Determining aroma differences among basil, parsley, and dill grown under varied supplemental light wavelengths using consumer sensory and flash gas chromatograph-electronic nose analyses

Anne Kalyn Seely

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Determining aroma differences among basil, parsley, and dill grown under varied supplemental light wavelengths using consumer sensory and flash gas chromatograph-electronic nose analyses

by

Anne Seely

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Food Science and Technology

Program of Study Committee:
Lester A. Wilson, Major Professor
Terri Boylston
Christopher Currey

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University
Ames, Iowa
2017

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DEDICATION

To all the Iowa State University alumni who came before me, but especially Claire Seely, Rick Seely, and Erin Seely.
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<tr>
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<td>High-pressure sodium</td>
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<td>MH</td>
<td>Metal halide</td>
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<td>LED</td>
<td>Light-emitting diode</td>
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<td>PPF</td>
<td>Photosynthetic photon flux</td>
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<td>DFT</td>
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<td>KI</td>
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ABSTRACT

Greenhouse herb producers may use artificial lighting to supplement the natural light available to their crops. High-pressure sodium (HPS) lights are the most common supplemental lighting systems employed in such operations, but light-emitting diode (LED) lighting is increasing in popularity because of its energy efficiency, customizability, and environmental friendliness. LED lights can be customized to emit specific proportions of light wavelengths, but many herb producers do not know how these “light recipes” affect their crops, specifically their crops’ aroma. This research utilized consumer sensory difference panels and flash gas chromatograph-electronic nose (GC-EN) analysis to evaluate the aroma of fresh basil, parsley, and dill herbs after cultivation under one of three supplemental light treatments: HPS, LED with a high proportion of blue to red diodes (high blue LED), or LED with a low proportion of blue to red diodes (low blue LED).

Consumer sensory panels using triangle difference tests found that consumers could not determine the difference between herbs grown under HPS and high blue LED. Preliminary work suggests a similar result for HPS and low blue LED, but further research is required to confirm this. GC-EN analysis revealed no significant chemical differences between lighting treatments among basil or parsley. Subtle chemical differences were uncovered in dill GC-EN data, especially when nonpolar and mid-polar column data were examined separately to prevent false correlation from multiple detections of a single compound. Consistent with literature findings, linear discriminant analysis of these data subsets revealed that multiple volatile compounds in dill are affected by the supplemental lighting wavelengths available to the herb.
In the scope of this study, there appears to be no overall aroma difference between herbs grown under HPS light and those grown under LED light, but more research must be conducted to confirm and expand upon these findings. Future research including sensory preference tests, descriptive analyses, GC-olfactometry, and GC-MS studies will make research like this more practical for herb farmers.
CHAPTER 1. INTRODUCTION

Herbs are prized for their desirable aromas and flavors and consumers are increasingly interested in purchasing fresh herbs in supermarkets, some of which are grown in greenhouses year-round (Resh, 2013; van Wyk, 2013). Many of these greenhouse herbs are cultivated via hydroponic methods that provide nutrients via salts dissolved in water (Resh, 2013). Hydroponic systems are the future of greenhouse crop production, and they are especially well suited for growing herbs (Resh, 2013). Growers have a high level of control in hydroponic systems, which can lead to “higher concentration of bioactive substances” in their crops (Canter, Thomas, & Ernst, 2005; Giurgiu et al., 2014). These bioactive substances provide herbs with their distinct aromas.

Supplemental lighting systems are often used in greenhouses alongside hydroponic systems, especially in regions with limited natural light like the Midwest United States. When supplemental lighting is used in herb production, high-pressure sodium (HPS) or metal halide (MH) lighting systems are most common because of their relatively low cost (Morrow, 2008; Gómez, Morrow, Bourget, Massa, & Mitchell, 2013; Olle & Virsile, 2013; Resh, 2013). Recently, light-emitting diode (LED) systems have gained popularity for their energy-efficiency and customizability (Morrow, 2008; Gómez et al., 2013; Kopsell & Sams, 2013; Son & Oh, 2015). However, there is a much greater capital investment involved in outfitting a greenhouse with an LED lighting system as opposed to an HPS or MH system (Massa, Emmerich, Morrow, Bourget, & Mitchell, 2006).

How does switching a greenhouse to an LED lighting system affect crops? Both small- and large-scale greenhouse herb producers need to know how their crops’ aromas will change under these LED systems before making such a steep investment. Many studies have been
conducted regarding the physiological and morphological effects of lighting conditions on hydroponic crops, but far fewer studies have investigated the effects of light on herb aroma (Briggs & Christie, 2002; Massa et al., 2006; Matsuda, Ohashi-Kaneko, Fujiwara, & Kurata, 2007; Li & Kubota, 2009; Hogewoning Trouwborst, Maljaars, Poorter, Van Ieperen, & Harbinson, 2010; Žukauskas et al., 2011; Samuoliūnė et al., 2012a; Samuoliūnė et al., 2012b; Kopsell & Sams, 2013; Olle & Virsile, 2013; Son & Oh, 2013; Taulavuori, Hyöky, Oksanen, Taulavuori, & Julkunen-Tiitto, 2016). The objective of this study is to determine if there is an aroma difference between herbs grown under conventional HPS greenhouse lighting and those grown under LED lights with a mixture of red and blue diodes, using basil, parsley, and dill as test herbs. This study includes two LED lighting treatments: one with a higher proportion of blue to red diodes, and another with a lower proportion of blue to red diodes. The scope of this study involves comparing HPS lighting to LED lighting; the LED treatments will not be directly compared because sample sizes were not large enough to accommodate this comparison.

Aroma analysis can utilize human sensory perception, an instrumental method, or both. This research takes advantage of both sensory evaluation and a flash gas chromatograph-electronic nose instrument, or GC-EN, to reveal any differences between lighting treatments.

Sensory difference tests are a common method used to determine if humans can perceive a difference between samples (Meilgaard, Civille, & Carr, 2016). They are statistically simple, relatively inexpensive, and easy to understand (Meilgaard et al., 2016). The triangle test, in particular, is a commonly used difference test because of its simplicity (Meilgaard et al., 2016). In the general format of a triangle test, two foods are compared. These samples are labeled with random 3-digit codes and presented in randomized serving orders to panelists (Meilgaard et al., 2016). A panelist is presented with three random samples coded with three different numbers.
Two of these samples are the same and one is different. They are asked to evaluate the samples and indicate which is the odd sample. Researchers determine significant differences based on the proportion of correct responses in the study.

Using sensory analysis can reveal information directly pertaining to consumers’ ability to tell the difference between herbs grown under HPS lighting and those grown under LED lighting, but flash gas chromatography-electronic nose (GC-EN) analysis is capable of detecting chemical differences that humans may miss. Using both GC-EN and sensory analysis will set this study apart as a resource for farmers, food scientists, horticulturalists, and engineers. Herbs have been analyzed by gas chromatography (GC) and electronic nose (eNose) instruments on multiple occasions, but this study appears to be the first opportunity for basil, parsley or dill to be analyzed on a GC-EN instrument (Callan, Johnson, Westcott, & Welty, 1991; Masanetz & Grosch, 1998; Yousif, Scaman, Durance, & Girard, 1999; Díaz-Maroto, Pérez-Coello, & Cabezudo, 2002; Chang, Alderson, Hollowood, Hewson, & Wright, 2007; Calín-Sánchez, Lech, Szumny, Figiel, & Carbonell-Barrachina, 2012; Ghasemi Pirbalouti, Mahdad, & Craker, 2013; Santos et al., 2014; Weisany, Raei, & Pertot, 2015; Carvalho, Schwieterman, Abrahan, Colquhoun, & Folta, 2016; El-Zaeddi, Calín-Sánchez, Martínez-Tomé, Noguera-Artiaga, Burló, & Carbonell-Barrachina, 2016; El-Zaeddi, Martínez-Tomé, Calín-Sánchez, Burló, Carbonell-Barrachina, & Buettner, 2016; El-Zaeddi, Martínez-Tomé, Calín-Sánchez, & Burló, 2017).

The GC-EN is a relatively new instrument designed to marry the detailed precision of a gas chromatograph with the pattern recognition capabilities power of an electronic nose, which is why it was used instead of a conventional GC or eNose instrument in this study (Heracles II odor analyzer, 2016). Since the introduction of the first GC-EN in 2005, it and its subsequent iterations have been used to analyze food packaging, olive oil pressing techniques, spirit
beverages, traditional Chinese vinegars, poultry shelf life, and more (Hinshaw, 2005; Stockwell, 2007; Ghosh, Chatterjee, Bhattacharjee, & Bhattacharyya, 2016; Wiśniewska, Śliwińska, Namieśnik, Wardencki, & Dymerski, 2016; Yaping et al., 2017; Wojnowski et al., 2017).

The GC-EN acts as a rapid gas chromatograph by simultaneously utilizing two columns and FIDs to analyze a single sample (*Heracles II odor analyzer*, 2016). This analysis cycle usually takes about 5 minutes, unlike the 30-45 minute long run of a conventional GC, but does not jeopardize resolution (*Heracles II odor analyzer*, 2016; Qian, Peterson, & Reineccius, 2010). The GC-EN serves to close the gap between GC and eNose analysis, incorporating both into a single instrument (*Heracles II odor analyzer*, 2016). This paper exists at the beginning of the GC-EN’s emergence into food analysis applications.

The following null hypotheses were tested as a part of this research:

1. There is no perceivable aroma difference between basil, parsley, or dill grown under conventional HPS lighting and basil, parsley, or dill grown under LED lighting with a high proportion of blue to red diodes.

2. There is no perceivable aroma difference between basil, parsley, or dill grown under conventional HPS lighting and basil, parsley, or dill grown under LED lighting with a low proportion of blue to red diodes.

3. Instrumental analysis will not reveal significant aroma differences between basil, parsley, or dill grown under conventional HPS lighting and basil, parsley, or dill grown under LED lighting with a high proportion of blue to red diodes.

4. Instrumental analysis will not reveal significant aroma differences between basil, parsley, or dill grown under conventional HPS lighting and basil, parsley, or dill grown under LED lighting with a low proportion of blue to red diodes.
CHAPTER 2: LITERATURE REVIEW

**Conventional Herb Cultivation**

Herb cultivation is extremely varied; small farms and large-scale producers alike operate in the U.S. (Rogers, 2012). Herbs and other essential oil crops like lavender and lemongrass are widely grown outdoors in warm climates (Adam, 2005). For example, much of the country’s fresh basil comes from California, Hawaii, Florida, or abroad (U.S. Department of Agriculture, 2016). Because of wide global commerce, herbs grown in greenhouses abroad could supply the entire U.S. herb market (Adam, 2005). But should it? Promoting the local production of herbs would supplement local farming economies and provide fresher, higher quality herbs to consumers. In the U.S., herbs are often grown as “a way to stabilize small farm ventures” (Miller, 1985). The same is true in greenhouse systems: growing herbs is an easy way to supplement income during the more predominant production of lettuce and other leafy greens (Currey & Flax, 2016). Because they are often supplementary crops, greenhouse herb production is not the focus in the United States. More greenhouse crop operations focus on vine crops like tomatoes, cucumbers, and peppers (U.S. Department of Agriculture, 2015).

When supplemental lighting is used in herb production, high-pressure sodium (HPS) or metal halide (MH) lighting systems are most common (Morrow, 2008; Resh, 2013). Most light given off by HPS systems is in the red-orange range, or, from 550-650 nm (Massa et al., 2006; Gómez et al., 2013). HPS lights give off heat as well as light, contributing to greenhouse temperature and demonstrating their inefficiency (Yeh & Chung, 2009; Gómez et al., 2013). Gómez et al. (2013) note that HPS lamps may contribute up to “41% of the heating requirements for a greenhouse operation.” The low cost of HPS systems relative to
Light-emitting diodes (LED) makes them the current gold standard of supplemental lighting in greenhouses (Morrow, 2008; Gómez et al., 2013; Olle & Virsile, 2013).

**Light-Emitting Diodes and Horticulture**

LEDs were developed in the early 20th century by H.J. Round, but made practical by the work of Nick Holonyak, Jr. in the early 1960s (Yeh & Chung, 2009). An LED is comprised of a chip of light-emitting semiconductor material attached to an electrical junction that excites the chip’s molecules (Yeh & Chung, 2009). The light wavelengths emitted from a diode depend on “the energy gap of the semi-conductor used, which is based on the semiconductor chemical composition,” so different colors of LEDs were invented at different times (Yeh & Chung, 2009; Mitchell et al., 2015). In fact, blue LEDs were invented relatively recently in 1993 (Yeh & Chung, 2009).

The first uses of LEDs as horticultural grow lighting occur in studies for space travel (Massa et al., 2006). Massa et al. (2006) concluded that LED lighting is the best lighting option for sustaining life during space travel because of their efficiency and intracanopy lighting potential. Since then, LEDs have only become more popular as horticultural grow lighting for their energy efficiency, durability, customizability, and environmental friendliness. Darko, Heydarizadeh, Schoefs, and Sabzalian (2014) predict that LED systems will not only replace HPS, fluorescent, and metal halide lighting systems, but also “revolutionize controlled growth environments.”

LED lighting systems require a steeper capital investment than other lighting systems, but they have lifespans of up to four times longer (Massa et al., 2006). With a longer lifespan comes the capacity to produce 25% less waste (Yeh & Chung, 2009; Olle & Virsile, 2013).
When LED lights do grow old, they do not burn out, but dim (Morrow, 2008; Yeh & Chung, 2009). Disposing of LED lights is simple because unlike HPS lights, they do not contain mercury (Yeh & Chung, 2009). Furthermore, LED lights are durable and shock resistant (Massa et al., 2006). LED systems are more energy efficient than all other common horticultural lighting systems. Yeh and Chung (2009) report that some LED products use only 60% of the energy input required to power a comparable fluorescent system. Unlike LED systems, HPS systems produce thermal energy that wastes ~75% of their total energy input (Gómez et al., 2013). LEDs are therefore cooler than HPS lights and don’t require bulky cooling systems or minimum hanging distances to prevent plant scorching (Gómez et al., 2013). For this reason, they can even be used as intracanopy lighting for vine crops (Gómez et al., 2013). HPS lamps need time to warm up and turn on gradually, while LED lights turn on and off instantly, contributing to their energy efficiency (Morrow, 2008). LED systems can also be dimmed as needed and connected to digital control systems (Morrow, 2008).

LEDs are also unique in that they can be tailored to give off an extremely specific light spectrum to a plant; in the future, LED systems could be customized to suit the needs of a certain producer, greenhouse, and even individual plant (Morrow, 2008; Kopsell & Sams, 2013; Son & Oh, 2015). Olle and Virsile (2013) point out that “light quality and quantity initiate signaling cascade[s] of specific photoreceptors,” that eventually impact the plant’s gene expression. These cascades are difficult to predict; the only reliable way to know how a plant will respond to certain light conditions is to conduct an experiment (Olle & Virsile, 2013). Plants are known to respond to wavelengths between 380 and 750 nm, so horticultural LED lighting must emit light within that range (Tibbits et al., 1994). Some wavelengths
within this range are more important to plant growth and development than others. According to Yeh & Chung (2009), “chlorophyll molecules absorb red and blue wavelengths most efficiently,” so these wavelengths are best suited to induce photosynthesis.

**Plant response to LED lighting systems**

Red LED lights are commonly used because plants respond well to the wavelengths they emit (Massa et al., 2006). The common red LED light wavelength of 640 nm “has a relative quantum efficiency for photosynthesis of ~96%” (Massa et al., 2006). Phytochrome, a common plant pigment and photoreceptor, is stimulated by red LED wavelengths (Darko et al., 2014). Red light has been shown to enhance antioxidant systems and phytochemical concentrations in leafy crops, including herbs like basil, parsley, marjoram, and dill (Li & Kubota, 2009; Žukauskas et al., 2011; Samolienė et al., 2012b; Olle & Virsile, 2013).

Blue LEDs are the most energy-efficient color available, so using them can reduce energy use and cost even more than switching to all red or green LEDs (Mitchell et al., 2015). Currey and Lopez (2013) also report that blue LEDs have a higher luminous efficiency than red LEDs. Plants absorb blue wavelengths with cryptochrome pigments that “impact plant development and physiological functions” (Mitchell et al., 2015). Most plants require a small percentage of blue light for normal growth (Massa et al., 2006). HPS lights contain around 5% blue light and fluorescent tubes contain about 23% (Hogewoning et al., 2010). Yorio, Goins, Kagie, Wheeler, and Sager (2001) proved that in order to match the weight of spinach and radishes achieved under cool fluorescent lighting, it takes at more than 10% blue to 90% red LED light. Blue light is responsible for “central processes such as phototropisms, suppression of stem elongation, chloroplast movements, stomatal operations,
and genetic expression” (Kopsell & Sams, 2013). Blue light has also been shown to increase phenol biosynthesis, which contributes to aroma and flavor compounds and antioxidant capacity (Taulavuori et al., 2016). Matsuda, et al. (2007) studied the effect of blue light in spinach photosynthesis and suggested that blue light plays a role in acclimating plants to low light. They also reported that on average, plants grown under blue light have a higher ratio of a/b chlorophyll, more cytochrome, and more Rubisco, an important photosynthetic enzyme, than plants grown under red light (Matsuda et al., 2007). This indicates a tendency toward vegetative growth under blue light. Blue light wavelengths also “maximize photosynthetic potential in weak light and prevent damage to the photosynthetic apparatus in excess light” (Briggs & Christie, 2002). They do so by a few different mechanisms: phototropism, stomata opening, chloroplast accumulation, and chloroplast avoidance (Briggs & Christie, 2002; Massa, Kim, Wheeler, & Mitchell, 2008). These mechanisms either protect plant organs from excess light or make it easier for plants to absorb and utilize light in dim conditions.

Growing sprouting broccoli under 100% blue light for 5 days before harvest tended to increase uptake of essential micro- and macronutrients like iron, calcium, and potassium compared to an 88% red and 12% blue light blend (Kopsell & Sams, 2013). Kopsell and Sams (2013) also noted that blue light increased concentrations of β-carotene, violaxanthin, and total xanthophyll pigments compared to the red and blue light blend. These pigments are important to human health, so blue light can help influence the nutritional value of vegetables and leafy greens (Kopsell et al., 2013). Samuoliene et al. (2012a) found that supplementing HPS lamps with blue LED increased leaf area, fresh weight, dry weight, and pigment content in vine crops. Taulavuori et al. (2016) concluded that blue light response is species-specific, but generally increases phytochemical concentrations in red leaf lettuce.
Blue light steers crops toward vegetative growth, allows for adaptation in adverse light conditions, and contributes to their nutritional value, antioxidant content, aroma, and flavor, especially in leafy greens. Red light provides efficient wavelengths for chlorophyll and phytochrome in plants and also may influence antioxidant and phytochemical concentrations. These similarities in plant response led Taulavuori et al. (2016) to conclude that red and blue light share some of the same mechanisms. However, growth under only red light often yields physical abnormalities like hypocotyl elongation and low dry weight and growth under 100% blue light seems to limit overall growth (Briggs & Christie, 2002; Yorio et al., 2001; Darko et al., 2014). Son & Oh (2015) note, “a combination of red and blue LEDs promoted the photosynthetic rate compared with the effect of monochromatic red or blue LEDs.”

