INFLUENCE OF ODOR INTENSITY AND MOOD ON HEDONIC REACTIONS TO AROMA COMPOUNDS IN A MULTI-ETHNIC SAMPLE OF YOUNG ADULTS

By

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and approved by

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ABSTRACT OF THE THESIS

Influence of Odor Intensity and Mood on Hedonic Reactions to Aroma Compounds

In a Multi-ethnic Sample of Young Adults

by LUMENG JIN

Thesis Director:

Beverly J. Tepper

This study was designed to investigate: 1) how nasal irritation influences the sensory perception of aromas; 2) the role of emotions in the preference and acceptance of aroma compounds; and 3) if ethnicity and genetic variation in taste sensitivity to the bitter compound 6-n-propylthiouracil (PROP) influence these outcomes. Ninety-six subjects (East Asian, n=53 and American Caucasians, n=43) were classified as PROP super-tasters; medium-tasters; and non-tasters. Subjects sniffed (orthonasally) aqueous solutions of cinnamaldehyde, methyl cinnamate, citral, citronellol, geraniol, and phenyl ethyl alcohol (PEA) at low (range =1-2.5 ppm) and mid-range (range =4-100ppm) concentrations in two separate sessions. Subjects rated intensity and overall liking for each aroma using 15-cm line scales. Pungency was rated for the mid-range concentrations only. Self-reported mood reactions to all aromas were collected using 8-point VAS scales. Additionally, subjects selected the most related mood descriptor of each aroma as an implicit measurement.

Data were analyzed using ANOVA, multiple linear regression, and principal component analysis (PCA). Nasal pungency contributed to intensity perception, however, pungency and intensity did not influence aroma liking. Liking of cinnamaldehyde and citral increased with increasing concentration ($p=0.05-0.003$), but concentration did not influence liking of the other aromas. At mid-range concentration, East Asians liked cinnamaldehyde less than American Caucasians. PROP did not influence liking alone, however, East Asian subjects liked cinnamaldehyde less with increasing sensitivity to PROP, as expected. Positive mood (the mean of happy and excited VAS ratings) was associated with greater liking of most of the samples ($p<0.002$ for all). In multiple regression, positive mood predicted liking of cinnamaldehyde, methyl cinnamate, citral, and PEA at both concentrations ($R^2=0.27-0.45$, $p<0.0001$ for all). PCA characterized the aromas by associating each with the sensory properties and mood(s) it elicited: PEA was associated with calm/relaxed mood and methyl cinnamate with negative moods (especially anxious/worried) at both concentrations; the most pungent aroma, cinnamaldehyde, was associated with exciting/energized at mid-range concentration. These data suggest that aromas eliciting positive moods were liked better. Also, differences in liking of cinnamaldehyde between ethnic groups could reflect cultural and PROP-related variability in the pleasantness of this aroma.

DEDICATION

I lovingly dedicate this thesis to my parents, Ping Jin and Lei Cheng, who encourage, support, and love me constantly throughout my life.

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I am most grateful to my advisor and mentor, Dr. Beverly Tepper for her time, encouragement, and expertise throughout this project. She taught me how to communicate as a scientist and it was a great honor to work with her for two years. My sincere thanks to Dr. Jeannette Haviland-Jones and Dr. James E. Simon for their encouragement and insightful suggestions on my work. Without their advice and assistance, this thesis would not have been possible.

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1. Introduction

1.1 The Importance of Aroma

Aromas are important contributors to food flavor and overall consumer experience. A positive aroma, such as the pleasant smell given off by freshly baked cookies, would attract consumers to the product; whereas a negative aroma, such as the rancid odor given off by an aged peanut, would be a warning cue and alert them to poor food quality. In the past, several studies have used the presence of either a single aroma compound or a complex aroma mixture as a tool to develop and characterize products. For example, aroma profiles have been used to determine the desirable processing parameters (roasting time and temperature) of roasted coffee beans (Schenker, Heinemann et al. 2002); to determine the key aromas responsible for sweet cream butter and oranges for future artifical flavor development (Peterson and Reineccius 2003, Mahattanatawee, Rouseff et al. 2005); and to charaterize different tomatillos or wine by comparing volatiles released in the mouth (Xu and Barringer 2010, Mayr, Parker et al. 2014). In addition, aromas are often associated with memories, which in turn influence emotions and the acceptance of a food or product. For example, a cinnamon-scented candle may evoke memories of the winter holidays or eating apple pie, presumably pleasant/happy experiences that the consumer may want to repeat or remember, and thus, feel more inclined to purchase the item.

Besides their olfactory properties, some aromas also stimulate nasal irritation (such as chili pepper) and cooling sensations (such as mint), though these factors are not well understood in terms of their influence on food acceptance. Thus, it is essential to understand how the aroma profiles influence an individual's perception and preference in order to direct researchers and attract consumers in the future.

1.2 Olfactory Sensation

Primarily, aromas elicit olfactory sensations. To detect a particular aroma, odorant molecules in the air must enter the nose and bind to the odorant receptors in the nasal cavity. The receptor activates olfactory neurons that send electrical signals to the olfactory bulb, which is located in forebrain. The olfactory bulb then passes the signals to the olfactory cortex, a higher region of the brain that decodes the signal and allows us to properly identify the odors. (Ihara, Yoshikawa et al. 2013, Secundo, Snitz et al. 2014, Shirasu, Yoshikawa et al. 2014). (Figure 1.1)

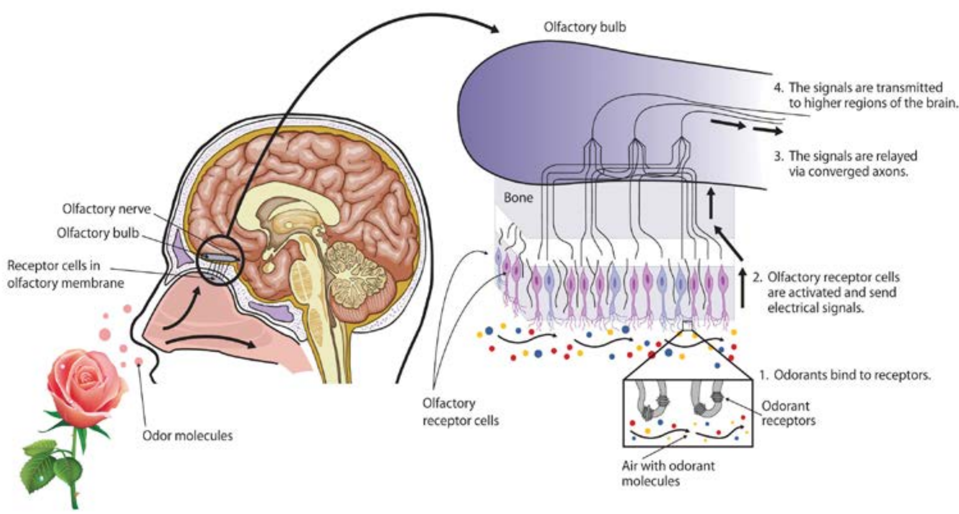


Figure 1.1 Odor molecules enter the nasal cavity and bind olfactory receptors. Olfactory neurons activated by the receptors send signals to the olfactory bulb, and further passes the signals to the brain, where the odors are identified (Krawiec, T., 1950).

1.3 Trigeminal Sensations

In addition to generating olfactory sensations, some aromas give rise to trigeminal sensations, such as pungency, prickling, irritating and/or burning (Cain 1974, Auvray and Spence 2008). For example, butyl acetate, an organic volatile compound with a fruity odor, is a good example of an olfactory compound that invokes nasal irritation (Cain 1974). Other organic volatile compounds such as some alcohols, acetates and terpenes also give rise to different degrees of nasal irritation (Cometto-Muniz, Cain et al. 1990, 1993, 1998, 2004). Cometto-Muñiz and his colleagues (1990, 1993, 1998, and 2004) observed that for a given aroma, the threshold for nasal pungency is typically lower than the threshold for aroma intensity. Thus, it can be difficult for untrained panelists to recognize pungency or rate reliably the pungency of a weak aroma. Understanding the role of pungency perception in aroma perception is one of the objectives of the current study.

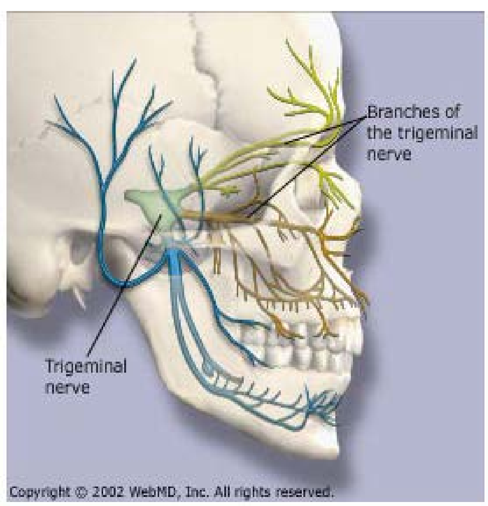


Figure 1.2 Three branches of trigeminal nerves cover 3 regions of human face: eyes, nose and mouth (WebMD.Inc., 2002).

The pungent, burning sensations felt throughout the nose by aromas are controlled by the trigeminal nerve, which is the largest cranial nerve. It consists of three branches that elicit trigeminal sensations of the mouth, nose and eyes (Figure 1.2). When the trigeminal receptors are activated by stimuli (such as capsaicin), nerve fibers carry the signal to the thalamus, and then further transmit the signal to the cortex in the brain. The family of receptors that mediates trigeminal sensations is referred as the Transient Receptor Potential (TRP) channel family (Silver 2010). These TRP receptors can be activated by different stimuli, such as temperature heat and cold, acid pH or food volatiles. Figure 1.3 illustrates the specificity of the individual TRP receptors. For example, TRPV1 is exclusively sensitive to capsaicin, acidity, and temperatures of 43°C and hotter; TRPA1 responds to a range of pungent stimuli such as horseradish and cinnamon; whereas TRPM8 is only responsive to cold temperature (20°C and cooler), menthol and other cooling agents. The array of trigeminal receptors works together and allows us to respond to a wide range of stimuli and sensations.

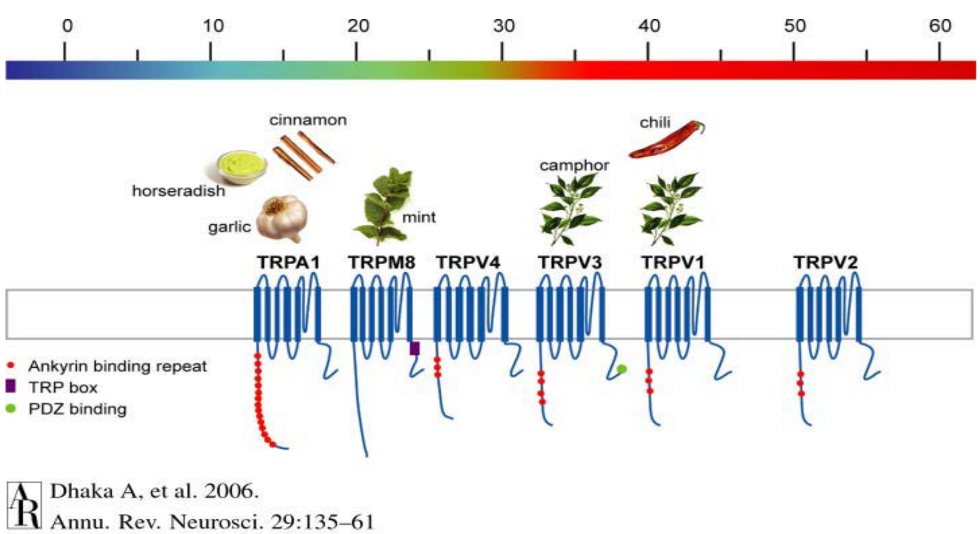


Figure 1.3 TRP family receptors response to a range of trigeminal stimuli.

Researchers have more often examined stimuli that elicit trigeminal sensations in the mouth than in the nose. These include the cooling sensation of menthol or cooling agents (Green 1985, Renneccius 2006, Klein, Carstens et al. 2011), heat irritation from pepper or mustard oil (Lawless 1984, Green and Lawless 1991, Prescott and Stevenson 1996, Simons, Carstens et al. 2003), and astringency from tannins (Guinard, Pangborn et al. 1986, Condelli, Dinnella et al. 2006, Soares, Sousa et al. 2012). Consequently, less is known about stimuli that cause nasal pungency. For example, capsaicin is the most widely studied trigeminal stimulus that irritates both the oral and nasal cavities. However, few researchers have studied its nasal pungency. Even less is known about the other trigeminal stimuli.

Trigeminal sensations from a food product can have a major impact on its acceptability. This is demonstrated by the growing economic impact of products such as carbonated beverages and hot/spicy salsa that have trigeminal sensations as their key sensory attributes (Lawless and Heymann 1998). According to the *Top 5 Flavor Trends* (Hensel 2014), hot and prickling sensations are among the top trending flavors, and consumers enjoy spicy and tangy products more than they did just a few years ago. The increasing number of hot chili varieties (such as chipotle, jalapeño and habanero) and tangy fermented foods (such as sriracha sauce, kimchi) introduced into the food business created opportunities and challenges to product developers. Thus, studying nasal trigeminal perceptions can help us gain a better understanding of the high consumer demand for these new flavors.

1.4 Influence of Olfactory and Trigeminal Sensations on Taste Perception

The overall sensory experience of a food arises from the complex interactions between olfactory, trigeminal and basic taste sensations such as sweet, salty, sour, bitter and umami. Researchers have investigated how these interactions among these three components can enhance or minimize the sensory impact of a food (Small and Prescott 2005, Auvray and Spence 2008). For example, two studies found that the addition of tomato aromas to tomato soup and chicken/beef aromas to chicken soup enhanced ratings of sweet and salty flavors, respectively (Baldwin, Goodner et al. 2008, Batenburg and Velden 2011). In contrast, the addition of capsaicin, to aqueous solutions had no impact on the intensity perception of salt, citric acid or umami flavors (Cowarts 1987a, Cowarts 1987b, Prescott, Allen et al. 1993, Prescott and Stevenson 1995) but did decrease the sweetness and tomato soup flavor ratings of tomato soup (Prescott, Allen et al. 1993, Prescott and Stevenson 1995). How aromas that give rise to olfactory and trigeminal sensations influence our perception, and thus, our overall sensory experience is important to explore.

1.5 PROP and Taste Perception Sensitivity

PROP, formally known as 6-n-propylthiouracil (Figure 1.4), is a bitter artificial compound similar in chemical structure to another artificial compound, PTC (phenylthiocarbamide). These two compounds are also chemically-similar to naturally occurring thiourea compounds that are responsible for the bitter taste in vegetables of the *Brassica* family. In 1931 Arthur Fox, a chemist from DuPont, accidentally discovered the existence of individual human taste variation for PTC.

