SENSORY EVALUATION OF FLAVORED BEVERAGES WITH COOLING INGREDIENT BLENDS

By

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ABSTRACT OF THE THESIS

Sensory evaluation of flavored beverages with cooling ingredient blends

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Derivatives of l-menthol are cooling ingredients that are widely used in confectionery, personal care, cosmetics, and pharmaceuticals. However, the psychophysics of cooling is rather complex and the sensory perception of cooling ingredients is not well understood.

We aimed to characterize the oral sensation of two different cooling ingredient blends in flavored beverages. In addition, we assessed the role of ethnicity, gender and familiarity in the sensory perceptions and hedonic reactions of flavored beverages containing cooling ingredient blends. 116 healthy subjects who were East Asian (n=54), Caucasian (n=43) or other (n=19) participated in the study. The stimuli were lemon-lime flavored beverages with two novel cooling ingredient blends: Coolact® 38D /Frescolat® ML blend or Coolact ®5 /Coolact ®10 blend. Each blend was tested at four concentrations: 0 (control), 75, 150, 300 ppm. Subjects rated intensity and liking of each sample for cooling, heat/burning, tingling, sweetness, bitterness and overall flavor on a

15-cm line scale at four time points over a 10-min period (0, 2.5, 5 and 10 min after tasting).

The intensity of all attributes was maximal immediately after tasting ($P < 0.0001$) and decreased with time ($P < 0.0001$). Both blends primarily delivered the sensations of cooling and tingling with minimal perception of heat/burning and bitterness. At time 0, East Asians perceived more heat/burning than Caucasians ($P < 0.01$). Female perceived more cooling from taste samples than male ($P = 0.004$). Also, subjects who were familiar with flavored beverages containing cooling ingredients (n=60) perceived more cooling, heat/burning and tingling from Coolact ®5 / Coolact ®10 blend ($p < 0.0001$) and more cooling ($p < 0.001$) and heat/burning ($p < 0.01$) from Coolact® 38D /Frescolat® ML blend compared to subjects who were not familiar with these products (n=56). In addition, our results demonstrated that subjects who had prior experience with cooling ingredient beverages had better ability to discriminate the concentration differences of cooling blends.

Our data suggest that the intensity of cooling ingredients in beverages is influenced by ethnicity, gender and prior experience with these types of beverages. These factors should be considered in future psychophysical studies of cooling ingredients and related product applications.

Dedication

I lovingly dedicate this thesis to my beautiful mother, who is the most important person in my whole life.

Acknowledgements

I owe my deepest gratitude to Dr. Beverly Tepper for being a fabulous advisor and mentor. She was always there whenever I needed advice or help. I appreciate very much all she has done to support and to encourage me to complete my research. In addition, she taught me to think independently and developed my ability of communication. It is a great honor to work with her for three years.

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Last but not the least; I would like to thank my family and friends for their care, support and love. Thank you for respecting my thoughts, my feelings, my doings and my life.

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List of Abbreviations

C	Caucasian
EA	East Asian
F	Familiar group
NF	Non- familiar group
NS	Not Significant
M	Mean Value

1. Introduction and Literature Review

1.1 Menthol background

Menthol is a naturally occurring compound found in the leaves of genus *Mentha,* especially in mint oils from *Menthapeperita* (peppermint oil) and *Menthaarvensis* (cornmint oil). Dating back to the late 1880s, commercial production of menthol from Japanese peppermint oil was used as cooling agents in cosmetic and pharmaceutical preparation. In the late 1950s and 1960s, there was an increasing interest in menthol-related cooling agents when some tobacco companies began to develop a variety of esters as potential menthol release agents (Eccles 1994).

Menthol is a $C_{10}H_{20}O$ terpenoid alcohol (MW 156.27) with three chiral centers leading to eight possible stereoisomers. Their structures are shown in Figure 1.1. However; only the (-)-menthol enantiomer, which is most widely found in nature, provides the desirable minty odor and intense cooling sensation. Other isomers of menthol do not have the same odor and cooling sensation as the (-)-menthol enantiomer. For instance, (+)-menthol enantiomer possesses an undesirable musty off note odor and delivers less cooling sensation (Eccles 1994; Leffingwell et al. 2009).

Menthol is the most widely used cooling agent in confectionery, tobacco, pharmaceuticals, oral care and other products. The effect of menthol depends on concentration, with a pleasant cooling sensation at low levels and inherent burning and bitterness at high concentrations, which are undesirable in most applications (Furrer et al. 2008). In addition, menthol is relatively volatile (vapor pressure 8.5 Pa at 25°C) and can lead to some side effects, such as eye irritation causing extreme tearing (Dewis 2005). Due to these disadvantages, its applications are somewhat limited.

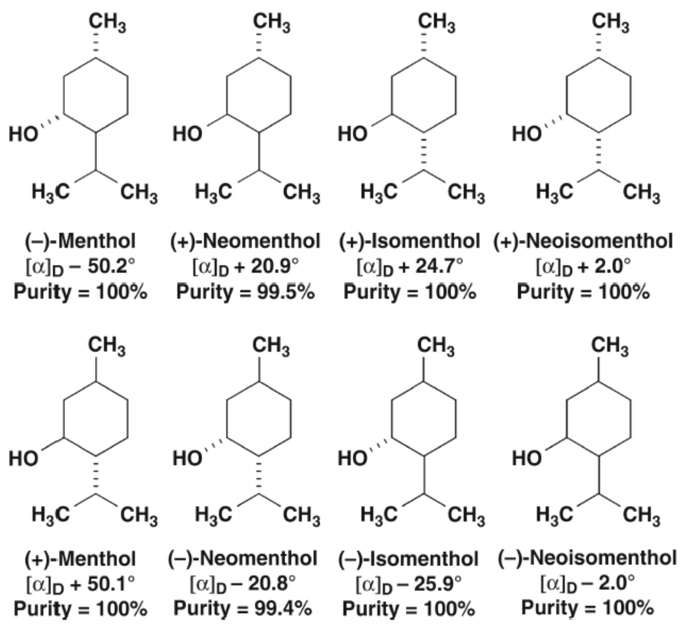


Figure 1.1 Stereoisomers of menthol (Leffingwell et al. 2009)

1.2 New development in the chemistry of cooling compounds

Over the last 30 years, a considerable number of cooling compounds have been synthesized (Eccles 1994; Leffingwell 2011). Commercially available cooling agents with FEMA (Flavor and Extracts Manufactures Association) GRAS (Generally Recognized As Safe) status can be divided into two major families: menthol-related cooling agents and carboxamide cooling agents (Leffingwell 2009).

In the family of menthol related cooling agents, l-menthyl lactate (FEMA 3748) is one of the most important cooling ingredients. L-menthyl lactate possesses a slight minty odor, but is tasteless with a pleasant and long-lasting cooling effect. Another example is 3-(l-Menthoxy) propane-1, 2-diol, known as Coolact®10 (Takasago International Corp), which is virtually odorless and shows 2.0-2.5 times stronger cooling sensation than (-)-menthol (Shiroyama 2001).

The discovery of carboxamides cooling ingredients dates back to the1970s. There was a strong commercial need for suitable cooling ingredients that deliver a pleasant cooling sensation without a minty flavor and other side effects of menthol. Laboratory research scientists at Wilkinson Sword Ltd. conducted extensive studies to discover and develop this new family of cooling agents, the menthane carboxamides (Stefan et al. 2008). Initially, WS-3® (N-ethyl-p-menthane-3-carboxamide) and WS-23 ®(menthane-3-carboxamide) and WS-14 ®[N- ([ethoxycarbonyl] methyl)-p-menthane-3-carboxamide] were commercialized. Currently, WS-5® [ethyl 3-(p-menthane-3-carboxamido) acetate] is the coldest of all commercial cooling agents. WS-3® and WS-23® are the two most commonly used cooling ingredients (Leffingwell 2009). WS- 5® and WS-3®are widely used in oral care products (Figure 1.2).

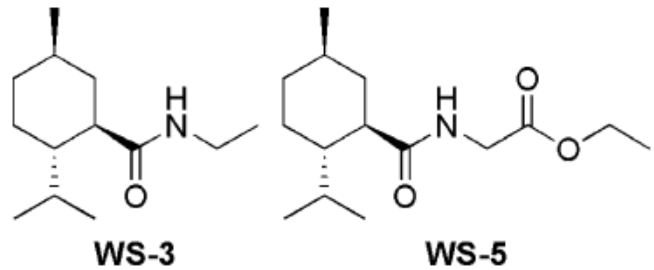


Figure 1.2 Chemical structures of WS-3 and WS-5

1.3 Cooling ingredients and their applications

Natural cooling ingredients such as menthol and peppermint oil have been used to provide a cooling sensation along with providing a minty flavor for a long time. Originally, the main end-application for cooling compounds was chewing gum and mints because of their strong minty flavor. Therefore, there is a strong need to develop alternative cooling ingredients without "burn", bitterness and minty flavor.

Many synthetic compounds providing cooling sensations have been known and used in the flavor and fragrance industry. Generally, cooling agents are soluble in flavor and fragrance oils and bases. The usage levels of cooling agents vary widely depending on the application. For flavors, the levels in toothpaste and chewing gum are the highest, followed by confectionary and tablets. Soft drinks and other water-based products have the lowest usage levels. Because of lower or absence of minty taste and bitterness, novel cooling ingredients can be used in non- mint flavoring systems. Currently, cooling agents are widely used in cosmetics, oral care (mouthwash/toothpaste), shampoo, chewing gums, pharmaceuticals, and alcoholic beverages (Dewis 2005).

Combinations of cooling ingredients with different sensory properties have been used in the industry to achieve very precise cooling temporal profiles. Synergies exist between cooling compounds to produce a particular set of sensation (Dewis 2005). Using cooling blends is a good solution to enhance positive sensory attributes while reducing the negative ones. For example, cooling blends can deliver a stronger cooling sensation than single cooling agents alone (Stefan 2008).

1.4 Cooling sensation

1.4.1 Trigeminal nerve

The Trigeminal nerve, known as the fifth cranial nerve, is the largest cranial nerve and the primary nerve responsible for sensation in the face and head. This nerve has three branches: the ophthalmic, the maxillary and the mandibular. The three braches come together at the trigeminal ganglion (Dewis 2005).