Blending different proportions of colored LEDs can provide unique advantages to plants grown under such systems (Massa et al., 2006). Sunlight contains about 31% blue and 34% red light, so it makes sense to blend colors in LED grow lights to better mimic natural light (Hernández & Kubota, 2014). The proportions of light used depend on the crop; according to Massa et al. (2006), “Some types of crops thrive under light with high blue fluxes, while others grow better under red-enriched light with minimum blue.” Some plants are capable of growing under completely red light, but most require at least a small percentage of blue (Massa et al., 2006). Tibbits (1994) reported that lettuce grown under 100% red light “developed excessive hypocotyl elongation, stem elongation, leaf extension, and reduced chlorophyll.” The hypocotyl elongation is of particular note because it shows that the plant was searching for different light wavelengths, presumably blue (Tibbits 1994). Hydroponic spinach, radish, and lettuce plants grown under 100% red LED lighting also
yield a significantly lower dry weight than those grown under fluorescent or blue-supplemented LED lighting (Yorio et al., 2001). Similarly, Hogewoning et al. (2010) grew cucumber plants under different percentages of blue to red LED light and found that they required at least 7% blue light to reach normal photosynthetic function. When Hernández and Kubota (2014) studied another cucumber cultivar, they found that as the blue light percentage increased, plant dry mass, leaf number, and leaf area decreased. Both of these studies found that increasing blue light percentage increased chlorophyll content per unit leaf area in cucumbers (Hogewoning et al., 2010; Hernández & Kubota, 2014). Son & Oh (2013) found that lettuce grown under increasing proportions of blue light had increasingly higher total phenol concentration. Darko et al., (2014) highlight the balance of red to blue light as it relates to the activity of phytochrome and cryptochrome. For example, the elongation of cotyledons and hypocotyls of leafy crops often seen under red LED light is “known to be phytochrome-dependent” (Darko et al., 2014). This phenomenon can be prevented by blue light supplementation, because blue light activates cryptochrome, which in turn “mediates reduction of hypocotyl length” (Darko et al., 2014).

Green light has also been used in some studies because it may penetrate canopies better than other wavelengths (Mitchell et al., 2015). It has been shown to increase plant dry mass when added to red-blue LEDs at 24% of total photosynthetic photon flux (PPF) (Kim, Goins, Wheeler, & Sager, 2004). However, most green and yellow wavelengths reflect off plants, indicating that they are not as vital to photosynthetic function as red and blue wavelengths are (Yeh & Chung, 2009; Son & Oh, 2015). Son & Oh (2015) found that adding green light to red/blue LEDs boosted leaf expansion and reduced the visual burden on workers, making it easier for them to see in growth chambers. Samuolienė et al. (2013) found
that an LED blend of green, yellow, and orange wavelengths increased phenolic compound and phytochemical content and, in some cases, α-carotene content.

**Hydroponic Herb Cultivation**

Hydroponic systems have been used for thousands of years. The first few incidences of hydroponic crop and ornamental plant production occur in ancient Babylon, Mexico, China, and Egypt (Resh, 2013). Since then, hydroponic systems were most often used to study plant nutrition until greenhouse growers in the mid-1920’s recognized hydroponics as an alternative to labor-intensive, pest-ridden soil greenhouse systems (Resh, 2013). W.F. Gericke coined the term “hydroponics” from the Greek words for “water” and “labor” or “work” (Resh, 2013; Giurgiu et al., 2014). Hydroponic systems put water to work as a substrate for plant growth. Utilizing hydroponics is a great way to conserve water as well (Adam, 2005). Growing lettuce hydroponically rather than in a field can reduce water use by 95% (Currey & Flax, 2016). Today, commercial hydroponic crop production operations exist in almost every country (Resh, 2013). Hydroponics reduce labor costs, increase grower control of pH and nutrient levels, nearly eliminate soil-borne pathogens, and produce higher yields than other cultivation methods (Adam, 2005; Resh, 2013; Giurgiu et al., 2014).

Herbs can be grown hydroponically in a few different ways, but the deep-flow (DFT) and nutrient-film techniques (NFT) are most common (Currey & Flax, 2016). The specifications of the system, including dimensions and spacing, depend on the specific herbs and cultivars (Resh, 2013; Currey & Flax, 2016).

DFT, also called the raceway or raft system, involves suspending the plants in a polystyrene raft over a large pool of nutrient solution, allowing their root systems to form in
the solution under the raft (Resh, 2013). The raft supports the plants and shields the nutrient solution from light, minimizing algae growth (Resh, 2013). Root aeration is an important consideration when using a DFT system (Resh, 2013). Yields can suffer greatly if ample concentrations of oxygen in the nutrient solution is not maintained (Currey & Flax, 2016).

NFT systems are comprised of a nutrient solution tank and slanted channels that drain into the tank. A pump provides a constant flow of nutrient solution to the roots of plants growing in the channels by pumping nutrient solution from the tank to the top of the slanted channels and allowing the solution to flow through and back into the tank (Resh, 2013). Channel lids support plants and provide darkness for root system, much like the Styrofoam rafts of DFT systems. NFT systems do not require aeration, because the roots are adequately aerated by the action of solution flowing through the channels (Currey & Flax, 2016).

Both NFT and DFT systems vary in size and depth, and managing the greater culture is also important for cultivating herbs. Understanding an herb’s optimum spacing, greenhouse temperature, light level, pH, and electrical conductivity, or EC (a measure of nutrient solution potency) is essential for successfully producing it hydroponically (Currey & Flax, 2016). Annual and perennial herbs differ in their optimum conditions. Perennial herbs like mint and rosemary have longer crop times than annual herbs and may require wider NFT channels or wider spacing (Currey & Flax, 2016).

Hydroponic systems are especially well-suited for growing herbs and other medicinal plants because the high level of grower control can lead to “higher concentration of bioactive substances” (Canter et al., 2005; Giurgiu et al., 2014). Giurgiu et al. also note that, “[flavor] compounds… are ones resulted in secondary metabolism which is influenced by electric conductivity, pH, humidity, that need to be carefully [monitored]” (Giurgiu et al., 2014).
“The aim is to increase potency, reduce toxin levels and increase uniformity and predictability of extracts,” or, the aromas of the herbs (Canter et al., 2005).

**A Closer Look At Three Herbs**

**Basil**

Sweet basil (*Ocimum basilicum*) is an annual herb that originated in tropical Asia, but it is used heavily in Mediterranean cuisine, most famously in Italian pesto and bruschetta (van Wyk, 2013). It is the most popular hydroponic herb (Currey & Flax, 2016). In addition to its culinary uses, basil can also repel insects, bacteria, fungi, and nematodes and treat ailments such as “headaches, coughs, diarrhea, worms, and kidney malfunctions” (Telci, Bayram, Yılmaz, & Avcı, 2006). Its aroma is attributed to linalool, 1,8-cineole, and methyl chavicol, from which is sweetness is derived (van Wyk, 2013). Methyl chavicol, also called estragole, is “a phenylpropanoid considered safe only when used in small amounts.” (van Wyk, 2013). Other varieties of basil include Thai (or purple) basil, lemon basil (*O. americanum*), holy basil (*O.tenuiflorum*), and Thai lemon basil (*O.xcitriodorum*) (van Wyk, 2013). They are used in many Asian and African cuisines (van Wyk, 2013). Some of these varieties’ unique aromas and flavors are attributed to eugenol (van Wyk, 2013). For the purposes of this paper, the term “basil” will henceforth refer exclusively to sweet basil except when otherwise specified.

Basil has been studied fairly extensively because of its wide variety of uses and even wider popularity. In the 1990s and early 2000s, many studies classified basil varieties into chemotypes, or groups with distinctive essential oil chemical composition (Grayer et al., 1996, Telci et al., 2006). Telci et al. (2006) studied various basils grown in various regions of
Turkey for differences in the essential oil composition. They characterized seven different chemotypes just within Turkish-grown basil (Telci et al., 2006). These papers combined chemical composition analysis and regional genetics to categorize basil for its content of linalool, methyl cinnamate, methyl eugenol, citral, and methyl chavicol (Telci et al., 2006). European-descended varieties were concluded to have more methyl chavicol, while tropical varieties have more methyl cinnamate (Telci et al., 2006). These chemotype studies just showed that basil essential oil composition varies greatly with genetics and region; selecting a basil cultivar directly depends on the aroma profile desired (Grayer et al., 1996; Telci et al., 2006). Basil essential oil composition also depends on the age of the plant (Nurzyńska-Wierdak, Bogucka-Kocka, Kowalski, & Borowski, 2012; Carvalho et al., 2016). Methyl chavicol and methyl eugenol concentrations decreased with basil plant age and linalool concentrations tended to increase with age in a study by Nurzyńska-Wierdak et al., (2012).

Many studies are devoted to retaining basil’s quality through processing, most often drying. In *The Potential of Herbs as a Cash Crop*, Richard Alan Miller (1985) explains, “after the crop is harvested, a little care in drying will more than repay the farmer for the work and expense involved.” Drying herbs, especially basil, which Shores (1999) calls, “the most tender herb,” increases the growers’ opportunity to profit from growing them. Grayer et al. (1996) even compared the chemotypes of fresh basil cultivars with their dried basil counterparts to see how chemotype changes with processing. They concluded that methyl chavicol and eugenol concentrations decreased after freeze-drying, but the not enough to impact the overall aroma of the herb (Grayer et al., 1996). In 1999, it was found that the newly devised vacuum-microwave dryer not only preserved basil’s volatile compounds, but increased volatile concentration through induced chemical reactions during the drying
process (Yousif et al., 1999). Díaz-Maroto, Palomo, Castro, Vinas, and Perez-Coello (2004) found that air drying basil at room temperature led to fewer volatile component losses than oven drying, but this method takes too long to be commercially viable. More recently, Calín-Sánchez et al. (2012) discovered that pre-drying basil in a convective drying process before finishing in a vacuum microwave “was the best option for drying sweet basil” because of its speed and aroma quality preservation. However, Ghasemi Pirbalouti et al. (2013) found that as drying temperature increases, essential oil content in basil decreases. They did not find any significant differences in methyl chavicol content between basil drying methods, but some monoterpane hydrocarbons were significantly reduced or lost in all drying methods (Ghasemi Pirbalouti et al., 2013). Oven drying at 60 °C caused the most essential oil loss (Ghasemi Pirbalouti et al., 2013). When storing fresh basil commercially, Anderson, Bower, and Bertling (2011) recommend using a micro-perforated polypropylene package stored “in a light environment” to prevent wilting and color loss.

Basil has long been used as a medicinal herb, but recent studies are learning more about the science behind basil’s medicinal properties and nutritional value. Basil was shown to lower plasma lipids and plasma cholesterol in rats with high blood lipids (Harnafi, Aziz, & Amrani, 2009). Harnafi et al. (2009) attributed these lipid metabolism properties to “phenolic compounds such as flavonoids and tannins.” They continued the research, concluding, “sweet basil contains phenolic products that are able to lower hyperlipidemia and prevent atherosclerosis” (Harnafi et al., 2013). They identified caftaric acid, cafeic acid, chicoric acid, and rosmarinic acid as the main compounds in the effective experimental drug they derived from basil (Harnafi et al., 2013). Wang, Wang, and Chan (2013) looked into treating mild to moderate type two diabetes with basil because of its ability to reduce carbohydrate
absorption and reduce fasting blood glucose levels. Berić, Nikolić, Stanojević, Vuković-Gačić, and Knežević-Vukčević (2008) linked basil and its linalool component to a reduction of oxidative cell damage in bacteria, demonstrating that basil and linalool have antioxidant properties. Dried basil shares this antioxidant activity at least to some degree (Lee, Umano, Shibamoto, & Lee, 2005). Basil was even successfully used as an antidepressant-like agent and a stroke prevention drug in rats (Bora, Arora, & Shri, 2011; Abdoly et al., 2012). Basil essential oil has been studied as a treatment for Salmonella dysenteriae, Giardia lambia, intestinal disorders, bronchitis, mouth epidermal carcinoma, and herpes simplex virus type-1, but it has also been shown to be carcinogenic (Raut & Karuppayil, 2014).

Other studies have made biodiesel from basil essential oil, studied sensory characteristics of goat’s milk after the goats were fed basil, used basil as an antimicrobial agent, learned more about beneficial flavones by studying their natural synthesis in basil, and tested the effectiveness of basil essential oil in acne medication (Šípalová & Kráčmar, 2011; Berim & Gang, 2016; Anastasiadou & Eriotou, 2015; Amini et al., 2017).

Basil is generally easy to grow. It requires warm conditions of 70-85°F and thrives in full sun (van Wyk, 2013). It tends to be a cold-sensitive plant that does well in hydroponic systems, so it is ideal for greenhouse growth (Shores, 1999; Currey & Flax, 2016).

Common pests that affect greenhouse basil cultivation include “aphids, whiteflies, spider mites, and various worms” (Shores, 1999). Outside, “slugs, flea beetles, and tarnished plant bugs” become a problem (Shores, 1999). Botrytis, Pythium, and Fusarium oxysporum f. sp. basilicum all affect basil as well. Fusarium is a particularly harmful fungus that only shows signs of infection when the basil plant is at least 6 inches tall (Shores, 1999). Alfalfa mosaic virus (AMV) affects both basil’s growth and its essential oil composition (Bruni,
Bellardi, & Parrella, 2015). Basil plants with AMV tend to yield essential oil with significantly less linalool and estragole and significantly more eugenol (Bruni et al., 2015). Other aspects of basil’s growth environment can affect the essential oil yield and composition too. Methyl jasmonate, which has been shown to induce secondary metabolite production in plants, increased eugenol and linalool concentrations in basil by 56 and 43%, respectively, when sprayed directly on the plants (Kim, Chen, Wang, & Rajapakse, 2006). Basil’s eugenol content increases with temperature, as do essential oil yields (Chang et al., 2007). Essential oil variation is also linked to leaf position and leaf age in basil (Fischer, Nitzan, Chaimovitsh, Rubin, & Dudai, 2011). According to Bufalo et al. (2015), organic fertilizer use does not have an affect on basil essential oil composition when compared to conventional fertilizer in a greenhouse setting. Klimankova et al., (2008) noted morphological differences between organically and conventionally grown basil varieties, but only one of the five cultivars they studied exhibited differences in essential oil composition and aroma. A red variety, Cinamette, exhibited higher levels of methyl chavicol but mere traces of eugenol (Klimankova et al., 2008). It also contained methyl cinnamate, which was not found in any other cultivar in the study (Klimankova et al., 2008). Santos da Costa et al. (2014) discovered that genetic hybrids of multiple basil varieties produce new aromas, and in one case, the previously unobserved in basil aroma compound (E)-caryophyllene.

Basil is well suited to soilless culture. Hydroponic systems are often used to conduct basil nutrition studies. For example, Kiferle, Maggini, and Pardossi (2013) showed that basil could thrive just as well on a reduced nitrate concentration of 5.0 mol m⁻³ as opposed to the standard 10.0mol m⁻³. Basil has been successfully grown in hydroponic, aeroponic, and aquaponic systems (Chandra et al., 2014; Saha et al., 2016). Basil yielded 19% more in an
aeroponic system compared to a conventional field system (Chandra et al., 2014). The aeroponic system had no affect on phenolic secondary metabolite content in basil (Chandra et al., 2014). Looking specifically at hydroponic production, both NFT and DFT systems work well for growing basil (Walters & Currey, 2015). The main consideration when growing basil in hydroponic systems must be selecting a suitable cultivar, but because “cultivar performance may vary across different locations, greenhouse environments, and cultural practices,” each grower should individually test cultivars in their growing conditions before selecting one (Walters & Currey, 2015).

**Basil under LED lighting**

Tarakanov, Yakovleva, Konovalova, Paliutina, and Anisimov (2012) studied basil under 75% red and 25% blue LEDs and found that the LED treatment “delayed or inhibited plant transition to flowering” compared to HPS (Olle & Virsile, 2013). A delay in flowering means that the basil plants were steered toward vegetative growth, and this could lead to increased herb and essential oil yields. Basil grown under blue light yielded up to 4.4 times more essential oil than basil grown under white light in a study by Amaki, Yamazaki, Ichimura, and Watanabe (2011).

Taulavuori et al. (2016) studied basil under different proportions of red and blue supplemental LED lighting and suggested, “both blue and red light may be needed to regulate the accumulation of phenolics in basil.” Carvalho et al. (2016) studied basil in growth chambers under six different LED treatments and found that 100% red and 50:50 red to blue increased the basil’s carboxylic acid esters and fatty acid esters, while red/blue blends including yellow, green, or far-red light increased its fatty alcohols, fatty aldehydes,
monoterpenoids, phenylpropanoids, and sesquiterpenoids. They also found that LED treatments on average produced smaller basil plants than natural light did (Carvalho et al., 2016). Carvalho et al. (2016) highlighted their research as a way to “increase the value and quality of high-value herbs grown for human sensory characteristics.” Loughrin and Kasperbauer (2001) found that basil is affected by light wavelengths reflected from colored mulches; basil grown on yellow and green surfaces had more aroma compounds and phenolic compounds than other colors (Loughrin & Kasperbauer, 2001). Using red mulch yielded basil with more surface area, higher moisture content, and greater fresh weight than basil grown on a black surface (Loughrin & Kasperbauer, 2001). Carruthers (2015) reported on a study by Phillips researchers that investigated basil under different proportions of red and blue LED light; basil plants grew larger under up to 16% blue light to 84% red (Carruthers, 2015). They did some sensory analysis that revealed that the basil grown under 32% blue light received the highest scores for taste, aroma, and spiciness (Carruthers, 2015).