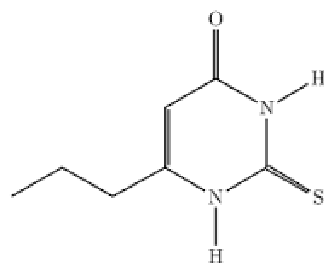


Figure 1.4 Chemical structure of PROP (6-n-propylthiouracil)

Fox found PTC powder tasted bitter to his colleague but was tasteless to himself (Fox 1931). Since this discovery, the ability to taste the bitterness of PROP and PTC has been studied extensively. We now know that this bitter taste sensitivity is inherited (Kalmus 1958), and that *TAS2R38* is the gene that controls the TAS2R38 bitter taster receptor, which binds PROP and PTC. The binding affinity of PROP and PTC to the TAS2R38 receptor is determined by three nucleotide polymorphisms in the DNA sequence of this gene. These three polymorphisms give rise to two forms: 1) PAV, resulting in strong binding of PROP/PTC; and 2) AVI, resulting in weak binding of PROP/PTC. Individuals with the AVI/AVI diplotype who experience weak or no taste from PROP are referred to as non-tasters (NT); individuals with the PAV/AVI diplotype who experience moderate bitterness from PROP are referred to as medium-tasters (MT); and individuals with the PAV/PAV diplotype who experience a strong bitterness from PROP are referred to as super-tasters (ST). It should be mentioned that not all PVA/PAV individuals are super-tasters and all PAV/AVI individuals are medium tasters due to strong phenotypic overlap between medium- and super-taster groups (Melis et al. 2013). Amongst American Caucasians in North America and Europe, the distribution of these taster groups is approximately: 30% non-tasters; 45% medium tasters and 25% super-tasters (Tepper

2008); though this distribution varies among other ethnicities. Guo and Reed (2001) reported that East Asians, including Chinese, Japanese and Korean have a low percentage of non-tasters (~10%) compared to Caucasians, including Americans, and Europeans.

PROP tasters (and particularly super-tasters) are more sensitive to other bitter substances and foods that do not contain the thiourea moiety. For example, Gayathri Devi et al. (1997) found that increasing PROP sensitivity was associated with increased perceived bitterness and decreased overall liking of green tea. Similarly, Drewnoski et al. (1997) found that PROP super-tasters perceived higher bitterness and gave lower acceptance to grapefruit juice, which gives a bitter taste. The PROP taster effect was also found for other products, such as bitter vegetables (Drewnowski, Henderson et al. 1999, Dinehart, Hayes et al. 2006) and beer (Intranuovo and Powers 1998).

The influence of PROP on other oral sensations has also been observed. PROP tasters are more sensitive to sweeteners (Gent and Bartoshuk 1983, Rankin, Godinot et al. 2004, Zhao and Tepper 2007), sourness (Prescott, Soo et al. 2004), fat from salad dressing (Tepper and Nurse 1997, Tepper and Nurse 1998) and creaminess of dairy products (Kirkmeyer and Tepper 2003, Kirkmeyer and Tepper 2004, Prescott, Soo et al. 2004). These findings suggest an important role of PROP as a general genetic marker for oral perceptions.

PROP sensitivity has also been used to examine individual variation in oral irritation perception. PROP tasters perceive higher oral intensity from the heat of chili peppers (Karrer and Bartoshuk 1991, Pickering, Simunkowa et al. 2004), the astringency of alcohol (Duffy, Davidson et al. 2004, Pickering, Simunkowa et al. 2004), and also the pungency of cinnamaldehyde (Prescott and Swain-Campbell 2000).

Not surprisingly, these sensitivities to basic tastes and other oral sensations have been shown to impact an individual's food preferences (Bartoshuk 1993, Tepper and Trail 1998, Drewnowski 2004). Super-tasters generally have a lower preference for strong-tasting foods than non-tasters. For example, super-tasters give lower preference ratings to bitter vegetables (Bell and Tepper 2006), bitter citrus (Drewnowski, Henderson et al. 1997), and sweet drinks (Looy and Weingarten 1992). To date, PROP screening has been used commonly to explore the individual differences in hedonic responses as a way to better understand a consumer's preference and selection for various products.

1.6 PROP, Olfactory, and Nasal Irritation

Since PROP sensitivity is associated with variation in a gene that controls the TAS2R38 bitter receptor, studies have rarely investigated the role of this trait in non-oral sensations such as olfaction and nasal irritation. One exception is the study by Yackinous and Guinard (2001) that examined the relationship between PROP taster status and detection of diacetyl, one of the major compounds in artificial butter flavor, that is known to elicit nasal pungency and causes inflammation in the nose (Hubbs, Battelli et al. 2002, Hubbs, Goldsmith et al. 2008). Yackinous and Guinard (2001) observed greater acuity for diacetyl amongst PROP medium- and super-tasters than for non-tasters. This finding led us to speculate that the nasal trigeminal impact of diacetyl may be driving the differences in olfactory perception across PROP status groups. Thus, the present study aimed to examine and understand the effect of PROP on the pungency perception of various aroma stimuli that elicit trigeminal sensations.

1.7 Aroma Effects on Mood, Physiology and Behavior

Aromas are known for their impact on individual's mood and cognitive performance. The scientific analysis of aroma effects on mood, physiology and behavior is known as *aromachology,* a term defined in 1982 by the Sense of Smell Institute (Herz 2009). According to the literature, many aromas can induce physiological changes in blood pressure, heart rate, and skin conductance (Alaoui-Ismaili, Vernet-Maury et al. 1997, Robin, Alaoui-Ismaili et al. 1999, Bensafi, Rouby et al. 2002a, Bensafi, Rouby et al. 2002b) which are indicators of mood changes. Moreover, olfaction, emotion and memory functions are all supported by a complex system called the limbic system in the brain. Thus odors are closely associated with emotion and memory by this connection (Cahill, Babinsky et al. 1995). In the past, aromas such as lavender and sandalwood have been used in treatments for depression, insomnia and other cognitive disorders (Buchbauer and Jirovetz 1994, Buchbauer 1996, Goel and Lao 2006) due to their physiological and psychological properties. Herz (2001, 2004) proposed that emotional, behavioral, and physiological responses to odors are learned through association with emotional experiences. For example, a calm and relaxed mood evoked by a cinnamon-scented candle might be due to a pleasant memory of a cinnamon-flavored dessert.

Aroma does not only impact our moods, but our behavior as well (Chu and Downes 2000, Herz, Beland et al. 2004, Chu 2008, Herz 2009, Castellanos, Hudson et al. 2010). A pleasant ambient aroma can evoke a positive mood and increase productivity (Baron 1997), while unpleasant odor can evoke a negative mood and lower the ratings for energy and well-being (Rotton 1983). Previously, essential oils of lemon, orange, peppermint, lavender and *Salvia (sage)* were studied in relation to mood and performance

(Tildesley, Kennedy et al. 2003, Lehrner, Marwinski et al. 2005, Scholey, Tildesley et al. 2008, Herz 2009, Moss, Rouse et al. 2010, Matsumoto, Asakura et al. 2013). Kuroda et al (2005) found sedative effects from jasmine tea, and Raudenbush et al (2001, 2002) found that peppermint odor can enhance athletic performance.

1.8 Emotional-Behavioral Effects of Aromas that Elicit Trigeminal Sensations

It has been observed that aromas that induce trigeminal sensations might have a stimulating effect. Raudenbush et al. (2002) found that peppermint aroma, which has a cooling sensation, had a stronger effect on enhancing vigor and performance in young athletes than jasmine aroma, which gives rise to only olfactory sensation. Another study by Ilmberger, et al. (2001) found that cinnamon aroma, which elicits a warming sensation, increased alertness compared to other odorants. Porcherot et al. (2010) also found an association between “energetic-invigorated” emotion and citrus fine fragrance/perfume oil, which also elicits nasal pungency. Although the relationship between the trigeminal property of an aroma and its stimulating effects on emotions and behaviors is not well established, the studies mentioned above suggest the presence of some interesting associations for the future researchers to investigate. No studies have examined the intensity of nasal pungency of a variety of aromas and how these sensations may influence emotional responses as far as we know.

1.9 Moods and Product Differentiation

Studies have shown that the emotional profile associated with a product provides extra information for understanding consumer acceptance beyond liking. For example, Ng et al. (2013) investigated the hedonic and emotion responses for eleven blackcurrant-flavored squashes. All the products elicited similar hedonic responses, but the emotional profiles were able to differentiate these 11 products. Similar results were found with other food products, such as strawberry, salty snack crackers, and chocolate/hazelnut spreads (King and Meiselman 2010, Porcherot, Raviot-Derrien et al. 2010, Spinelli, Masi et al. 2014). These results suggest that emotion measurement provides extra advantages to understand consumer behavior. To our knowledge, no studies have examined the connection between an emotional response and hedonic response to aromas to understand consumer acceptance.

1.10 Culture, Familiarity and Consumer Perception and Preference

The associations learned within different cultures may mediate consumer perception and acceptance of flavors and fragrances. For example, wintergreen mint, a common odor associated with medicine in Britain, was rated as the least pleasant aroma in a British study (Moncreiff 1966); whereas a mint odor, which is often associated with candy in the United States, was rated as the most pleasant aroma in a United States study (Cain and Johnson 1978).

Cross-cultural studies have also observed the impact of ethnicity and familiarity on consumer perception and acceptance. Laing and Prescott (1994) observed similar sensory ratings for food products between Japanese and Australian subjects, however, each ethnic group gave higher hedonic ratings to their own domestic products than to unfamiliar products. In a biscuit study, Pages et al. (2007) found that consumers from

France gave higher hedonic ratings to French biscuits, and Pakistanis gave higher hedonic ratings to biscuits from Pakistan.

Studies from our laboratory have also shown relationships between ethnicity, familiarity and liking for oral cooling ingredients. Tepper et al. (2008) and Su et al. (2013) found that East Asians perceived more heat/burning from beverage samples that contained cooling ingredients (Coolact®5, Coolact®10, Coolact®38D, Coolact®5/Coolact®10 blend, and Coolact®38D/Frescolat®ML blend) than American Caucasians. Moreover, Su et al. (2013) found that the group, which was more familiar with beverages with cooling ingredients (mostly East Asians) perceived higher cooling than the group that was unfamiliar with these beverages (mostly American Caucasians). Together, these data suggest that culture and familiarity may be important determinants to explain consumer perception and selection of a range of different products, and deserve further attention in research studies.

1.11 Ethnicity and Mood Response

Individual emotional responses to situations and events are greatly influenced by ethnicity and cultural background (Eid and Diener 2001, Tsai, Knutson et al. 2006, Chentsova-Dutton, Chu et al. 2007, Porter and Samovar 1998, Uchida and Kitayama 2009, Bastian, Kuppens et al. 2012). For example, Tsai et al. (2006) observed that European American couples showed more positive emotional response than Chinese American couples during conversations about conflicts in their relationships.

Many studies have utilized dimensional theory to examine the impact of culture on emotion response (Lang, Greenwald et al. 1993, Tsai 2007, Porcherot, Raviot-Derrien et al. 2010). In this theory, moods have either a positive or negative valence, and either a high or low arousal. For instance, 'angry' is categorized as high arousal negative valence mood, and in contrast, 'calm' is categorized as low arousal positive valence mood. Tsai (2007) compared American and East Asian views for low-arousal positive emotions (such as calm) and high-arousal positive emotions (such as excitement). Her findings demonstrated that Americans value high-arousal positive emotions more and put a lesser value on low-arousal positive emotions compared to East Asians. In line with Tsai's (2007) findings, Imada and Ellsworth (2011) observed that Japanese selected a low-arousal word 'luck' and American selected a high-arousal word 'proud' when asked to recall a successful event. Davis et al (2012) found that Chinese subjects reported lower levels of emotion than American subjects after being exposed to an event that was intended to elicit negative emotions. It is already known that culture shapes an individual's emotion response towards different situations. However, studies have not investigated individual emotional responses to various aromas, taking into consideration the role of subjects' culture and ethnicity.

1.12 Research Gap

As described, we know that aromas impact an individual's moods and emotions. However, it is not known how the trigeminal-stimulating properties of aromas influence these outcomes. The finding of Yackinous and Guinard (2001) showing that PROP sensitivity may influence the perception of the aroma compound, diacetyl, led us to speculate that there may be an association between PROP status and nasal irritation. Furthermore, no studies have examined the trigeminal impact of an aroma on an individual’s intensity perception and acceptance of that aroma. Further investigation of these relationships will provide valuable insight for both researchers and product developers, alike, as to why individuals prefer certain aromas to others, perform better under certain aroma conditions over others, or purchase certain scented products over others.

1.13 Objectives and Hypotheses

This study was designed to investigate the how nasal irritation influences the sensory perception of aromas and the role of emotions in the preference and acceptance of aroma compounds. We tested a panel of six pure aroma compounds with different sensory qualities including cinnamaldehyde, methyl cinnamate, citral, citronellol, geraniol, and PEA. The following objectives and hypotheses were addressed:

Objective 1: To understand the contribution of an aroma’s nasal pungency and it’s perceived intensity to the liking of aromas with different sensory qualities (such as citrus, cinnamon, and floral).

Hypothesis 1: We hypothesize that the nasal pungency of an aroma will contribute to its overall intensity perception and will be the major driver for liking.

Objective 2: To understand how different aromas modulate an individual’s mood and the contribution of mood to preference for the aromas.

Hypothesis 2a: We hypothesize that each of the six aromas will evoke different mood profiles. Aromas with lower perceived nasal pungency and intensity ratings will be associated with low arousal moods; whereas aromas with higher perceived nasal pungency and intensity will be associated with higher arousal moods.

Hypothesis 2b:We hypothesize that aromas that evoke positive moods will also receive a higher liking rating.

Objective 3: To determine the role of genetic variation in the bitter taste marker, PROP, as well as ethnicity in individual differences in perception and liking of the six aromas.

Hypothesis 3: We hypothesize that the liking rating of an aroma will be negatively correlated with PROP sensitivity and positively correlated with familiarity (by way of ethnic background).

2. Method

2.1 Design Overview

The purpose of this study was to examine consumer reactions to common aroma compounds varying in sensory quality and nasal pungency. The six aroma compounds selected for this study have bioactive properties in essential oil form, such as antimicrobial, insecticidal, etc. Since bioactive compounds provide a range of benefits to consumers, they are economically important to the industry and warrant further study.

We conducted a consumer-based study wherein untrained subjects came to the laboratory to evaluate pure odor compounds prepared in aqueous solutions. The results of this study will provide direction for future studies testing model foods or other consumer products (such as fragrances, personal care products, etc.)

2.2 Subjects

Healthy subjects between ages 18-47 were recruited for this study via flyers and email from Rutgers University. Previous work from our laboratory suggested that East Asians perceived higher heat/burning sensation from cooling ingredients than American Caucasians (Tepper, Koelliker et al. 2008, Su, Tepper et al. 2013); thus, we focused our recruitment on East Asian and Caucasian ethnic groups. None of the subjects were allergic to fragrances or taking medication that could interfere with taste or smell. Subjects completed a general questionnaire, which includes demographic information. The experimental protocol was approved by the Rutgers University Institutional Review Board for the protection of human subjects in research. They were asked to not eat or drink for one hour before their scheduled sessions. Informed consent was obtained in advance, and each subject was compensated for his/her participation.

2.3 PROP Screening

The screening session was designed to classify subjects into groups by their sensitivity to PROP (NT, MT, and ST). The paper disk method was used to assess an individual’s taster status (Zhao, Kirkmeyer et al. 2003). Subjects were asked to place a filter paper impregnated with NaCl or PROP on the tip of the tongue until it was thoroughly wet. Then they rated the intensity of each disk on the labeled magnitude scale (LMS), a 100mm vertical scale. Subjects always tasted NaCl first and rinsed their mouth thoroughly between disks.

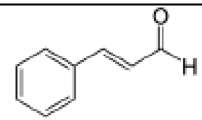
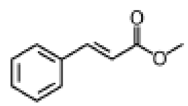
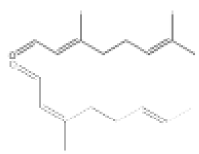
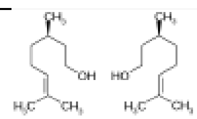
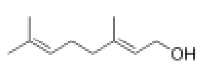
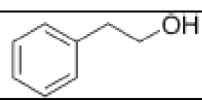
A cheek swab was used to collect cells for future DNA analysis of the *TAS2R38* gene, which controls PROP taste sensitivity (Kim, Jorgenson et al. 2003). Genotyping is typically used, to confirm the taster status of the subjects.

