensity ratings **(C, D)** for hot black tea sweetened with sugar (Sugar control) or the unit equivalent of clover honey in mass (Honey EM), volume (Honey EV), and kcals (Honey EC). Columns represent individual cohorts of consumers (right n= 104; left n=79). *** Indicates significance at $p<0.001$ as determined by paired t-tests. Fisher's LSD method was used to determine post hoc means separation groups. Error bars represent the standard error of the mean (±1 se).

In the final testing session, we examined the relative sweetness of honey in hot black teas using two additional units of equivalence (volume and kcal) compared to the sugar-sweetened counterpart. The honey-sweetened conditions with an equivalent mass or equivalent calories were less sweet than the sugar control, but the tea sweetened with an

equivalent volume of honey was as sweet as the sugar control (Figure 4C, D). However, there was no significant difference in overall liking between the sugar control and any of the honey-sweetened conditions (Figure 4A, B).

These results reveal that consumers, on average, overuse honey relative to even the most conservative relative sweetness results. Density disparities between the sweeteners mean that honey contains less total sugar and calories than sugar when measured by equivalent mass. However, when measured by volume, honey contains more total sugar and calories. With that, for consumers who aim to match the sweetness intensity of sugar using honey, replacing sugar with an equivalent volume of honey is sufficient. Additionally, for consumers who aim to optimize added sugar use for health, substituting sugar with an equivalent mass of honey will generate a similar liking (for most food and beverages) while reducing total added sugars and calories.

**Figure 4:** The overall liking and sweetness intensity as a function of the most-used sweetener by consumers for products served in experiment 1. Significant differences in liking and sweetness intensity ratings for sugar- or honey-sweetened conditions are indicated by * ($p<0.05$), ** ($p<0.01$), and *** ($p<0.001$).




Another factor we found to impact consumer acceptance of sugar- or honey-sweetened foods is the sweetener an individual consumer uses most often. Figure 4 shows liking and

sweetness intensity ratings for consumers who use “sugars” or “naturally derived sweeteners” (such as honey) the most often; any tested product that did not meet a minimum criterion n≥10 natural sweetener users were excluded from analysis (i.e., all vinaigrette and salads, experiment 1&2; Greek yogurt with walnuts, experiment 2). Consumers that use sugar the most often found all sugar-sweetened products to be significantly sweeter than the honey-sweetened counterpart ($p \leq 0.001$), and consumers that use natural sweeteners the most generally rated sugar-sweetened products as sweeter than the honey-sweetened counterpart. Consumer consumption behaviors appear to have a much larger impact on overall liking than sweetness perception. Consumers who use sugar the most often liked the sugar-sweetened products significantly more than the honey-sweetened counterpart ($p \leq 0.001$), except for the hot black tea. In contrast, consumers who most often use natural sweeteners had no significant difference in overall liking of all honey- or sugar-sweetened products, except for the honey-sweetened oatmeal being less liked ($p \leq 0.05$). Sugar-sweetened products were generally sweeter and more liked. However, among natural sweetener users, the gap in liking between honey- and sugar-sweetened products closes, even though the gap in perceived sweetness remains. Familiarity or appreciation of honey flavor may result in increased liking, even at lower sweetness levels.

While this study offers valuable insights into the sweetness of honey and its potential to make bitter foods more palatable, there are several limitations. First, most products were tested with only one concentration of honey. Exploring a range of honey concentrations compared to sugar will provide a more comprehensive understanding of honey's relative sweetness. Additionally, our study only examined sweeteners at low to moderate

concentrations, which does not encompass the full range of sweetness used in commercial products. Furthermore, different consumer groups evaluated each product set, with some overlap between the groups, so consumer differences cannot be fully disentangled from product effects. Finally, reported sweetener usage for honey and sugar added to an eight-ounce cup of tea should be interpreted with caution, as consumers were required to answer these questions regardless of whether they would add these sweeteners to tea. Despite these limitations, this study offers valuable insight into using honey in foods.