Ultraviolet (UV) radiation, or light with wavelengths slightly less than visible blue light, has been shown to “enhance the levels of most of the major volatiles” in basil, including eugenol, methyl eugenol, linalool, 1,8-cineole, and trans-β-ocimene (Johnson, Kirby, Naxakis, & Pearson, 1999). Ghasemzadeh et al. (2016) found that post-harvest UV-B irradiation is “a promising technique to improve the healthy-nutritional and pharmaceutical properties of sweet basil leaves.” The UV-B treatment increased total phenol content and induced production of cinnamic acid and luteolin, which were only present in UV-B treated basil (Ghasemzadeh et al., 2016).
**Parsley**

Parsley (*Petroselinum crispum*) is a biennial herb utilized in many cuisines as a flavoring and garnish (van Wyk, 2013). Though parsley is a biennial, it is usually grown hydroponically as an annual (Currey & Flax, 2016). It produces leaves the first year and a stem with flowers the next year, so it is harvested in the first year for its aromatic leaves (van Wyk, 2013; Currey & Flax, 2016). Parsley comes from “Mediterranean parts of southern Europe and western Asia,” but it is now cultivated in most of the world (van Wyk, 2013). There are two common varieties: *neapolitanum* and *tuberosum* (van Wyk, 2013). *Neapolitanum* has wide, flat leaves, so it is commonly called flat-leaved, or Italian, parsley (van Wyk, 2013). Variety *tuberosum* has curly leaves (van Wyk, 2013). It is commonly called curly, celery-leaved, or French parsley (van Wyk, 2013).

The main flavor compounds in parsley are 1,3,8-\textit{p}-methatriene, apiole, myristicin, and tetramethoxyallylbenzene, of which apiole and myristicin “are toxic at high doses” (van Wyk, 2013). Some of these flavor compounds act as aroma compounds as well. Masanetz et al., quantified the odorants of parsley and found that 1,3,8-\textit{p}-methatriene is the most abundant aroma compound in parsley (Masanetz et al., 1998). One Italian parsley variety that Masanetz and Grosch (1998) tested “was much richer in 1,3,8-\textit{p}-methatriene” than the French parsley they sampled. 1,3,8-\textit{p}-methatriene was described as “‘terpeny’, ‘parsley-like’-smelling” (Masanetz & Grosch, 1998). “The ‘green, grassy’ and the ‘fruity’ notes” in parsley are due to (\textit{Z})-hex-3-enal, (\textit{Z})-hex-3-enol, and (\textit{Z})-hex-3-enyl acetate (Masanetz & Grosch, 1998).

Parsley keeps fairly well as fresh cuttings; Santos et al. (2014) observed that its color, soluble phenolic compounds, flavonoids, macronutrients, and minerals were stable during
10-day storage at 3 °C but a 25% reduction in antioxidant activity occurred during this time. Tubes of parsley paste have become popular in produce sections as a more shelf stable alternative to fresh cut parsley. Kaiser et al., (2012) explored water and steam blanching as a way to preserve phenolic compounds, including antioxidants, present in these products. Water and steam blanching for 1 minute were “the most suitable measures to ensure polyphenol retention” (Kaiser et al., 2012). Catunescu, Rotar, Vidican, Bunghez, and Rotar (2016) investigated the effect of irradiation on fresh Italian parsley and found that it destroyed some vitamin C but increased total polyphenols. They concluded, “fresh products have a higher bioactivity provided they undergo minimal processing and storage” (Catunescu et al., 2016). Unfortunately, minimal processing and storage is not always an option. Like basil, parsley is commonly dried to make it shelf stable. Díaz-Maroto et al. (2002) determined that “air drying at ambient temperature resulted in few losses in volatile compounds” while freeze-drying and oven drying caused more substantial volatile compound losses. In a later study, air-drying at ambient temperature also led to the least aroma differences compared to fresh parsley (Díaz-Maroto, González Viñas, & Cabezudo, 2003). Both freezing and drying decrease the concentrations of β-phellandrene and 1,3,8-p-menthatrine in Italian parsley (Petropoulos, Daferera, Polissiou, & Passam, 2010). Dry storage and freezer storage caused further losses of parsley’s aroma compounds (Petropoulos et al., 2010).

Parsley is prized for its health benefits as well as its flavor. Parsley is a good source of vitamin C with 190 mg per 100 g of parsley (van Wyk, 2013). In Turkey, parsley is commonly used to treat high blood glucose levels in diabetics, so Ozsoy-Sacan et al. (2006) studied treating diabetic rats with parsley extract. They and other researchers found that
parsley extract has a protective effect against hepatotoxicity, or chemically induced liver damage, caused by diabetes (Bolkent, Yanardag, Ozsoy-Sacan, & Karabulut-Bulan, 2004; Ozsoy-Sacan et al., 2006). In similarly treated rats, parsley extract “eliminated accumulation of lipid peroxides and returned the glutathione levels towards normal” in the heart (Sener, Saçan, Yanardag, & Ayanoglu-Dülger, 2003). Parsley was found to be an effective “nutraceutical intervention in inflammatory bowel disease” in mice (Jia et al., 2014). Parsley extract contains an iron-chelating antioxidant, which quenches harmful free radicals (Wong & Kitts, 2006). These antioxidant activities have the potential to prevent DNA damages that cause cancer (Zhang, Chen, Wang, & Yao, 2006; Tang, Rajarajeswaran, Fung, & Kanthimathi, 2015). Tang et al. (2015) suggested that parsley should become a component in functional foods because of its cancer-fighting potential. Parsley has also been shown to significantly increase sleeping time in mice treated with the anesthetic ketamine (Bursac, Popovic, Mitic, Kaurinovic, & Jakovljevic, 2005).

Parsley grows well in both conventional and soilless systems. El-Zaeddi, Calín-Sánchez, et al. (2016) recently studied the essential oil content in relation to plant density and irrigation dose to learn more about the optimum conditions for parsley sensory quality. El-Zaeddi et al. (2017) also studied parsley’s essential oil content with respect to its harvest date and found that the earliest harvest yielded the most essential oil. Ulrich, Bruchmüller, Krüger, and Marthe (2011) compared resistance to Septoria blight (Septoria petroselini) with the occurrence of different flavor and aroma compounds in various parsley cultivars. Resistance to Septoria blight is associated with “several negative sensory characteristics such as bitter, grassy, herbaceous, pungent, chemical, and harsh” (Ulrich et al., 2011). Applications of nickel fertilizer to Italian parsley up to 50 mg/kg of soil “strongly improve
not only parsley leaf yield and quality (i.e., leaf area, mineral content, oil yield, and flavor) but also the leaves are safer for human consumption since their nitrate and ammonium contents are significantly reduced” (Atta-Aly, 1999). Petropoulos, Daferera, Polissiou, & Passam (2009) showed that both Italian and French parsley are moderately sensitive to salinity.

Soilless growth of parsley began as a way to control growth factors during research, but parsley is now a common hydroponic, aeroonic, and aquaponic crop (Currey & Flax, 2016). Álvaro, Lao, Urrestarazu, Baghour, and Abdelmajid (2016) studied hydroponic parsley’s essential oil content and yield after altering nutrient solution salinity and ionic concentration. They concluded that the salinity treatments did not affect growth, but recommended an EC of 1.2–2.2 dS m⁻¹ for optimizing essential oil production of parsley in soilless systems (Álvaro et al., 2016). Similarly, parsley yields were shown to increase by 21% in aeroonic systems compared to conventional field systems (Chandra et al., 2013). Parsley also successfully accumulated selenium in an aeroonic growth system to make selenium more available in the human diet (Mazej et al., 2007). Aquaponic parsley was found to grow well with either low or high flow of water from the aquaculture component of the system (Buzby, Waterland, Semmens, & Lin, 2016).

Parsley, its flavor profile, its aroma compounds, or its extracted essential oil, have been studied in many other contexts as well. Sbai, Saad, Ghezal, Greca, and Haouala (2016) have studied the phytotoxicity of parsley’s bioactive compounds, finding that some of parsley’s secondary metabolites could be used as natural herbicides. Parsley essential oil and phenolic compounds have antibacterial effects against *Bacillus subtilus, Escherichia coli, Staphylococcus aureus, Listeria monocytogenes,* and *Salmonella enterica* (Wong & Kitts,
2006; Linde et al., 2016). It is also useful as an antifungal against *Penicillium ochrochloron* and *Trichoderma ciride* at lower concentrations than the common antifungal agent ketoconazole (Linde et al., 2016).

**Parsley Under LED Lighting**

Parsley has not been studied under LED light as much as basil. Logemann, Tavernaro, Schulz, Somssich, and Hahlbrock (2000) conducted a parsley cell study to clarify the pathways of UV-light induced flavonoid production. When parsley was grown under red LED supplemental lighting 3 days prior to harvest, Bliznikas et al. (2012) found that it increased antioxidant capacity and saccharide content, including glucose, fructose, and sucrose. This red LED lighting treatment also decreased nitrate accumulation in parsley cells (Bliznikas et al., 2012). Samuolienė et al. (2017) found that parsley had higher tocopherol content when cultivated without blue light supplementation. Chlorophyll and total carotenoid components were consistent between lighting treatments (Samuolienė et al., 2017). Lutein, α-carotene, β-carotene, neoxanthin, and violaxanthin contents all varied sporadically, but sometimes significantly, between LED treatments of various blue light percentages (Samuolienė et al., 2017).

**Dill**

Dill (*Anethum graveolens*) is an annual herb used as a flavoring worldwide (van Wyk, 2013). It is often used to season pickles, fish dishes, and processed meats (van Wyk, 2013). Both its leaves and fruits can be used; dill leaves are often referred to as “dill weed” while the fruits are called “dill seed” (van Wyk, 2013). Dill essential oil can be used as a
commercial ingredient in “confectionary, desserts, condiments, beverages, and meat seasonings” (van Wyk, 2013). The typical dill weed flavor comes from α-phellandrene and (+)-dill ether (van Wyk, 2013). Dill fruits have lower levels of α-phellandrene and (+)-dill ether, but higher levels of (+)-carvone and (+)-limonene that make up their flavor profile (van Wyk, 2013). (+)-Carvone is said to have a “caraway-like, cooling” characteristic, while phellandrene and (+)-dill ether are described as “dill-like, fragrant, fresh” (Callan et al., 2006). For the purposes of this paper, “dill” will henceforth refer to dill weed unless otherwise specified.

(S)-α-phellandrene was discovered to be “the character-impact compound of the dill flavor” based on gas chromatography (GC) and sensory data (Blank, Sen, & Grosch, 1992). Dill ether also significantly contributes to the rounded flavor of dill (Blank et al., 1992). Blank & Grosch (1991) also analyzed dill herb using high performance liquid chromatography (HPLC) and GC-mass spectrometry (GC-MS) to find that “[dill ether], methyl 2-methylbutanoate, (+)-(4S)-α-phellandrene, and myristicin were the most important odorants of dill herb.”

As with basil and parsley, adding value to dill often involves processing. Kruma et al. (2011) published the ideal microwave dryer settings that yield dried dill as chemically comparable to fresh dill as possible. Madhava Naidu et al. (2016) determined that the best method for drying dill was low humidity air-drying. However, all drying methods tested affected the dill leaves’ color, flavor, and yield (Madhava Naidu et al., 2016).

Freezing is another common method for preserving dill. Blanching dill prior to freezing significantly improves the chlorophyll and beta-carotene retention after 3 months of frozen storage (Lisiewska, Kmiecik, & Słupski, 2003). Lisiewska et al. (2003) recommend
blanching dill before freezing for more than 6 months. Kmiecik, Lisiewska, and Słupski (2004) note that blanching also reduces nitrates, nitrites, and oxalates, which can be “unfavorable for human nutrition.” Additionally, blanching also reduces potassium, calcium, magnesium, phosphorus, zinc, copper, cadmium, sodium, and iron (Słupski, Lisiewska, & Kmiecik, 2005).

Tsamaidi & Passam (2015) studied the use of modified atmosphere storage for fresh dill. Modified atmosphere packaging is the practice of flushing food packaging with a gas other than air to preserve its quality or shelf life (Vaclavik & Christian, 2008). For example, potato chip bags are flushed with nitrogen gas to prevent lipid oxidation. Tsamaidi & Passam (2015) found that modified atmosphere packaging fresh dill resulted in a decrease in respiratory activity, but also vitamin C and chlorophyll losses.

Historically, dill was used as a natural medicine to relieve digestive symptoms (van Wyk, 2013). More recently, dill has been studied as a treatment for primary dysmenorrhea, hepatotoxicity, female fertility, and abnormal lipid metabolism in rats (Takahashi et al., 2013; Heidarifar et al., 2014; Rabeh & Aboraya, 2014; Monsefi, Ghasemi, Alaee, & Aliabadi, 2015). Goodarzi, Khodadadi, Tavilani, and Oshaghi (2016) cited dill as an “antihyperlipidemic, antihypercholesterolemic, antidiabetic, anticancer, antioxidant, antistress, antisecretory, cardioprotective, antispasmodic, and diuretic.” Dill was proven to lower cholesterol at the low-density lipoprotein level by binding sterols (Danesi, Govoni, D'Antuono, & Bordoni, 2016).

Both dill weed and dill seed extracts act as antioxidants, but dill weed extract is more effective than dill seed extract (Shyu et al., 2009; Isbilir & Sagiroglu, 2011). The main phenolic acid in dill extract, rosmarinic acid, is known to be a very effective antioxidant
(Erdogan Orhan et al., 2013). Low humidity air-dried dill extract retains the free-radical scavenging ability of fresh dill (Madhava Naidu et al., 2016). This antioxidant activity is linked to lowered lipid levels and total cholesterol levels in rats (Oshaghi, Khodadadi, Tavilani, & Goodarzi, 2016).

The antimicrobial activity of dill seed oil has been confirmed by multiple studies, revealing that it disrupts the plasma membrane of the pathogenic fungus *Aspergillus flavus* (Tian et al., 2011; Tian et al., 2012; Eleiwa & El-Diasty, 2013). Babri, Khokhar, Mahmood, and Mahmud (2012) concluded that the essential oil of dill seeds has the potential to work “as a natural insecticide against various insects.”

Erdogan Orhan et al. (2013) compared dill’s functionality after growth in conventional and organic systems. They found no differences between the enzyme inhibitory and antioxidant functionality of conventional and organic dill essential oils (Erdogan Orhan et al., 2013). However, the minor components of the dills’ essential oil composition did vary significantly (Erdogan Orhan et al., 2013).

Increased plant density has a negative affect on essential oil yield, because dense planting reduces the dry weight and thus the essential oil yield (Callan et al., 2006). Callan et al. (2006) also found that low plant density produced essential oil with higher carvone content, while oil from plants cultivated at a high density had “more herbaceous characteristics.” This is because essential oil yield of dill depends on plant maturity (Callan et al., 2006). As dill matures, its essential oil yield decreases relative to the plant dry weight because as it matures, the plant starts to put more energy into reproductive growth rather than vegetative growth (Callan et al., 2006). Plants at lower densities have the capacity to mature faster than those planted at higher densities (Callan et al., 2006). El-Zaeddi, Martínez-Tomé,
et al. (2016) found that moderate irrigation dose and plant density led to the highest dill yield. In another paper, El-Zaeddi et al., (2017) determined that a later harvest date yielded more essential oil from dill. They also discovered that the highest sensory quality dill plants were grown at a density of 5.56 per meter with a higher than normal irrigation dose of 2180 m$^3$/ha (El-Zaeddi, Martínez-Tomé, et al., 2016).

Nejatzadeh-Barandozi et al., (2014) compared the effect of no fertilizer, chemical fertilizer, and biological fertilizer (beneficial bacteria) on dill and found that biological fertilizer application gave a significantly higher essential oil content and yield. Biofertilizer is generally more environmentally friendly than chemical fertilizer because there is no risk of chemical runoff (Nejatzadeh-Barandozi et al., 2014). Micorrhizal relationships can also affect the essential oil of dill (Weisany et al., 2015). Micorrhizae are a type of fungi that live in and around plant roots, and are recently being studied as a way to minimize agricultural risks (Weisany et al., 2015). When dill root systems are inoculated with micorrhizal fungi, the essential oil yield increases and the essential oil contains higher phelandrene, limonene, cryptone, and carvone and lower terpinene, cymene, terpinolene, dimethylstyrene, dill ether, and other minor compounds (Weisany et al., 2015).

**Dill Under LED Lighting**

Hälvä, Craker, Simon, and Charles (1992a) used sunlight to study how light level affected dill plants’ growth and development and found that light level has a proportional relationship to leaf number, leaf area, and plant height. They also found that essential oil content was greatest under higher sunlight conditions (Hälvä et al., 1992a). That same year, they studied dill under different light qualities (Hälvä, Craker, Simon, & Charles, 1992b).
Hälvä et al. (1992b) treated dill with red, far-red, or blue light at the end of each day of the study and found that blue light supplementation produced the highest yield. The red light treatment increased plant growth and the far-red treatment had the highest essential oil concentration (Hälvä et al., 1992b). Under the longest 4-hour treatment of red and far-red light, the dill yielded more volatile essential oil constituents (Hälvä et al., 1992b).

Bliznikas et al. (2012) found that dill grown under red LED lighting 3 days prior to harvest had increased vitamin C content, antioxidant activity, and carbohydrate content, including fructose, sucrose, and glucose. The light treatment also decreased nitrates via increasing nitrate-reducing enzyme activity (Bliznikas et al., 2012).

Frąszczak (2013) experimented with supplementing dill with red or blue light at the end of day or end of night and found that red light generally stimulated growth while blue light generally stunted it. When light was supplemented at the end of the day, no matter the wavelength, it inhibited the dill plants’ growth (Frąszczak, 2013). Frąszczak, Gąsecka, Golc, and Zawirska-Wojtasiak (2016) studied dill under various proportions of red and blue LED light, sometimes incorporating orange and green diodes as well. They found that as blue light proportion increased, the dill exhibited increased elongation, leaf area, dry mass, glucose content, and fructose content (Frąszczak et al., 2016). As red light proportion increased, essential oil and phytochemical contents also increased (Frąszczak et al., 2016). They essentially proved that “the proportion of red and blue light has significant influence on the morphological qualities, chemical composition, and dynamics of photosynthesis” in dill (Frąszczak et al., 2016). In another publication, Frąszczak (2016) grew dill under different proportions of red and blue LED light and found that blue light supplementation increased...
the plants’ leaf area up to 20%. She noted that dill grown under 30% blue had “the greatest photosynthesis intensity” (Frąszczak, 2016).

**Aroma Analysis**

Food aroma analysis began with the human nose, which is still the gold standard of aroma analysis because of its impressive sensitivity (Reineccius & Peterson, 2013). Human olfactory neurons are able to react with as little as “8 molecules of a potent odorant” (Reineccius & Peterson, 2013). Reineccius and Peterson (2013) state that the human nose “has a theoretical detection limit of about $10^{-19}$ moles.” This detection limit is far more sensitive than any aroma analysis instrument to date (Reineccius & Peterson, 2013).