2.4 Aroma Samples

The six aroma compounds used in this study were: cinnamaldehyde; methyl cinnamate; citral; citronellol; geraniol; and phenyl ethyl alcohol (PEA) (See Table 2.1). These compounds exhibit a range of bioactive properties, different olfactory qualities and various degrees of pungency. Their aroma qualities are also well known to consumers.

Samples were tested at two concentrations: near threshold (low concentration) and at mid-range intensity. Mid-range concentrations were intended to mimic intensities commonly encountered in everyday products. We surveyed the literature to estimate the appropriate test concentrations and conducted pilot studies with lab personnel to determine the final test concentrations. The low and mid-range concentrations tested for each aroma are listed in Table 2.1.

Table 2.1 Selected aroma compounds.

Aroma Compounds	Chemical Structure	Low Concentration (ppm)	Mid-Range Concentration (ppm)	Aroma Quality	Bioactivity	Rerferences
Cinnamaldehyde		2	100	Cinnamon, sweet, spicy	Antimicrobial, insecticide	(Inouye, Takizawa et al. 2001, Lee, Kim et al. 2008, Cheng, Liu et al. 2009, Ali, Murphy et al. 2013)
Methyl Cinnamate		2	100	Cinnamon, sweet	Antimicrobial, antiadipogenic activity, insect repellent	(Huang, Zhu et al. 2009, Dekker, Ignell et al. 2011, Chen, Lee et al. 2012)
Citral		1	4	Citrus, fresh, lemon, lime	Antibacterial, insecticide, anti-inflammatory	(Dorman and Deans 2000, Inouye, Takizawa et al. 2001, Katsukawa, Nakata et al. 2010, Ponce-Monter, Fernandez-Martinez et al. 2010, Ali, Murphy et al. 2013)
Citronellol		1	4	Citrus, floral, green scent	Insecticide, anti-inflammatory	(Katsukawa, Nakata et al. 2011, Ali, Murphy et al. 2013)
Geraniol		1	100	Geranium plant, sweet, fruity	Antibacterial, insecticide, anti-inflammatory	(Dorman and Deans 2000, Inouye, Takizawa et al. 2001, Katsukawa, Nakata et al. 2011, Ali, Murphy et al. 2013)
Phenyl Ethyl Alcohol (PEA)		2.5	10	Rose, sweet, floral	None	

All aroma compounds are approved for use in food and fragrances, were food grade and purchased from Sigma-Aldrich (St. Louis, MO). Compounds were stored in a food grade refrigerator before use. Each compound was solubilized with propylene glycol, a common diluent in fragrance oil industry, and then stabilized with the food grade emulsifier TWEEN20 (1.25%), and then further diluted with water to the proper concentrations. Samples were prepared on the morning of each test session and presented in brown glass jar with screw cap at room temperature.

2.5 Ballots and Questionnaires

A 15-cm line scale was utilized to rate intensity and liking for low concentration samples. The same scale was used to rate intensity, pungency and liking for mid-range concentration samples.

Self-Reported Mood Questionnaire

Various questionnaires have been used in previous studies on self-reported mood (Haviland-Jones, Rosario et al. 2005, King and Meiselman 2010), but due to the length of these questionnaires, Haviland-Jones developed a brief questionnaire for self-reported mood state. The questionnaire uses 8-point Visual Analog Scales (VAS), to measure enjoyment/happiness; interest/excitement; surprise; anger; contempt/disgust; fear; anxiety; frustration; sadness/despair; shame/shyness; tension; and guilt.

Mostly Mood Signature Questionnaire

It is common in aroma studies for individuals to ‘manage’ their moods and report that an aroma has not influenced their mood. Nevertheless, aromas are known to elicit moods even when the aromas are too weak to be noticed or recognized. Thus, aromas can affect mood even in circumstances when individuals are not conscious of an aroma and they do not believe the aroma has influenced their mood (Larsen et al. 2008). To overcome this report bias, a second measure of mood was developed by Haviland-Jones to capture each odor's "mood signature" (unpublished). This questionnaire asked subjects to assign a mood to each aroma rather than report how the aroma makes them feel – just as one can assign a mood to music or visual art. A list of nine mood terms were provided, including: anxious/worried; frustrated/angry; calm/relaxed; attentive/interested; depressed/upset; embarrassed/ashamed; stressed; pleasant/confident; and exciting/energized. Subjects were instructed to select one term that 'mostly' described the mood of each aroma. This mood term was considered the 'mood signature' of that aroma.

Ballots and questionnaire were computerized and presented to the subjects by utilizing FIZZ software (Biosystemes, Couternon, France, see Appendix).

2.6 Procedure

All sessions were conducted in the Sensory Evaluation Laboratory in the Food Science Building at Rutgers University. Subjects were tested in individual booths. The experimental design/presentation order of samples was developed by FIZZ software. Subjects participated in a total of four sessions on separate days: a screening session, a training session and two sensory evaluation sessions. Each subject was expected to complete the entire testing procedure in a three-week period.

Training Sessions

The training session was designed to familiarize subjects with the testing procedure and the ballots they would be using to evaluate aroma samples. After subjects came to the Sensory Evaluation Lab, they were asked to self-report their mood state, and then assign a mood signature (the most related mood descriptive term) to a cartoon picture (A boy reading a book, see Appendix for questionnaires and picture). A cheek swab of each subject was collected at the end of the training session.

Sensory Sessions

During the sensory sessions, subjects were asked to complete the aroma ballots, self-report their mood and then answer the Mood Signature questionnaire for the six aroma samples. Low-concentration samples were evaluated in the first session and mid-range concentrations were evaluated in the second session. Samples were presented in random order within each session.

All samples were presented in brown glass jars with screw-caps. Subjects were instructed to remove the screw-cap from each sample, sniff the sample, and replace the cap. They could re-sniff the sample if they wished. Subjects were given a 60 second break before evaluating the next sample. Each of the two sensory sessions took around 30-45 min to complete.

2.7 Data Handling and Statistical Analysis

PROP Taster Status

Subjects were classified into 3 groups based on their intensity ratings on LMS (0-100mm) for NaCl and PROP disks (Zhao, Kirkmeyer et al. 2003). Individuals who gave ratings of PROP between 0-13mm were classified as PROP non-tasters; between 13-67mm were classified as PROP medium tasters; and between 67-100mm were classified as PROP super tasters. The NaCl intensity rating was used as a reference when the PROP intensity rating was given a borderline rating.

Self-Reported Mood Rating

The mean rating and standard error (SE) of self-reported mood (enjoyment/happiness, interest/excitement, surprise, anger, contempt/disgust, fear, anxiety, frustration, sadness/despair, shame/shyness, tension and guilt) were calculated for each aroma.

Mostly Mood Signature

The number of times each 'mostly mood' descriptive term (including anxious/worried, frustrated/angry, calm/relaxed, attentive/interested, depressed/upset, embarrassed/ashamed, stressed, pleasant/confident, and exciting/energized) was selected by subjects was counted for each aroma.

Statistical Analysis

Mean sample ratings for intensity and liking, and mood reactions were analyzed by analysis of variance (ANOVA), repeated measures ANOVA, principal component analysis (PCA) and linear regression. ANOVA was used to investigate the influence of aroma type, taster status, gender, ethnicity, mood reaction and their interactions on the intensity, pungency and liking ratings. Significant ANOVA results were followed by Duncan's post-hoc tests. Repeated measures ANOVA was performed to assess changes in intensity and liking ratings over low and mid-range concentrations.

PCA was performed to reduce and summarize the large number of mood terms into fewer, more interpretable mood dimensions. PCA models were rotated by orthogonal projection to enhance the interpretability of the data. Only the factors that have an eigenvalue larger than 1.0 and can explain 75% and above of the total variation (cumulated) were selected. The resulting factor scores were then standardized to a -1 to 1 scale. The standardized (i.e., weighted) scores were used to construct the PCA plots to better visualize the results. Mapping of the six aromas on the PCA plots was performed to determine the associations between the samples, intensity, pungency, liking, and moods. Based on the PCA results, self-reported mood ratings were categorized and used in the further analyses.

Multiple regression modeling was used to examine the predictors of liking for each aroma sample. The models included the following predictor variables: intensity, pungency (for mid-range aroma concentrations), taster status, gender, ethnicity, and the categorized mood terms obtained from the PCAs.

All data are reported as means ± SEM. Statistical significance was set as $p < 0.05$ for all tests. SAS statistical software (SAS® Software 9.4) was used.

3. Results

3.1 Subjects

A total of 96 subjects participated in this study. All subjects were between 18-47 years of age. Fifty-three were East Asians and 43 were American Caucasians. Sixty-six were females and 30 were males.

PROP Taster Status

Based on the method stated in data handling section, N=26 individuals were classified as PROP non-tasters (NT); N=41 individuals were classified as PROP medium-tasters (MT); and N=29 individuals were classified as PROP super-tasters (ST). This classification method led to 3 groups (NT, MT, and ST) that accounted for 27%, 43%, and 30% of the sample. The PROP ratings for NT. MT and ST were 6.77±1.16, 41.34±1.88, and 79.79±1.77, respectively [$F_{(2,93)} = 71.54$, $p<0.0001$)]; NaCl ratings for NT. MT and ST were 37.21±4.23, 34.42±3.21, and 31.91±3.59, respectively [$F_{(2,93)} = 0.47$, $p=0.63$)].

3.2 Intensity and Liking Ratings at Low Concentrations

Aroma intensity and liking ratings at low concentrations were compared across the six aroma compounds (Figures 3.1a and 3.1b). One-way ANOVA was performed and then followed by Duncan's post-hoc test. At low concentrations, intensity ratings did not differ across aroma compound types [$F_{(5, 570)} = 1.66$, $p = 0.14$] suggesting that they were well matched for intensity. However, as expected, significant differences in liking were observed across compounds [$F_{(5, 570)} = 7.47$, $p < 0.0001$]. Citral (8.56±0.32) was liked more than cinnamaldehyde (7.33±0.35) and methyl cinnamate (5.99±0.31) ($p=0.05$). Geraniol (7.65±0.29), citronellol (7.65±0.30) and PEA (7.96±0.31) were liked as well as citral and cinnamaldehyde but less than methyl cinnamate that was liked less than all the other samples ($p=0.05$).

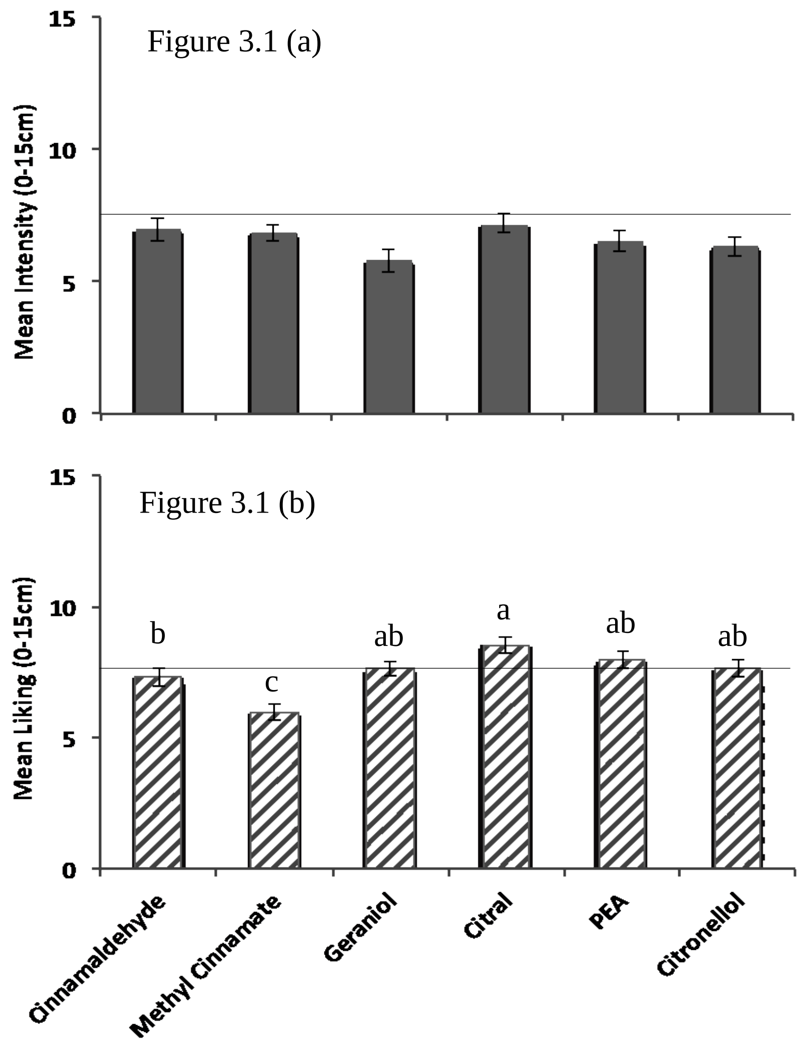


Figure 3.1 Intensity (top) and liking (bottom) ratings for the six aromas at low concentration. Superscripts indicate differences among samples ($p<0.001$). The 7.5-midpoint lines are shown in the graphs. Data are presented as means ± SEM.

3.3 Intensity, Pungency and Liking Ratings at Mid-Range Concentrations

The intensity and liking ratings at mid-range concentration were also compared across the six aromas. One-way ANOVA was performed and then followed by Duncan's post-hoc test. Pungency was also assessed to determine its contribution to aroma intensity and liking. Intensity ratings were perceived differently across aroma compounds [$F(5, 570) = 29.53$, $p < 0.0001$, Figure 3.2a]. Cinnamaldehyde was the most intense aroma (11.58±0.24), followed by geraniol (10.62±0.28). Methyl cinnamate (10.30±0.34). PEA (8.08±0.34) and citronellol (6.98±0.37) were perceived as two of the least intense aromas.

Pungency ratings were also perceived differently across aroma compounds [$F(5, 570) = 26.48$, $p<0.0001$, Figure 3.2b]. Similar to the pattern for intensity, cinnamaldehyde (9.66±0.41), geraniol (8.67±0.40) and methyl cinnamate (8.74±0.40) were perceived as the most pungent samples, followed by citral (6.97±0.41), PEA (4.98±0.39), and citronellol (4.78±0.39). The similarity in the patterns observed for aroma intensity and pungency suggests that pungency contributes to overall aroma intensity.

Liking was rated differently across the samples [$F(5, 570) = 20.33$, $p<0.0001$] as shown in Figure 3.2c. Citral (9.60±0.36) was liked the best at mid-range concentration and liked as well as cinnamaldehyde (8.73±0.46) and PEA (8.82±0.41), followed by geraniol (7.1±0.36) and citronellol (7.54±0.27). Methyl cinnamate (4.86±0.37) was liked the least at mid-range concentration.