## Conclusion

While the relative sweetness of honey was lower in most foods than when measured in aqueous solutions (1.0x per unit mass), the hot black tea (experiment 1), vinaigrette (experiments 1 and 2), leafy greens salad (experiments 1 and 2), and farro salad (experiment 2) sweetened with honey were liked at parity or better than the sugar-sweetened counterpart. Variability in liking of sugar- or honey-sweetened products may be impacted by the cohort of consumers rating the samples. At the same time, variations in relative sweetness appear to be mainly affected by product characteristics, such as exposure to high temperatures or degree of required oral processing. Additional studies will need to be conducted to confirm these hypotheses; until then, consumers should sweeten to taste since the relative sweetness of honey varies by product.

Adding low levels of either sweetener to nutritious, bitter foods effectively increases liking and sweetness while simultaneously reducing bitterness. Since honey has a lower energy density than sucrose, the use of honey has potential as a strategy to increase acceptance of nutrient-dense foods while reducing added sugars. However, consumers report using higher volumes of honey than sugar to sweeten products. It is vital to communicate to

consumers that honey is significantly denser than sugar (1.7x), so a lower volume of honey must be used instead of sugar to reduce added sugars and kcals.

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## APPENDIX

**Table A1:** Sweetener and sweetened product consumption questionnaire.

1. On average, how many servings of each of the following sweet products do you consume daily: regular soft drinks, sugars and candy, cakes, cookies, pies, fruit drinks (e.g., fruitades and fruit punch), dairy desserts and milk products (e.g., ice cream, sweetened yogurt, and sweetened milk), other grains (e.g., cinnamon toast and honey nut waffles)?

2. Which type of sweeteners do you use to sweeten your foods or beverages? (select all that apply)
    a. Sugar (white table sugar, brown sugar, cane sugar, etc.)
    b. Artificial sweeteners (Equal, Sweet'N Low, Splenda)
    c. Naturally derived low-calorie sweeteners (Stevia, Monk Fruit, etc.)
    d. Other naturally derived sweeteners (honey, agave, maple syrup, etc.)
    e. Other (please list)
    f. Not Applicable/ I don't sweeten my foods or beverages

3. Which type of sweetener do you consume the most often?
    a. Sugar (white table sugar, brown sugar, cane sugar, etc.)
    b. Artificial sweeteners (Equal, Sweet'N Low, Splenda)
    c. Naturally derived low-calorie sweeteners (Stevia, Monk Fruit, etc.)
    d. Other naturally derived sweeteners (honey, agave, maple syrup, etc.)
    e. Other (please list)
    f. Not Applicable/ I don't sweeten my foods or beverages

4. If you were to sweeten an **8-ounce cup of tea** with **white table sugar**, approximately how many **teaspoons** would you add?

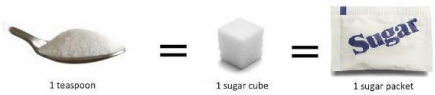


**Table A1 (cont'd).**

5. If you were to sweeten an **8-ounce cup of tea** with **honey,** approximately how many **teaspoons** would you add?

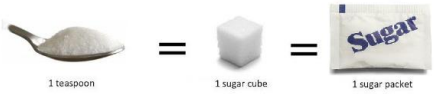


**Table A2:** Mediterranean diet adherence questionnaire.

| |
|---|
| 1. Do you use olive oil as the principal source of fat for cooking? |
| 2. How much olive oil do you consume per day (including that used in frying, salads, meals eaten away from home, etc.)? Please answer with the number of tablespoons. |
| 3. How many servings of vegetables do you consume per day? |
| 4. How many servings of fruit (including fresh-squeezed juice) do you consume per day? |
| 5. How many carbonated and/or sugar-sweetened beverages do you consume per day? |
| 6. How many servings of nuts do you consume per week? |