This study couples data collected by the human nose with flash gas chromatograph-electronic nose (GC-EN) analysis. The following sections detail how each method functions in the scope of food aroma analysis. For more detailed information about their functions in this particular study, see Chapter 3.

**Aroma sensory evaluation**

Sensory difference tests are a common method used to determine if consumers can perceive a difference between samples (Meilgaard et al., 2016). They are statistically simple, relatively inexpensive, and easy for panelists to understand (Meilgaard et al., 2016). Triangle tests, in particular, are “useful in situations where treatment effects may have produced product changes that cannot be characterized simply by one or two attributes” (Meilgaard et al., 2016). In a triangle test, there is a 33.3% chance that a panelist will guess the correct response, as opposed to other difference tests like paired comparisons wherein there is a 50%
chance of the panelist guessing correctly (Meilgaard et al., 2016). Thus, triangle tests are characterized by a higher statistical efficiency than many other difference tests (Meilgaard et al., 2016).

Triangle tests are inexpensive largely because they utilize consumers as panelists, who often require no training prior to participating. If consumer panelists are trained, this training usually takes the form of a short information session (Meilgaard et al., 2016). Triangle tests are occasionally used to determine if panelists can detect certain attributes in doctored samples during the selection process for more intense trained panels (Meilgaard et al., 2016). Consumer panels like triangle difference panels require a larger number of panelists than a trained panel; Meilgaard et al. (2016) recommend 20-100 people. However, a consumer panel takes far less time. Table 19.7 in Meilgaard et al. (2016) Sensory Evaluation Techniques helps researchers determine how many participants are required to yield statistical clarity at desired α- and β-levels.

In the general format of a triangle test, two foods are compared at a time, but three samples are presented to panelists. Two samples are identical and one is different. These samples are each labeled with a different random 3-digit code and presented in a randomized serving order (Meilgaard et al., 2016). The panelist is asked to evaluate the samples in a certain order (often left to right) and indicate which they believe to be the odd sample. Using table 19.8 in Sensory Evaluation Techniques, researchers can total the number of correct responses in the context of the total number of panelists and determine if the consumers could identify a difference between the samples (Meilgaard et al., 2016). In a triangle test, a statistically significant result at an α-risk of 0.05 “indicates strong evidence that a difference is apparent” between the two samples (Meilgaard et al., 2016). For example, in a study with
30 participants at an α-risk of 0.05, 17 of the 30 responses must be correct in order to conclude that panelists could perceive a difference between the two samples (Meilgaard et al., 2016).

**Gas chromatography**

Gas chromatography (GC) is a chemical analysis method that separates molecules by their polarity, size, and boiling point (Ismail & Nielsen, 2010). GC can only be used for thermally stable volatile compounds, so it is well suited for flavor and aroma analysis (Ismail & Nielsen, 2010; Qian et al., 2010). All chromatography methods are used to separate the components of a sample by carrying it via a mobile phase through a stationary phase that brings about this separation (Ismail & Nielsen, 2010). In GC, the mobile phase is an inert gas and the stationary phase is an immobilized liquid or solid adhered to the inside of a long, thin column (Ismail & Nielsen, 2010). The stationary phase can vary in polarity, sensitivity, and optimum temperature range depending on the parameters desired (Qian et al., 2010).

To analyze an aroma using GC, the volatile aroma compounds must be collected to form a sample. The simplest way to collect food aromas is called direct headspace sampling (Qian et al., 2010). This method involves using a gastight syringe to collect air from the headspace of a food sample at equilibrium and injecting it directly into the GC instrument (Qian et al., 2010). Direct headspace sampling is rapid and inexpensive but it is not the most sensitive method, as it does not concentrate the aroma (Qian et al., 2010). Other more expensive and time-consuming methods include cryogenic trapping and adsorbent trapping, which use condensation and adsorption, respectively, to immobilize and concentrate volatile
aroma compounds before further preparation in the form of extraction and concentration (Qian et al., 2010).

Samples can also be distilled with moisture or steam to collect volatiles, but they must be solvent extracted following distillation to reduce the moisture content (Qian et al., 2010). Solvent extraction alone is widely used to directly collect samples, but it presents some challenges for GC analysis (Qian et al., 2010). Solvents can dilute samples and yield a large peak, so it is important to choose a solvent that elutes a peak at a different range than that of the sample (Qian et al., 2010). Solvent extraction has the tendency to concentrate impurities in the sample as well (Reineccius & Peterson, 2013). Furthermore, solvents extract both aroma compounds and lipids, which require separation before final analysis (Reineccius & Peterson, 2013). Solvents like methylene chloride also give rise to environmental and lab safety concerns (Moldoveanu, 2004).

The newest methods for collecting samples for GC analyses are solid-phase extractions, which utilize solid coatings on fibers, on stir bars, and inside gastight syringes (Qian et al., 2010). The method using coated fibers, called solid-phase microextraction, or SPME, is widely used in food aroma analysis (Qian et al., 2010). It is more accurate and precise than solvent methods, requires fewer preparatory steps, can be automated, and does not produce a peak in the sample’s chromatogram (Qian et al., 2010). SPME fibers vary in size, coating material, and polarity to best suit the needs of individual research goals (Qian et al., 2010).

Once inside the instrument’s injection port, inert gas carries the GC sample into the column (Qian et al., 2010). In the case of solid phase extractions, the sample is vaporized in the heat of the injection port over a set amount of time (Qian et al., 2010). From there, the
sample travels into the column inside the oven. The oven adheres to a set temperature program designed to elute the sample’s components clearly and independently over time (Qian et al., 2010). The oven temperature program’s ramp speed determines the resulting chromatogram’s resolution (Qian et al., 2010). When the temperature ramp is quick, resolution is low; when it is slow, resolution is high (Qian et al., 2010). GC columns vary in diameter, length, polarity, and optimum temperature range (Qian et al., 2010). Longer columns have higher resolutions, but require exceptionally long analysis times (Qian et al., 2010). Today, nearly all GC columns are capillary columns (Qian et al., 2010). Capillary columns are thin, flexible fused silica glass tubes coated on the inside with the desired stationary phase and on the outside with polyamide to improve flexibility (Qian et al., 2010). The most common stationary phase utilized in capillary columns is 95% polar dimethylpolysiloxane and 5% phenyl. These columns have “a very wide temperature range (−60 °C to 325 °C) and [are] very stable” (Qian et al., 2010). More nonpolar 100% dimethylpolysiloxane columns are available for extremely polar compounds like alcohols or fatty acids, but they operate within a smaller temperature range because of their instability and susceptibility to residual oxygen in the carrier gas (Qian et al., 2010).

As substances leave the column, they can be detected by a great number of methods (Qian et al., 2010). The most common detection method is the flame ionization detector, or FID (Qian et al., 2010). It works by burning a substance in a hydrogen flame as it exits the column and recording the electrical current produced (Qian et al., 2010). This current is “proportional to the organic ions present in the flame from the burning of an organic compound” (Qian et al., 2010). Because of this methodology, it records the response of compounds with carbon-carbon and carbon-hydrogen bonds best, but gives “virtually no
response” for water, nitrogen dioxide, carbon dioxide, and hydrogen sulfide (Qian et al., 2010). FIDs have good sensitivity and a linear response useful for quantification of volatiles (Qian et al., 2010). They are fairly durable as well (Qian et al., 2010). Other detectors use ionization, electrical conductivity, or thermal energy principles (Qian et al., 2010). GC detectors vary in sensitivity, selectivity, ease of use, and price (Qian et al., 2010).

A relatively new innovation in GC detection is GC-Olfactometry (GC-O), in which the incredibly sensitive human nose is used alongside a conventional GC detector (D’acampora Zellner, Dugo, Dugo, & Mondello, 2008; Reineccius & Peterson, 2013). There are many substances eluted during a sample’s GC run that are not detectable by the human senses but are by the instrumental detector and vice versa (D’acampora Zellner et al., 2008; Reineccius & Peterson, 2013). GC-O investigates those discrepancies by splitting the GC elute so that half goes to the conventional detector, and the other half goes to an olfactory port for human sensory evaluation (D’acampora Zellner et al., 2008). GC-O serves as a direct link between sensory evaluation and chromatography. GC-O panels are time consuming and expensive because panelists must be heavily trained to sit through long GC runs, observing aromas as they elute (D’acampora Zellner et al., 2008). Other challenges associated with this method include panelist fatigue and lack of consistency between panelist responses (D’acampora Zellner et al., 2008). Like any other sensory evaluation method, GC-O panels depend on the sensitivity of the panelists. GC-O can be incorporated into a GC-MS system as well, forming the comprehensive GC-MS-O instrument (Cheng, Chen, Chen, Wu, Liu, & Ye, 2015).

A successful GC run should yield a chromatogram with “narrow-based peaks and ideally, but not essential to quality of data, baseline separation of compounds” (Qian et al.,
Narrow-based peaks indicate very precise elution times for a sample component, while baseline separation of compounds makes chromatograms easier to interpret by reducing the chance that compositionally close compounds are mistaken for one another (Qian et al., 2010). The real challenge of GC analysis is to achieve the best possible peak separation in the shortest amount of time, or maximizing separation efficiency (Qian et al., 2010). To do so, Qian et al. (2010) recommend using hydrogen carrier gas at its maximum velocity, short columns with small diameters, and lower oven temperatures.

A chromatogram plots the detector response vs. the component’s retention time. In order to quantify a sample, researchers can measure the area under the substance’s peak using an integral that includes the sample’s initial weight (Qian et al., 2010). Many GC software programs do these calculations automatically (Qian et al., 2010).

Hundreds of food products have been analyzed using gas chromatographs, and herbs and spices are no exception. Pesek, Wilson, and Hammond (1985) correlated sensory to GC data when studying spice quality after cryogenic milling and found that cryogenically milled spices retained more volatile compounds than conventionally milled spices. To learn more about GC analysis as applied to basil, parsley, and dill, see the previous sections on each of these herbs.

**Electronic nose analysis**

An electronic nose, or eNose, is an instrument designed to mimic the human nose (Reineccius & Peterson, 2013). The idea for such an instrument was proposed in the early 1960s, but eNoses did not become readily available until the 1990s (Reineccius & Peterson, 2013; Wardencki, Chimel, & Dymerski, 2013). There is no perfect instrument that acts
exactly like the human nose, because A, the human nose is a widely variable organ that functions differently from person to person, and B, matching the sensitivity of the human nose is extremely difficult (Reineccius & Peterson, 2013). Essentially, an eNose is a chemical sensor with software for pattern recognition (Reineccius & Peterson, 2013). This software is usually programmed with a database of volatile chemicals called a neural network to help it identify chemical sensations (Reineccius & Peterson, 2013; Wardencki et al., 2013).

eNoses can use a variety of sensors, from the common semiconductor gas sensors to complex mass spectrometer (MS) instruments (Reineccius & Peterson, 2013). Semiconductor gas sensors often take the form of metal oxide semiconductors, more commonly called MOS sensors (García-González & Aparicio, 2010). MOS sensors work by measuring the resistance change as the sensor’s metal coating interacts with the aroma’s volatile molecules (Wilson & Baietto, 2009). They can even be connected to GC-MS, which together provide the most comprehensive aroma analysis instrumentation available to date (Reineccius & Peterson, 2013).

An eNose responds to patterns of stimuli via prediction (Reineccius & Peterson, 2013). Based on the affinity of its sensors, absorption/desorption slopes are created and analyzed to characterize a certain aroma (García-González & Aparicio, 2010). Reineccius and Peterson (2013) use coffee oxidation as an example of an eNose coming to the wrong conclusion. An eNose would measure oxidation in coffee the same way it measures lipid oxidation, because its prediction software assumes the oxidations are correlated (Reineccius & Peterson, 2013). If they are not correlated, the eNose will make critical errors as it analyzes the data (Reineccius & Peterson, 2013). Another complication with using an eNose
is that some instruments detect carbon dioxide and water vapor along with volatile compounds, diluting the data (Reineccius & Peterson, 2013). eNose sensors can also deteriorate over time, becoming fatigued just like human panelists (Reineccius & Peterson, 2013). Frequent calibration may be necessary to ensure the sensor’s effectiveness, especially during shelf-life studies (Reineccius & Peterson, 2013). In literature, most eNose data analysis is conducted through statistical principal component analysis (PCA) of Kovats retention indices (Na ayudhaya, Klinbumrung, Jaroensutasinee, Pratontep, & Kerdcharoen, 2009; Cheng et al., 2015). The Kovats index (KI) is the result of a statistical model used to normalize chromatographic data for easy comparison across instruments with different columns and settings (Alpha MOS, 2014). KI values are based on the established peaks of a specific group of alkanes and do not vary between instruments as much as retention times do (Alpha MOS, 2014). This method can also account for variation in the same instrument and aid in compound identification (Alpha MOS, 2014).

eNoses analyze complete aromas, so complicated preparatory steps used in GC analysis are not necessary (Reineccius & Peterson, 2013). Analyzing a complete food aroma gives a more comprehensive view of the food’s aroma, unlike the split aroma analysis of the GC (Reineccius & Peterson, 2013). Additionally, when researchers wish to correlate sensory data with an instrumental method, the eNose stands out because it experiences aroma in the same way a human sensory panelist does (Reineccius & Peterson, 2013). This is especially true when the eNose utilizes static headspace sampling. Static headspace sampling is a simple sampling technique that involves isolating a small amount of an odorous material in a sealed vial and using air from the headspace of the vial as the injected sample (Da Costa & Eri, 2005). This method is quick, relates well to sensory analyses, and does not involve
complicated preparation. However, the sample’s composition depends heavily on its
temperature (Tudor, 1997). Headspace sampling can skew data toward the most volatile
aroma components, because more volatile compounds release more readily into the
headspace than less volatile components (Da Costa & Eri, 2005).

An eNose can also be trained to recognize patterns between samples. eNoses are often
used in the food and beverage industry as quality assurance devices because they can
distinguish between a standard and a deviant (Reineccius & Peterson, 2013). For example, a
milk processor can program an eNose to recognize a standard for fresh milk and a standard
for spoiled milk (Reineccius & Peterson, 2013). If the eNose detects spoiled milk aromas in a
certain processing day’s sample, it can alert quality assurance professionals to a potential
problem, saving money, time, and possibly consumers’ lives (Reineccius & Peterson, 2013).

GC runs can be lengthy because they require gradual temperature programs to elute
compounds with satisfactory resolution, but eNose runs are much faster because they do not
endeavor to separate volatile components (Reineccius & Peterson, 2013). It is essential that
eNoses analyze aromas quickly because humans analyze aromas quickly.

eNose analysis is common in the world of food and beverage analysis. Shen et al.
(2001) used eNose and sensory analysis to study the oxidative shelf life of vegetable oils,
finding that the eNose “is capable of measuring changes in volatile compounds associated
with oil oxidation.” They recommend using eNose data to complement sensory evaluation
data (Shen et al., 2001). Du et al. (2002) also used an eNose in a shelf-life study of salmon
fillets. García-González and Aparicio (2010) used olive oil to demonstrate the relationship
between electronic nose analysis and gas chromatography. GC elute was split before the
instrument’s detector; half went to an eNose, and the other half went on to the GC’s own
detector (García-González & Aparicio, 2010). The resulting GC chromatogram and eNose absorption/desorption curves could be directly compared (García-González & Aparicio, 2010). They concluded that some eNose sensors are better at detecting the quality attributes of olive oil than others, but stressed that this research is specific to olive oil and other eNose sensors may be better for other foods (García-González & Aparicio, 2010). Nurjuliana, Che Man, and Mat Hashim (2011) successfully used an eNose to differentiate halal lard from other fat samples. Yang, Baldermann, and Watanabe (2013) suggested eNose analysis for the aroma of tea, citing its speed and similarity to the human nose to be advantageous over GC-MS analysis. Cheng et al. (2015) used an eNose to differentiate between the aromas of several Chinese bayberry cultivars. Their analysis was paired with GC-MS-O to obtain sensory and exact chemical makeup data as well as the pattern identification of the eNose (Cheng et al., 2015).

Herbs have been subjected to eNose analysis. Na ayudhaya et al. (2009) determined that a low-cost eNose could adequately differentiate between the aromas of fresh Thai herbs. Basil has been analyzed by eNose on multiple occasions, but neither parsley nor dill has. This is not surprising, as basil is the most heavily researched of the three herbs in almost every aspect. Ground spice mixtures containing basil, cinnamon, and garlic were used to prove that the eNose can “quickly predict the compositions of mixtures” as they vary by aroma (Zhang, Balaban, Principe, & Portier, 2005). Lieberzeit, Rehman, Iqbal, Najafi, and Dickert (2009) used an eNose prototype to measure basil freshness as it grew. They found that their prototype, which used “six sensors coated with molecularly imprinted materials,” was capable of detecting volatile terpene levels of basil and mint in the air as they grew (Lieberzeit et al., 2009). The detected terpene levels increased with plant maturity, making it
possible to select a harvest time based on the concentration volatile aroma compounds in the air (Lieberzeit et al., 2009). Iqbal et al. (2010) used an eNose to evaluate the differences between fresh and dry basil. The concentration of volatile aromas in dry basil is greater than fresh, but the researchers were most impressed with the “remarkable sensitivity and selectivity” of the eNose (Iqbal et al., 2010).

**Flash gas chromatography-electronic nose analysis**

In 2005, Alpha MOS introduced an instrument that combines the detailed precision of a GC with the pattern recognition capabilities of an eNose: the Heracles analyzer (Hinshaw, 2005). Since then, a newer version, the Heracles II flash gas chromatograph-electronic nose (GC-EN) was introduced (Heracles II odor analyzer, 2016). The GC-EN acts as a rapid gas chromatograph by simultaneously utilizing two columns and FIDs to analyze a single sample (Heracles II odor analyzer, 2016). This analysis cycle usually takes about 5 minutes, unlike the 30-45 minute long run of a conventional GC, but does not “compromise on resolution” (Heracles II odor analyzer, 2016; Qian et al., 2010). The data collected can be analyzed like that of a conventional chromatograph, using KI values and Alpha MOS’ AroChemBase neural network to identify specific compounds (Heracles II odor analyzer, 2016; Alpha MOS, 2014). To do so, the AroChemBase compares the retention indices of a sample’s peaks to those of known standards to give a list of compounds that could be responsible for each particular peak. Essentially, the software calculates how different an unknown compound is from the nearest standard.