A variety of chemicals can stimulate trigeminal nerve endings in the mouth, nose or eyes and induce a range of sensations. The trigeminal nerves can carry the cooling sensations from menthol and other cooling agents, the heat irritation from chili pepper and hot tea, the astringency from red wine and black tea or tingling sensation from carbonated beverages and mint. Many of these chemesthetic sensations are mediated by a family of receptor proteins called Transient Receptor Potential (TRP) channels (Silver 2008).

1.4.2. Temperature cooling sensation

There are two different types of cooling sensations: temperature cooling and chemical cooling. These sensations are typically associated with cleanliness and freshness (Erman 2007).

In general, most cold sensitive neurons in the human body are sensitive to decreases in temperature (McKemy 2007). The sensation of non-painful coldness is reported to appear when human skin is cooled as little as 1 °C from normal body temperature (Campero et al. 2001). Psychophysiological studies indicate that phasic temperature decreases from 22°C and 16 °C produced cooling sensation, whereas decreases from 10°C and 6 °C produced painful sensation that is mediated through a different receptor system (Chen et al. 1996; Dewis 2005). Morin and Bushnell (1998) found the perception of cold pain that is described as burning, aching and pricking is evoked once temperatures are reduced to 15°C. Compared to skin, the human mouth is far more sensitive to cooling (Dewis 2005).

1.4.3 Chemical cooling sensation

Most of the cold sensitive neurons are also sensitive to cooling agents such as menthol and icilin (McKemy 2007). Different concentrations of cooling agents can elicit different intensities of cooling sensations. Moderate concentrations of menthol can deliver a pleasant sensation of coolness; however higher doses can cause noxious cold with qualities of pain, burning and aching (McKemy 2007). Studies show that these sensations are associated with the presence of two types of receptors: receptors for innocuous cold that mediate coldness and receptors for noxious cold that medicate pain (Sawada et al.2007).

1.5 Potential cooling activated receptors

1.5.1 TRPM8: the minty-cool ion channel

Although it has long been known that menthol produces a sensation of coldness, the molecular site of this action was poorly understood. Hensel and Zotterman (1951) put forward a hypothesis that menthol elicits cooling sensation by increasing the temperature threshold and that it exerts its actions on a protein that is specifically involved in cold transduction. The recent identification of several cooling- activated ion channels gives a molecular framework to better understand the transduction mechanisms for cold temperature. Hensel and Zotteman's original hypothesis was finally confirmed in 2002 by the concurrent cloning of a cold- and menthol- sensitive ion channel by two independent groups (McKemy 2002; Peier et al. 2002). The first group used menthol to expression-clone a complementary DNA (cDNA), from rat trigeminal (TG) ganglion neurons that could confer menthol sensitivity in heterologous expression systems (Mckemy 2002). The other group used a genomics approach to identify TRP channels expressed in mouse cultured dorsal root (DRG) neurons (Peier et al. 2002). Surprisingly, both groups identified transient receptor potential melastatin 8 (TRPM 8), an ion channel of the transient receptor potential (TRP) sub family.

TRPM8 is considered the primary receptor candidate to explain cold transduction since it is activated by low temperatures (threshold of ~25 °C) and by cooling compounds such as menthol (Madrid et al., 2006). In cold-sensitive (CS) primary sensory neurons, cooling opens a cation current (I_{cold}) with properties consistent with those of TRPM8-dependent currents in transfected cells. Also, the same neurons are excited by menthol (McKemy et al., 2002; Reid et al., 2002). However, it is not known if TRPM 8 is localized to nerve terminals in the trigeminal ganglia (TG). Abe et al. (2005) found that TRPM 8 was expressed in lingual nerve fibers reaching fungiform papillae of the tongue, but weakly expressed in foliate and circumvallate papillae.

In addition to menthol and icilin (AG-3-5), Behrendt and his colleagues (2004) identified ten substances, including linalool, geraniol, hydroxycitronellal, WS-3 (Givaudan), WS-23, Frescolat MGA, Frescolat ML (Haarmann & Reimer GmbH), PMD38, Coolact P and Cooling Agent 10 (Takasago) as TRPM 8 agonists in vitro. Among these ten substances, WS-3, Coolact P, Cooling Agent 10, and PMD38 are used as cooling agents in the food and cosmetics industry. It is entirely possible that TRPM8 partly mediates their cooling sensation.

1.5.2 TRPA 1, a noxious cold sensor?

TRPA 1(Transient receptor potential cation channel, subfamily A, member 1), another potential cooling-activated receptor, has been postulated to mediate our perception of noxious cold temperature; however many conflicting reports have suggested that the role of the TRPA 1 in cold sensation needs to be confirmed.

Story et al. (2003) first found that a TRP-like channel TRPA 1(or ANKTM1) was activated by noxious cold temperatures. Later, it was determined that noxious cold increased cytosolic Ca^{2+} levels and elicited whole-cell current in TRPA 1 expressing Chinese hamster ovary (CHO) cells as well as dorsal root ganglion neurons. Thus, one group of researchers holds the view that TRPA 1 is a deep cooling-activated channel (Story et al. 2003; Bandell et al. 2004; Macpherson et al. 2006; Karashima et al. 2008). In contrast, other research groups concluded that TRPA 1 is not activated by deep cooling (Jordt et al. 2004; Nagata et al.2005; Bautista et al. 2006).

In addition, mouse behavioral experiments withTRPA1 knock-out animals are also conflicting with some studies showing a deficit and some showing no deficits in these animals. Bautista et al. (2006) suggested that TRPA 1 knockout mice had normal sensitivity to cooling. They found no difference in the latency of first paw lift or first shiver. Nevertheless, Karashima et al. (2009) reported that TRPA1-deficient mice lost a specific subset of cold-sensitive trigeminal ganglion (TG) neurons. They found that wild-type mice always jump with a latency of 20 s on a cold plate chilled to 0 °C; however, only 12% of TRPA $1^{-/-}$ mice jumped, with a latency three times longer. In a tail-flick experiment, wild-type mice flicked their tails out of a -10 °C solution in 10-15 s. However, one third of TRPA $1^{-/-}$ mice did not response at all, and the rest took 30 – 40 s to flick their tails out (Karashima et al. 2009).

1.6 Current research on sensory evaluation of cooling ingredients

Although cooling ingredients such as menthol have been utilized for many years, the sensory perception of cooling ingredients is complex and not completely understood. Cliff and Green (1994) conducted an experiment to understand the temporal characteristics of the oral perception of menthol solutions. Fifteen adults received ten menthol solutions of either 0.03% or 0.3% at 1- min intervals and rated the perceived intensity of cooling and irritation using the oral labeled magnitude scale (LMS). Panelists also reported the quality of irritation by choosing descriptors: burning, tingling, stinging/pricking, numbing, aching, itching and pain. This experiment revealed that cooling was the dominant sensation at a relatively low concentration of 0.03%, while at the higher concentration of 0.3%, irritation was dominant. They also found that the intensity of burning and stinging sensations increased at the higher concentration. In addition, a significant decrease in perceived intensity of irritation over time was perceived at higher concentration, but not for the cooling.

Gwartney and Heymann (1994) did a study to describe and compare the temporal properties of l-and d-menthol. Eleven trained panelists using time intensity methodology evaluated the cooling, heat/burning and bitterness of two menthol isomers (l-, d-) each at four concentrations [0.01, 0.02, 0.04 and 0.08% (w/v)] in aqueous solution. Subjects put the entire 15 ml sample in the mouth at time 0 and rated attribute intensities on an unstructured line scale anchored with “none” and “extreme”. Samples were expectorated at 10 s and panelists continued to rate attribute intensity until termination of sensation. They found that l-menthol samples had a greater maximum intensity and longer total duration of cooling and burning compared to the d-menthol samples. Additionally, maximum intensity and total duration of cooling, burning and bitterness increased with concentration.

The studies by Gwartney and Heymann (1994) and Cliff and Green (1994), cited above, were the first experiments looking at temporal perception of menthol over the time. This motivated our laboratory to look at how sensory characteristics of cooling ingredients change over a period of time. Tepper et al. (2007, 2008 and 2010) evaluated the sensory perception and characterized eight cooling ingredients:

- Coolact 5®,
- Coolact 10®,
- Coolact 38D®,
- (L)-menthy lactate,
- Mono – (l) menthyl succinate,
- Mono- (l)-menthylglutarate
- Coolact 5® /Coolact 10® blend
- Coolact 38D®/ (l)-menthy lactate blend.

Subjects rated three concentrations (75, 150 and 300 ppm) of each compound using 15-cm line scales at four time points over a 10-min period (0, 2.5, 5 and 10 min). They also indicated the locations (lips, tongue, gums, roof of the mouth, cheeks, throat, and nose) where they perceived attributes (cooling, heat/burning, tingling and bitterness). All taste stimuli were tested in solutions. They found that cooling and tingling were the predominant attributes across cooling ingredients; heat/burning and bitterness were minimal.

Based on anecdotal evidence from Takasago Intl. Corp (unpublished), Tepper et al. (2008, 2010) also investigated and found that East Asians perceived more heat/burning from the samples and perceived most of the attributes in more locations in the mouth compared to Caucasians. These findings indicated that the role of ethnicity might affect the sensory perception of cooling ingredients.

1.7 Cross-cultural sensory studies

Cross-cultural studies make direct comparisons between cultural groups, by investigating individuals in cross national surveys and migration studies, and by collecting units in holocultural comparisons and globalization analyses. Holocultural analysis aims to identify cultural traits in different cultures, and then compares the cultural characteristics across cultures. The overall idea is to examine the patterns of cultural characteristics (Sobal 1998). Globalization analyses attempts to examine the world patterns of change over the time (McMichael 1990). There has been little research conducted on cross-cultural differences, particularly with respect to foods. However, there is an increasing interest in cross-cultural research since the international market in processed foods between Western and Asian countries is growing (Jaeger *et al*. 1998). Understanding cultural values is very important to help companies understand consumer preferences in different countries. There are reasons to believe that culture influences consumer perceptions of sensory qualities that could underline differences in taste or food preference. Therefore, cross-cultural research is an essential step to ensure that product development is targeted and focused.