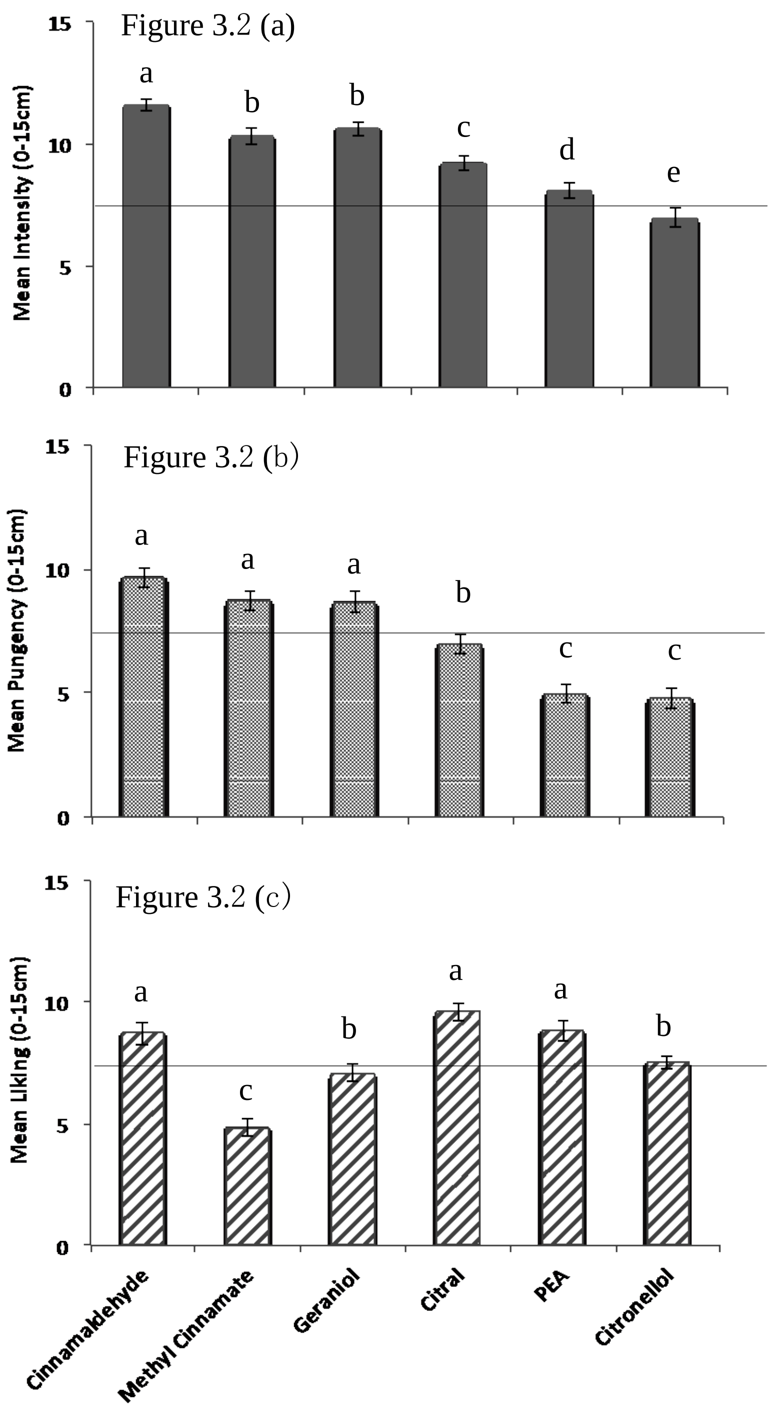


Figure 3.2 Intensity (top), pungency (middle) and liking (bottom) ratings for six aromas at mid-range concentration. Superscripts indicate differences among samples within each attribute ($p<0.001$ for all). The 7.5-midpoint lines are shown in the graphs. Data are presented as means ± SEM.

3.4 Liking Ratings at Low and Mid-Range Concentrations

Figure 3.3 compared the liking ratings between low and mid-range concentrations across the six aromas. Repeated measures of ANOVA was performed. The liking ratings for cinnamaldehyde and citral increased as concentration increased [F (1, 93) =7.21, p =0.009; and F (1, 93) =6.35, p =0.01]. Geraniol, PEA, and cintronellol were liked the same at low and mid-range concentrations, and only methyl cinnamate became disliked at higher concentration [F (1, 93) =3.15, p=0.08].

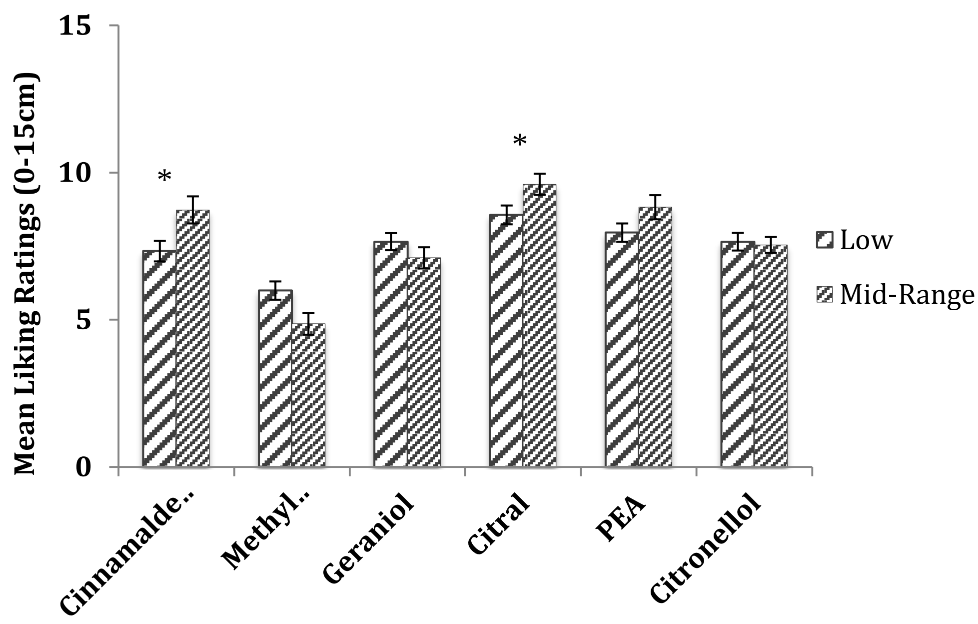


Figure 3.3 Liking ratings for low and mid-range concentrations across six aroma compounds. Only the liking ratings for cinnamaldehyde and citral increased as concentration increased ($p \leq 0.01$ for both). Data are presented as means ± SEM.

3.5 Effect of Ethnicity and PROP Status on Attribute Ratings

Statistical results for the effects of ethnicity, PROP status, and their interaction on the aroma ratings are shown in Table 3.1. Except for cinnamaldehyde, ethnicity alone and PROP taster status alone had only a few isolated effects on aroma intensity, pungency or liking.

In the case of cinnamaldehyde, we observed an effect of ethnicity on liking of this compound [$F (1, 90) = 16.42$, $p=0.0001$]. To further illustrate this effect, we examined the influence of ethnicity on liking of cinnamaldehyde across concentrations. At low concentration, there was no difference in liking of cinnamaldehyde across ethnic groups, but at mid-range concentration, cinnamaldehyde was more liked by Caucasian (10.59 ± 0.56) than East Asian (7.22 ± 0.62) subjects. This outcome is shown in Figure 3.4. This result suggests a specific cultural effect on liking of cinnamaldehyde that was not observed for the other compounds.

We then examined the interaction between PROP status and ethnicity for liking of cinnamaldehyde at mid-range concentration. This interaction was statistically significant ($p<0.04$) and is shown in Figure 3.5). Liking ratings decreased across PROP groups for East Asian subjects but not for Caucasian subjects. This finding suggests that PROP status plays a role in liking of cinnamaldehyde in East Asian subjects but not Caucasian subjects.

Table 3.1

Attribute	**Ethnicity**		**PROP Status**		**PROP Status x Ethnicity**	
	F-value	P-value	F-value	P-value	F-value	P-value
Cinnamaldehyde						
Low Concentration						
Intensity	4.68	**0.03**	0.01	NS	0.82	NS
Liking	3.24	0.08	0.68	NS	3.4	NS
Mid-Range Concentration						
Intensity	1.41	NS	1.72	NS	0.82	NS
Pungency	3.88	**0.05**	0.68	NS	0.96	NS
Liking	16.42	**0.0001**	1.58	NS	3.53	**0.03**
Methyl Cinnamate						
Low Concentration						
Intensity	0.58	NS	0.34	NS	0.24	NS
Liking	6.26	**0.01**	0.41	NS	0.57	NS
Mid-Range Concentration						
Intensity	0.27	NS	1.03	NS	1.23	NS
Pungency	0.45	NS	0.46	NS	0.36	NS
Liking	0.31	NS	1.30	NS	3.08	**0.05**
Citral						
Low Concentration						
Intensity	1.5	NS	0.56	NS	1.09	NS
Liking	0.18	NS	4.08	**0.02**	0.62	NS
Mid-Range Concentration						
Intensity	2.79	NS	0.08	NS	0.87	NS
Pungency	3.13	NS	0.04	NS	0.48	NS
Liking	0.42	NS	2.21	NS	1.21	NS

Table 3.1 (Continued)

Attribute	**Ethnicity**		**PROP Status**		**PROP Status x Ethnicity**	
	F-value	P-value	F-value	P-value	F-value	P-value
Citronellol						
Low Concentration						
Intensity	0.00	NS	0.17	NS	0.36	NS
Liking	0.23	NS	0.84	NS	0.35	NS
Mid-Range Concentration						
Intensity	3.26	NS	2.96	NS	0.85	NS
Pungency	8.19	**0.005**	1.82	NS	0.57	NS
Liking	0.06	NS	1.66	NS	2.01	NS
Geraniol						
Low Concentration						
Intensity	4.47	**0.04**	0.29	NS	1.33	NS
Liking	1	NS	2.87	NS	0.69	NS
Mid-Range Concentration						
Intensity	0.23	NS	0.65	NS	3.46	**0.04**
Pungency	5.83	**0.02**	0.12	NS	0.96	NS
Liking	0.17	NS	0.55	NS	1.00	NS
PEA						
Low Concentration						
Intensity	3.14	**0.05**	0.31	NS	0.50	NS
Liking	0.00	NS	0.54	NS	0.73	NS
Mid-Range Concentration						
Intensity	0.07	NS	0.27	NS	4.37	**0.02**
Pungency	0.48	NS	0.75	NS	0.95	NS
Liking	0.66	NS	0.94	NS	3.00	0.06

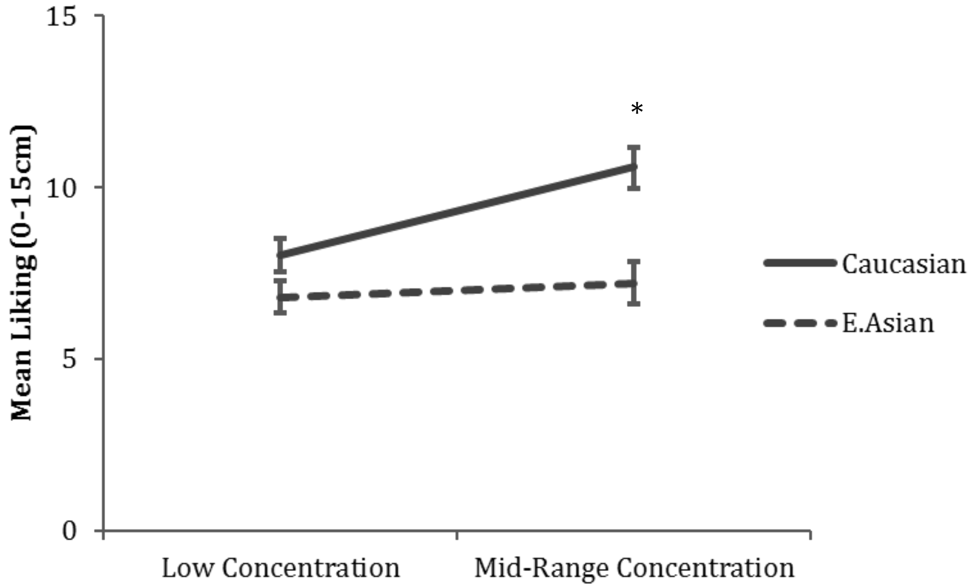


Figure 3.4 Liking ratings of cinnamaldehyde at low and mid-range concentrations by Caucasian and East Asian subjects. Data are presented as means ± SEM.

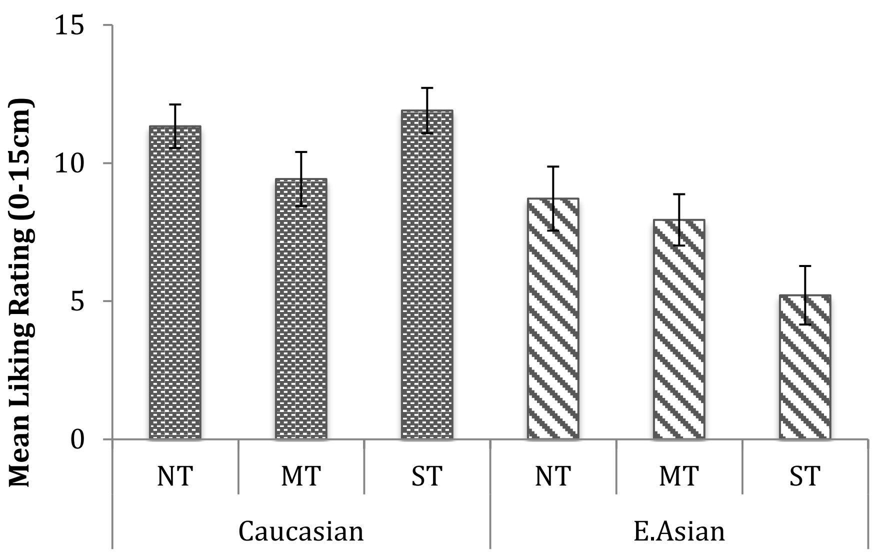


Figure 3.5 Mean liking ratings for for cinnamaldehyde of super-, medium-, and non-tasters with in American Caucasians and East Asians subjects. Data are presented as mean ± SEM.

3.6 Self-Reported and Mostly Mood Data

3.6.1 Self-Reported Mood

Mean self-reported mood ratings for the six aromas are summarized in Table 3.2. Subjects gave similar mood ratings to the six aroma compounds regardless of concentration. However, in general, the aromas elicited higher positive moods (enjoyment/happiness and interest/excitement) than negative moods (sadness/despair, anger, contempt/disgust, shame/shyness, anxiety, tension, frustration and guilt).

3.6.2 Mostly Mood Distribution

The selection frequency of each “mostly mood” term for the six aromas at low and mid-range concentrations are listed in Table 3.3. By general impression, at low concentration, the mostly mood terms “calm/relaxed” and “pleasant/confident” were predominately selected. In contrast at mid-range concentration, the selection frequency of mostly mood “exciting and energized” increased, and the selection frequency of “calm and relaxed” decreased for the top three pungent aromas (cinnamaldehyde, methyl cinnamate and geraniol).