A GC-EN can also be programmed for quality control screening, sensory data comparison, and pattern recognition like a regular eNose (Heracles II odor analyzer, 2016;
Alpha MOS, 2014). The GC-EN serves to close the gap between GC and eNose analysis, incorporating both into a single device (Heracles II odor analyzer, 2016).

In 2007, Alpha MOS researchers began presenting work using the Heracles analyzer for shelf life studies and food-grade packaging innovations (Stockwell, 2007). In more recent years, food science researchers are starting to use GC-EN analysis in everyday research. Ghosh et al. (2016) explored how coconut oil pressing method affects the oil’s aroma using sensory evaluation and GC-EN analysis. They used a GC-EN for validating doctored sensory samples, but used a conventional MOS eNose for sensory correlation analysis (Ghosh et al., 2016). Wiśniewska et al. (2016) validated the use of GC-EN analysis for spirit beverage analysis. A GC-EN was used to analyze the flavor of traditional Chinese vinegars, validating that different fermentation methods produced different flavors (Yaping et al., 2017). Wojnowski et al. (2017) conducted a shelf life study on fresh poultry using a GC-EN and found that it supplemented “the established methods of chicken meat quality assessment.”

**Conclusion**

LED light is the future of greenhouse lighting, and herbs are a common greenhouse crop. Determining if sensory differences occur between herbs grown under conventional HPS lighting and those grown under LED lighting is essential for the implementation of more energy-efficient lighting. More research is required in this field before recommending a lighting system to greenhouse herb producers when it comes to aroma quality of their crops. There seem to be no published sensory or comparable instrumental data on the subject, so this study endeavors to provide both using triangle sensory difference tests and GC-EN analysis.
CHAPTER 3: CONSUMER SENSORY EVALUATION AND FLASH GAS CHROMATOGRAPH-ELECTRONIC NOSE ANALYSIS OF HERB AROMA AFTER GROWTH UNDER VARIED PROPORTIONS OF RED AND BLUE LED SUPPLEMENTAL LIGHT

Abstract

Greenhouse herb producers may use artificial lighting to supplement the natural light available to their crops. High-pressure sodium (HPS) lights are the most common supplemental lighting systems employed in such operations, but light-emitting diode (LED) lights are increasing in popularity because of their energy efficiency, customizability, and environmental friendliness. LED lights can be customized to emit specific proportions of light wavelengths, but many herb producers do not know how these varied wavelengths affect their crops, specifically their crops’ aroma. This study utilized consumer sensory difference panels and flash gas chromatograph-electronic nose (GC-EN) analysis to evaluate the aroma of fresh basil, parsley, and dill herbs after cultivation under one of three supplemental light treatments: HPS, LED with a high proportion of blue to red diodes (high blue LED), or LED with a low proportion of blue to red diodes (low blue LED).

Consumer sensory panels using triangle difference tests found that consumers could not determine the difference between herbs grown under HPS and high blue LED. Preliminary research suggests a similar result for HPS and low blue LED, but further research is required to confirm this. GC-EN analysis revealed no significant chemical differences between lighting treatments among basil or parsley. Subtle chemical differences were uncovered in dill GC-EN data, especially when nonpolar and mid-polar column data
were examined separately to prevent false correlation from multiple detections of a single compound. Consistent with literature findings, linear discriminant analysis of these data subsets revealed that multiple volatile compounds in dill are affected by the supplemental lighting wavelengths available to the herb.

In the scope of this study, there appears to be no overall aroma difference between herbs grown under HPS light and those grown under LED light, but more research must be conducted to confirm and expand upon these findings. Future research including sensory preference tests, descriptive analyses, GC-olfactometry, and GC-MS studies will make research like this more practical for farmers.
Introduction

Supplemental lighting systems are often used in greenhouses. When supplemental lighting is used in herb production, high-pressure sodium (HPS) or metal halide (MH) lighting systems are most common because of their relatively low cost (Morrow, 2008; Gómez et al., 2013; Olle & Virsile, 2013; Resh, 2013). Recently, light-emitting diode (LED) lighting systems have gained popularity for their energy-efficiency and customizability (Morrow, 2008; Gómez et al., 2013; Kopsell & Sams, 2013; Son & Oh, 2015). HPS lights give off ~75% of their total energy input as heat, contributing to greenhouse temperature and demonstrating their inefficiency compared to LEDs (Gómez et al., 2013; Yeh & Chung, 2009). Most light given off by HPS lamps is in the red-orange range, or, “peak 550-650 nm,” while LED lighting systems can be tailored to give off an extremely specific light spectrum (Massa et al., 2006; Gómez et al., 2013). In the future, LED systems’ emitted wavelengths could be customized to suit the needs of a certain producer, greenhouse, and even individual plant (Massa et al., 2006; Morrow, 2008; Gómez et al., 2013; Kopsell & Sams, 2013; Son & Oh, 2015). Olle and Virsile (2013) point out that “light quality and quantity initiate signaling cascade[s] of specific photoreceptors,” that eventually impact the plant’s gene expression, and in the case of herbs, their flavor and aroma. These cascades are difficult to predict; the only reliable way to know how a plant will respond to certain light conditions is to conduct an experiment (Olle & Virsile, 2013). Plants are known to respond to wavelengths between 380 and 750 nm, so horticultural LED lighting would need to emit light within that range (Tibbits et al., 1994). Some wavelengths within this range are more important than others. According to Yeh & Chung (2009), “chlorophyll molecules absorb red and blue wavelengths most efficiently,” so these wavelengths are best suited to induce photosynthesis.
These red and blue wavelengths have different affects on the plants that utilize them. Blue light steers crops toward vegetative growth, allows for adaptation in adverse light conditions, and contributes to their nutritional value, antioxidant content, aroma, and flavor (Briggs & Christie, 2002; Matsuda et al., 2007; Hogewoning et al., 2010; Samuolienè et al., 2012a; Kopsell & Sams, 2013; Olle & Virsile, 2013; Son & Oh, 2013; Taulavuori et al., 2016). Red light provides efficient wavelengths for chlorophyll and phytochrome in plants and also may influence antioxidant and phytochemical concentrations (Massa et al., 2006; Matsuda et al., 2007; Li & Kubota, 2009; Žukauskas et al., 2011; Samolienè et al., 2012b; Olle & Virsile, 2013). These similarities in plant response led Taulavuori et al. (2016) to conclude that red and blue light share some of the same mechanisms.

Growth under completely red light often yields physical abnormalities like hypocotyl elongation and low dry weight, while growth under 100% blue light seems to limit overall growth (Briggs & Christie, 2002; Yorio et al., 2001; Darko et al., 2014). Son & Oh (2015) note, “a combination of red and blue LEDs promoted the photosynthetic rate compared with the effect of monochromatic red or blue LEDs.”

It is evident from the literature that varied light wavelengths can make a difference in herb phenolic compound and essential oil contents, but we do not know how varied light wavelengths affect the perceived aroma of basil, parsley, or dill (Carruthers, 2015; Frąszczak et al., 2016; Taulavuori et al., 2016; Samuolienè et al., 2017). If there are significant aroma differences, sensory evaluation and flash gas chromatograph-electronic nose (GC-EN) analysis can reveal them.

Sensory difference tests are a common method used to determine if humans can perceive a difference between samples. They are statistically simple, relatively inexpensive,
and easy for panelists to understand. The triangle test, in particular, is a commonly used
difference test because of its statistical efficiency. In a triangle test, there is a 33.3% chance
that a panelist will select the correct response when they are unable to tell a difference, as
opposed to other difference tests like paired comparisons wherein there is a 50% chance of
the panelist guessing correctly when they are unsure. In the general format of a triangle test,
two foods are compared. These samples are each labeled with a different random 3-digit code
and presented in a randomized serving order. A panelist is presented with three random
samples coded with three different numbers. Two of these samples are the same and one is
different. They are asked to evaluate the samples and indicate which is the odd sample.
Triangle tests are inexpensive largely because they utilize untrained consumers as panelists.
Consumer panels like triangle difference panels require a larger number of panelists but a
shorter amount of time than a trained panel. In a triangle test, a statistically significant result
at an \( \alpha \)-risk of 0.05 “indicates strong evidence that a difference is apparent” between the two
samples (Meilgaard et al., 2016).

Instrumental analysis of herb volatiles can lend information regarding the chemical
differences that may contribute to possible aroma differences. The relatively new GC-EN
instrument is designed to marry the detailed precision of a gas chromatograph (GC) with the
pattern recognition capabilities power of an electronic nose (eNose) (\textit{Heraclès II odor
analyser}, 2016). Since the introduction of the first GC-EN in 2005, it and its subsequent
versions have been used to analyze food packaging, olive oil pressing techniques, spirit
beverages, traditional Chinese vinegars, poultry shelf life, and more (Hinshaw, 2005;
Stockwell, 2007; Ghosh et al., 2016; Wiśniewska et al., 2016; Yaping et al., 2017;
Wojnowski et al., 2017). It did not appear to be used for fresh herb aroma analysis prior to this research.

The GC-EN acts as a rapid gas chromatograph by simultaneously utilizing two columns and FIDs to analyze a single sample (Heracles II odor analyzer, 2016). This analysis cycle usually takes about 5 minutes, unlike the 30-45 minute long run of a conventional GC, but does not “compromise on resolution” (Heracles II odor analyzer, 2016; Qian et al., 2010). The data collected can be analyzed like that of a conventional chromatograph, using KI values and Alpha MOS’ AroChemBase neural network to identify specific compounds (Heracles II odor analyzer, 2016; Alpha MOS, 2014). To do so, the AroChemBase compares the retention indices of a sample’s peaks to those of known standards to give a list of compounds that could be responsible for each particular peak; the software calculates how different an unknown compound is from the nearest standard.

The objective of this study is to ascertain if there is an aroma difference between herbs cultivated under HPS lamps and those cultivated using one of two different LED light treatments with different proportions of blue and red diodes using sensory evaluation and eNose analysis. In doing so, researchers can make recommendations to herb producers regarding LED supplemental lighting system investment and settings. No study of this scope has ever been attempted, so some of the data remain preliminary.

**Materials and Methods**

This study was conducted from January through May of 2017 at Iowa State University in Ames, Iowa. Basil, parsley, and dill grown in an on-campus research greenhouse were evaluated for aroma by consumer sensory panelists in an on-campus
research facility. Approval by Iowa State University’s Institutional Review Board was granted on September 23, 2016 (Appendix A). The same herbs were subjected to analysis by a GC-EN housed in a lab near the sensory evaluation facility.

**Plant growth conditions**

Before transplantation into hydroponic deep-flow technique (DFT) systems, ‘Nafur’ basil (*Ocimum basilicum* L.), ‘Fernleaf’ dill (*Anethum graveolens* L.), and ‘Giant of Italy’ flat-leaved parsley (*Petroselinum crispum* L.) from Johnny’s Seeds in Winslow, MA were propagated in phenolic foam within a growth chamber. During this 3-week nursery stage, the seedlings were watered with deionized water, given 100 ppm nitrogen, and provided 450 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) of light for 16 hours per day from both incandescent and fluorescent sources. Individual phenolic foam cubes were thinned to one seedling per cube before transplantation into nine individual 227 L DFT systems inside a metal and glass greenhouse at Iowa State University in Ames, IA (42° 02’ N latitude). Each hydroponic system housed all three herb species at a rate of 24 plants per species. The plants floated in nutrient solution with the help of net pots spaced 15 cm apart in polystyrene rafts; this apparatus allowed for the plants’ roots to be in direct contact with the nutrient solution at all times. The nutrient solution was made by dissolving 53 mg of MgSO\(_4\) in 227 L deionized water and adding a complete balanced water-soluble fertilizer to an electrical conductivity (EC) of 1.5 dS·m\(^{-1}\). This EC was maintained through the remaining 3 weeks of growth. In each DFT system, water was circulated to maintain a temperature of 22.5 ± 0.5 °C, while air was provided to the plants’ roots through six air stones and a 110 L air pump. Appropriate pH in the DFT systems was maintained through potassium bicarbonate or citric acid and phosphoric acid adjustments.
Daytime greenhouse temperature was maintained at 24 °C; it was allowed to drop to 20 °C at night.

Herb plants were provided with roughly 8 mol·m⁻²·d⁻¹ of light, using automatic overhead shades in the greenhouse working in tandem with supplemental lighting timers. This comes from ambient light plus one of three possible supplemental lighting systems: HPS, low blue LED, and high blue LED. The HPS treatment consisted of one 400 W high-pressure sodium lamp (see Figure 1 in Appendix B). The treatment referred to as low blue LED employed a 7:93 ratio of blue to red diodes in a Phillips GreenPower LED Toplighting unit (see Figure 2 in Appendix C). The high blue LED treatment also utilized Phillips GreenPower LED Toplighting unit, but this time with a 30:70 ratio of blue to red (see Figure 3 in Appendix D). When observed in the greenhouse, high blue LEDs provide a violet light, while low blue LEDs provide a pink or magenta light. All three treatments provided 100 µmol·m⁻²·s⁻¹ of light. Opaque white plastic liners separated each treatment, hanging above the light system and below the DFT system. The plastic was 6 mm thick and folded to provide maximum separation.

**Sensory evaluation**

Ten sensory panels were conducted using triangle difference tests, the procedure of which is outlined in *Sensory Evaluation Techniques* (Meilgaard et al., 2016). Seven of the ten panels tested herbs grown under conventional HPS lighting against those grown under high blue LED systems. The other three panels tested herbs grown under HPS lights against those grown under low blue LED lights. Each sensory panel tested basil, parsley, or dill.
individually. In accordance with Institutional Review Board approval, each sensory panelist was required to complete the informed consent form provided in Appendix F.

Herbs samples were harvested and placed into 1 oz. plastic containers with lids to equilibrate the morning of each sensory panel. Herbs from different hydroponic systems and different individual plants were mixed together to negate differences in system and individual plant placement within DFT systems.

Each individual panel consisted of 33 panelists who participated in two triangle tests for a total of 66 observations per panel. An example of the sensory test ballot presented to each panelist is available in Appendix G. Each triangle test within the panels was independently randomized so that a panelist received different sample codes and a different serving order every time. A typical sensory panel worksheet used for randomization can be found in Appendix H.

**Flash gas chromatograph-electronic nose analysis**

An Alpha MOS Heracles II GC-EN with an Odorscanner headspace autosampler and its corresponding AlphaSoft V14 software were used to analyze herb samples. Figure 4 in Appendix E is a color photograph of the GC-EN apparatus. Samples were prepared at the same time as sensory samples from the same harvest. Five replications of four samples were collected from every harvest. The four samples included: an empty vial to serve as a blank, an HPS sample, a low blue LED sample, and a high blue LED sample. All samples were sealed in glass vials with magnetic lids and left to equilibrate for approximately 1 hour before beginning headspace analysis. Each sample incubated in the 40°C autosampler oven for 20 minutes with 500-rpm agitation cycling through 5 seconds on, 2 seconds off, and so on. The
syringe was heated 50°C and used a fill speed of 500 µl/s. The nonpolar MXT-5 and mid-
polar MXT-1701 columns utilized in the eNose were both 10 meters long and 0.18 mm in
diameter. The eNose program began with an oven temperature of 40°C with an initial
isotherm of 2 seconds and continued to increase the temperature at a rate of 3°C/s. A hold of
20 seconds occurred once the temperature reached 260°C. KI values were generated using
C6-C16 external standards.

**Statistical analysis**

Sensory results were analyzed using table 19.8 in *Sensory Evaluation Techniques*
(Meilgaard et al., 2016). A correct response is recorded in a triangle test when the consumer
panelist indicates the correct odd sample of the three they were presented. With 66
observations at an α-level of 0.05, 29 correct responses or more indicate that consumer
panelists observed significant aroma differences between an HPS herb and an LED herb
(Meilgaard et al., 2013).

SAS version 9.4 was used to conduct further statistical analysis on the GC-EN data.
First, a Pillai’s Trace analysis was conducted on each herb’s samples to determine if there
were significant differences between peak areas of samples grown under different lighting
treatments. Once significance was established, individual peaks were analyzed via
MANOVA (multivariate analysis of variance) for significance between lighting treatment
groups. The significant compounds were subjected to pairwise comparison analysis two
treatment groups at a time to clarify the relationships between individual groups. Finally,
Linear Discriminant Analysis (LDA) was used to visualize the differences between the three
lighting treatments. LDA is used to reveal underlying patterns from data much like PCA.
Unlike PCA, LDA searches for differences in the context of treatment groups as opposed to the data set as a whole.

**Results and Discussion**

**Sensory evaluation**

Table 1 in Appendix I compiles the results of all consumer panels that tested herbs grown under HPS light against those grown under high blue LED light. Both basil panels yielded fewer correct responses than 29, so they are not statistically significant. 29 correct responses out of 66 is the threshold for significance based on statistical tables in Meilgaard et al. (2013). The two HPS vs. high blue LED parsley panels also resulted in no significant differences between lighting treatments. HPS and high blue LED dill required three consumer panels to come to a conclusion, because consumers could not perceive aroma differences between HPS and high blue LED dill in the first dill panel, but could in the second. A third HPS and high blue LED dill panel was conducted and generated results below the significance threshold with 24 correct responses. Overall, panelists were unable to detect the difference between basil, parsley, or dill grown under HPS and the same herb grown under high blue LED lighting.

Herbs grown under low blue LED light could only be tested against HPS herbs for one replication, so the following data are merely exploratory (Table 2 in Appendix I). Based on these data, panelists were unable to tell the difference between HPS and low blue LED basil or dill, but they were able to distinguish between HPS and low blue LED parsley.
**Flash gas chromatography-electronic nose analysis**

This study utilized a GC-EN because of the unmatched sensitivity and speed of the instrument; it marries the advantages of a gas chromatograph with those of a more conventional electronic nose. GC-EN runs yielded retention times and estimated Kovats indices (KI) for each peak in each sample just like a conventional GC, amounting to a staggering amount of data. Statistical analyses revealed some interesting differences between lighting treatments, but only in dill. No significant differences between treatments occurred in basil and parsley. P-values from Pillai’s Trace analysis comparing all three lighting treatments revealed p-values of 0.3253 and 0.4563 for basil and parsley, respectively. Significant differences are indicated by a p-value of 0.05 or less. These results are consistent with the sensory panel findings.