**Table A3:** Oatmeal preparation.

| | |
|---|---|
| **Ingredients** | Quick Cook Oats (Meijer, Grand Rapids, MI), Water Besco Water Treatment, Inc. Battle Creek, MI), Sweeteners: Sugar (Domino pure cane sugar, West Palm Beach, FL), Honey Dutch Gold, Lanc. CO) |
| **Preparation** | 1:2.5:13.2 parts sweetener, raw oats, and water by mass.<br>Boiling water was added to raw oats and stirred. Sweeteners were then added to the cooked oatmeal and stirred. |
| **Serving** | Served warm in ¼ cup portions of cooked oats (60g) that were scooped on site to prevent cooling and gelatinization of the oats.<br>Stored in an aluminum chaffing dish until served. |

**Table A4:** Greek yogurt preparation.

| | |
|---|---|
| **Ingredients** | 0% milkfat Plain Greek yogurt (Fage Total, Johnstown, NY), Sweeteners: Sugar (Domino pure cane sugar, West Palm Beach, FL), Honey Dutch Gold, Lanc. CO)<br>Chopped walnuts (Meijer, Grand Rapids, MI)- phase 2 |
| **Preparation** | 1:15 parts sweetener to yogurt in grams.<br>Sweetener stirred into yogurt. |
| **Serving** | Yogurt scoop in ¼ cup portions (~62g)<br>12g (2 tablespoons) walnuts placed in black 2oz cups<br>Styrofoam cups were kept in a cooler and taken out ≤5 min before serving.<br>In phase 2 testing, walnuts were served with yogurt cups (consumers were instructed to empty the entire portion into yogurt and mix them in). |

**Table A5:** Vinaigrette preparation.

| | |
|---|---|
| Ingredients | First cold pressed extra virgin olive oil-Robust (Pompeian, Baltimore, Maryland), White wine Vinegar (Pompeian, Baltimore, Maryland), Dijon Mustard (Meijer, Grand Rapids, Michigan), Sweeteners: Sugar (Domino pure cane sugar, West Palm Beach, FL), Honey Dutch Gold, Lanc. CO) |
| Preparation | A simple vinaigrette comprises a 1:1:1.5.5 ratio (sweetener, mustard, vinegar, olive oil). The sweetener, mustard, and vinegar were first whisked together, followed by continuous whisking as oil was slowly added. |
| Serving | 2tbs portions were transferred into black 2oz cups and stored in the fridge.<br>Dressings were brought to room temperature before serving. |

**Table A6:** Leafy greens preparation.

| | |
|---|---|
| Ingredients | Power greens- a blend of baby chard, baby spinach, and baby kale (Earthbound Farm organic, Carmel Valley, California) |
| Preparation | ½ cup portions placed in clamshells along with a dressing cup. |
| Serving | Pre-portioned clam shells were kept in coolers and taken out within 5 minutes of serving |

**Table A7:** Farro salad preparation.

| | |
|---|---|
| Ingredients | 50/50 blend by weight of cooked farro of Bob's Red Mill Farro (Milwaukie, Oregon) and Earthly Choice Italian Pearled Farro (Umbria Region of Central Italy), Chickpeas (Fresh Thyme, Downers Grove, IL), Parsley, Cucumber |
| Preparation | 1:3:3:8 volumetric ratio of parsley, chickpea, chopped cucumber, and farro |
| Serving | Served in 1/3cup portions with 2 tbs vinaigrette. |

**Table A8:** Hot black tea preparation: equivalent mass of honey and sugar.

| | |
|---|---|
| Ingredients | Lipton Hot black tea (Unilever, Englewood Cliffs NJ), Sweeteners: Sugar (Domino pure cane sugar, West Palm Beach, FL) |
| Preparation | 1 tea bag added to (1 cup) 8 oz boiled water and steep for 3-5 minutes. Sweetener then added.<br>5.73 g sweetener |
| Serving | Stored in insulated 6-gallon Cambro's that kept the teas warm for the entirety of testing. 4oz portions were dispensed on site. |