For example, Druz and Baldwin (1982) found that Koreans and Nigerians preferred apple sauce and tomato juice sweetened with 8% and 2% sucrose, while North American Caucasians preferred the unsweetened products.

Bertino et al. (1983) compared the sweetness intensity and pleasant ratings of U.S. students of European ancestry and Taiwanese students of Chinese ancestry for cookies in which sucrose levels ranging from 8 to 37% wet weight of the ingredients. The Taiwanese panelists rated sucrose as tasting sweeter, either in solution or in a cookie, than U.S. students. However, the hedonic reaction of the Taiwanese group to cookies was sucrose concentration dependent. They rated the lower concentrations of sucrose in the cookies as more pleasant than the U.S. group and gave lower hedonic ratings at higher concentrations.

Prescott et al. (1997) examined Japanese and Australian panelists responses to sweetness within three foods – orange juice, cornflakes and ice cream. All the subjects were instructed to give ratings of sweetness intensity, sweetness liking, sweetness just right, and overall liking of all samples. There were few cross-cultural differences in the sensory perception of sweetness intensity. However, hedonic responses to manipulating sucrose levels in the three foods indicated cross- cultural differences, with the pattern of responses varying from food to food.

Jaeger *et al.* (1998) examined sensory preferences for three apple varieties varying in degree of mealiness among British and Danish consumers. Mealiness was considered a negative quality attribute associated with floury and granular texture. There was no evidence of cross-cultural differences in consumer preferences for apples.

1.8 Current research on the effect of familiarity with test products

Some authors suggest that familiarity with test products might be a major influence in cross-cultural research. In the study by Bertino et al. (1983) cited earlier, the authors suggested that U.S. students rated cookies as more pleasant, due to their greater familiarity with this type of baked food with a high sucrose concentration compared to Taiwanese students. In addition, Taiwanese students gave higher pleasantness ratings to the NaCl solutions because they were more familiar with salty solutions, such as soy sauce, in Chinese cuisine.

Laing et al. (1994) compared the responses of Japanese and Australians to the sweetness of 36 products from 6 food categories, namely beverages, cereals, chocolates, biscuits, fruit juices and jams. They found that consumers gave higher liking and sweetness liking ratings to foods from their own culture. This indicated that liking for the sweetness levels may be dependent on the familiarity with the products. It is likely that the lack of familiarity with a product may reduce 'sweetness liking' ratings.

Another example is a cross-cultural study conducted by Prescott et al. (1997) who found that Australian panelists gave higher sweetness hedonic ratings than Japanese panelists to four cornflake samples at varying sucrose levels. Cornflakes are less familiar to Japanese consumers. In contrast, Australian and Japanese consumers gave similar liking ratings to orange juices and ice cream that are both highly familiar products.

Similarly, Pages et al. (2007) compared the hedonic responses of eight biscuits from France and Pakistan by consumers in both countries. They found that consumers liked biscuits from their own country more than biscuits from the other country.

To sum up, it is necessary to take the role of product familiarity into consideration when conducting cross-cultural research.

1.9 Objectives and Hypothesis

To my knowledge, no one has investigated intensity ratings of cooling ingredients over the time in a beverage, or the effects of ethnicity and prior experience on the sensory perception of cooling ingredient beverages. By looking at a beverage (instead of solutions), we can also address hedonic reactions. These are the motivations for conducting the current study.

Objective 1: to characterize the oral sensation of two different cooling blends in lemon-lime flavored beverages. Time-intensity studies will be utilized to characterize the time course, intensity and liking of each perceived attribute

Objective 2: to assess the role of ethnicity and gender in the sensory perception of flavored beverages with cooling ingredients. This study will compare East Asian subjects to Caucasian subjects with respect to attribute ratings

Objective 3: to determine whether familiarity with beverages containing cooling ingredients influences sensory perceptions and hedonic reactions

Hypothesis 1: Intensity ratings of cooling, heat/burning and tingling attributes will increase with concentrations of cooling ingredient blends.

Hypothesis 2: East Asian subjects will perceive more heat/burning from flavored beverages containing cooling blends compared to the Caucasian subjects.

Hypothesis 3: Subjects who are familiar with beverages that deliver cooling sensation will perceive the cooling blends differently than subjects who are not familiar with these products.

2 Methods

2.1 Subjects

Participants were recruited via flyers and email from Rutgers University and the local community. Subjects were healthy men and women, 18-45 years old. Subjects were excluded if they had oral or nasal disease, a chronic disease or severe food allergies, or if they were pregnancy or taking medication that could affect taste or smell. We aimed to recruit equal numbers of East Asians and Caucasians. Subjects completed two questionnaires: a general questionnaire to collect demographic data and a product usage questionnaire to understand usage patterns of several products with cooling ingredients (tooth paste, gum, mouth wash etc.). We also asked some questions about their familiarity with cooling ingredient beverages at the end of product usage questionnaire. The experimental protocol was approved by the Rutgers University Institutional Review Board for the protection of human subject in research. All participants gave written informed consent for their participation and received compensation after the study.

2.2 Taste Stimuli

Four cooling ingredients were utilized in the current study: Coolact® 38D, Coolact ® 5 and Coolact ®10 (proprietary compounds provided by Takasago International Corp) and Frescolat® ML from Symrise Inc. (see Table 2.1).

The two cooling blends were: Coolact® 38D/ Frescolat® ML (1:1 by weight) and Coolact ®5 /Coolact ®10 (also 1:1 by weight). The Coolact® 38D/Frescolat® ML blend is considered nature identical; Coolact ®5 /Coolact ®10 blend is considered artificial. These two cooling blends were the same blends Tepper et al. (2010) tested previously in aqueous solutions.

A lemon lime flavored beverage was used. The syrups were made to deliver 300 ppm, 150 ppm, 75 ppm and 0 ppm of cooling ingredients in the finished beverages. The syrups were formulated by Takasago International Corp and were delivered to the Rutgers's Sensory Evaluation Laboratory. To make the finished beverage, 50 ml of syrup was added to spring water to a final volume of 300 ml, for a total of 8 samples (Table 2.2). All samples were sealed in 1-liter glass bottles and stored at 5^{o} C; they were brought to room temperature prior to use. The safety profile of Coolact was described under risks/benefits assessment. Altoid ® peppermints (Callard & Bowser, Chicago, IL) were used in a practice session to familiarize the subjects with the experimental protocol.

Table 2.1 Cooling ingredients utilized in the study

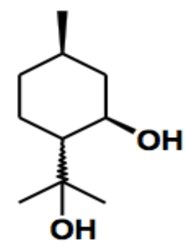
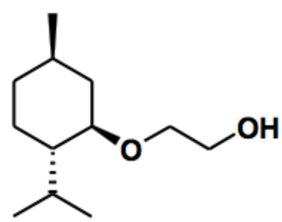
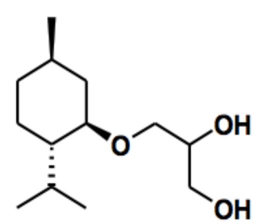
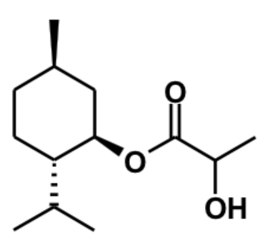
Cooling Ingredients	Chemical Name	Chemical Structure
Coolact® 38D	P-menthane 3,8-diol	
Coolact ®5	2-l-Menthoxy-ethan-1-ol	
Coolact ®10	3-l-Menthoxy-propane-1,2-diol	
Frescolat® ML	(L)-menthyl lactate	

Table 2.2 Sample description

Sample Number	Cooling Ingredient Blend	Concentration (ppm)
1 (Control)	Coolact® 38D /Frescolat® ML	0
2	Coolact® 38D /Frescolat® ML	75
3	Coolact® 38D /Frescolat® ML	150
4	Coolact® 38D /Frescolat® ML	300
5 (Control)	Coolact ®5 /Coolact ®10	0
6	Coolact ®5 /Coolact ®10	75
7	Coolact ®5 /Coolact ®10	150
8	Coolact ®5 /Coolact ®10	300

2.3 Experimental Procedure

All testing was conducted in the Sensory Evaluation Laboratory in the Food Science Building at Rutgers University. The experimental design and ballots were developed utilizing FIZZ sensory software (Biosystemes, Couternon, France).

Subjects participated in a total of 3 sessions on separate days. Two sessions were scheduled in one week separated by at least 1 day. Each subject was expected to complete the entire testing sequence in a 2-week period. The first session was a practice session to familiarize subjects with the test procedure using the computerized ballot. Subjects practiced rating the intensity and liking of each attribute using the scales provided. Altoid® peppermints were used as taste stimuli at this session because Altoid® has many of the same sensory characteristics as the test samples including cooling, heat/burning, tingling, sweetness and bitterness. Subjects also completed a product usage questionnaire. The practice session ensured that all subjects understood the experimental procedure and scored the ballot correctly. At the end of this session, each subject provided a cheek swab to collect genetic information (if they were interesting in participating in this part of the study).

Subjects evaluated flavored beverages containing cooling blends during session 2 and session 3. They were randomly assigned to receive one cooling ingredient blend [Coolact® 38D /Frescolat® ML blend or Coolact ®5 /Coolact ®10 blend] in each session. The serving order within each session was also randomly determined. Subjects were instructed to take the whole 20 ml sample into the mouth, swish it for 15 seconds, and then swallow it. They rated each sample for perceived intensity and liking at 0 min (immediately after tasting), 2.5, 5 and 10 min. All samples were rated for intensity and liking of 6 attributes on a 15-cm line scale end-anchored with the words “very weak” (0) at the left end and “very strong” (15) at the right end. The attributes included: cooling, heat/burning, tingling, bitterness, sweetness and overall flavor. Subjects pointed and clicked with a mouse on a computer screen to record their responses. The subjects were given a 5- minute break in between tasting each sample and were instructed to rinse their mouths with water in between each tasting. Subjects could see a countdown clock on the screen during in the timed wait and the system prompted them when it was time to take the next sample. Each session took around 55 min to complete.