Table 3 compiles the most abundant basil GC-EN peaks that the AroChemBase could roughly identify (Appendix J). Note that these peaks are not significantly different between lighting treatments; they are the most abundant and easily identifiable across all basil samples. These compounds probably serve to supplement basil’s primary aroma compounds of linalool, 1,8-cineole, and methyl chavicol (van Wyk, 2013). Maltol, 2-phenylethanol, 1-nonanol, benzyl acetate, and p-anisaldehyde were detected by both columns. The GC-EN detected maltol in all three herbs in this study, and it contributes to the herbs’ sweet aromas. 2-phenylethanol, 1-nonanol, benzyl acetate, and p-anisaldehyde generally smell sweet and floral.

Table 4 serves the same purpose for parsley, compiling the most abundant GC-EN peaks that were readily identifiable by the AroChemBase software (Appendix K). Maltol, 2-phenylethanol, 1-nonanol, and benzyl acetate all contributed their sweet, floral aromas to
parsley as well as basil. Also notable in parsley samples is terpinen-4-ol, nerol, and decanal. Terpinen-4-ol smells peppery, woody, and citrus, while decanal provides sweet, waxy, and orange aromas. Nerol contributes to parsley’s sweet aroma.

The results of dill sample statistical analyses were more complicated. The Pillai’s Trace analysis yielded a p-value of 0.0289. This indicates that there were chemical differences between the three treatments, but further analysis was required to reveal where the differences occurred.

The individual peaks of each lighting treatment were compared, and eight peaks had p-values below 0.05, indicating that these compounds were significantly different when compared through the context of lighting treatment groups. Using the AroChemBase and literature values, these eight compounds were roughly identified via their Kovats retention indices, or KI values (see Table 5 in Appendix L). Some peaks have multiple corresponding compounds; the GC-EN is not always capable of precisely identifying each peak, so it provides a few probable options. As in the AroChemBase, they are listed from most probable to least. These peaks do not necessarily correspond to the most common or most heavily contributing dill aroma compounds; they are merely the peaks that differed significantly by peak area. This is not to say the main dill aroma compounds listed in Chapter 2 are not present, but most of them did not differ significantly. These significant compounds may serve to round the aroma of fresh dill. For example, artificial vanilla is largely composed of the compound vanillin, while natural vanilla flavor from vanilla beans contains over 250 flavor and aroma compounds that serve to complete the vanilla aroma (Kennedy, 2015).

The software proposed that the four nonpolar peaks most likely correspond to ethyl butyrate, myrcene, benzyl alcohol, and benzyl salicylate. Ethyl isobutyrate and benzyl
salicylate were both the only proposed compounds for their respective peaks, so this indicates a level of clarity not present in the other two nonpolar peaks. Ethyl isobutyrate is described as sweet, ethereal, and fruity, while benzyl salicylate smells balsamy, herbaceous, clean, and oily (The Good Scents Company, 2015; El-Zaeddi, Martínez-Tomé, et al., 2016). The peak eluted at a retention time (RT) of 42.93 could be either myrcene or (-)-β-pinene. Myrcene has notes of pepper, terpene, and balsam (El-Zaeddi, Martínez-Tomé, et al., 2016). Descriptors of (-)-β-pinene include dry, woody, resinous, and pine (El-Zaeddi, Martínez-Tomé, et al., 2016). The AroChemBase software indicated that myrcene was only slightly more probable than (-)-β-pinene to truly correspond with the peak, so both compounds must be considered. Seven compounds must be considered for the nonpolar peak at 45.60; Benzyl alcohol, acetylpyrazine, benzeneacetaldehyde, (Z)-2-octenal, trans-hex-2-enyl acetate, α-terpene, and p-cymene were all listed as the possible corresponding compound. These two peaks with multiple possible compounds are the most significantly different ones, already prompting a call for further research.

Analysis of peaks from the mid-polar column also yielded four significant peaks. The first one, at 20.49, was not identified by the AroChemDatabase or by searching literature and databases. Its p-value was the highest among those of significance. This is not to say that it is insignificant, but further research must be done to determine if it is an aroma-contributing compound, an odorless compound, or perhaps noise from the environment reflecting different base odors for different testing days. The next mid-polar peak was also unidentifiable by the AroChemBase, but using the Flavornet online database (Datu Inc., 2004), it can be roughly identified as mercaptoacetaldehyde, a sulfurous compound found in cabbage and other vegetables of the Brassica family. The AroChemBase listed five possible
compounds for the mid-polar peak at 40.05: (-)-β-pinene, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, and (Z)-4-heptenal. Because (-)-β-pinene was also identified as a possibly significant compound by the nonpolar column, it is the most likely compound for this peak as well. 3-methylbutanoic acid is probably the compound that corresponds with the final peak in Table 5. It was estimated to be responsible for this peak in every dill sample, whereas the AroChemBase only listed myrcene and (+)-(4S)-α-phellandrene as possibilities for a few individual samples.

Table 5 gives us more information about the possible aroma descriptors that can change when the supplemental lighting system is changed in a greenhouse. For dill, it seems that the balsamy, woody, and piney aromas are most impacted (El-Zaeddi, Martínez-Tomé, et al., 2016; The Good Scents Company, 2015; Datu Inc., 2004).

The data were divided into nonpolar and mid-polar data to minimize the possibility of repetitive detection skewing the data. Pillai’s Trace analyses were conducted on both of these groups. The analysis of nonpolar data yielded a p-value of 0.0604, an insignificant result. Statistical analysis revealed no significant differences between lighting treatments when dill samples were analyzed through the nonpolar GC-EN column. The same result occurred with the mid-polar data. The Pillai’s Trace analysis resulted in a p-value of 0.334. For a closer look, the 20 most abundant peaks detected on each column were separated from their larger data sets. It is important to note that these 20 most abundant compounds do not necessarily correspond to the most odorous or most important compounds, nor are these 20 compounds the same for both columns. These subsets were also subjected to Pillai’s Trace analysis to a far different result. For the nonpolar column, a p-value of 0.0026 indicated significant
differences between the three treatment groups. A p-value of 0.0001 for the mid-polar column revealed strong significant differences between the lighting treatments.

All the analysis up to this point gave us no indication of which treatment groups differed from which. Pairwise comparisons of the peaks from Table 5 in Appendix L are available in Tables 6-8 in Appendix M. Table 6 compares HPS and low blue LED lighting treatments. All eight compounds of significance examined were significantly different between the two treatments. Table 7 compares the HPS and high blue LED treatments, revealing a lone significantly different compound: Benzyl salicylate. Table 8 compares the two LED treatments; they differ significantly at six of the eight peaks, or all but the unknown chemical and benzyl salicylate. In fact, benzyl salicylate seems to react to both LED treatments in the same way, because the p-value of the benzyl salicylate peak in Table 8 is 1. The unknown compound may act similarly, but more research is needed. The low blue LED treatment is certainly chemically different from both HPS and high blue LED treatments because it differs from them at nearly every other significant peak.

In the case of these data, LDA was able to depict the chemical differences between dill plants grown under the three lighting treatments (Figures 5-9 and Table 9 in Appendices M-R). Each figure was constructed using canonical scores calculated by centering and standardizing each log-transformed peak area, multiplying each standard peak area by its coefficient, and adding that over all the peaks; they allow us to see exactly where the most striking differences lie between treatments. These scores are arbitrary, so a point’s location does not necessarily correspond to the same chemical difference from figure to figure.

In Figure 5 (Appendix N), the first canonical score along the x-axis roughly separates HPS from the LED treatments, while the second score on the y-axis distinguishes between
the LED treatments. There is overlap between all three treatments, so there are still chemical similarities between them. This is not unexpected; the chemical differences should be subtle among samples of the same plant species and cultivar. The x-axis of Figure 5 is defined by canonical score 1 for each peak. As canonical score 1 becomes more negative, it is defined by the mid-polar peak eluted at 69.68 and the nonpolar peaks at 52.93 and 54.85. The AroChemBase software could not identify the mid-polar compound at 69.68, but the nonpolar peaks at 52.93 and 54.85 could correspond to a number of compounds. See Table 9 in Appendix S for the full list of possible compounds that impact for Figures 5-9. As the x-axis becomes more positive, the mid-polar peaks at 51.42 and 53.18 define the axis.

Citronellal is probably responsible for the peak at 53.18, but 51.42 has three possible compounds: n-nonanal, ethyl 3-methylthiopropanoate, and benzyl alcohol. That multiple peaks, and thus compounds, contribute to the differences between lighting treatments reflects both the nature of both LDA and plant response to LED lighting.

The y-axis of Figure 5 is less characteristic of LDA and plant response. The mid-polar peak at 53.18 defines its positive end and the negative end is related to the nonpolar peak at 54.85. These two peaks are so individually correlated that they must correspond to the same compound, isomers, or degradation products of the same compound. Figure 5 is severely skewed by repetitive data; both columns are able to detect some of the same compounds, creating these false correlations. To combat this, the same LDA procedure was conducted on each column individually, as well as on each column’s 20 most abundant peaks, similar to the statistical analyses performed on the peak areas earlier in this section.

LDA analysis of all nonpolar peaks yielded Figure 6 (Appendix O). The separation is even clearer than in Figure 5, which is very promising. In theory, Figure 6 should look less
separated if it truly eliminated all correlation. Instead it eliminated false correlation due to overlapping data from both columns and revealed patterns much closer to the true relationships between the lighting treatment groups. Low blue points do not overlap with either HPS or high blue points. Some overlap occurs between HPS and high blue. This is consistent with the pairwise comparison results; low blue differs readily from both of the other lighting treatments, but fewer differences occur between HPS and high blue.

Six main compounds define the axes of Figure 6, so the differentiation should not rely on unwanted correlation between hyper-related compounds like isomers or degradation products. An unknown compound at 18.47 and a compound estimated to be decanal at 54.85 define the x-axis’ negative end. Its positive end is influenced by peaks at 51.87 and 42.50, both of which could correspond to up to four compounds (see Table 9 in Appendix S). Peaks at 18.47, 49.61, and 54.85 pull dill samples toward the negative end of Figure 6’s y-axis. The peak at 42.50 pulls samples toward the positive end. Interestingly, this peak is only measurably abundant in two dill samples in the entire study, both of which were cultivated under low blue LED lighting and harvested on March 30, 2017. This peak could be a compound of some interest, or it could be meaningless noise. Both of the low blue samples that contain it were harvested and analyzed on the same day, so it may be something as simple as an aroma compound from the perfume or shampoo of the research assistant that prepared the low blue GC-EN samples that day. There are three other compounds that contribute to the dill samples’ behavior along the y-axis to varying degrees, so Figure 6 is still the most promising of the LDA figures.

Figure 7 in Appendix P, based on mid-polar data, depicts some separation as well. However, its y-axis is defined almost exclusively by the relationship between peaks at 53.18
and 54.15. Citronellal, the spicy-sweet citrusy compound that contributes to the distinctive aroma of citronella oil, is responsible for the peak at 53.18 (The Good Scents Company, 2015; van Wyk, 2013). It is also among the possibilities for the compound at 54.15 (Appendix S). These peaks could easily be related, so any vertical separation should be viewed with skepticism as a result. The canonical coefficients for the x-axis are very small, meaning that many compounds contribute to the overall behavior and its separation is quite promising. It seems to separate all three groups: HPS samples exist toward the negative end of the x-axis, followed by low blue samples around zero, and high blue samples gather at the positive end. There are overlapping samples among the groups along the x-axis. Peaks at 53.18, 54.83, and others contribute to the axis’ negative end, while 51.42, 55.85 and others pull samples toward its positive end. Estimated compounds for each of these peaks are available in Table 9 in Appendix S.

Figures 8 and 9 are the result of analyzing only the 20 most abundant compounds from GC-EN analysis, separated by column. Again, these compounds do not necessarily correspond to those with those that contribute most to the aroma of the samples, and the 20 most abundant compounds according to the nonpolar column are not the same as those according to the mid-polar column. There is some overlap, but the groups were compiled independently.

Figure 8 was generated using the 20 most abundant peaks according to the nonpolar column. The treatment groups are not as nicely separated in Figure 8 as they were in the previous figures (Appendix Q). Figure 8’s x-axis is formed from many peaks, but the y-axis uses almost exclusively peaks at 54.85 and 55.67. These compounds are so greatly correlated that they must be considered structurally similar if not identical. According to the
AroChemBase, they both could correspond to decanal, a citrusy, sweet-smelling substance common in herbs, especially cilantro (The Good Scents Company, 2015; van Wyk, 2013). These compounds also contribute to the separation along the x-axis, but not as heavily. This misleading correlation and the poor separation between the treatment groups makes Figure 8 unfit for interpreting these data.

Figure 9 in Appendix R draws similar conclusions. It too is defined by two peaks along its y-axis: 53.18 and 54.15. These may be the result of the exact same compounds that define the y-axis in Figure 8. Figure 9’s x-axis is well divided among many peaks, and it seems to separate the HPS from the LED treatments. The negative end is defined by peaks at 47.00 and 55.85. Compounds that could be responsible for the peak at 55.85 include decanal, sweet and nutty maltol, or the floral smelling compounds benzyl acetate and 2-phenylethanol (The Good Scents Company, 2015). Acetylpyrazine is responsible for the peak at 47.00, contributing a nutty, popcorn-like aroma (The Good Scents Company, 2015). The positive end of the x-axis reflects the contribution of peaks at 54.15 and 76.51. According to the AroChemBase, 1-nonanol is the compound most likely to correspond to the peak at 54.15 (see Table 9 in Appendix S for a full list of possible compounds). The peak at 76.51 may correspond to δ-decalactone, but the observed retention index is so high that it is difficult to confirm the AroChemBase identification with literature.

Integrating Sensory and GC-EN Data

Many of the findings from GC-EN analysis require further research or validation through more intense sensory methods than those applied in this study, but GC-EN data and basic sensory findings can still be related to one another. For example, PCA, global testing,
and Pillai’s Trace analyses of basil and parsley GC-EN data were consistent with sensory findings; no significant differences occurred between herbs grown under HPS lighting and those grown under LED lighting. The notable exception is the preliminary observation that one sensory panel was capable of detecting the difference between HPS parsley and low blue parsley, but this conclusion has been weakened by the GC-EN result, especially because the sensory panel was not replicated.

From the one panel comparing HPS to low blue dill samples, the panelists could not detect the difference between the treatments, but sensory panelists fell just short of detecting significant differences between HPS and high blue LED dill samples. Statistical analysis of dill GC-EN data did detect significant differences between treatments, but it was unclear where these differences laid once the data from the nonpolar and mid-polar columns were separated. However, when the 20 most abundant compounds from each column were analyzed, significant differences were evident. These differences probably do not contribute greatly to the comprehensive aroma of dill herbs, because the sensory panels did not detect them. Perhaps a more in-depth descriptive sensory panel would be able to pinpoint these subtle differences, if these compounds contribute to aroma as well as chemical differences.

Pairwise comparisons of eight significantly different peaks from both columns attempted to pinpoint the specific groups that differed in previous analyses. HPS dill samples differed from high blue samples in only one peak, while they differed from low blue in all eight peaks. This is yet another reason to continue this research by replicating the HPS vs. low blue LED sensory difference panel. When the LED treatments were compared, they differed in 75% of the peaks. The most interesting aspect of this comparison, however, came from a compound that was not significantly different between the LED treatments. The
nonpolar peak at 88.59 acted the same way in both LED treatments because this analysis revealed a p-value of 1. Future researchers should wonder why, and how, such a similar response is possible.

LDA allowed these subtle chemical differences, and possible sensory differences, to be visualized. Disregarding Figures 5 and 8, which exhibit false correlations between peaks that may correspond to the same compound or a group of closely related compounds, Figures 6, 7, and 9 show promising chemical separation between the three groups of dill samples, but all three of these figures show overlap between the treatments as well. This overlap may be the key to understanding why sensory panelists did not detect a difference between HPS and LED treatments. These figures also demonstrate that multiple substances contribute to the chemical differences between lighting treatments; plant response to lighting is complicated, and most likely is the result of many changes in plant physiology, photoreception, and metabolism. These responses are unique to dill, as each plant reacts uniquely to its growth conditions. For this reason, more crops must be subjected to studies like this. Experimentation is the only way to truly predict how a plant will respond to specific stimuli. In the case of herb producers, subtle chemical differences like those revealed in dill GC-EN analysis are capable of severely impacting their success. Chemical differences are what cause aroma differences, and aroma differences can be the difference between a successful herb business and an unsuccessful one.

A Note on Error

Error can always play a role in science, no matter how much researchers prepare. Errors are particularly common in the headspace sampling method utilized for GC-EN
analysis in this study. Da Costa and Eri (2005) point out that, “the relative concentration of components in the headspace does not reflect the concentration in the sample due to the differences in volatility of aroma compounds.” Additionally, the aroma released by the sample into the vial’s headspace depends on the sampling temperature (Da Costa & Eri, 2005). It is unclear how much the lab’s temperature fluctuated as measured in this 4-month study.

Another incidence of error occurred during GC-EN sampling. Sample weights were not recorded prior to analysis, which made it impossible to accurately calculate the concentrations of the identified chemicals in the herb samples (Qian et al., 2010). Recording sample weights would have improved the accuracy and the caliber of conclusions drawn from these data. This is by far the greatest shortcoming of this study; further research must be done in this area to determine how aroma compound concentrations change when herbs are cultivated under varied light wavelengths.

**Conclusion**

Within the scope of this study, basil, parsley, and dill plants grown under LED lights with a high proportion of blue to red light do not have a significantly different aroma than basil, parsley, and dill plants grown under HPS lights. Furthermore, a similar albeit preliminary conclusion can be drawn when it comes to basil, parsley, and dill grown under LED lights with a low proportion of blue to red light; They do not seem to significantly differ in aroma from those herbs grown under HPS lights. Subtle chemical differences appear in dill plants cultivated under these three lighting treatments, but this study cannot draw any specific conclusions about how these differences contribute to the aroma of dill plants.
Further research in horticulture, sensory science, and chromatography must be conducted with herbs and all kinds of food crops in order to confirm these findings and supplement them for the benefit of herb farmers, horticulturalists, and food scientists alike.
CHAPTER 4: SUMMARY, CONCLUSIONS, AND FUTURE WORK

This research aims to start a larger conversation about plant response under LED light, starting with providing herb farmers with guidance for their urgent concerns. The objective of this study was to ascertain if there is an aroma difference between herbs cultivated under HPS lamps and those cultivated using one of two different LED light treatments with different proportions of blue and red diodes via sensory evaluation and GC-EN analysis. The high blue LED system had a 30:70 proportion of blue to red diodes, while the low blue LED system had a proportion of blue to red of 7:93.