Table 3.2: Mean (± SEM) mood ratings for six aroma compounds from the self-reported mood questionnaire

	Enjoyment/ Happiness	Interest/ Excitement	Surprise	Sadness/ Despair	Anger	Contempt/ Disgust
	Mean ± SE					
Low Concentration						
Cinnamaldehyde	3.01±0.20	2.98±0.21	1.70±0.20	0.5±0.11	0.39±0.10	0.82±0.18
Methyl Cinnamate	3.01±0.20	2.77±0.20	1.59±0.18	0.67±0.12	0.46±0.11	1.06±0.17
Citral	3.92±0.18	3.71±0.19	1.63±0.19	0.30±0.09	0.19±0.08	0.41±0.12
Citronellol	3.58±0.18	3.15±0.20	1.41±0.18	0.5±0.13	0.31±0.10	0.42±0.12
Geraniol	3.39±0.18	3.09±0.20	1.52±0.18	0.33±0.07	0.21±0.05	0.39±0.11
PEA	3.57±0.20	3.05±0.20	1.49±0.19	0.41±0.10	0.29±0.10	0.35±0.10
Mid-Range Concentration						
Cinnamaldehyde	3.45±0.20	3.25±0.21	1.51±0.19	0.5±0.11	0.59±0.16	0.79±0.18
Methyl Cinnamate	2.75±0.20	2.52±0.19	1.43±0.18	0.76±0.12	0.61±0.13	1.5±0.21
Citral	3.93±0.18	3.58±0.19	1.67±0.20	0.29±0.08	0.21±0.07	0.27±0.08
Citronellol	3.49±0.18	2.82±0.19	1.17±0.16	0.47±0.12	0.30±0.09	0.44±0.11
Geraniol	3.27±0.20	2.95±0.21	1.44±0.19	0.56±0.12	0.40±0.10	0.59±0.13
PEA	3.63±0.20	3.04±0.21	1.31±0.18	0.46±0.09	0.33±0.10	0.29±0.08

Table 3.2 (continued)

	Fear	Shame/ Shyness	Anxiety	Tension	Frustration	Guilt
	Mean ± SE					
Low Concentration						
Cinnamaldehyde	0.28±0.08	0.30±0.08	0.98±0.16	0.90±0.14	0.59±0.13	0.21±0.06
Methyl Cinnamate	0.26±0.07	0.28±0.07	0.91±0.13	0.93±0.12	0.56±0.10	0.16±0.05
Citral	0.16±0.04	0.13±0.04	0.79±0.13	0.69±0.12	0.45±0.10	0.10±0.04
Citronellol	0.25±0.08	0.31±0.11	0.85±0.14	0.79±0.15	0.49±0.12	0.22±0.08
Geraniol	0.21±0.07	0.25±0.06	0.76±0.12	0.72±0.11	0.42±0.10	0.11±0.04
PEA	0.18±0.07	0.25±0.07	0.74±0.14	0.78±0.13	0.44±0.11	0.23±0.08
Mid-Range Concentration						
Cinnamaldehyde	0.40±0.10	0.28±0.08	0.95±0.15	0.91±0.15	0.67±0.14	0.24±0.07
Methyl Cinnamate	0.40±0.09	0.36±0.09	1.30±0.18	1.29±0.17	1.03±0.16	0.36±0.10
Citral	0.22±0.08	0.19±0.06	0.63±0.12	0.66±0.12	0.39±0.09	0.16±0.05
Citronellol	0.23±0.07	0.29±0.08	0.80±0.13	0.77±0.12	0.42±0.09	0.22±0.08
Geraniol	0.28±0.08	0.21±0.06	0.96±0.15	0.91±0.14	0.68±0.12	0.20±0.06
PEA	0.24±0.07	0.35±0.10	0.68±0.11	0.66±0.11	0.52±0.10	0.28±0.08

Table 3.3: Selection frequency for the 'mostly mood' terms from the Mood Signature questionnaire. [1]

	Anxious or Worried	Frustrated	Calm and Relaxed	Attentive and Interested	Depressed	Embarrassed or Ashamed	Stressed	Pleasant and Confident	Exciting and Energized
	Selection Frequency								
Low Concentration									
Cinnamaldehyde	10	4	26	14	5	4	5	10	18
Methyl Cinnamate	9	2	23	10	16	7	11	10	8
Citral	1	2	33	6	5	1	4	21	23
Citronellol	7	4	43	7	4	1	8	17	5
Geraniol	2	5	48	4	8	5	6	12	6
PEA	5	3	38	9	7	2	1	21	10
Mid-Range Concentration									
Cinnamaldehyde	6	6	10	11	7	0	6	14	36
Methyl Cinnamate	17	8	7	12	18	5	6	9	14
Citral	4	1	25	11	2	0	2	15	36
Citronellol	6	2	43	11	4	2	5	12	11
Geraniol	11	4	24	10	4	1	11	4	27
PEA	8	1	38	8	6	0	3	27	5

[1] Each cell shows the selection frequency for the 'mostly mood' terms for individual aromas. For example, 10 subjects selected anxious/worried as the term that mostly described the mood of cinnamaldehyde at low concentration.

3.7 PCAs for the Mood Responses

Two sets of PCA were developed in this study using the reported moods and the mostly mood responses from the mood signature questionnaire. The PCA analyses of the self-reported moods provided better results for reducing and condensing the total number of mood terms to calculate regression models for predicting liking of the aroma compounds. In contrast, the PCA analyses of the mostly mood terms provided more complex perceptual maps and a richer interpretation of the emotional impact of the aromas. Thus, both sets of PCAs will be presented in the following sections.

3.7.1 Self-Report Mood Rating

3.7.1.1 Low Concentration

Results of the PCA showed that 90% of the total variability in aroma perception could be accounted for by the first two principal components (69% and 21% respectively, see Table A. in Appendix). The first principal component was explained by a dimension described by positive moods (enjoyment/happiness and interest/excitement) on one pole; and negative moods (sadness/despair, anger, contempt/disgust, fear, shame/shyness, anxiety, tension, frustration, and guilt) on the other pole. The correlations of these moods with the first component are shown in Figure 3.6 (a).

3.7.1.2 Mid-Range Concentration

Results of the PCA showed that 89% of the total variability was explained by the first two principal components (69% and 20% respectively, see Table B. in Appendix). Similar to the PCA for the low concentrations, the first principal component was explained by a dimension described by positive (enjoyment/happiness and interest/excitement) moods on one pole and negative moods (sadness/despair, anger, contempt/disgust, fear, anxiety, tension, frustration, shame/shyness, and guilt) on the other pole. The correlations of these moods with the first component are shown in Figure 3.6 (b).

Based on these two PCAs, self-reported moods were grouped into two categories then further analyzed by multiple regression analysis: *positive moods* (the average of enjoyment/happiness and interest/excitement) and *negative moods* (the average of sadness/despair, anger, contempt/disgust, fear, anxiety, tension, frustration, shame/shyness, and guilt).

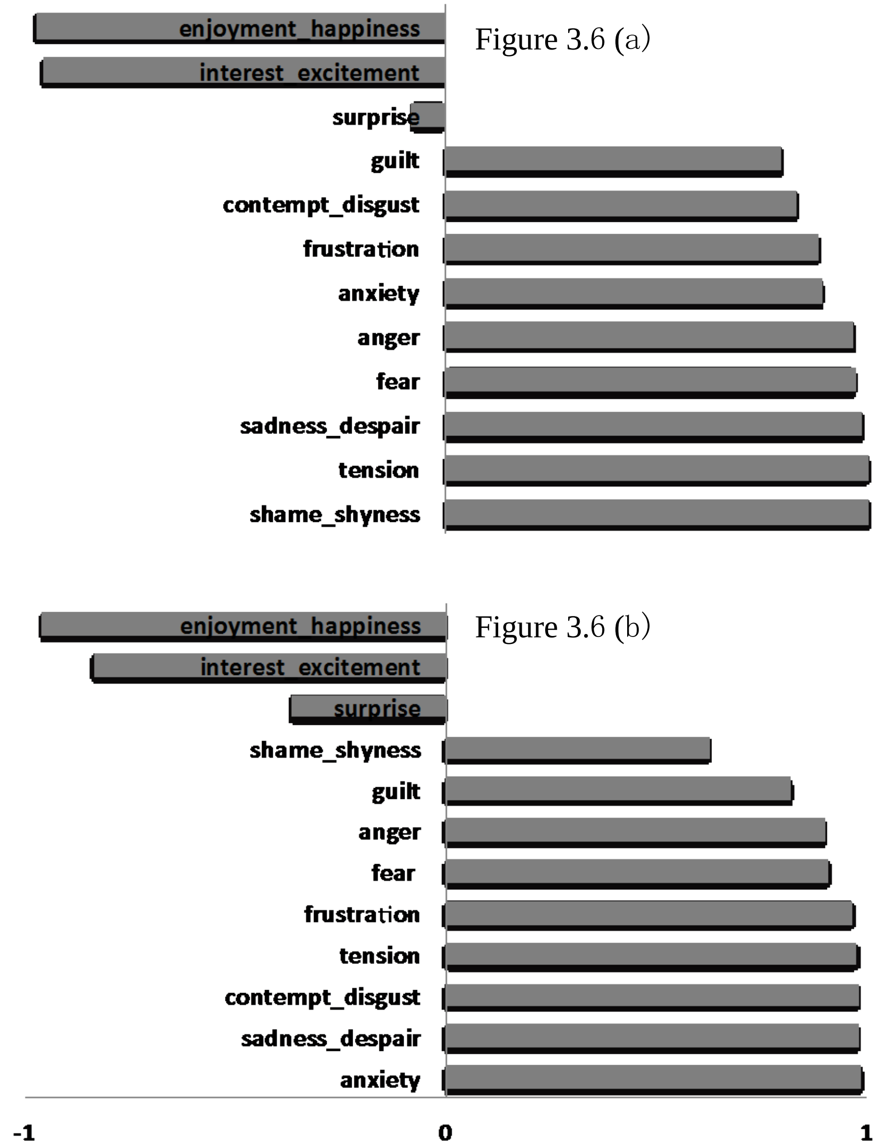


Figure 3.6 The correlations between moods and first component at low (top) and mid-range (bottom) concentrations.

3.7.1.3 Multiple Regression Analysis - Liking Prediction

Multiple regression modeling was used to determine how intensity, pungency, PROP status, ethnicity, and *positive* and *negative* mood influenced liking of the six aromas. In the regression model, higher *positive moods* predicted higher liking ratings of cinnamaldehyde, methyl cinnamate, citral and PEA at both low and mid-range concentrations. Also, the higher *negative moods* predicted lower liking ratings of cinnamaldehyde, methyl cinnamate, and PEA at low concentration, as well as lower liking ratings of cinnamaldehyde at mid-range concentration (R^2=0.28-0.45, $p \leq 0.0001$ for all, see Table 3.4). Agreeing with the previous ANOVA results, ethnicity was a strong predictor for liking rating of cinnamaldehyde (R^2=0.45, $p<0.0001$)(See Table 3.1), where American Caucasians rate liking of cinnamaldehyde higher than East Asians.

Table 3.4 Positive mood predicted liking of cinnamaldehyde, methyl cinnamate, citral and PEA at mid-range concentration.

	Coefficients			
	Beta±SE	P	adj R2	P
Cinnamaldhyde				
Low Conc				
Positive Mood	0.81±0.17	<0.0001	0.38	<0.0001
Negative Mood	*neg*0.75±0.26	0.005		
Mid-Range Conc				
Ethnicity	*neg* 1.04±0.36	0.0047	0.45	<0.0001
Positive Mood	1.14±0.21	<0.0001		
Negative Mood	*neg* 0.79±0.41	0.06		
Methyl Cinnamate				
Low Conc				
Positive Mood	0.68±0.16	<0.0001	0.29	<0.0001
Negative Mood	*neg* 0.59±0.27	0.0318		
Mid-Range Conc				
Positive Mood	0.89±0.19	0.0001	0.28	<0.0001
Citral				
Low Conc				
Positive Mood	0.80±0.18	<0.0001	0.29	<0.0001
Mid-Range Conc				
Positive Mood	0.94±0.18	<0.0001	0.27	<0.0001
PEA				
Low Conc				
Positive Mood	0.74±0.15	<0.0001	0.30	<0.0001
Negative Mood	*neg* 0.63±0.26	0.0185		
Mid-Range Conc				
Positive Mood	1.19±0.19	<0.0001	0.29	<0.0001

3.7.2 Mostly Mood PCAs -Association between Intensity, Pungency, Liking, Mood and Aroma

3.7.2.1 Mood Signature: Mostly Mood at Low Concentrations

Results of the PCA showed that 76% of the total variability in aroma perception was explained by the first two principal components (44% and 32% respectively, PCA biplot see Figure 3.7). The first principal component described a contrast between positive mood (pleasant/confident) and liking of the aroma together (to the left), and a group of negative moods (embarrassed/ashamed, stressed, depressed/upset and anxious/worried, to the right). The second principal component described a calm to energized dimension that was explained by calm/relaxed mood (on the bottom), and intensity, exciting/energized, and attentive/interested moods (on the top). The six samples were distributed across these four main sensory dimensions (top, bottom, left and right).

After examining the positions of the mood terms on the map, we observed that methyl cinnamate was strongly associated with negative moods (anxious/worried, depressed/upset, embarrassed/ashamed and stressed, on the right of the map) and PEA was characterized by pleasant/confident mood (on the left). Geraniol and citronellol were positioned closely with a calm/relax mood.

3.7.2.2 Mood Signature: Mostly Mood at Mid-Range Concentrations

Results of the PCA showed that 79% of the total variability was explained by the first two principal components (41% and 38% respectively, PCA biplot see Figure 3.8). The first principal component was explained by the contrast between a calm/relaxed mood (to the left), and intensity and pungency (to the right). The second principal component was explained by a contrast from liking of the aroma (on the bottom), to a group of negative moods (embarrassed/ashamed, depressed/upset and anxious/worried, on the top).

The six samples were distributed across these four main mood dimensions (top, bottom, left and right). Methyl cinnamate was still strongly associated with negative moods (on the top); whereas citronellol and PEA were strongly associated with positive moods (calm/relaxed and pleasant/confident, on the left). Cinnamaldehyde and geraniol were equally characterized by intensity and pungency (on the right). Citral was linked with liking.

3.7.2.3 Overall Findings from the Mostly Mood PCAs

On the perceptual map of aroma samples at mid-range concentrations, the six aromas were located at the end of each dimension suggesting a higher clarity and quality of the analysis. At mid-range concentration, cinnamaldehyde and geraniol were associated with higher intensity and pungency, as well as evoked exciting/energized mood. PEA and citronellol were associated with calm/relaxed mood at both concentrations. Citral became more associated with liking with increased concentration, matching with our previous result of repeated measures of ANOVA. At both low and mid-range concentrations, methyl cinnamate was grouped with negative moods indicating an unpleasant sensory experience.

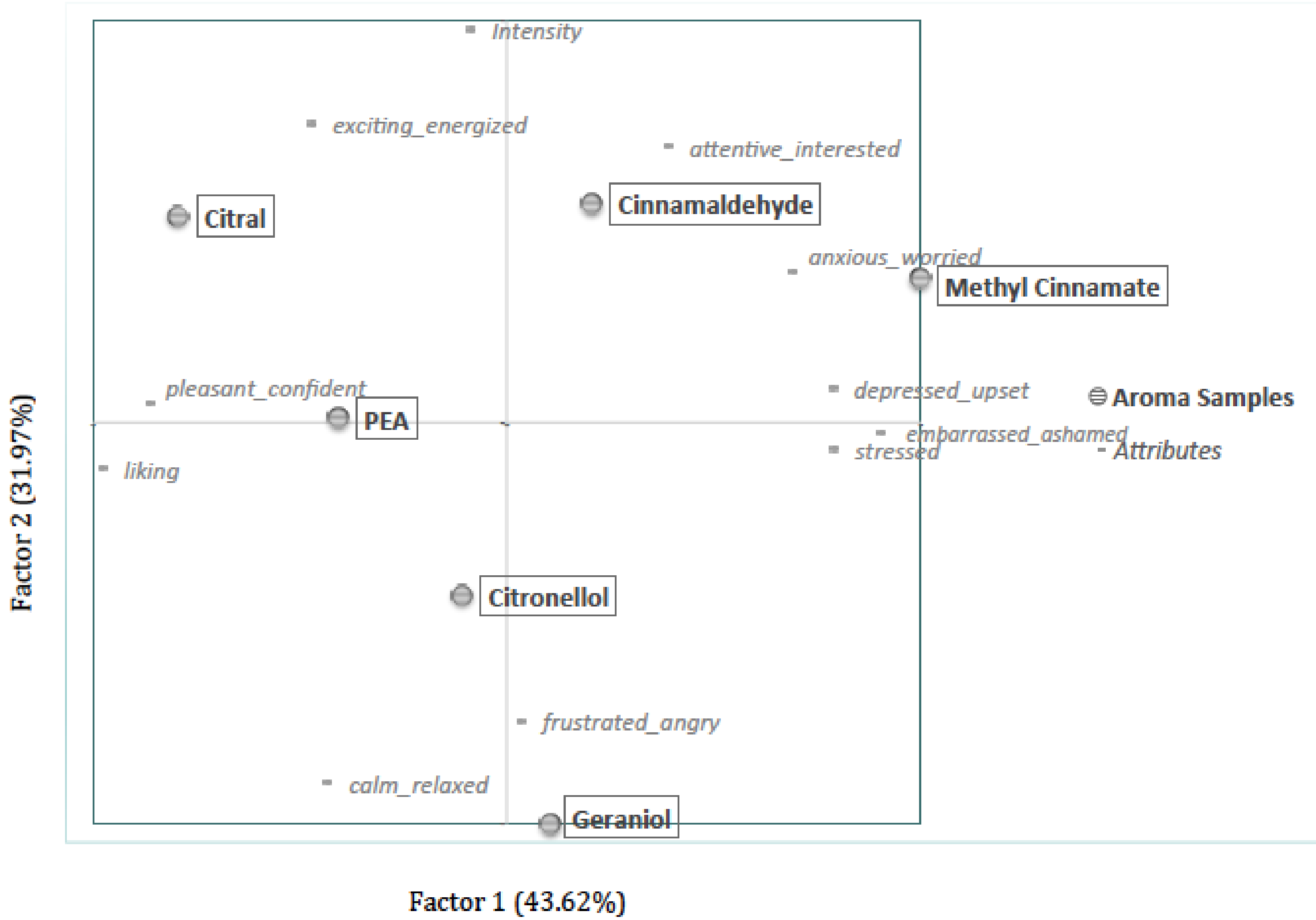


Figure 3.7 Perceptual map of six aromas based on mostly mood PCA at low concentration.