2.4 Statistical Analysis

Data (presented as mean values ± SEM) were analyzed using SAS for Windows (version 9.2 SAS institute, Cary, NC). Repeated measures analysis of variance (ANOVA) was used to assess changes in the attribute ratings over time. A nested model was used in which concentration was nested within cooling blend type. ANOVA was also used to investigate the influence of ethnicity, gender, familiarity and their interactions on the attribute ratings. Analysis of covariance (ANCOVA) was further conducted to control for familiarity with cooling ingredient beverages. Post-hoc comparisons were made using Duncan's Multiple Range Test. Due to the large subject population, a stringent statistical cutoff criterion $p \leq 0.01$was used for all tests.

3 Results

3.1 Subjects

One hundred and sixteen subjects participated in the study. All subjects were between 18 and 45 years of age. Forty seven percent of subjects were East Asians (Chinese and Korean), 37% were Caucasians and 16% were other. The "other" group was made up of South Asians, Blacks, Hispanics and those of Middle-Eastern descent. Seventy-four were females and 42 were males. Fifty two percent of participants were familiar with flavored beverages that deliver cooling sensation, and 48% were not familiar with this type of beverage. Subject demographics are shown in Figure 3.1.

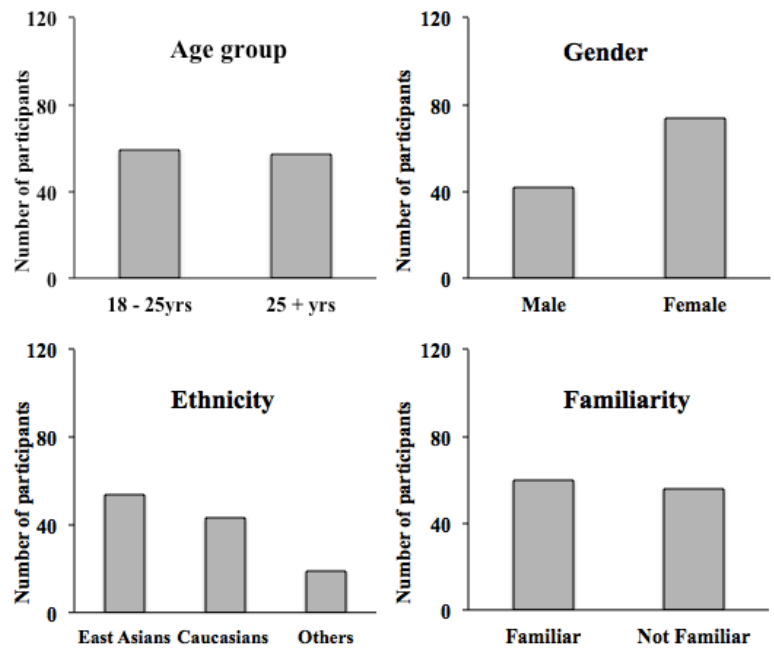
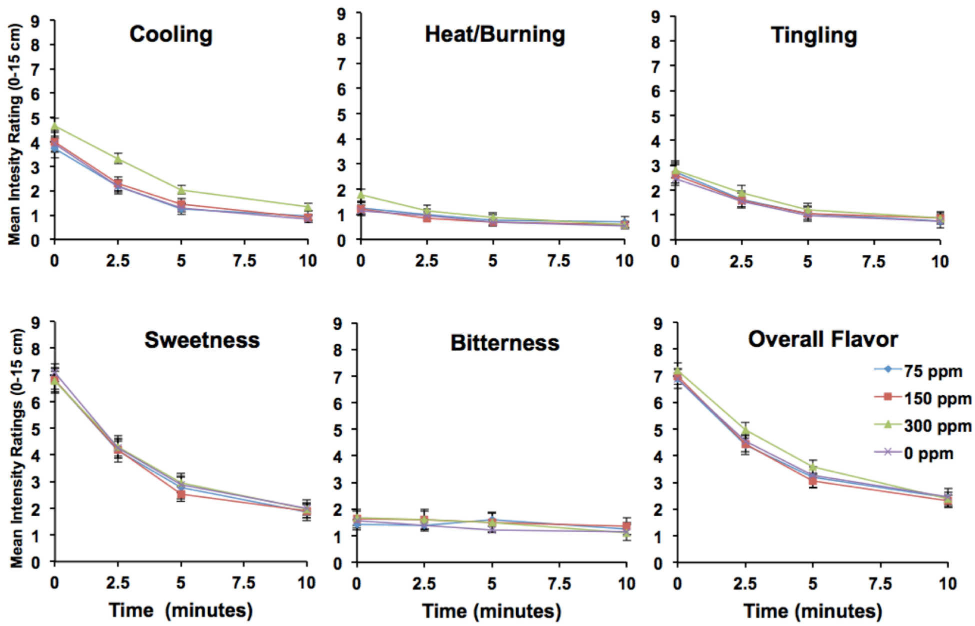


Figure 3.1 Subject Demographics

3.2 Effects of cooling blend type by concentration over time

Attribute intensity ratings were examined as a function of cooling blend concentration and time. Figure 3.2a and 3.2b shows that both cooling blends followed the same pattern. They primarily delivered the sensations of cooling and tingling, with minimal perception of heat/burning and bitterness. All perceived intensities (except bitterness) reached a maximum immediately after tasting (Time 0) and declined to baseline by 10 min ($P \leq 0.0001$). Intensities of bitterness were flat over the entire 10 min time period. Figure 3.2 also indicated that concentration (0ppm, 75ppm, 150ppm and 300ppm) did not affect cooling, heat/burning, tingling, bitterness, sweetness and overall flavor intensity ratings at any time point (0 min, 2.5 min after tasting, 5 min after tasting and 10 min after tasting). However, there was a non-significant trend for cooling intensity to be higher at 300 ppm when compared to the other concentrations. There were no differences between the blends for any of the intensity attributes.

3.2 a) Coolact® 38D/Frescolat® ML blend



3.2 b) Coolact ®5 /Coolact ®10 blend

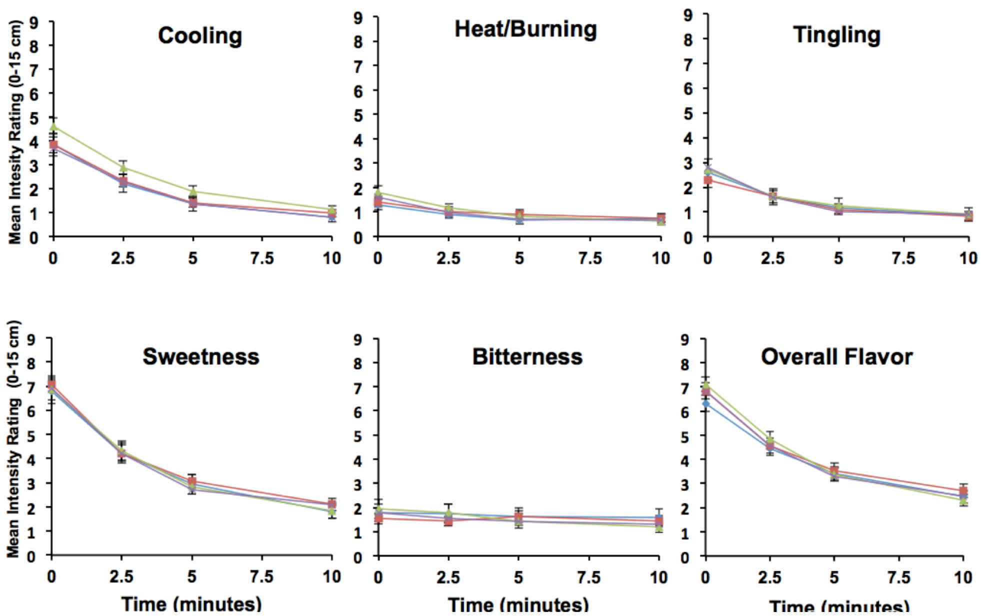
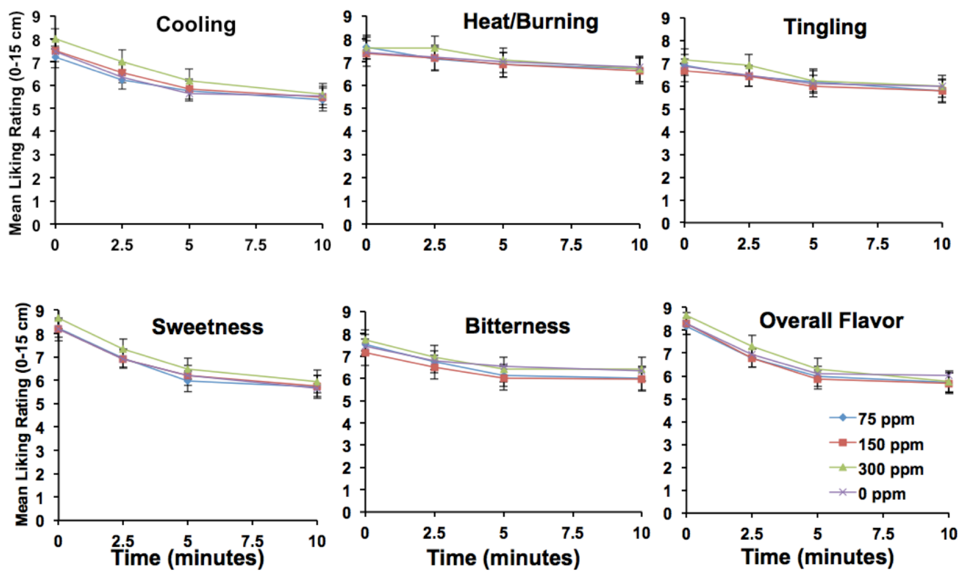


Figure 3.2 Attribute intensity ratings by time and cooling blend concentration. The x-axis of each figure is time; the y-axis represents mean intensity ratings on a 15-cm line scale.