**Table A9:** Iced black tea preparation.

| | |
|---|---|
| Ingredients | Lipton iced black tea (Unilever, Englewood Cliffs NJ), Sweeteners: Sugar (Domino pure cane sugar, West Palm Beach, FL), Honey Dutch Gold, Lanc. CO) |
| Preparation | Added 1 tea bag to 1 quart of boiled water and brewed for 3-5 minutes, and then sweetener added. Add 3 quarts of cold water.<br>5.73 g sweetener added per 4oz of tea. |
| Serving | Stored in insulated 6-gallon Cambro's that kept the teas cold for the entirety of testing. 4oz portions were dispensed on site. |

**Table A10:** Hot herbal tea preparation.

| | |
|---|---|
| Ingredients | Roasted dandelion root tea (Traditional Medicinals, Sebastopol, California), Sweeteners: Sugar (Domino pure cane sugar, West Palm Beach, FL), Honey Dutch Gold, Lanc. CO) |
| Preparation | 1 tea bag added to (1 cup) 8 oz boiled water and steep for 10-15 minutes. Sweetener then added.<br>5.73 g sweetener added per 4oz of tea. |
| Serving | Stored in insulated 6-gallon Cambro's that kept the teas warm for the entirety of testing. 4oz portions were dispensed on site. |

**Table A11:** Hot black tea preparation: equivalent calories of honey and sugar.

| | |
|---|---|
| Ingredients | Lipton Hot black tea (Unilever, Englewood Cliffs NJ), Sweeteners: Honey (Dutch Gold, Lanc. CO) |
| Preparation | 1 tea bag added to (1 cup) 8 oz boiled water and steep for 3-5 minutes. Sweetener then added.<br>5.73 g sugar, 7.27 g honey |
| Serving | Stored in insulated 6-gallon Cambro's that kept the teas warm for the entirety of testing. 4oz portions were dispensed on site. |

**Table A12:** Hot black tea preparation: equivalent volume of honey and sugar.

| | |
|---|---|
| Ingredients | Lipton Hot black tea (Unilever, Englewood Cliffs NJ), Sweeteners: Honey (Dutch Gold, Lanc. CO) |
| Preparation | 1 tea bag added to (1 cup) 8 oz boiled water and steep for 3-5 minutes. Sweetener then added.<br>5.73 g sugar, 9.88 g honey |
| Serving | Stored in insulated 6-gallon Cambro's that kept the teas warm for the entirety of testing. 4oz portions were dispensed on site. |

## Chapter 5: Conclusions

This research utilizes sensory methodologies to explore honey and its potential to increase acceptance of nutritious foods. To fully understand this potential, it was necessary to determine how sweet honey is. In aqueous solutions, honey and sucrose are equivalently sweet by mass, and the aromas present in the honey samples significantly contributed to sweetness perception (chapter 3). When prepared in foods, the sweetness of honey varied by food product and its preparation (chapter 4). Liking of foods prepared with honey also varied by food product and were highly impacted by the main source of sweetener a consumer currently uses.

When consumers rated bitter but nutritious foods, honey—or sugar-sweetened conditions were liked significantly more than the control. Honey increased acceptance of bitter foods with fewer added sugars and total calories than table sugar. Honey has showcased legitimate potential as a part of a strategy to improve the health of many individuals. However, this work was only tested on a sample of consumers with a small number of food items. Future research should broaden the range of tested foods to include a wider variety of ready-to-eat processed foods and a more diverse group of consumers.

It is important to note that determining the health impact of honey use requires a well-designed dietary intervention study. Such a study could involve examining the effects of consuming nutritious bitter foods from the Mediterranean diet sweetened with various sweeteners, including sucrose and honey. Prior to the intervention, baseline data should be gathered, and specific attributes monitored, in order to assess the potential impact of these sweeteners on health outcomes. Subsequently, the intervention should involve monitoring

participants to evaluate how consistently they incorporate these foods into their diet, aiming to determine whether a particular sweetener is preferred and to assess the long-term acceptance of these foods based on the choice of sweetener.