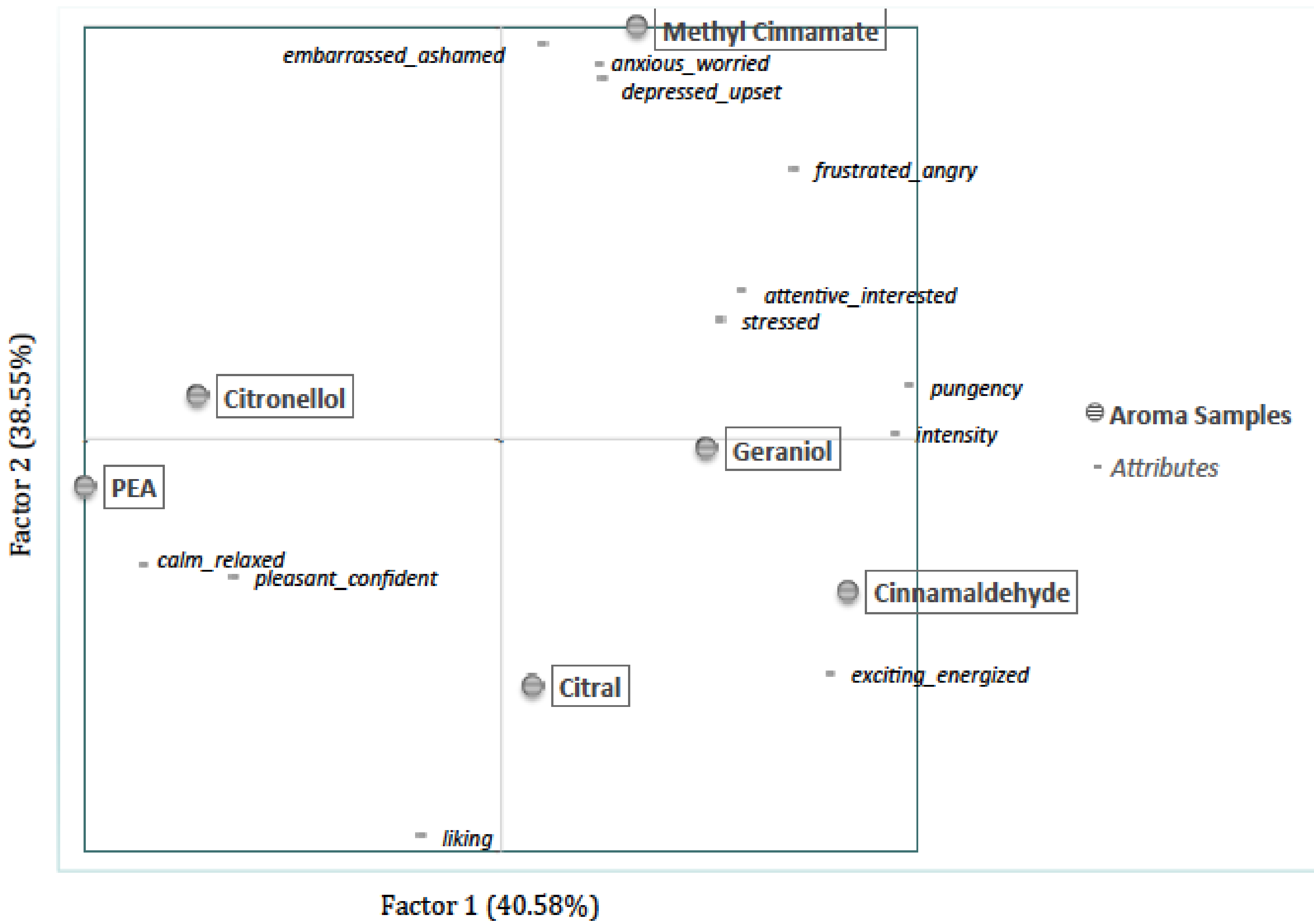


Figure 3.8 Perceptual map of six aromas based on mostly mood PCA at mid-range concentration.

3.8 Effect of Ethnicity and PROP Sensitivity on Mood Respond

American Caucasians reported a higher positive mood than East Asians for cinnamaldehyde, methyl cinnamate and citronellol at both low and mid-range concentrations [F (1,94) >5.17, p< 0.03 for all, see Figure 3.9 (a) and 3.9 (b)). We did not observe any significant differences for reported negative moods between American Caucasians and East Asians. PROP taster status had no impact on mood response.

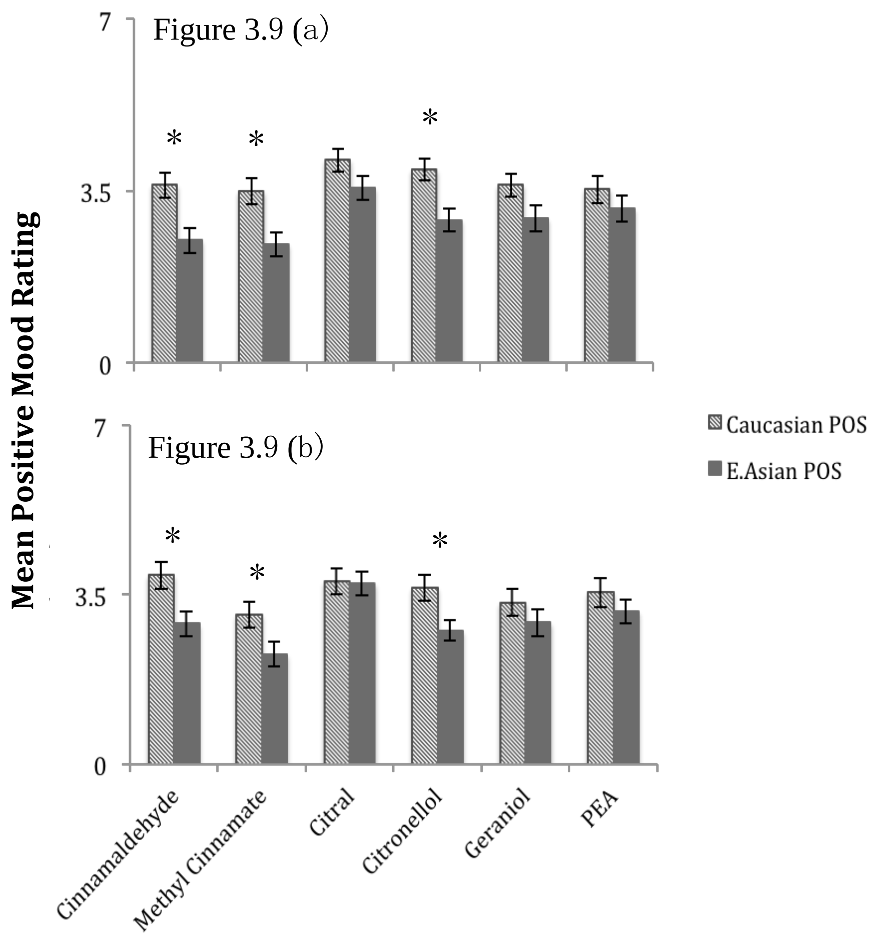


Figure 3.9 Positive mood ratings for the six aromas by American Caucasians and East Asians at low (top) and mid-range (bottom) concentrations. Asterisk (*) indicates signaificant differences between Caucasian and East Asian ratings. Data are presented as mean± SEM.

4. Discussion

The aims of our study were: 1) to investigate how an aroma intensity and nasal pungency impact hedonic responses to individual aroma compounds; 2) to investigate whether PROP sensitivity and ethnicity impact aroma perception and liking; 3) to determine the relationship between mood responses and hedonic responses to aromas; and 4) to map the sensory perceptions (intensity, pungency), mood and hedonic responses to aromas.

4.1 The nasal pungency and intensity of an aroma do not significantly impact its hedonic response

The six samples were given similar aroma intensity ratings at low concentrations, suggesting that they were well matched for intensity. At mid-range concentrations, the samples produced a distinct pattern of aroma intensities: cinnamaldehyde was the most intense aroma, followed by same-intensity methyl cinnamate and geraniol, which elicited similar aroma intensities; citronellol was the least intense sample. This suggests that the mid-range concentration were less well matched for intensity as compared to the low concentrations. This may be due to the pungency of these aromas, which appeared to mimic the pattern of aroma intensities, but at lower values. These data suggest that nasal pungency contributes to overall aroma intensity for most of the aromas. We did not ask subjects to rate the pungency of the low concentrations because the concentrations might be too low for subjects to detect any nasal pungency.

The six aromas were moderately well-liked at both concentrations except for methyl cinnamate, which was disliked at both concentrations. At mid-range concentration, cinnamaldehyde, methyl cinnamate and geraniol were rated similar in nasal pungency and intensity. Despite these similarities in sensory ratings, perceived intensities of the samples did not predict liking. For example, cinnamaldehyde and methyl cinnamate, two chemically-related compounds, were among the most pungent and intense aromas that were studied. Nevertheless, cinnamaldehyde received high liking ratings and methyl cinnamate received the lowest liking ratings in the study.

In accordance with the ANOVA and multiple linear regression analyses, nasal pungency and perceived intensity had no significant impact on the hedonic ratings of the aromas. These data suggest that at the relatively mild intensities studies here, liking was not a function of aroma impact and nasal pungency. It seems likely that sensory perceptions would make a much greater contribution to liking at higher concentrations. This should be tested in future studies.

4.2 Positive mood strongly predicts hedonic response

In contrast to intensity judgments, positive moods were associated with higher liking for several, but not all of the aromas. Specifically, positive moods were associated with higher liking ratings for cinnamaldehyde, methyl cinnamate, citral and PEA at both low and mid-range concentrations. This outcome partially supports our hypothesis that positive moods correlate with liking. Positive mood was not associated with liking of citronellol or geraniol. These two aromas produced the most neutral liking responses (~7.5 on the 15cm line scale) suggesting that subjects may have been indifferent to these aromas.

4.3 Cultural differences impact an individual's liking and mood for an aroma

We found that American Caucasians preferred cinnamaldehyde more than East Asians at both low and mid-range concentrations, which could be due to cultural differences regarding the familiarity and/or use of this aroma. We speculate this is because American Caucasians are frequently exposed to the cinnamon flavor and aroma across a wide range of products, and they often associate this aroma with many pleasant experiences (holidays, desserts, etc.). Thus, the acceptance of this aroma is high in that group. In comparison to American Caucasians, East Asians are rarely exposed to cinnamon within their daily lives. For example, cinnamon is typically not used alone as a cooking spice in Chinese or Korean cooking. These findings agree with previous cross-cultural studies showing that subjects tend to give higher hedonic ratings to products they are more familiar with (Laing, Prescott et al. 1994, Pages, Bertrand et al. 2007). Thus, our findings for one aroma, cinnamaldehyde, confirmed that cultural differences impact aroma liking (Herz 2009).

In contrast to the results for cinnamaldehyde, we found no ethnic differences for any of the other aromas. We speculated that this is due to a stronger cultural impact on cinnamaldehyde compared to the other aromas. In addition, cinnamaldehyde was the most intense aroma and it is possible that both American Caucasians and East Asians subjects could identify its sensory quality better than the other compounds. Citrus aromas are used commonly in citrus flavored beverages, air refreshener and cleaning products, and it is possible consumers are becoming more alike as they become increasingly exposed to these products.

Contrary to the findings of Tsai et al. (2006), there were no observed differences in negative mood ratings between Caucasian and East Asian groups in our study. However, American Caucasians gave higher positive mood ratings than East Asians. A cross-cultural study by Eid and Diener (2001) observed much lower intensity ratings for emotion for Chinese and Taiwanese individuals compared to American and Australian individuals. The results from the present study are similar to these earlier findings. This may be due to the different emotion-regulation strategies by Caucasian and East Asians as suggested by Davis et al. (2012). In addition, the ratings for negative moods were low in our study (~0.50 points on an 8-point scale), except for methyl cinnamate, which was higher than the other aromas. Since negative moods were low in our study, it might not have been possible to observe ethnic differences between groups.

The mood term selected by our subjects did not differ between Caucasian and East Asian subjects. Ferdenzi et al. (2013) conducted a series of odor emotion studies in 5 countries (Switzerland, UK, Singapore, Brazil and China) and found that the emotions elicited by odors were common across different cultures, which supports our findings from this study as well.

4.4 PROP sensitivity impacts the hedonic response for cinnamaldehyde

Tepper et al. (2009) proposed that PROP sensitivity influences both our perception and liking for various foods, especially those with oral trigeminal effects such as capsaicin (Karrer and Bartoshuk 1991, Pickering, Simunkowa et al. 2004) and cinnamaldehyde (Prescott and Swain-Campbell 2000). In particular, Prescott and Swain-Campbell showed that oral cinnamaldehyde was perceived as more irritating in PROP super- and medium-taster groups than non-taster group. However, findings from some studies showed no relationship between PROP status and perception and liking of trigeminal stimulants (Tornwall, Silventoinen et al. 2012). These controversial findings point to the possibility that PROP taster status, alone, may not determine a consumer's hedonic response and preference for a food (Drewnowski, Henderson et al. 1997a, 1997b, 1998, Tepper and Nurse 1998, Ullrich, Touger-Decker et al. 2004). Indeed, the present study found no relationship between PROP status and perception and liking of any of the aroma compounds. However, we did find a relationship between PROP status and liking of cinnamaldehyde when subjects were divided by ethnicity. Specifically, Caucasian PROP super-tasters liked cinnamaldehyde much more than East Asian PROP super-tasters. Thus classifying subjects by ethnicity and PROP status unmasked the specific relationship between PROP sensitivity and liking in East Asian subjects that was not present in Caucasian subjects.

The role of ethnic/cultural differences in taste perception and food preferences has rarely been studied in PROP-classified subjects. One study in Italy (Tepper, White et al. 2009) reported a high degree of liking for vegetables regardless of PROP sensitivity. However; super-tasters gave lower liking ratings than non-tasters to foods that stimulate trigeminal sensations (e.g., pungent and spicy foods, certain alcoholic beverages). These results suggest that culture expressed through frequent exposure to certain foods, can sometimes override one's genetically determined taste preferences. Within this cultural context, PROP status may have had very little influence on liking of cinnamaldehyde aroma in Caucasian subjects in our study but it did have a strong impact on liking in East Asian subjects. Additional studies should be conducted to confirm these finding.