Attribute liking ratings were also examined as a function of concentration and time. Figure 3.3 shows that all liking ratings reached a maximum immediately after tasting and declined over time. In addition, there were no significantly differences among concentration (0ppm, 75ppm, 150ppm and 300ppm) at any time point (0 min, 2.5 min, 5 min or 10 min after tasting) with respect to attribute liking ratings. Both cooling blends followed the same pattern.

3.3 a) Coolact® 38D/Frescolat® ML blend



3.3 b) Coolact ®5 /Coolact ®10 blend

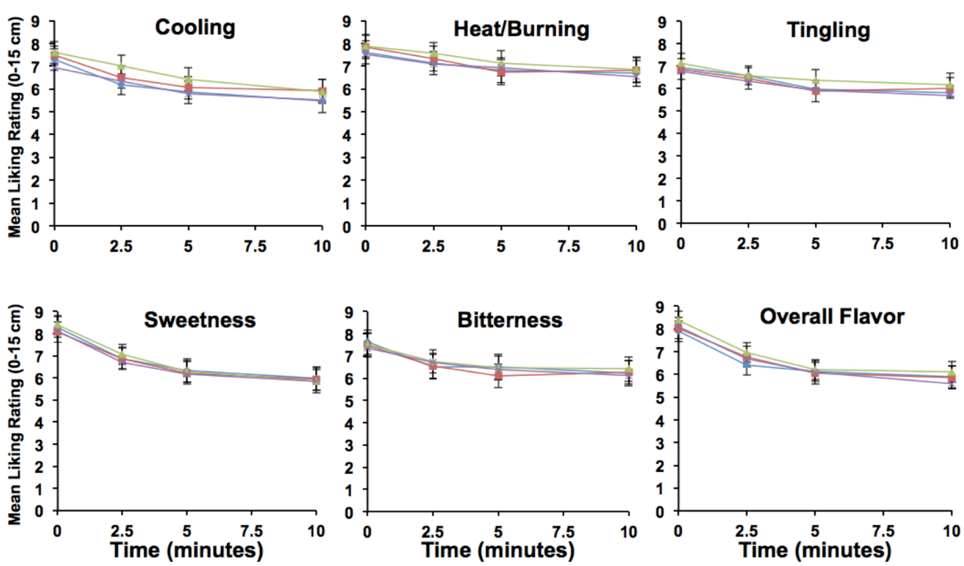


Figure 3.3 Attribute liking ratings by time and cooling blend concentration

The x-axis of each figure is time; the y-axis represents mean liking ratings on a 15-cm line scale.

3.3 Effects of ethnicity and gender on attribute ratings

Data analyses were conducted comparing only the East Asian (n= 54) and Caucasian (n=43) groups because they were the main ethnic groups of interest. ANOVA was calculated on the attribute intensity and liking ratings at time 0 because both types of ratings were maximal at this time. Also, the data were collapsed across concentrations and cooling blend types since these factors did not influence the results of the previous analyses. The statistical results can be seen in Table 3.1. There was a significant difference between East Asians and Caucasians with respect to the heat/burning attribute [$F (1, 577) = 6.37$, $p = 0.01$]. East Asians gave higher intensity ratings to heat/burning (1.88 ± 0.14) compared to Caucasians (1.35 ± 0.16). There were no significant differences between ethnic groups for the intensity ratings of cooling, tingling, sweetness, bitterness and overall flavor attribute.

Regarding gender effects, females (4.41 ± 0.17) perceived more cooling from the samples [$F (1,691) = 8.23$, $P = 0.004$) compared to males (3.61 ± 0.20). In addition, the interaction between gender and ethnicity showed significant differences for cooling [$F (1,575) = 10.53$, $P = 0.001$] and heat/burning [$F (1,575) = 8.05$, $P = 0.005$] intensity ratings. There were twenty-nine East Asian women, 31 Caucasian women, 25 East Asian men and 12 Caucasian men. Caucasian men gave significantly lower intensity ratings (2.74 ± 0.34, $P < 0.002$-0.0001) than East Asian women (4.25 ± 0.27), Caucasian women (4.73 ± 0.28) and East Asian men (4.28 ± 0.28) for the cooling attribute. The heat/burning attribute showed similar patterns; Caucasian men (0.55 ± 0.13, $P < 0.002$-0.0001) gave lower intensity ratings than East Asian women (1.81 ± 0.20), Caucasian women (1.66 ± 0.21) and East Asian men (1.98 ± 0.20). Similarly, Caucasian men rated the intensity of bitterness (0.91±0.21) lower than East Asian women (1.94 ± 0.20, P= 0.004) and Caucasian women (1.97 ± 0.22, P = 0.003). Overall, Caucasian men significantly differed in the perception of cooling, heat/burning and bitterness from the other groups. Thus Caucasian men gave consistently lower ratings to these attributes than did the other groups.

Table 3.1 P value and F value of attribute intensity ratings by gender, ethnicity, and their interactions (Time = 0).

Attribute	**Gender**		**Ethnicity**		**Gender ×Ethnicity**	
	F-value	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**
Cooling	8.23	**0.004**	0.09	NS	10.53	**0.001**
Heat/burning	0.18	NS	6.37	**0.01**	8.05	**0.005**
Tingling	5.29	NS	0.00	NS	0.09	NS
Sweetness	0.01	NS	0.99	NS	0.38	NS
Bitterness	3.70	NS	0.37	NS	2.71	NS
Overall Flavor	0.08	NS	1.98	NS	0.00	NS

The effects of gender and ethnicity on the hedonic ratings are shown in Table 3.2.There were no significant differences between East Asians and Caucasians for any of the liking ratings. However, there were significant differences among females and males for the cooling attribute [$F(1, 691) = 18.82$, $P < 0.0001$), heat/burning attribute [$F(1, 691)$

= 11.5, P = 0.007], tingling [F (1, 691) = 13.34, P = 0.0003) and bitterness [F (1, 691) = 6.59, P = 0.01]. Females ($M_{cooling}$= 7.91 ± 0.15; M_{heat} = 7.99 ± 0.16; $M_{tingling}$= 7.28 ± 0.15; $M_{bitterness}$= 7.76 ± 0.17) gave significantly higher liking scores for cooling, heat/burning, tingling and bitterness than males ($M_{cooling}$= 6.79 ± 0.22; M_{heat} = 7.06 ± 0.24; $M_{tingling}$= 6.34 ± 0.22; $M_{bitterness}$= 7.03 ± 0.23). There were no effects of gender and ethnicity interaction on any of the attribute liking ratings.

Table 3.2 P value and F value of attribute liking ratings by gender, ethnicity, familiarity and their interactions (Time = 0)

Attribute	**Gender**		**Ethnicity**		**Familiarity**		**Gender ×Ethnicity**		**Gender × Ethnicity ×Familiarity**	
	F-value	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**
Cooling	18.82	**< 0.0001**	0.32	NS	0.40	NS	1.98	NS	11.30	**0.0008**
Heat/burning	11.50	**0.0007**	3.22	NS	4.44	NS	1.44	NS	0.07	NS
Tingling	13.34	**0.0003**	0.34	NS	0.17	NS	0.02	NS	5.21	NS
Sweetness	0.13	NS	0.12	NS	0.05	NS	0.03	NS	20.36	**<0.0001**
Bitterness	6.59	**0.01**	4.51	NS	0.02	NS	0.00	NS	2.28	NS
Overall Flavor	0.04	NS	0.13	NS	1.33	NS	0.28	NS	4.23	NS

3.4 Effects of familiarity with cooling ingredient beverages on the attribute ratings

The subjects were then divided into two groups based on their familiarity with cooling ingredient beverages. Subjects who were familiar with flavored beverages that deliver cooling sensations were considered the “familiar” group (F), and those who were not familiar with flavored beverages that deliver cooling sensations were considered the “non- familiar” group (NF). Sixty participants were in the “familiar” and 56 were in the “non-familiar” group. This analysis included all subjects who participated in the study (n=116).

As before, the data were examined at time zero and collapsed across concentrations and blend types. Table 3.3indicates that “familiar group” gave higher intensity ratings for all attributes compared to “non-familiar group”. The “Familiar group” rated the intensity of cooling [$F (1, 691) = 29.29$, $P < 0.0001$], heat/burning [$F (1, 691) = 24.19$, $P < 0.0001$], tingling [$F (1, 691) = 14.65$, $P < 0.0001$], sweetness [$F (1, 691) = 10.57$, $P = 0.0012$] and bitterness [$F (1, 691) = 6.53$, $P = 0.01$] higher than the “non-familiar group”.

Table 3.3 Attribute intensity ratings as a function of familiarity (n= 116). [1]Values are Means ± SEM. [2]Means in the same row with different superscripts (a,b) are significantly different ($p \leq 0.01$).

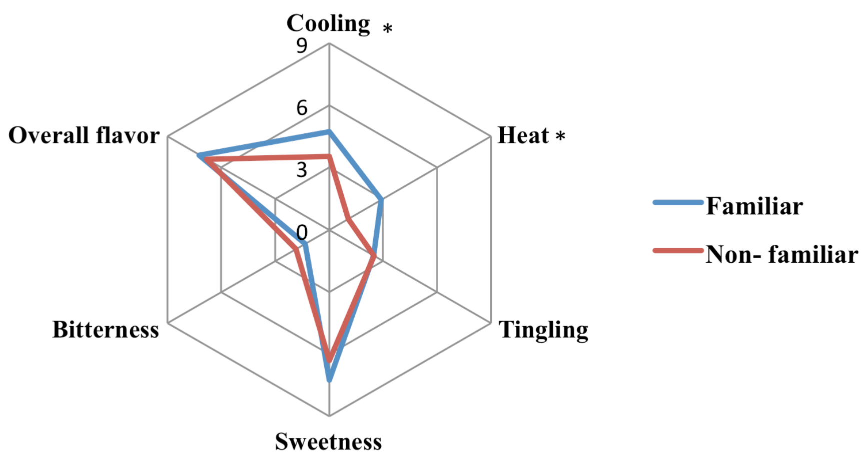
	Familiar Group	**Non- familiar Group**
Cooling	4.81 ± 0.18^{a}	3.38 ± 0.19^{b}
Heat/Burning	1.88 ± 0.14^{a}	1.0 ± 0.12^{b}
Tingling	3.08 ± 0.19^{a}	2.12 ± 0.16^{b}
Sweetness	7.28 ± 0.18^{a}	6.39 ± 0.21^{b}
Bitterness	1.43 ± 0.11^{a}	1.92 ± 0.16^{b}
Overall Flavor	7.13 ± 0.16	6.61 ± 0.19

3.5 Attribute ratings as a function of familiarity and cooling blend type

The two cooling blends showed the same general pattern (Figure 3.4). The familiar group perceived more cooling, heat/burning and tingling from the Coolact ®5 / Coolact ®10 blend ($p< 0.0001$) and more cooling ($p < 0.001$) and heat/burning ($p< 0.01$) from the Coolact® 38D /Frescolat® ML blend compared to the " Non – familiar group". The only difference between these profiles was that the Coolact ®5 / Coolact ®10 blend produced more tingling for the familiar group compared to the non-familiar group. The Coolact® 38D /Frescolat® ML blend produced no differences between the groups for tingling.