This tandem approach to aroma analysis utilizes both human sensory perception and a state of the art instrumental method. Using sensory evaluation and a GC-EN treats the herb’s aroma both as a comprehensive property and a chemical cocktail of volatile compounds. This dual-analysis technique revealed far more than one method could alone.

Ten consumer difference panels were conducted for the study, three each for basil and parsley, and four for dill. Four panels were conducted for dill because the results of the first couple panels contradicted one another. An extra panel was added to settle this inconsistency. Because of their relative simplicity, cost effectiveness, and ease of analysis, a set of two triangle tests was used in each panel. The first two parsley, two basil, and three dill panels revealed that consumers were unable to tell the difference between an herb grown under conventional HPS supplemental lighting from an herb of the same cultivar grown under high blue LED lights at an $\alpha$-level of 0.05 and when the herb in question is either basil, parsley, or dill. It is important to note this result applies only to these three herbs because plant response to light is highly plant dependent; other species and cultivars may react to these lighting treatments differently (Morrow, 2008; Kopsell & Sams, 2013; Olle & Virsile, 2013).
The final sensory panel for each herb asked consumers to distinguish between HPS light and low blue LED light treatments. Consumers could not tell the difference between these treatments in basil and dill, but they could detect differences between HPS and low blue LED parsley. The findings of these low blue panels must be understood as unofficial and preliminary; these panels could not be replicated because of temporal and budgetary constraints. Thankfully, GC-EN data lent some clarity to sensory study conclusions.

The GC-EN is the future of aroma analysis because it marries the precision and translatability of the gas chromatograph with the pattern recognition and statistical power of the electronic nose. The instrument utilized in this study was also capable of searching the AroChemBase to roughly identify compounds that could correspond to specific peaks, which helped compile Tables 3, 4, 5, and 9 in Appendices J, K, L, and S, respectively.

Simultaneous with each sensory panel, GC-EN runs analyzed the same herbs from the same harvest. The GC-EN is equipped with both nonpolar and mid-polar columns that both collected peak area data on each sample. These peak areas were subjected to statistical analysis in many forms.

Initial statistical analysis of peak areas from both GC-EN columns revealed significant chemical differences between light treatments in dill, but none in parsley or basil. Separate analysis of dill nonpolar peaks and mid-polar peaks showed no significant differences, but when the 20 most abundant peaks from each column were tested, both revealed sound statistical evidence of significant differences between treatment groups.

Initial statistical analysis revealed eight significantly different peaks in the overall dill analysis, and pairwise comparisons of these peaks gave a more detailed view of where the differences between lighting treatments occurred. High blue LED and HPS herbs only differ
at one of the eight compounds, while low blue LED and HPS herbs differ at all eight compounds. Similarly, high blue and low blue LED treatments differ at six of the eight compounds. At least at these compounds, it seems that low blue LED is the source of many of the differences between lighting treatments observed in previous statistical analysis.

Linear discriminant analysis (LDA) conducted on dill GC-EN data helped visualize these differences. Four of the five LDA-generated figures suffered from false correlation, but there were promising differences in Figure 6. Figures 5, 7, 8, and 9 all experienced false correlation between peaks that correspond to either the same compound or closely related compounds like isomers or degradation products. Figure 6 separated all nonpolar dill GC-EN peaks in the context of the lighting treatment groups. Multiple compounds define each axis of Figure 6, demonstrating that plant response to varied lighting treatments is not defined by a single pathway or a single aroma compound.

Relating consumer sensory panels to GC-EN analysis proved more difficult than initially anticipated. Neither method was particularly consistent. The data generally indicate that the null hypotheses should not be rejected; no perceivable aroma difference occurred between herbs cultivated under HPS and herbs cultivated under LED lighting treatments. This conclusion must be treated with a fair amount of skepticism, as not all sensory panels could be repeated, and not all GC-EN statistical analyses were free of false correlation. Additionally, this study falls short of drawing conclusions about aroma compound concentration or prevalence in the overall herb aroma. Further research must build on this preliminary work.

This study sought to give herb producers peace of mind when facing lighting system changes in their greenhouse operations; unfortunately, a study of this scope is beyond the
capabilities of one research team in a two-year time limit. This research must be seen as a springboard for future studies. Greenhouse farmers, of herbs and other crops, deserve the tools they need to succeed, and those tools begin with cutting-edge research.

Because of the subtle differences discovered in dill, other herbs must be subjected to parameters like this study, comparing LED lights of various wavelengths to industry standards like HPS or metal halide systems. In fact, more research must be done with all kinds of food crops under LED lighting, because response to lighting treatment is extremely species-dependent. The only way to discover how lighting treatments affect a certain crop is to experiment. LED lights come in more colors than red and blue; they are now available in almost every color. Green, yellow, and purple LED lights may have unexpected effects on greenhouse crops. Do they produce aroma differences in herbs? What light wavelengths are perfect for basil, roses, tomatoes, or poinsettias?

These future inquiries should make use of different sensory methods. Consumer preference tests can help herb producers learn which lighting treatments produce the most desirable herbs to consumers. Descriptive analysis methods with trained panelists are capable of finding aroma descriptors and qualitatively describing sensory differences instead of merely detecting them.

Other chromatographic methods will also shed new light on herbs grown under LED. There are many substances eluted during a GC run that are not detectable by the human senses but are by the instrumental detector and vice versa; GC-olfactometry (GC-O) investigates those discrepancies (D’acampora Zellner et al., 2008; Reineccius & Peterson, 2013). GC-O analysis involves splitting a GC sample before detection, and letting half the elute flow through an olfactory port for a sensory panelist to smell (D’acampora Zellner et
al., 2008). The process is tedious and fatiguing for panelists, but GC-O is the only true way to link GC and sensory analyses (D’acampora Zellner et al., 2008). If confident compound identification is desired, perhaps GC-MS analysis may be a better choice. It is also possible to combine all three into GC-MS-O analysis (Cheng et al., 2015). Regardless of the analysis method used, it should be more in depth than the scope of this study, because this research only scratched the surface of possibility.

At this point in time, it is difficult to make a lighting system recommendation to hydroponic herb farmers unless their operation solely rests on basil, parsley, and dill. Even then, this research does not claim to be anything but preliminary. However there seems to be no impact on the perceivable aroma of basil, parsley, or dill under the specific LED light systems used in this study when compared with the same herbs grown under a conventional HPS light fixture. If a greenhouse basil, parsley, or dill farmer wishes to switch to more energy efficient LED lighting, it should not affect their herbs if they adhere to this study’s lighting parameters. If that farmer also produces lettuce, rosemary, cilantro, and tomatoes in the same greenhouse, they should demand further research before making the switch; each plant reacts differently to lighting changes and should be tested before a large investment like an LED lighting system is made. Just because an LED system works for this study’s test herbs doesn’t mean that it will work for another crop. Furthermore, since is not an immediately evident difference between HPS and LED lighting, farmers should use the system that works best for their business’ needs, whether that means saving money by continuing to use HPS lights, or switching to LED lighting to minimize their carbon footprint for moral or marketing purposes.
It is the duty of horticulture, food science, and sensory researchers to provide opportunities for this research to take place. Academia is fueled by the need of communities, and the controlled environment agriculture community is in need of answers from inquisitive, enthusiastic, and comprehensive researchers.
REFERENCES


APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL

Date: 9/23/2016

To: Dr. Lester Wilson
2312 Food Sciences Bldg

From: Office for Responsible Research

Title: Determining Sensory Differences Between Culinary Herbs Cultivated Under Varied Light Wavelengths

IRB ID: 16-246

Study Review Date: 9/22/2016

The project referenced above has been declared exempt from the requirements of the human subject protections regulations as described in 45 CFR 46.101(b) because it meets the following federal requirements for exemption:

- (6) Tests and studies of the safety, effectiveness, and biological characteristics of materials where required by the Federal Food, Drug, and Cosmetic Act (FDCA) or any other Federal law to which the material is subject, or tests and studies of the quality of these materials; or
- (7) Research activities which do not involve controlled human participation and which are conducted in non-interactive settings, where the investigation involves observing existing conditions or events, such as the collection of biological specimens, or activities involving the use of data that has been de-identified or are exempted from full IRB oversight due to their nature.

The determination of exemption means that:
- You do not need to submit an application for annual continuing review.
- You must carry out the research as described in the IRB application. Review by IRB staff is required prior to implementing modifications that may change the exempt status of the research. In general, review is required for any modifications to the research procedures, method of data collection, nature or scope of information to be collected, changes in confidentiality measures, etc., modifications that result in the inclusion of participants from vulnerable populations, and/or any change that may increase the risk or discomfort to participants. Changes to key personnel must also be approved. The purpose of review is to determine if the project still meets the federal criteria for exemption.

Non-exempt research is subject to many regulatory requirements that must be addressed prior to implementation of the study. Conducting non-exempt research without IRB review and approval may constitute non-compliance with federal regulations and/or academic misconduct according to ISU policy.

Detailed information about requirements for submission of modifications can be found on the Exempt Study Modification Form. A Personnel Change Form may be submitted when the only modification involves changes in study staff. If it is determined that exemption is no longer warranted, then an Application for Approval of Research Involving Humans Form will need to be submitted and approved before proceeding with data collection.

Please note that you must submit all research involving human participants for review. Only the IRB or designees may make the determination of exemption, even if you conduct a study in the future that is exactly like this study.

Please be aware that approval from other entities may also be needed. For example, access to data from private records (e.g., student, medical, or employment records, etc.) that are protected by FERPA, HIPAA, or other confidentiality policies requires permission from the holders of those records. Similarly, for research conducted in institutions other than ISU (e.g., schools, other colleges or universities, medical facilities, companies, etc.), investigators must obtain permission from the institution(s) as required by their policies. An IRB determination of exemption in no way implies or guarantees that permission from these other entities will be granted.

Please don't hesitate to contact us if you have questions or concerns at 515-294-4566 or irb@iastate.edu.
Figure 1. High Pressure Sodium supplemental lighting system in place over an individual deep-flow hydroponic system housing young basil, parsley, and dill plants.
Figure 2. LED supplemental lighting system with a 7:93 ratio of blue to red diodes in place over an individual deep-flow hydroponic system housing young basil, parsley, and dill plants.
APPENDIX D

COLOR PHOTOGRAPH OF THE LED LIGHT TREATMENT WITH A HIGH PROPORTION OF BLUE TO RED LIGHT

Figure 3. LED supplemental lighting system with a 30:70 ratio of blue to red diodes in place over an individual deep-flow hydroponic system housing young basil, parsley, and dill plants.
Figure 4. Heracles II flash gas chromatograph-electronic nose (GC-EN) instrument with attached headspace autosampler and gas tanks.
APPENDIX F

SENSORY PANEL INFORMED CONSENT FORM

Informed Consent Document

Title of study: Consumer Evaluation of Culinary Herbs

Investigators: Lester Wilson, Ph.D. and Anne Seely, B.S.

You are being asked to participate in a research study of the sensory differences between three samples of three different culinary herbs. Please take your time deciding if you would like to participate. Feel free to ask questions at any time. All products have been grown at an Iowa State University on-campus greenhouse facility. If you agree to participate you will be asked to attend up to 3 test sessions that will take approximately 15 minutes. There will be no names on the ballot. The study will be conducted in Room 2595 Food Sciences Building.

You should not participate if you have an allergy or sensitivity to basil, parsley, or dill.

The general benefit derived from this study for society is that information will be gained regarding the sensory differences between treatments of different culinary herbs. You will receive a honorarium in the form of cash of $5 at the end of the final test session on Friday, February 17th, if you attend all three test sessions.

Your participation in this study is completely voluntary and you may withdraw or leave the study at any time without penalty or repercussions, by notifying one of the investigators. You may skip any questions you are uncomfortable answering. If you have any questions at any time regarding the study, contact one of the investigators listed below.

Principal Investigator
Dr. Lester Wilson, 2541 FSB, 294-3889, lawilson@iastate.edu

Key Personnel
Anne Seely, 2551 FSB, aseely@iastate.edu

If you have any questions about the rights of research subjects or research-related injury, please contact the IRB Administrator, (315) 294-4566, IRB@iastate.edu or Director, (315) 294-3115, Office for Responsible Research, Iowa State University, Ames, Iowa 50011.

Your signature indicates that you are 18 years of age or older, that you voluntarily agree to participate in this study, that the study has been explained to you, that you have been given the time to read the document and that your questions have been satisfactorily answered. You will receive a copy of the signed and dated written informed consent upon request.

Participant’s Name (printed)

Participant’s Signature                         Date
APPENDIX G

SAMPLE SENSORY EVALUATION TEST BALLOT

<table>
<thead>
<tr>
<th>Triangle Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test code: B1</td>
</tr>
<tr>
<td>Taster number:</td>
</tr>
<tr>
<td>Type of Sample: Fresh basil leaves</td>
</tr>
<tr>
<td>Instruction</td>
</tr>
<tr>
<td>Smell the samples on the tray from left to right. Two samples are identical and one is different. Select the odd/different sample and indicate it by placing an X next to the code of the odd sample. If you wish to comment for reasons on your choice or if you wish to comment on the product characteristic, you may do so under Remarks.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample on Tray (Left to Right)</th>
<th>Indicate odd sample with ‘X’</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
</tbody>
</table>
### SAMPLE SENSORY EVALUATION PANEL WORKSHEET

<table>
<thead>
<tr>
<th>Worksheet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test code: B1</td>
</tr>
<tr>
<td>Post this sheet in the area where trays are prepared. Code scoresheets ahead of time label serving containers ahead of time.</td>
</tr>
<tr>
<td>Type of samples: Fresh basil</td>
</tr>
<tr>
<td>Type of test: Triangle</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample Identification</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control HPS basil</td>
<td>A</td>
</tr>
<tr>
<td>High blue LED basil</td>
<td>B</td>
</tr>
</tbody>
</table>

Code serving containers as follows:

<table>
<thead>
<tr>
<th>Panelist Numbers:</th>
<th>Order (TEST 1):</th>
<th>Order (TEST 2):</th>
</tr>
</thead>
<tbody>
<tr>
<td>4, 8, 25, 27, &amp; 29</td>
<td>AAB</td>
<td>ABB</td>
</tr>
<tr>
<td>6, 7, 12, 15, 20, &amp; 24</td>
<td>ABB</td>
<td>AAB</td>
</tr>
<tr>
<td>2, 5, 10, 14, 18, &amp; 19</td>
<td>BAA</td>
<td>BBA</td>
</tr>
<tr>
<td>1, 3, 9, 17, 30, &amp; 33</td>
<td>BBA</td>
<td>ABA</td>
</tr>
<tr>
<td>16, 21, 22, 26, &amp; 28</td>
<td>ABA</td>
<td>BAB</td>
</tr>
<tr>
<td>11, 13, 23, 31, &amp; 32</td>
<td>BAB</td>
<td>BAA</td>
</tr>
</tbody>
</table>

1. Mark tray/plate with panelist's number.
2. Select containers of “A” or “B” from those previously coded and place on tray/plate from left to right.
3. Write codes selected on panelist’s score sheet.
4. Serve samples.
5. Receive filled-in score sheet and note on it the order of presentation used and whether reply was correct (c) or incorrect (i).
APPENDIX I

CONSUMER SENSORY ANALYSIS OF BASIL, PARSLEY, AND DILL UNDER VARIED LIGHT WAVELENGTHS

Table 1. High-pressure sodium vs. high blue LED herb consumer difference sensory results.

<table>
<thead>
<tr>
<th>Herb</th>
<th>Correct Responses (out of 66 total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rep 1</td>
</tr>
<tr>
<td>Basil</td>
<td>19</td>
</tr>
<tr>
<td>Parsley</td>
<td>27</td>
</tr>
<tr>
<td>Dill</td>
<td>25</td>
</tr>
</tbody>
</table>

* indicates statistical significance at an α-level of 0.05.

Table 2. High-pressure sodium vs. low blue LED herb consumer difference sensory results.

<table>
<thead>
<tr>
<th>Herb</th>
<th>Correct Responses (out of 66 total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basil</td>
<td>26</td>
</tr>
<tr>
<td>Parsley</td>
<td>31*</td>
</tr>
<tr>
<td>Dill</td>
<td>19</td>
</tr>
</tbody>
</table>

* indicates statistical significance at an α-level of 0.05.
APPENDIX J

THE MOST ABUNDANT FLASH GAS CHROMATOGRAPHY-ELECTRONIC NOSE PEAKS PRESENT IN FRESH BASIL


<table>
<thead>
<tr>
<th>Polarity</th>
<th>Peak (RT)</th>
<th>Retention Index (KI)</th>
<th>Probable compound(s)</th>
<th>Descriptors from Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average Observed</td>
<td>Literature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonpolar</td>
<td>49.62</td>
<td>1102</td>
<td>1092.2</td>
<td>Maltol</td>
</tr>
<tr>
<td></td>
<td>1092</td>
<td>1153.6</td>
<td>1-Nonanol</td>
<td>Floral, rose, fresh, sweet</td>
</tr>
<tr>
<td></td>
<td>1092</td>
<td>1072</td>
<td>Ethyl 3-methylthiopropanoate</td>
<td>Sulfurous, pineapple, fruity</td>
</tr>
<tr>
<td></td>
<td>1138</td>
<td>1153.6</td>
<td>1-Nonanol</td>
<td>Fresh, floral, rose, orange</td>
</tr>
<tr>
<td></td>
<td>1125</td>
<td>(E,Z)-2,6-Nonadienal</td>
<td>Green, fatty, dry, cucumber</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1236</td>
<td>p-Anisaldehyde</td>
<td>Sweet, powdery, floral, vanilla</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1178</td>
<td>Epoxy-p-methene</td>
<td>Mint, dill</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1267</td>
<td>(E,E)-2,4-Decadienal</td>
<td>Oily, cucumber, melon, citrus, nut</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1248</td>
<td>1,2,3-Trimethoxybenzene</td>
<td>Dry, musty, dusty</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1608</td>
<td>1621</td>
<td>Demeton thiono</td>
<td>--</td>
</tr>
<tr>
<td>Mid-polar</td>
<td>47.03</td>
<td>1120</td>
<td>1195</td>
<td>cis-decalin</td>
</tr>
<tr>
<td></td>
<td>1095</td>
<td>Terpinolene</td>
<td>Fresh, woody, sweet, pine, citrus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1072</td>
<td>Ethyl 3-methylthiopropanoate</td>
<td>Sulfurous, pineapple, fruity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1195</td>
<td>n-Nonanal</td>
<td>Waxy, aldehydic, citrus, fresh</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1092</td>
<td>1245</td>
<td>1-Nonanol</td>
<td>Fresh, floral, rose, orange</td>
</tr>
<tr>
<td></td>
<td>1176</td>
<td>Terpinen-4-ol</td>
<td>Peppery, woody, citrus, spice</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1125</td>
<td>(E,Z)-2,6-Nonadienal</td>
<td>Green, fatty, dry, cucumber</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1180</td>
<td>Ethyl octanoate</td>
<td>Fruity, wine, apricot, banana</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1138</td>
<td>1299</td>
<td>Benzyl acetate</td>
<td>Sweet, floral, fruity, jasmine</td>
</tr>
<tr>
<td></td>
<td>1092</td>
<td>1138</td>
<td>2-Phenylethanol</td>
<td>Floral, rose, fresh, sweet</td>
</tr>
<tr>
<td></td>
<td>1092.2</td>
<td>1092</td>
<td>Maltol</td>
<td>Sweet, caramel, cotton candy</td>
</tr>
<tr>
<td></td>
<td>1092.2</td>
<td>1092</td>
<td>Maltol</td>
<td>Sweet, caramel, cotton candy</td>
</tr>
<tr>
<td></td>
<td>1332</td>
<td>p-Methyladecenone</td>
<td>Sweet, creamy, fruity, cherry</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1159</td>
<td>Benzoic acid</td>
<td>Faint balsam, urine</td>
<td></td>
</tr>
<tr>
<td>63.41</td>
<td>1457</td>
<td>1375</td>
<td>(E,E)-2,4-Decadienal</td>
<td>Oily, cucumber, melon, citrus, nut</td>
</tr>
<tr>
<td></td>
<td>1236</td>
<td>p-Anisaldehyde</td>
<td>Sweet, powdery, floral, vanilla</td>
<td></td>
</tr>
<tr>
<td>86.33</td>
<td>1956</td>
<td>1945</td>
<td>δ-Dodecylactone</td>
<td>Fresh, sweet, metallic, peach, oily</td>
</tr>
<tr>
<td>93.38</td>
<td>2120</td>
<td>1931</td>
<td>Ronnel</td>
<td>--</td>
</tr>
</tbody>
</table>

RT = retention time
APPENDIX K

THE MOST ABUNDANT FLASH GAS CHROMATOGRAPHY-ELECTRONIC NOSE PEAKS PRESENT IN FRESH PARSLEY

Table 4. Most abundant peaks observed through flash gas chromatograph electronic nose and statistical analysis of parsley grown under one of three supplemental lighting treatments (Datu Inc., 2004; El-Zaeddi, Martínez-Tomé, et al., 2016; The Good Scents Company, 2015; Khan et al., 2006; National Center for Biotechnology, 2017; Royal Society of Chemistry, 2015; Shellie & Marriott, 2003).