4.5 Characteristics of aromas

Principal component analyses of the self-reported mood ratings allowed us to reduce and organize the emotion terms into *positive mood* and *negative mood* dimensions. As expected, liking was positively correlated with positive moods (e.g. enjoyment/happiness) and negatively correlated with negative moods (e.g. anxiety). This also matches the results we obtained from the multiple regression analysis.

The mood signature questionnaire was superior to the self-reported mood-rating questionnaire because it allowed more variables to load onto the principal components, providing a better emotional characterization for the six aromas. Overall, the 2-dimensional spaces for the low and mid-range concentrations were similar. Each plot consisted of a dimension characterized by liking on one end and negative moods (especially anxious/worried) on the other end. The other dimension was characterized by calm mood on one end and excited/interested/attentive moods on the other end. The 'excited' pole of this dimension was associated with intensity/pungency ratings for the samples. Thus, the overall emotional spaces characterizing these aromas did not vary across concentrations.

However, the plot for the low concentrations was less interpretable than the plot for the mid-range concentrations. At low concentrations, methyl cinnamate was associated with negative emotions (especially anxious/worried), PEA was associated with pleasant/confident, and geraniol was associated with calm/relaxed. The other aromas did not show strong associations with the emotional terms.

In contrast, the plot for the mid-range concentrations better characterized the emotional reactions to these aromas. At mid-range concentrations, methyl cinnamate was strongly associated with the terms anxious/worried, similar to what we observed at the low concentration for this aroma. In addition, cinnamaldehyde was characterized by exciting/energized mood, citronellol and PEA were characterized by calm/relaxed and pleasant/confident moods, and citral was modestly associated with liking. Cinnamaldehyde and geraniol were also linked closely with nasal pungency and intensity perception, which agrees with the previous ANOVA results.

These findings suggest that methyl cinnamate, which is generally unpleasant, elicits negative emotions even at lower concentrations. At mid-range concentrations, cinnamaldehyde elicits excitement, and citronellol and PEA elicit a calm/relaxed mood.

Interestingly, the exciting/energized mood was associated with cinnamaldehyde at mid-range concentration. We speculate that this may be due to the trigeminal sensation it elicited that excites the mood. Porcherot et al. (2010) found that mandarin essential oils (sniffed from a bottle) evoked a “energetic-invigorated” mood. However, we did not observe this same result in this study. Citral and citronellol, two of the citrus-like aromas, were not associated with the exciting/energized mood. Instead, citronellol was associated with calm/relaxed mood. Nevertheless, the present study tested pure, single aroma compounds rather than fine fragrance essential oils, and it is possible that the concentrations used by Porcherot et al. (2010) may have been higher than those used here. These differences could have caused the opposite result. On the other hand, one study by Lehrner (2005) did find that ambient orange oil decreased anxiety in dental patients, which agrees with the calm/relaxed mood effect of citronellol.

Chrea et al. (2009) found that common everyday emotions did not seem to be relevant to describe the moods elicited by aromas. We also found this is to be the case in the present study. For example, frustrated, angry and stressed are very common emotions in everyday life. However, none of the aromas at the concentration tested in the present study were associated with these mood terms on the PCA plots. In addition, Chrea et al. (2009) found that the moods elicited by aromas did not follow dimensional theory, wherein moods have either a positive or negative valence, and either a high or low arousal. In their study, moods elicited by odors were also clearly separated into positive and negative dimensions only, with no high or low arousal dimension. We observed similar results in the present study where the PCAs generated only by a positive moods group and negative moods group. Additional studies need to be conducted with other aromas over a wider range of concentrations to support these findings.

5. Conclusions and Future Directions

This study showed that the liking for the six aromas we studied is a function of the unique sensory properties (nasal pungency and intensity perception) of each aroma and the mood(s) it elicits. Ethnicity contributed to liking of cinnamaldehyde, but not to the liking of the other aromas. PROP status alone had no influence on perception or liking of any of the aromas These findings suggest that the PROP phenotype is not a reliable marker for individual differences in nasal pungency, aroma perception and liking, at least at the moderate concentrations used here. Results may be different at higher concentrations. When combined with ethnicity, however, PROP status unmasked differences in liking for cinnamaldehyde in East Asian subjects. Specifically, East Asian super-tasters disliked the aroma of cinnamaldehyde, whereas Caucasian super-tasters liked this aroma as well as Caucasian subjects in the other taster groups. Each aroma was unique and characterized by different mood(s) and sensory properties. These data suggest that elucidating the unique characteristics of each aroma is necessary for predicting consumer acceptance.

Our study was novel in that it linked PROP sensitivity and mood response to consumer acceptability of a range of pure aroma compounds in aqueous solutions. Future studies are needed to: 1) examine the emotion response and acceptability of complex aromas when they incorporated into real products such as foods, fine fragrances and other products; and 2) examine a array of chemically-related aroma compounds and how they impact hedonics and mood(s).

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APPENDIX I
Additional Tables

Table A. Eigenvalue, proportion, and cumulative values for PCA at low concentration.

	Initial			After Rotation		
	Eigenvalue	Proportion	Cumulative	Eigenvalue	Proportion	Cumulative
1	10.5645	0.7043	0.7043	10.2996	0.6866	0.6866
2	2.9731	0.1982	0.9025	3.2380	0.2159	0.9025
3	0.8606	0.0574	0.9599			
4	0.3566	0.0238	0.9836			
5	0.2453	0.0164	1.0000			

Table B. Eigenvalue, proportion, and cumulative values for PCA at mid-range concentration.

	Original			After Rotation		
	Eigenvalue	Proportion	Cumulative	Eigenvalue	Proportion	Cumulative
1	10.3908	0.6927	0.6927	10.387511	0.6925	0.6925
2	3.0640	0.2043	0.8970	3.067227	0.2045	0.8970
3	0.8188	0.0546	0.9516			
4	0.5914	0.0394	0.9910			
5	0.1350	0.0090	1.0000			

APPENDIX II
Consent form

ID# ___

CONSENT FORM – SCREENING PROCEDURE

Genetic Markers for Flavor & Aroma Perception and Mood

Principal Investigator: Beverly J. Tepper, Ph.D.
Sensory Evaluation Laboratory (Room 211)
Department of Food Science, Rutgers University
65 Dudley Road, New Brunswick, NJ 08901
848-932-5417 email: tepper@aesop.rutgers.edu

PURPOSE: Genetic differences in taste are believed to play an important role in the perception and liking of aroma and flavor. The overall goal of this project is to better understand how genes that control aroma and flavor perception differ among people. In order to participate in this research, you must complete a screening procedure to see if you quality for this study.

PROCEDURES: You will be asked to taste filter paper disks that may or may not have a taste to you. The ability to taste one of these substances (called PROP) is a genetic trait. You also will be asked to complete brief questionnaires about your health and eating habits. These activities will take ~10 min for you to complete. You will be notified whether or not you qualify for the main study.

RISKS: The activities you will be participating in pose no foreseeable risks to your health.

BENEFITS: Although you will receive no direct benefits from participating in this study, this research will benefit society by providing a better understanding of the genetic basis of aroma perception.

COMPENSATION: No monetary compensation will be provided to you for participating in the screening procedure.

CONFIDENTIALITY: The information collected in this screening will be kept strictly confidential. 'Confidential' means that your name and the information collected about you will be linked by a code number, and the code number will be used to identify your data. All data will be kept in a locked filing cabinet or on a pass-word protected computer in the Principal Investigator's laboratory. Only research staff involved in this study or the Institutional Review Board (a committee that reviews research studies in order to protect research participants) at Rutgers University will be allowed to see the data, except as may be required by law. If a report of this study is published, or the results are presented at a professional conference, only group results will be stated. You will not be personally identified in any report of this research. If you are not selected to participate in the main study or you decline to participate, the information you provide will be deleted.

YOUR RIGHTS AS A RESEARCH PARTICIPANT: Your participation in this screening is completely voluntary and you have the right to withdraw at any time without explanation or penalty.

DISCLAIMER: Rutgers University has made no general provision for financial compensation or medical treatment for any physical injury resulting from this research.

CONTACT INFORMATION: You can contact the Principal Investigator at the number listed above if you have any questions about this study.

If you have any questions about your rights as a research subject, you may contact the IRB Administrator at Rutgers University at:

Rutgers University, the State University of New Jersey
Institutional Review Board for the Protection of Human Subjects
Office of Research and Sponsored Programs
3 Rutgers Plaza
New Brunswick, NJ 08901-8559
Tel: 848-932-0150
Email: humansubjects@orsp.rutgers.edu

______________________	______________________
Name of participant (print)	Date
______________________	______________________
Signature of Participant	Signature of Investigator

Please confirm that you received a copy of this statement for your records ______
(initials)

This informed consent form was approved by the Rutgers Institutional Review Board for the Protection of Human Subjects on ______; approval of this form expires on ______.

ID# ___

CONSENT FORM

Genetic Markers for Flavor & Aroma Perception and Mood

Principal Investigator: Beverly J. Tepper, Ph.D.
Sensory Evaluation Laboratory (Room 211)
Department of Food Science, Rutgers University
65 Dudley Road, New Brunswick, NJ 08901
848-932-5417 email: tepper@aesop.rutgers.edu

PURPOSE: Genetic differences in taste are believed to play an important role in the selection of food, perfumes and personal care products. The overall goal of this project is to better understand how genes control our perception and liking of aromas. You are invited to participate in this research because you have already participated in a screening procedure and you qualify for this study.

PROCEDURES: You will be asked to participate in a brief practice session (~15 min) and two test sessions on different days. During the test sessions, you will be asked to smell and evaluate several aroma compounds and the moods associated with these aromas. Each test session will take ~30 minutes to complete. The sessions will be scheduled over a two week period.

RISKS: The activities you will be participating in pose no foreseeable risks to your health.

BENEFITS: Although you will receive no direct benefits from participating in this study, this research will benefit society by providing a better understanding of the genetic basis of aroma perception and mood.

COMPENSATION: At the completion of the study, you will receive a single payment of $ 30. If you withdraw from the study prior to its completion, your payment will be pro-rated for each session completed.

CONFIDENTIALITY: The information collected in this screening will be kept strictly confidential. 'Confidential' means that your name and the information collected about you will be linked by a code number, and the code number will be used to identify your data. All data will be kept in a locked filing cabinet or on a pass-word protected computer in the Principal Investigator's laboratory. Only research staff involved in this study or the Institutional Review Board (a committee that reviews research studies in order to protect research participants) at Rutgers University will be allowed to see the data, except as may be required by law. If a report of this study is published, or the results are presented at a professional conference, only group results will be stated. You will not be personally identified in any report of this research.

YOUR RIGHTS AS A RESEARCH PARTICIPANT: Your participation in this study is completely voluntary and you have the right to withdraw at any time without explanation or penalty.

DISCLAIMER: Rutgers University has made no general provision for financial compensation or medical treatment for any physical injury resulting from this research.

CONTACT INFORMATION: You can contact the Principal Investigator at the number listed above if you have any questions about this study.

Initials_______

ID# ___

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Email: humansubjects@orsp.rutgers.edu

Name of participant (print)	Date
Signature of Participant	Signature of Investigator

Please confirm that you received a copy of this statement for your records _______
(initials)

This informed consent form was approved by the Rutgers Institutional Review Board for the Protection of Human Subjects on ______; approval of this form expires on ______.

ID# ___

Genetic Markers for Flavor & Aroma Perception and Mood

Genetic Testing

Cells will be collected by gently brushing the inside of the cheek with a soft brush. There is no discomfort from this procedure. The genetic material you provide will allow the researchers to determine whether you are positive or negative for gene that controls bitter taste (PROP sensitivity) and other genes involved in nasal irritation. This information will help confirm the results of the behavioral tests and lead to a better understanding of the inheritance of these traits. The genetic material you provide will be used solely for this purpose and will not be sold or donated to a third party for unrelated purposes. This information will be kept strictly confidential with your identity protected by a code number. If you agree to participate in this procedure, you should sign and date below. If you decline to participate in this procedure you can still participate in the main study.

____________________ ____________

Signature of participant Date

APPENDIX III
Questionnaires

I.D. _____________
Date: _____________

Demographic and Health Information

Instructions

Please answer these questions about you to the best of your knowledge and make sure you answer every question. Thank you for your time.

A. GENERAL INFORMATION ABOUT YOU

Please provide the following information:

1. Name:

2. Date of birth: ☐☐ month ☐☐ day ☐☐☐☐ year

3. Age: ___________

4. Gender: ☐ 1 male ☐ 2 female

5. Contact Telephone Number:

6. Email Address:

7. Home Address:

8. Occupation:

9. Were you born in the United States? Yes ☐ 1 No ☐ 2

If "No," Please write in the country in which you were born: ___________________________

10. To which of the following races do you consider yourself to belong? (You may choose all that apply)

☐ 1 Black or African-American
☐ 2 White
☐ 3 American Indian or Alaska native
☐ 4 Asian or Pacific islander
☐ 5 Hispanic or Latino
☐ 6 Other *(please specify):*

Continued on next page

11. In addition, which of the following groups describes your ethnicity? (You may choose all that apply)

- ☐ 1 African ***(please specify):*** ______________________________
- ☐ 2 West Indian / Caribbean ***(please specify)***: __________________________
- ☐ 3 Mexican / Mexican-American/ Chicano
- ☐ 4 Puerto Rican
- ☐ 5 Cuban
- ☐ 6 Central American
- ☐ 7 Other Latino/Hispanic **(*please specify):*** ______________________________
- ☐ 8 Asian Indian
- ☐ 9 Japanese
- ☐ 10 Chinese
- ☐ 11 Korean
- ☐ 12 Filippino
- ☐ 13 Vietnamese
- ☐ 14 Other Asian, **(*please specify):*** ______________________________
- ☐ 15 Native Hawaiian
- ☐ 16 Guamanian or Chamorro
- ☐ 17 Samoan
- ☐ 18 Tongan
- ☐ 19 Other, **(*please specify):*** ______________________________
- ☐ 20 None of the above

B. HEALTH INFORMATION

12. Do you have a history of or are currently being treated for any of the following medical conditions? (Please check all that apply)

- ☐ 1 Diabetes (Type I or Type II)
- ☐ 2 Heart problems
- ☐ 3 Blood problems (haemophilia)
- ☐ 4 Kidney problems
- ☐ 5 Hypertension
- ☐ 6 Stroke
- ☐ 7 PKU (phenylketonuria)
- ☐ 8 Otiitis Media (chronic ear infection, especially as a young child)
- ☐ 9 Severe hayfever or allergies
- ☐ 10 Asthma
- ☐ 11 Cancer
- ☐ 12 Sinusitis

Continued on next page

13. Are you currently pregnant or nursing? (please check one)

☐1 YES ☐2 NO

14. Have you had a cold/flu or ear infection in the past 2 weeks? (Please check one)

☐1 YES ☐2 NO

If yes, please describe:

____

15. What, if any, prescription medications are you currently taking (including birth control) and how often?

____

16. Have you been to the dentist in the past 2 weeks? (Please check one)

☐1 YES ☐2 NO

17. Have you had hay fever/ nasal allergies in the past two weeks? (Please check one)

☐1 YES ☐2 NO

18. Do you dislike or avoid eating certain foods? (Please check one)

☐1 YES ☐2 NO

If yes, please describe:____

19. Do you have any food allergies? (Please check one)

☐1 YES ☐2 NO

If yes, please describe:____

20. How often do you try unfamiliar foods?

☐ Never ☐2 Rarely ☐3 Sometimes ☐4 Often ☐5 Very Often

Continued on next page

21. Have you taken multi-vitamins or vitamin A, C, or E supplements in the past month?

☐1 YES ☐2 NO

22. On average, how many hours do you sleep per night? _______________

23. Are you currently dieting to lose weight? (Please check one)

☐1 YES ☐2 NO

24. ***How many times have you been on a diet to lose weight over the past six months?________***

25. Have you unintentionally gained or lost more than five pounds in the past six months? (Please check one)

☐1 YES ☐2 NO

26. What is your current height?

☐ FT. ☐ IN. **OR** ☐ M.