3.4 a) Coolact® 38D / Frescolat® ML blend

Attribute ratings with “ * ” are significantly different at $p < 0.01$.



3.4 b) Coolact ®5 / Coolact ®10 blend

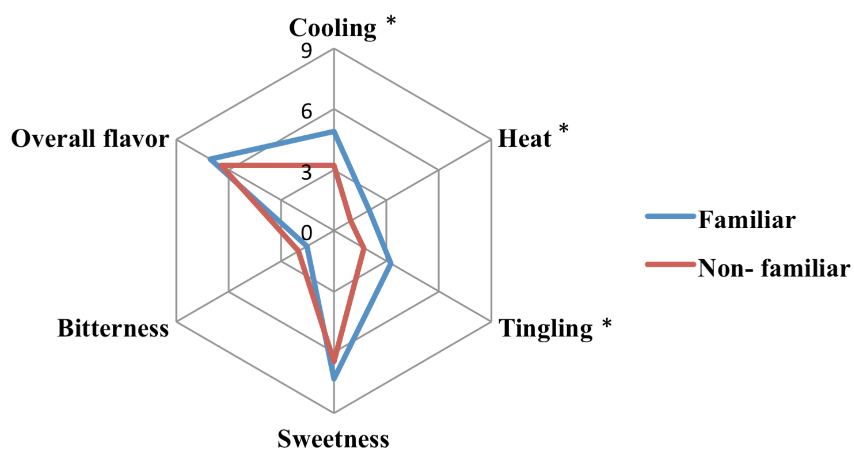
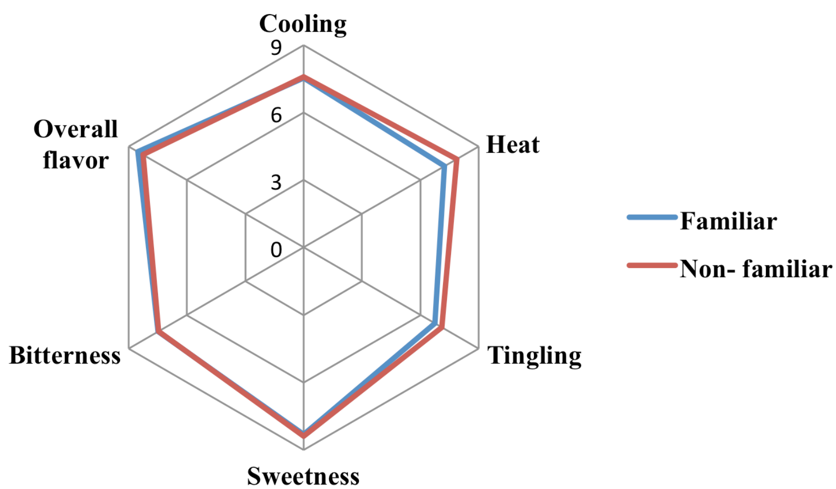


Figure 3.4: Attribute intensity ratings as a function of familiarity (Time = 0)

Despite differences in the attribute ratings for the familiar and non-familiar groups, there were no differences in liking ratings between blends as a function of familiarity. The two cooling blends showed same liking profiles (Figure 3.5).

3.5 a) Coolact® 38D / Frescolat® ML blend



3.5 b) Coolact ®5 / Coolact ®10 blend

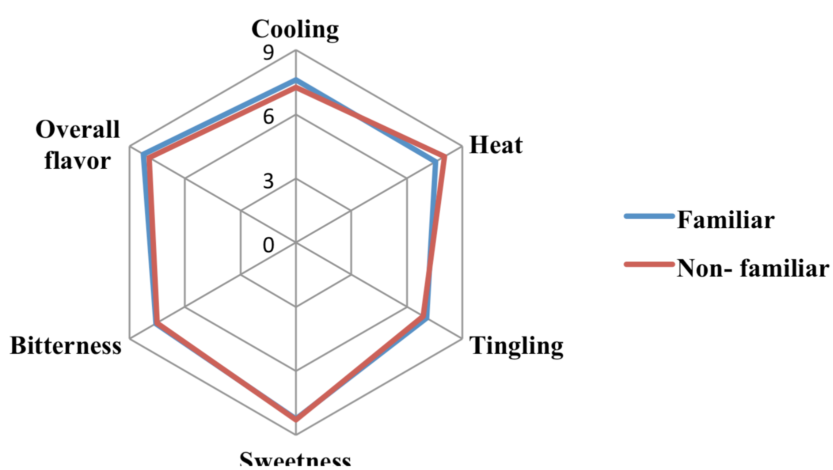
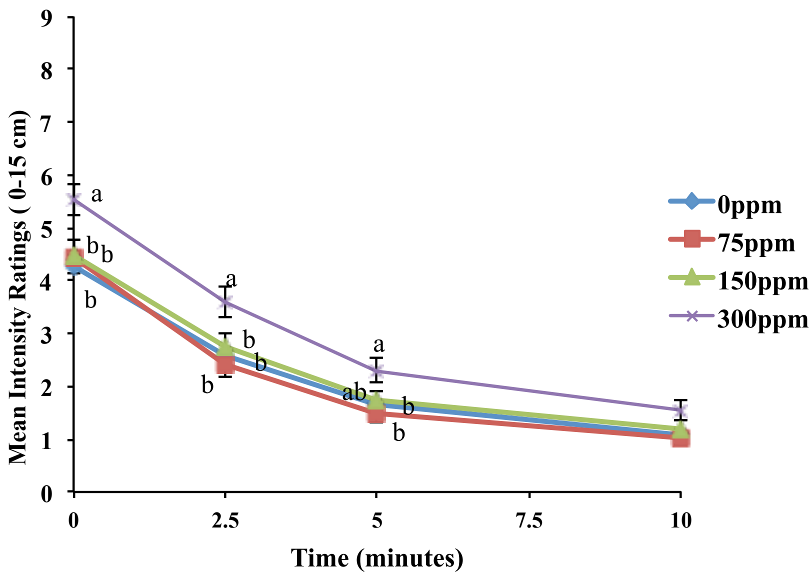


Figure 3.5 Attribute liking ratings as a function of familiarity (Time = 0)

3.6 Attribute ratings by cooling blend familiarity, concentration, and time

Finally, the effect of familiarity was assessed as a function of concentration and time, after collapsing the data across cooling blend types. At time 0 and 2.5 minute after tasting, the familiar group perceived more cooling from the 300 ppm sample compared to the other three concentrations (0 ppm, 75 ppm and 150 ppm) ($p < 0.01$). Also, at 5 minutes after tasting, the familiar group perceived more cooling from the 300 ppm than from the 0 ppm and 75 ppm samples ($p < 0.01$) (Figure 3.6 a). In contrast, the 'Non – familiar group' (NF) gave similar cooling intensity ratings to all concentrations over time (Figure 3.6 b).

3.6 a) Familiar group



3.6 b) Non – Familiar Group

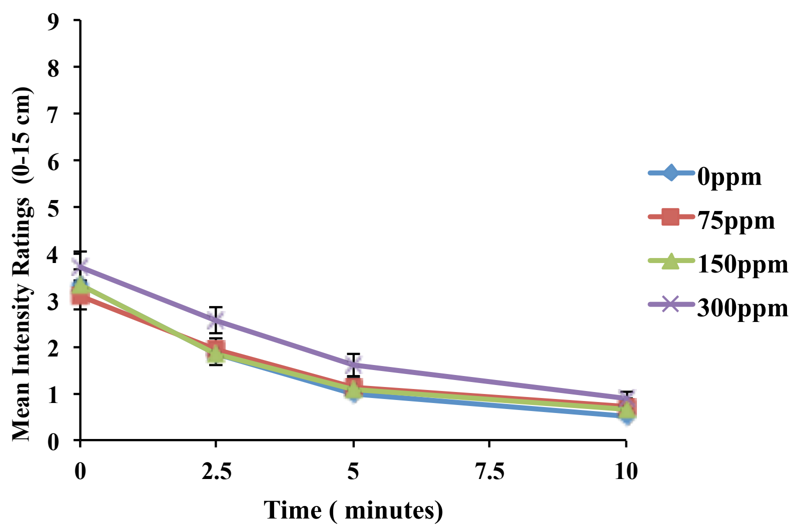
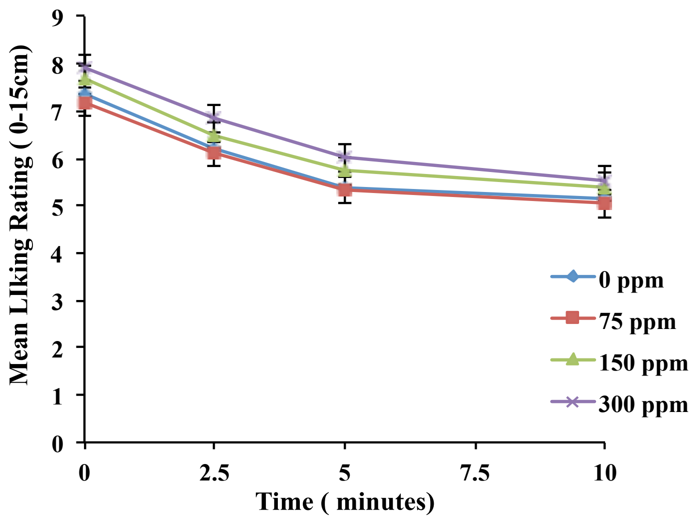


Figure 3.6 Intensity ratings of cooling attribute by concentration and time averaged across cooling blend type. Ratings at a single time point with different superscripts are different at $p < 0.01$.