<table>
<thead>
<tr>
<th>Polarity</th>
<th>Peak (RT)</th>
<th>Retention Index (KI)</th>
<th>Probable compound(s)</th>
<th>Descriptors from Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average Observed</td>
<td>Literature</td>
<td></td>
</tr>
<tr>
<td>Nonpolar</td>
<td>49.63</td>
<td>1101</td>
<td>1092</td>
<td>2-Phenylethanol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1153.6</td>
<td>1-Nonanol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1072</td>
<td>Ethyl 3-methylthiopropanoate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1092.2</td>
<td>Maltol</td>
</tr>
<tr>
<td></td>
<td>53.03</td>
<td>1164</td>
<td>1153.6</td>
<td>1-Nonanol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1138</td>
<td>Benzyl acetate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1176</td>
<td>Terpinen-4-ol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1125</td>
<td>(E,Z)-2,6-Nonadienal</td>
</tr>
<tr>
<td></td>
<td>54.84</td>
<td>1199</td>
<td>1180</td>
<td>Ethyl octanoate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1179</td>
<td>Methyl salicylate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1187</td>
<td>Decanal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1235</td>
<td>Ethyl phenylacetate</td>
</tr>
<tr>
<td></td>
<td>56.90</td>
<td>1239</td>
<td>1235</td>
<td>Ethyl phenylacetate</td>
</tr>
<tr>
<td></td>
<td>60.93</td>
<td>1318</td>
<td>1356</td>
<td>Decanoic acid</td>
</tr>
<tr>
<td></td>
<td>62.68</td>
<td>1352</td>
<td>1360</td>
<td>Eugenol</td>
</tr>
<tr>
<td>Mid-polar</td>
<td>47.06</td>
<td>1120</td>
<td>1118</td>
<td>Acetylpyrazine</td>
</tr>
<tr>
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<td>51.43</td>
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<td>1245</td>
<td>1-Nonanol</td>
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<tr>
<td></td>
<td>53.20</td>
<td>1242</td>
<td>1246</td>
<td>Citronellal</td>
</tr>
<tr>
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<td>54.87</td>
<td>1276</td>
<td>1092</td>
<td>2-Phenylethanol</td>
</tr>
<tr>
<td></td>
<td>55.76</td>
<td>1294</td>
<td>1138</td>
<td>Benzyl acetate</td>
</tr>
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<td></td>
<td>59.15</td>
<td>1365</td>
<td>1343</td>
<td>Ethyl phenylacetate</td>
</tr>
<tr>
<td></td>
<td>61.77</td>
<td>1421</td>
<td>1424</td>
<td>4-Ethylguaiacol</td>
</tr>
<tr>
<td></td>
<td>64.54</td>
<td>1478</td>
<td>1236</td>
<td>Decanoic acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1464</td>
<td>Decanoic acid</td>
</tr>
</tbody>
</table>

RT = retention time


### ELECTRONIC NOSE PEAKS PRESENT IN FRESH DILL THAT DIFFER SIGNIFICANTLY BETWEEN LIGHTING TREATMENTS

**Table 5.** Significantly different peaks observed through flash gas chromatograph electronic nose and statistical analysis of dill grown under one of three supplemental lighting treatments (El-Zaeddi, Martínez-Tomé, et al., 2016; The Good Scents Company, 2015; Jalali-Heravi, Zekayat, & Sereshti, 2006; National Center for Biotechnology, 2017; Royal Society of Chemistry, 2015; Schwob, Bessiere, Masotti, & Viano, 2004; Tzakou & Couladis, 2001).

<table>
<thead>
<tr>
<th>Polarity</th>
<th>Peak (RT)</th>
<th>Retention Index (K1)</th>
<th>Probable compound(s)</th>
<th>Descriptors from Literature</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Observed</td>
<td>Literature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonpolar</td>
<td>29.27</td>
<td>770.5</td>
<td>751</td>
<td>Ethyl isobutyrate</td>
<td>Sweet, ethereal, fruity</td>
</tr>
<tr>
<td></td>
<td>42.93</td>
<td>985.5</td>
<td>991</td>
<td>Myrcene</td>
<td>Peppery, terpene, balsam</td>
</tr>
<tr>
<td></td>
<td>45.60</td>
<td>1032</td>
<td>1031</td>
<td>Benzyl alcohol</td>
<td>Floral, fruity, balsamic, rose</td>
</tr>
<tr>
<td></td>
<td>45.60</td>
<td>1032</td>
<td>1017</td>
<td>Acetylpurazine</td>
<td>Dry, woody, resinous, pine</td>
</tr>
<tr>
<td></td>
<td>45.60</td>
<td>1032</td>
<td>1039</td>
<td>Benzeneacetalddehyde</td>
<td>Floral, fruity, balsamic, rose</td>
</tr>
<tr>
<td></td>
<td>45.60</td>
<td>1032</td>
<td>1043</td>
<td>(Z)-2-Octenal</td>
<td>Roasted, nutty, popcorn</td>
</tr>
<tr>
<td></td>
<td>45.60</td>
<td>1032</td>
<td>1093</td>
<td>Trans-hex-2-enyl acetate</td>
<td>Sweet, green, fresh, apple</td>
</tr>
<tr>
<td></td>
<td>45.60</td>
<td>1032</td>
<td>1017</td>
<td>α-Terpinene</td>
<td>Woody, terpene, lemon, herb</td>
</tr>
<tr>
<td></td>
<td>45.60</td>
<td>1032</td>
<td>1026</td>
<td>p-Cymene*</td>
<td>Citrus, terpene, woody, spice</td>
</tr>
<tr>
<td></td>
<td>88.59</td>
<td>1872</td>
<td>1850.1</td>
<td>Benzyl salicylate</td>
<td>Balsam, clean, herbal, oily</td>
</tr>
<tr>
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<td>20.49</td>
<td>648</td>
<td>--</td>
<td>Unknown</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>26.81</td>
<td>771</td>
<td>768</td>
<td>Mercaptoacetaldehyde*</td>
<td>Cabbage, sulfurous</td>
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<td>40.05</td>
<td>994.5</td>
<td>980</td>
<td>(-)-β-Pinene</td>
<td>Dry, woody, resinous, pine</td>
</tr>
<tr>
<td></td>
<td>40.05</td>
<td>994.5</td>
<td>894</td>
<td>2,5-Dimethylpyrazine</td>
<td>Cocoa, nutty, roasted, musty</td>
</tr>
<tr>
<td></td>
<td>40.05</td>
<td>994.5</td>
<td>894</td>
<td>2,6-Dimethylpyrazine</td>
<td>Cocoa, roasted, nutty, meaty</td>
</tr>
<tr>
<td></td>
<td>40.05</td>
<td>994.5</td>
<td>894</td>
<td>2,3-Dimethylpyrazine</td>
<td>Nutty, coffee, tasty, roasted</td>
</tr>
<tr>
<td></td>
<td>40.05</td>
<td>994.5</td>
<td>913</td>
<td>(Z)-4-Heptenal</td>
<td>Oily, fatty, green, dairy, milk</td>
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<td>42.14</td>
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<td>1033</td>
<td>3-Methylbutanoic acid</td>
<td>Cheesy, dairy, acidic, sour</td>
</tr>
<tr>
<td></td>
<td>42.14</td>
<td>1031.5</td>
<td>991</td>
<td>Myrcene</td>
<td>Peppery, terpene, balsam</td>
</tr>
<tr>
<td></td>
<td>42.14</td>
<td>1031.5</td>
<td>1006</td>
<td>(+)-(4S)-Phellandrene*</td>
<td>Citrus, herbal, terpene, green</td>
</tr>
</tbody>
</table>

RT = retention time

*Identification from literature.
APPENDIX M

PAIRWISE COMPARISONS OF THREE LIGHTING TREATMENTS APPLIED TO DILL

Table 6. Wilcoxon tests for pairwise comparison of dill cultivated under high-pressure sodium or low blue LED lighting.

<table>
<thead>
<tr>
<th>Polarity</th>
<th>Peak</th>
<th>Avg. Observed KI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpolar</td>
<td>29.27</td>
<td>770.5</td>
<td>0.008104*</td>
</tr>
<tr>
<td></td>
<td>42.93</td>
<td>985.5</td>
<td>0.00556*</td>
</tr>
<tr>
<td></td>
<td>45.60</td>
<td>1032</td>
<td>0.000509*</td>
</tr>
<tr>
<td></td>
<td>88.59</td>
<td>1872</td>
<td>0.040161*</td>
</tr>
<tr>
<td>Mid-polar</td>
<td>20.49</td>
<td>648</td>
<td>0.004302*</td>
</tr>
<tr>
<td></td>
<td>26.81</td>
<td>771</td>
<td>0.021318*</td>
</tr>
<tr>
<td></td>
<td>40.05</td>
<td>994.5</td>
<td>0.00886*</td>
</tr>
<tr>
<td></td>
<td>42.14</td>
<td>1031.5</td>
<td>0.000758*</td>
</tr>
</tbody>
</table>

* Indicates statistical significance

Table 7. Wilcoxon tests for pairwise comparison of dill cultivated under high-pressure sodium or high blue LED lighting.

<table>
<thead>
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<th>Polarity</th>
<th>Peak</th>
<th>Avg. Observed KI</th>
<th>P-value</th>
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</thead>
<tbody>
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<td>45.60</td>
<td>1032</td>
<td>0.285305</td>
</tr>
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<td></td>
<td>88.59</td>
<td>1872</td>
<td>0.040161*</td>
</tr>
<tr>
<td>Mid-polar</td>
<td>20.49</td>
<td>648</td>
<td>0.187795</td>
</tr>
<tr>
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<td>26.81</td>
<td>771</td>
<td>0.080631</td>
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<td></td>
<td>40.05</td>
<td>994.5</td>
<td>0.485885</td>
</tr>
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<td></td>
<td>42.14</td>
<td>1031.5</td>
<td>0.218406</td>
</tr>
</tbody>
</table>

* Indicates statistical significance

Table 8. Wilcoxon tests for pairwise comparison of dill cultivated under high blue LED or low blue LED lighting.

<table>
<thead>
<tr>
<th>Polarity</th>
<th>Peak</th>
<th>Avg. Observed KI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
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<td>770.5</td>
<td>0.017844</td>
</tr>
<tr>
<td></td>
<td>42.93</td>
<td>985.5</td>
<td>0.000179*</td>
</tr>
<tr>
<td></td>
<td>45.60</td>
<td>1032</td>
<td>0.001782*</td>
</tr>
<tr>
<td></td>
<td>88.59</td>
<td>1872</td>
<td>1</td>
</tr>
<tr>
<td>Mid-polar</td>
<td>20.49</td>
<td>648</td>
<td>0.261393</td>
</tr>
<tr>
<td></td>
<td>26.81</td>
<td>771</td>
<td>0.00937*</td>
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<td></td>
<td>40.05</td>
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<tr>
<td></td>
<td>42.14</td>
<td>1031.5</td>
<td>0.003057*</td>
</tr>
</tbody>
</table>

* Indicates statistical significance
Figure 5. Dill peaks from both nonpolar and mid-polar columns in discriminant space as calculated by linear discriminant analysis.
Figure 6. Nonpolar dill peaks in discriminant space via linear discriminant analysis.
Figure 7. Mid-polar dill peaks in discriminant space via linear discriminant analysis.
APPENDIX Q

LINEAR DISCRIMINANT ANALYSIS OF THE 20 MOST ABUNDANT NONPOLAR DILL PEAKS FROM ELECTRONIC NOSE ANALYSIS

Figure 8. Twenty most abundant nonpolar dill peaks in discriminant space via linear discriminant analysis.
Figure 9. Twenty most abundant mid-polar dill peaks in discriminant space via linear discriminant analysis.
## Table 9. Dill peaks used to differentiate treatment groups in Figures 5-9 (El-Zaedi, Martinez-Tomé, et al., 2016; The Good Scents Company, 2015; Shawl, Srivastava, Syamasundar, Tripathi, & Raina, 2002; Takeoka, Buttery, & Flath, 1992).

<table>
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<tr>
<th>Polarity</th>
<th>Peak</th>
<th>Retention Index (KI)</th>
<th>Probable compound(s)</th>
<th>Descriptors from Literature</th>
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<td></td>
<td>Avg. Observed</td>
<td>Literature</td>
<td>Probable compound(s)</td>
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<td>18.47</td>
<td>577.5</td>
<td>Myrcene</td>
<td>--</td>
</tr>
<tr>
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<td>42.50</td>
<td>979</td>
<td>(E)-β-Pinene</td>
<td>Dimethyl trisulfide</td>
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<tr>
<td></td>
<td>997</td>
<td></td>
<td>Hexanoic acid</td>
<td>Sour, fatty, sweat, cheese</td>
</tr>
<tr>
<td></td>
<td>949</td>
<td></td>
<td>Ethyl 3-methylthiopropanoate</td>
<td>Sulfurous, pineapple, fruity</td>
</tr>
<tr>
<td></td>
<td>950</td>
<td></td>
<td>2-Phenylethanol</td>
<td>Floral, rose, fresh, sweet</td>
</tr>
<tr>
<td></td>
<td>49.61</td>
<td>1102</td>
<td>n-Nonanal</td>
<td>Waxy, citrus, fresh, green</td>
</tr>
<tr>
<td></td>
<td>1072</td>
<td></td>
<td>Ethyl 3-methylthiopropanoate</td>
<td>Sulfurous, pineapple, fruity</td>
</tr>
<tr>
<td></td>
<td>1092</td>
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<td>2-Phenylethanol</td>
<td>Floral, rose, fresh, sweet</td>
</tr>
<tr>
<td></td>
<td>1092.2</td>
<td></td>
<td>Maltol</td>
<td>Sweet, caramel, cotton candy</td>
</tr>
<tr>
<td></td>
<td>1091</td>
<td></td>
<td>Nonan-2-one</td>
<td>Fruity, sweet, fresh, herbal</td>
</tr>
<tr>
<td></td>
<td>51.87</td>
<td>1146</td>
<td>(E,Z)-2,6-Nonadienal</td>
<td>Green, fatty, dry, cucumber</td>
</tr>
<tr>
<td></td>
<td>1125</td>
<td></td>
<td>Citronellal</td>
<td>Sweet, floral, herbal, citrus</td>
</tr>
<tr>
<td></td>
<td>1153</td>
<td></td>
<td>Benzoic acid</td>
<td>Faint balsam, urine</td>
</tr>
<tr>
<td></td>
<td>1159</td>
<td></td>
<td>E-Limonene oxide</td>
<td>Fresh, clean, citrus, minty</td>
</tr>
<tr>
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<td>1130</td>
<td></td>
<td>1-Nonanol</td>
<td>Fresh, floral, rose, orange</td>
</tr>
<tr>
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<td>52.93</td>
<td>1145</td>
<td>(E,Z)-2,6-Nonadienal</td>
<td>Green, fatty, dry, cucumber</td>
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<tr>
<td></td>
<td>1125</td>
<td></td>
<td>Benzyl acetate</td>
<td>Sweet, floral, fruity, jasmine</td>
</tr>
<tr>
<td></td>
<td>1138</td>
<td></td>
<td>Terpinen-4-ol</td>
<td>Peppery, woody, citrus, spice</td>
</tr>
<tr>
<td></td>
<td>1159</td>
<td></td>
<td>Benzoic acid</td>
<td>Faint balsam, urine</td>
</tr>
<tr>
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<td>54.85</td>
<td>1163</td>
<td>Decanal</td>
<td>Sweet, waxy, citrus, orange</td>
</tr>
<tr>
<td></td>
<td>1180</td>
<td></td>
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<td>Fruity, wine, apricot, banana</td>
</tr>
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<td>1179</td>
<td></td>
<td>Methyl salicylate</td>
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<td>Benzoic acid</td>
<td>Faint balsam, urine</td>
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<td>Maltol</td>
<td>Sweet, caramel, cotton candy</td>
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<td>2-Phenylethanol</td>
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<td>Faint balsam, urine</td>
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<td>δ-Decalactone</td>
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