27.What is your current weight?

☐ LBS. **OR** ☐ KG

28. What is the highest weight you have ever been?

☐ LBS. **OR** ☐ KG

29. What is the lowest weight you have ever been?

☐ LBS. **OR** ☐ KG

30. Do you currently smoke? (Please check one)

☐1 YES ☐2 NO

If yes, please specify cigarettes, cigar, or pipe:

____

Continued on next page

31. If you smoke, how many:

cigarettes per day? ___________

cigars per day? ______________

pipes per day? _______________

32. Have you smoked in the past?

☐ 1 YES ☐ 2 NO

If yes, how many years ago did you quit? ______________________

C. OTHER INFORMATION

Please answer the following questions about your family.

33.What is the highest education level you have finished? (Please "X" only one answer)

☐ 1 6th grade or less

☐ 2 8th grade or less

☐ 3 Attended some High School

☐ 4 High School Graduate or GED

☐ 5 Technical School

☐ 6 Some College

☐ 7 College Graduate

☐ 8 Post Graduate Study

34.What was the approximate total income, before taxes, of your household last year? Please include wages, salaries, social security, interest, child support, public assistance, unemployment compensation, rent from property and all other income.(Please "X" only one answer)

☐ 1 Less than $5,000

☐ 2 $5,000 - $9,999

☐ 3 $10,000 - $19,999

☐ 4 $20,000 - $29,999

☐ 5 $30,000 - $39,999

☐ 6 $40,000 - $49,999

☐ 7 $50,000 - $59,999

☐ 8 $60,000 - $69,999

☐ 9 $70,000 - $79,999

☐ 10 $80,000 - $89,999

☐ 11 $90,000 - $99,999

☐ 12 Over $100,000

Thank you. You are done with this form. Please return this form to the test administrator.

I.D. ______________
Date: ______________

Paper Disc Samples

Instructions:

You will receive two paper discs to taste. Rinse your mouth thoroughly with water before you begin. Place the disc that matches the number below on the tip of the tongue for 30 second or until it is wet. Rate the **intensity of the taste** of the paper disc by drawing a mark on the scale for your answer. You can draw your mark on any place on the scale. For the next sample, go to the next page.

First Sample:

Strongest Imaginable

Very Strong

Strong

Moderate

Weak

Barely Detectable

I.D. ________________
Date: ________________

Please rinse with water and wait for 45 seconds before you begin.

Second Sample:

Strongest Imaginable

Very Strong

Strong

Moderate

Weak

Barely Detectable

APPENDIX IV
FIZZ Network Ballot

Training Session

Welcome to the Sensory Evaluation Training Session on Flavor and Fragrance!

Instructions

You will be given a picture to rate and to familiarize yourself with the ballot you will be using later in this study.
Please read and follow all of the instructions carefully!
(Next page)

Pictures or songs have moods. It is possible to say "what a happy picture" or "that is a sad song".
As you look at the picture, think about its unique mood "**signature**".
(Next page)

Please look at this picture and click "Next Screen" to evaluate.
(Next page)

Please check the term that "mostly" describes the mood of the picture. If it does not evoke any emotion for you, please give the best guess:

❑Anxious or worried ❑Frustrated or angry

❑Calm and relaxed ❑Attentive and interested

❑Depressed or upset ❑Embarrassed or ashamed

❑Stressed ❑Pleasant and confident

❑Exciting and energizing

(Next page)

Please check the minor moods of the scent (Check as many as apply):

The mood of this scent is	Very little or none	Not much	Somewhat
Anxious or worried	❑	❑	❑
Frustrated or angry	❑	❑	❑
Calm and relaxed	❑	❑	❑

Attentive and interested	❑	❑	❑
Depressed or upset	❑	❑	❑
Embarrassed or ashamed	❑	❑	❑
Stressed	❑	❑	❑
Pleasant and confidant	❑	❑	❑
Exciting and energizing	❑	❑	❑

(Next page)

Finally rate your own mood. Does the odor affect you?

The mood of this scent is	Very little or none	Not much	Somewhat	Mostly
This odor affects my mood	❑	❑	❑	❑

(Next page)

Let’s review – did you check one mood that “mostly” describes the odor?
Did you check one or more minor moods?
Did you rate your own mood?
(Next page)

Thank you!
You have finished Session I.
Please notify your server!
(Next page)

Session I

Welcome to the Sensory Evaluation Session I on Flavor and Fragrance!

Instructions

You will be given six samples of aroma to smell **one at a time**.
You will evaluate each sample for the **overall intensity** and **overall liking**.
Then you will give "**mood signatures**" to the aroma.
Please read and follow all of the instructions carefully!
(Next page)

Here we are asking about how you feel: please indicate the extent to which each of the following words or phrases reflects your current state:

	Not at all	Little			Moderate			Extremely
Enjoyment/Happiness	0	1	2	3	4	5	6	7
Interest/Excitement	0	1	2	3	4	5	6	7
Surprise	0	1	2	3	4	5	6	7
Sadness/Despair	0	1	2	3	4	5	6	7
Anger	0	1	2	3	4	5	6	7
Contempt/Disgust	0	1	2	3	4	5	6	7
Fear	0	1	2	3	4	5	6	7
Shame/Shyness	0	1	2	3	4	5	6	7
Anxiety	0	1	2	3	4	5	6	7
Tension	0	1	2	3	4	5	6	7
Frustration	0	1	2	3	4	5	6	7
Guilt	0	1	2	3	4	5	6	7

(Next page)

Make sure the code of the sample you are sniffing matches the code on the screen!
Please open the lid and smell the sample and then place the lid back on immediately.
Please place a single mark on the line scale below corresponding to your answer.
How strong is the **OVERALL INTENSITY** of the aroma?

None **Very Strong**

(Next page)

Make sure the code of the sample you are sniffing matches the code on the screen!
Please rate your **OVERALL LIKING** of the aroma:

Please rate the **OVERALL LIKING** of the sample:

Extremely Dislike **Extremely Like**

(Next page)

Please write comments or other perceptions in the text box below:

K

(Next page)

Many scents have moods just as many pictures or songs have moods. It is possible to say "what a happy picture" or "that is a sad song". The same is true for aromas. You can say "what a depressing smell". Some aromas are happy or angry or smoothing or sad maybe a little of several moods.
As you sniff each aroma, think about its unique mood "**signature**".
(Next page)

After you sniff the aroma, first decide what mood it "mostly" conveys, and check that box. Even if you think you are guessing, check one box as "mostly" the right mood for that aroma.
(Next page)

Second, consider whether the aroma has other minor moods. Just as a song can be scary and humorous, an aroma can be two or more moods. These moods might seem related or opposites - you can choose what fits the best. To show this, check other moods as "somewhat" or "not much" or "very little" (you can check multiple boxes)

Before you check the categories, read all the possibilities. You will notice that each selection has two descriptions such as "anxious or worried". If either or the words (for example worried) is a good fit for the aroma you can choose it and ignore the other word (for example, anxious).
(Next page)

Please check the term that "mostly" describes the mood of the aroma. If it does not evoke any emotion for you, please give the best guess:

❑Anxious or worried	❑Frustrated or angry
❑Calm and relaxed	❑Attentive and interested
❑Depressed or upset	❑Embarrassed or ashamed
❑Stressed	❑Pleasant and confident
❑Exciting and energizing	

(Next page)

Please check the minor moods of the scent (Check as many as apply):

The mood of this scent is	Very little or none	Not much	Somewhat
Anxious or worried	❑	❑	❑
Frustrated or angry	❑	❑	❑
Calm and relaxed	❑	❑	❑
Attentive and interested	❑	❑	❑
Depressed or upset	❑	❑	❑
Embarrassed or ashamed	❑	❑	❑
Stressed	❑	❑	❑
Pleasant and confidant	❑	❑	❑
Exciting and energizing	❑	❑	❑

(Next page)

Finally rate your own mood. Does the odor affect you?

The mood of this scent is	Very little or none	Not much	Somewhat	Mostly
This odor affects my mood	❑	❑	❑	❑

(Next page)

Let’s review – did you check one mood that “mostly” describes the odor?
Did you check one or more minor moods?
Did you rate your own mood?
Good, go to the next page.
(Next page)

Here we are asking about <u>how you feel</u>: please indicate the extent to which each of the following words or phrases reflects your <u>current</u> state:

	Not at all	Little		Moderate			Extremely	
Enjoyment/Happiness	0	1	2	3	4	5	6	7
Interest/Excitement	0	1	2	3	4	5	6	7
Surprise	0	1	2	3	4	5	6	7
Sadness/Despair	0	1	2	3	4	5	6	7
Anger	0	1	2	3	4	5	6	7
Contempt/Disgust	0	1	2	3	4	5	6	7
Fear	0	1	2	3	4	5	6	7
Shame/Shyness	0	1	2	3	4	5	6	7
Anxiety	0	1	2	3	4	5	6	7
Tension	0	1	2	3	4	5	6	7
Frustration	0	1	2	3	4	5	6	7
Guilt	0	1	2	3	4	5	6	7

(Next page)

Before evaluating the next sample you must: wait 60 seconds
Once the 60 seconds have passed, **please notify your server!**
1:00 countdown
(Next page, proceed to the rest of the samples)

Thank you!
You have finished Session I.
Please notify your server!

Session II

Welcome to the Sensory Evaluation Session II on Flavor and Fragrance!

Instructions

You will be given six samples of aroma to smell **one at a time**.
You will evaluate each sample for the **intensity** of several attributes, select **descriptor(s)** for each sample, and rate the **overall liking**.
Then you will give "**mood signatures**" to the aroma.
Please read and follow all of the instructions carefully!
(Next page)

Here we are asking about how you feel: please indicate the extent to which each of the following words or phrases reflects your current state:

	Not at all	Little			Moderate			Extremely
Enjoyment/Happiness	0	1	2	3	4	5	6	7
Interest/Excitement	0	1	2	3	4	5	6	7
Surprise	0	1	2	3	4	5	6	7
Sadness/Despair	0	1	2	3	4	5	6	7
Anger	0	1	2	3	4	5	6	7
Contempt/Disgust	0	1	2	3	4	5	6	7
Fear	0	1	2	3	4	5	6	7
Shame/Shyness	0	1	2	3	4	5	6	7
Anxiety	0	1	2	3	4	5	6	7
Tension	0	1	2	3	4	5	6	7
Frustration	0	1	2	3	4	5	6	7
Guilt	0	1	2	3	4	5	6	7

(Next page)

Make sure the code of the sample you are sniffing matches the code on the screen!
Please open the lid and smell the sample and then place the lid back on immediately.
Please place a single mark on the line scale below corresponding to your answer.
How strong is the **OVERALL INTENSITY** of the aroma?

None **Very Strong**

(Next page)

Make sure the code of the sample you are sniffing matches the code on the screen!
Nasal pungency is a sharp irritation in the nose that is given by smelling or tasting food, or smelling cosmetic products. For example, products that can give this sensation are chili pepper, citronella candles, cinnamon, etc.
How strong is the **PUNGENCY LEVEL** of the aroma?

How strong is the **PUNGENCY LEVEL** of the sample?

None **Very Strong**

(Next page)

Make sure the code of the sample you are sniffing matches the code on the screen!
Check the **descriptor(s)** that best describe the aroma (Check as many as apply):

- ❑ Citrus
- ❑ Floral
- ❑ Sweet
- ❑ Minty
- ❑ Musty
- ❑ Cinnamon
- ❑ Spicy
- ❑ Rose
- ❑ Foul
- ❑ Other:

Please write comments or other perceptions in the text box below:

K

(Next page)

Make sure the code of the sample you are sniffing matches the code on the screen!
How **confident** are you on the descriptor(s) that you checked in previous question?

Not Confident **Very Confident**

(Next page)

Make sure the code of the sample you are sniffing matches the code on the screen!
Please rate your **OVERALL LIKING** of the aroma:

Extremely Dislike **Extremely Like**

(Next page)

What does the aroma remind you of? (e.g., you can say it reminds you of a food/non-food product, a place, or an experience, etc.)

K

(Next page)

Many scents have moods just as many pictures or songs have moods. It is possible to say "what a happy picture" or "that is a sad song". The same is true for aromas. You can say "what a depressing smell". Some aromas are happy or angry or smoothing or sad maybe a little of several moods.
As you sniff each aroma, think about its unique mood "**signature**".
(Next page)

After you sniff the aroma, first decide what mood it "mostly" conveys, and check that box. Even if you think you are guessing, check one box as "mostly" the right mood for that aroma.
(Next page)

Second, consider whether the aroma has other minor moods. Just as a song can be scary and humorous, an aroma can be two or more moods. These moods might seem related or opposites - you can choose what fits the best. To show this, check other moods as "somewhat" or "not much" or "very little" (you can check multiple boxes)

Before you check the categories, read all the possibilities. You will notice that each selection has two descriptions such as "anxious or worried". If either or the words (for example worried) is a good fit for the aroma you can choose it and ignore the other word (for example, anxious).
(Next page)

Please check the term that "mostly" describes the mood of the aroma. If it does not evoke any emotion for you, please give the best guess:

- ❑Anxious or worried
- ❑Calm and relaxed
- ❑Depressed or upset
- ❑Stressed
- ❑Exciting and energizing
- ❑Frustrated or angry
- ❑Attentive and interested
- ❑Embarrassed or ashamed
- ❑Pleasant and confident

(Next page)

Please check the minor moods of the scent (Check as many as apply):

The mood of this scent is	Very little or none	Not much	Somewhat
Anxious or worried	❑	❑	❑
Frustrated or angry	❑	❑	❑
Calm and relaxed	❑	❑	❑
Attentive and interested	❑	❑	❑
Depressed or upset	❑	❑	❑
Embarrassed or ashamed	❑	❑	❑
Stressed	❑	❑	❑
Pleasant and confidant	❑	❑	❑
Exciting and energizing	❑	❑	❑

(Next page)

Finally rate your own mood. Does the odor affect you?

The mood of this scent is	Very little or none	Not much	Somewhat	Mostly
This odor affects my mood	❑	❑	❑	❑

(Next page)

Let’s review – did you check one mood that “mostly” describes the odor?
Did you check one or more minor moods?
Did you rate your own mood?
Good, go to the next page.
(Next page)

Here we are asking about <u>how you feel</u>: please indicate the extent to which each of the following words or phrases reflects your <u>current</u> state:

	Not at all	Little			Moderate			Extremely
Enjoyment/Happiness	0	1	2	3	4	5	6	7
Interest/Excitement	0	1	2	3	4	5	6	7
Surprise	0	1	2	3	4	5	6	7
Sadness/Despair	0	1	2	3	4	5	6	7
Anger	0	1	2	3	4	5	6	7
Contempt/Disgust	0	1	2	3	4	5	6	7
Fear	0	1	2	3	4	5	6	7
Shame/Shyness	0	1	2	3	4	5	6	7
Anxiety	0	1	2	3	4	5	6	7
Tension	0	1	2	3	4	5	6	7
Frustration	0	1	2	3	4	5	6	7
Guilt	0	1	2	3	4	5	6	7

(Next page)

Before evaluating the next sample you must: **<u>wait 60 seconds</u>**
Once the 60 seconds have passed, **please notify your server!**
1:00 countdown
(Next page, proceed to the rest of the samples)

Thank you!
You have finished Session II.
Please notify your server!