There were no significant differences between the familiar and non-familiar group in the liking ratings as a function of concentration and time (Figure 3.7)

3.7 a) Familiar Group



3.7 b) Non – Familiar Group

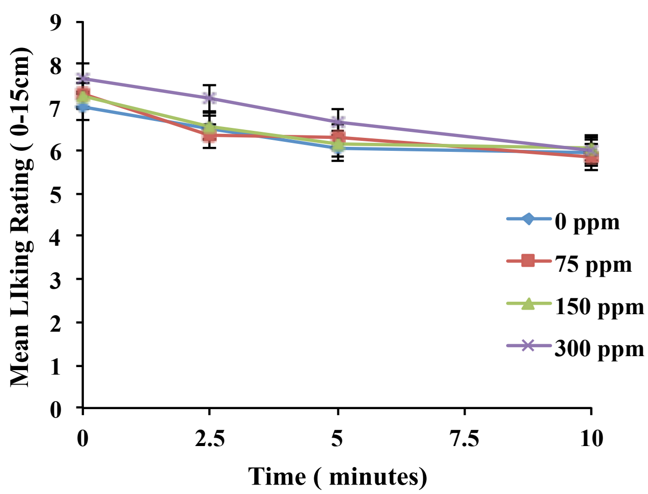


Figure 3.7 Attribute liking ratings by concentration and time averaged across cooling blend type.

3.7 The effect of the use of products with cooling ingredients on attribute ratings

Based on subjects' consumption frequencies of some products containing cooling ingredients (gum, mints, mouthwash, toothpaste and flavored beverages that deliver cooling sensation), we divided them into two sub-groups, "Not often" group and "Often" group. Regarding the use of gum, mints, mouthwash and toothpaste, there were no significant differences between the "Not often" and "Often" groups with respect to any of the attribute ratings.

However, frequencies of consuming flavored beverages that deliver cooling sensation affected the oral perception of the test samples. The "Often" group perceived more cooling [4.92 ± 0.29, $F(1,691) = 11.86$, $P = 0.0006$] and heat/burning [2.14 ± 0.23, $F(1,691) = 19.14$, $P < 0.0001$] than the "not often group" ($M_{cooling} = 3.86 \pm 0.15$; $M_{tingling} = 2.52 \pm 0.09$). Also, the "not often" group gave higher liking ratings to tingling [7.13 ± 0.14, $F(1, 691) = 7$, $P = 0.0083$] compared to "often" group (6.38 ± 0.26).

3.8 The effects of ethnicity, gender and familiarity on the Altoids® Peppermint attribute ratings

Attribute ratings of Altoids® Peppermint were assessed by ethnicity, gender and familiarity. There were no significant differences between East Asians and Caucasians for any of the attribute intensity ratings at any time points. Also, there was no effect of gender on attribute intensity ratings at any time points. However, compared to the non-familiar group, the familiar group perceived more cooling at 2.5 and 5 min, more heat/burning at 0 and 2.5 min, more sweetness at 0 min and more overall flavor at 2.5 and 5 min (See Table 3.4). Thus, the familiar group perceived more intense sensations (heat/burning, sweetness and overall flavor) from the Altoids® Peppermint as they also did from the flavored beverages with cooling. This suggests that subjects' prior experience with cooling in one class of products may generalize to other products with cooling, and may sensitize these individuals to differences in cooling intensity.

Table 3.4 Altoids® Peppermint Attribute intensity ratings as a function of familiarity (n= 116). [1]Values are Means ± SEM. [2]At each time point, means in the same row with different superscripts (a,b) are significantly different ($P \leq 0.01$).

	0 min		2.5 min		5 min		10 min	
	Familiar	Non-familiar	Familiar	Non-familiar	Familiar	Non-familiar	Familiar	Non-familiar
Cooling	10.87 ± 0.39	10.71 ± 0.38	9.58 ± 0.34[a]	8.22 ± 0.40[b]	6.74 ± 0.45[a]	5.3 ± 0.45[b]	3.9 ± 0.43	2.8 ± 0.35
Heat/Burning	7.48 ± 0.52[a]	5.66 ± 0.62[b]	4.98 ± 0.53	3.39 ± 0.50	2.92 ± 0.38	1.97 ± 0.38	1.56 ± 0.31	0.90 ± 0.25
Tingling	7.44 ± 0.45	6.79 ± 0.58	5.14 ± 0.46	5.02 ± 0.48	3.26 ± 0.38	2.89 ± 0.38	1.76 ± 0.31	1.28 ± 0.23
Sweetness	6.18 ± 0.49[a]	4.76 ± 0.47[b]	4.59 ± 0.40	3.99 ± 0.46	3.43 ± 0.41	2.46 ± 0.35	1.96 ± 0.36	1.61 ± 0.28
Bitterness	5.21 ± 0.54	3.90 ± 0.52	4.62 ± 0.52	3.46 ± 0.49	3.92 ± 0.49	2.23 ± 0.45	2.78 ± 0.41	2.15 ± 0.39
Overall Flavor	10.80 ± 0.29	10.49 ± 0.38	8.93 ± 0.34[a]	7.37 ± 0.38[b]	6.86 ± 0.43[a]	4.95 ± 0.42[b]	4.23 ± 0.44	3.4 ± 0.44

Regarding liking ratings, there were no effects of ethnicity, gender and familiarity on the attribute liking ratings of Altoids® Peppermint.

4 Discussion

4.1 Sensory perception of cooling ingredient blends in flavored beverages

The primary objective of this study was to characterize the sensory perception of two different cooling blends in flavored beverages. Results based on the entire cohort (n=116 subjects) showed that the Coolact® 38D /Frescolat® ML blend and the Coolact ®5 /Coolact ®10 blend were perceived in a similar manner. They primarily delivered the sensations of cooling and tingling with minimal perception of heat/burning and bitterness. All perceived intensities (except bitterness) reached a maximum immediately after tasting (Time 0) and declined over the time. Intensities of bitterness were flat during the 10 min period.

Contrary to our hypothesis, we found that there was no significant concentration effect on attribute intensity and liking ratings over time for the cohort as a whole. There are several possible explanations for this finding. It is possible that the concentration differences used in this study were not large enough for the subjects to distinguish the samples. However, the concentrations of citrus flavor and cooling ingredients selected for study were based on typical use levels for commercial beverages. Consumption of a full serving of these beverages would be expected to provide robust flavor and cooling sensation. However, to maintain the integrity of the sensory test, only small samples were tasted and evaluated. This is an inherent limitation of sensory evaluation that does not fully mimic the consumer experience. In previous cooling studies, Tepper et al. (2007, 2008, 2010) used the same blends at the same concentrations as used here, except solutions were used, and these samples were easily distinguished from each other. This is often what happens when model beverages are used that have more complex attributes than solutions.

Another issue is that the control sample (0 ppm) was rated as having cooling intensity, even though it had no cooling ingredient. It is possible that this outcome is due to expectation bias of the panelists; they were not specifically told that some samples had no cooling ingredients. Another possible explanation is that our subjects were untrained consumers and they might interpret the 'refreshing' sensation of the lemon-lime beverages as a cooling sensation. It is also possible that this outcome is due to carryover effects between samples. This explanation seems unlikely, however, since subjects waited for 5 minutes in between samples. Moreover, we saw no carryover effects in our prior studies with solutions. Despite these weaknesses, the present study found important sensory differences in the perception of cooling ingredient beverages in different subgroups of our study population.

4.2 Sensory perception of cooling ingredients by ethnic groups

We found that East Asians perceived more heat/burning from the samples than Caucasians at time 0. This finding confirms our previous findings showing that that East Asians gave higher heat/burning ratings to (l)- menthyl lactate or Coolact® 38D /Frescolat® ML blend when they were presented in solutions compared to Caucasians

(Tepper et al. 2010).

4.3 Sensory perception of cooling ingredients by gender

Gender had a significant influence on ratings of cooling in the samples, but not any of the other attributes. Specifically, females perceived more cooling than males. One possible explanation of this finding is that females may be more sensitive to cooling ingredients compared to males due to a gender differences in cold sensitivity. Kondrats'kyi et al. (2009) found that sex steroid hormones can cause differences in the functional properties of the TRMP 8 cold receptor in mice and rats. Whether these effects are present in humans is currently unknown, but deserves further consideration.

4.4 Sensory perception of cooling ingredients by previous experience

Previous experience with flavored beverages that deliver cooling sensations affected the oral perception of cooling ingredients in the flavored beverages. As shown in Figure 3.5, the familiar group perceived more cooling and heat/burning from Coolact® 38D /Frescolat® ML blend and more cooling, heat/burning and tingling from Coolact ®5 / Coolact ®10 blend than the non-familiar group. Our findings agree with studies by Laing et al. (1993) showing that Australian panels gave higher sweetness strength scores to Rice Bubbles (a cereal from Australia) than Japanese panels and that the Japanese panel rated the sweetness of Chokowa (a chocolate-flavored cereal from Japan) higher than Australian panels). Together, these findings show that familiarity with a product or a specific set of attributes may enhance the ability of panelists to detect subtle differences between products.

Some research also indicated that the degree of familiarity with products play an important role in influencing consumers' liking or preference (Birch, 1979; Bertino et al., 1983; Prescott et al., 1998). However, our study did not find any liking differences between two groups. It is possible that our samples did not meet the expectation of the familiar group based on their previous experience with flavored beverages with cooling ingredients. Anderson (1973) reported that expectation is strongly related to consumer satisfaction, and is often measured by the degree of discrepancy between expectations and product performance. Also, Cardello (1992) suggested that consumer evaluation of a product's disconfirmation experience, which comes from the difference between consumer expectations and actual product performance, was due to the comparison of expected hedonic acceptability with perceived hedonic acceptability. Our test samples were intended to be model beverages for research purposes and they were not optimized. Only sweetness and overall flavor intensity ratings were slightly above neutral. Therefore, our test samples might be low in cooling sensation and weak in flavor compared to some beverage products on the market. Generally speaking, attribute liking ratings were above neutral, but the samples were not highly liked. In this case, subjects may evaluate samples to be less pleasant if actual product performance doesn't match their expectation.

Findings from the research of Tu et al. (2010) also support our explanation. They suggested that Vietnamese consumers who were frequent users of soy products gave lower hedonic ratings to soy yogurts because they expect authentic soy-related sensory attributes when tasting soy yogurts but they could not find these attributes in the tasted samples. Based on their internal reference, the soy flavor of the samples might have been too different from their expectations.

4.5 Conclusions and future directions

The two cooling blends used in this study Coolact® 38D /Frescolat® ML blend and Coolact ®5 /Coolact ®10 blend provided similar sensory profiles. They primarily delivered the sensations of cooling and tingling with minimal perception of heat/burning and bitterness, which is desirable in most applications. This confirmed our first hypothesis.

We used ethnicity gender and familiarity to better understand individual differences in the sensory perception of cooling in flavored beverages. Our findings indicated that East Asians perceived more heat/burning from the samples compared to Caucasians, which confirmed the results from a previous cooling ingredient study done in our laboratory (Tepper et al. 2008). Also, we found that females perceived more cooling than males, which may suggest a gender difference in the cold sensitivity to cooling ingredients. Further research is needed to support this possibility.

In addition, the results of our study indicated that prior experience with beverages that deliver cooling sensation and frequency of consumption of these beverages might influence the sensory perception of cooling blends in the laboratory setting. Specifically, we found that subjects who had prior experience with beverages that deliver cooling sensations had better ability to discriminate concentration differences of cooling blends.

Finally, we also found that there was an overlap between ethnicity and familiarity. Sixty-nine percent of our East Asian subjects were familiar with cooling beverages but 70% of Caucasians were not. Familiarity is highly related to food exposure and ethnicity may relate to genetic differences. The relative contributions of culture and genetic differences to these results are currently unknown.

As mentioned earlier in the introduction section, two receptors may be involved in cooling sensations: TRPM8 and TRPA1. TRPA1 has diverse functional properties such as a sensor of irritation and cell damage signaling (Nilius et al. 2012). Presently, it is not known if sequence variations in the TRPM8 or TRPA1 genes play a role in individual differences in cooling sensations in humans, although some progress has been made in identifying the polymorphisms involved. For example, Carreno et al. (2011) suggested that single nucleotide polymorphisms (SNPs) within the Vanilloid TRPV subfamily of receptors were associated with the sensation of pain (migraine) in the Spanish Population. Another study done by Knaapila et al. (2012) to investigate if genotype affects chemosensory responses to some commonly used taste and smell stimuli. They detected the associations between the liking of cilantro and variants in three genes (TRPA 1, GNAT3 and TAS2R50). These finding might explain the person-to-person differences in the perception of cilantro. They demonstrated that genetic variation within chemosensory pathways could partially determine the individual differences in the perception of taste and smell stimuli. Once the functional variants associated with cooling perception have been identified, we will extract DNA from the cheek swab samples from our subjects to look for genetic associations.

In summary, our study provided in-depth knowledge of the psychophysical properties of novel cooling ingredients in a model beverage. Our findings show that when developing beverages containing cooling ingredients, ethnicity and prior experience may be important factors to consider. Understanding cultural values is very necessary in trying to predict target consumers' responses for companies.

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APPENDIX 1

Consent Form

CONSENT FORM

Study on Flavor Enhancers – Screening Procedure

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

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

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

RISKS/BENEFITS: The activities I will be participating in pose no foreseeable risks to my health. Although I will receive no direct benefits from participating in this study, this research will benefit society by providing a better understanding of the relationship between taste and diet.

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

MY RIGHTS AS A RESEARCH SUBJECT/CONFIDENTIALITY: My participation in this screening is completely voluntary and I have the right to withdraw at any time without explanation or penalty. The information collected in this experiment will be kept strictly confidential, my identity protected by a code number, and all data kept in a locked filing cabinet or on a pass-word protected computer. Only research staff involved in this study will have access to these files.

AGREEMENT: I have read the above description. All my questions have been answered to my satisfaction and I agree voluntarily to participate. I understand that I have the right to leave the experiment at any time without penalty. I also understand that Rutgers University has made no general provision for financial compensation or medical treatment for any physical injury resulting from this research. If I have questions about this research, I can contact the Principal Investigator at the number listed above. If I have any questions about my rights as a research subject, I may contact the Sponsored Programs Administrator at Rutgers University at:

Rutgers University Institutional Review Board for the Protection of Human Subjects
Office of Research and Sponsored Programs, 3 Rutgers Plaza
New Brunswick, NJ 08901-8559
Tel: 732-932-0150 ext. 2104 Email: humansubjects@orsp.rutgers.edu

___________________________________ ____________________________

Name of participant (print) Date

___________________________________ ____________________________

Signature of Participant Signature of Investigator

I have received a copy of this statement for my 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 ______.

CONSENT FORM

Study on Flavor Enhancers

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

PURPOSE: Flavor enhancers are novel ingredients that boost the flavor of foods. This study will help us to better understand how people with different taste perceptions and food experiences perceive these new ingredients. I am invited to participate in this research because I have already participated in a screening procedure and I qualify for this study.

PROCEDURES: I will be asked to participate in 3 test sessions on different days. During the sessions, I will be asked to taste and evaluate flavored drinks. I will be asked to rate the samples at timed intervals after swallowing. These procedures will be explained to me in the first session which is a training session. I will taste test samples during the remaining sessions. Each session will take ~ 60 minutes to complete. The sessions will be scheduled over a two week period.

RISKS/BENEFITS: The activities I will be participating in pose no foreseeable risks to my health. Although I will receive no direct benefits from participating in this study, this research will benefit society by providing a better understanding of the relationship between taste and diet.

COMPENSATION: At the completion of the study, I will receive a single payment of $ 60. If I withdraw from the study prior to its completion, my payment will be pro-rated for each session completed.

MY RIGHTS AS A RESEARCH SUBJECT/CONFIDENTIALITY: My participation in this screening is completely voluntary and I have the right to withdraw at any time without explanation or penalty. The information collected in this experiment will be kept strictly confidential, my identity protected by a code number, and all data kept in a locked filing cabinet or on a pass-word protected computer. Only research staff involved in this study will have access to these files.

AGREEMENT: I have read the above description. All my questions have been answered to my satisfaction and I agree voluntarily to participate. I understand that I have the right to leave the experiment at any time without penalty. I also understand that Rutgers University has made no general provision for financial compensation or medical treatment for any physical injury resulting from this research. If I have questions about this research, I can contact the Principal Investigator at the number listed above. If I have any questions about my rights as a research subject, I may contact the Sponsored Programs Administrator at Rutgers University at:

Rutgers University Institutional Review Board for the Protection of Human Subjects
Office of Research and Sponsored Programs, 3 Rutgers Plaza
New Brunswick, NJ 08901-8559
Tel: 732-932-0150 ext. 2104 Email: humansubjects@orsp.rutgers.edu

_______________________________ _______________________________

Name of participant (print) Date

_______________________________ _______________________________

Signature of Participant Signature of Investigator

I have received a copy of this statement for my 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 ______.

Flavor Enhancers Study

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 I provide will allow the researchers to determine whether I am positive or negative for a gene that controls bitter taste sensitivity. This information will help confirm the results of the behavioral tests and better understand the inheritance of this gene. The genetic material I 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 my identity protected by a code number. If I agree to participate in this procedure I should sign and date below. If I decline to participate in this procedure I can still participate in the main study.

__________________________ ______________

Signature of participant Date

APPENDIX II

Questionnaires

I.D. ____________
Date: ____________

Demographic and Health Information

Instructions

Please answer these questions about you <u>to the best of your knowledge</u> 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	☐ 4 Asian or Pacific islander
☐ 2 White	☐ 5 Hispanic or Latino
☐ 3 American Indian or Alaska native	☐ 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 Filipino
- ☐ 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. ________________

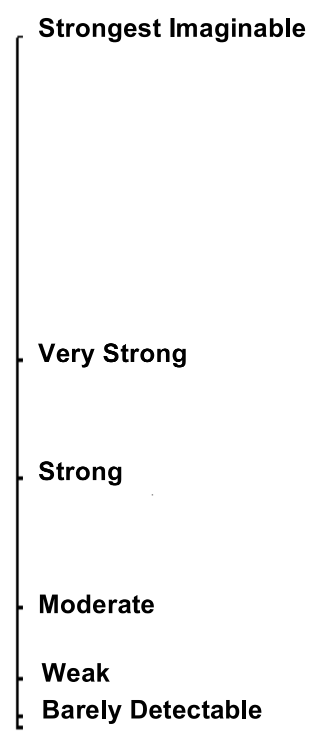
Date: ________________

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: **151**



I.D. _______________

Date: _______________

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

First Sample: **627**

Strongest Imaginable

Very Strong

Strong

Moderate

Weak

Barely Detectable

Product Usage Questionnaire

You will now be asked to answer some questions about yourself and your use of a few different products.

Please check the answer that best corresponds to your usage of mints or breath mints.

Are you a regular user of mints or breath mints?

☐ Yes ☐ No

How often do you use mints or breath mints?

☐ Daily
☐ Weekly
☐ Monthly
☐ N/A

What brand and flavor mints or breath mints do you usually use? (Example: Altoids, Breath Savers, Tic Tacs). If you do not know the exact brand and type, write as much as you can remember. If you have no preference, please write “no preference.”

____

Please check the answer that best corresponds to your usage of breath strips or breath freshener.

Are you a regular user of breath strips or breath freshener?

☐ Yes ☐ No

How often do you use breath strips or breath freshener?

☐ Daily
☐ Weekly
☐ Monthly
☐ N/A

What brand and flavor breath strips or breath freshener do you usually use? (Example: Listerine Pocketmist, Cool Mint). If you do not know the exact brand and type, write as much as you can remember. If you have no preference, please write "no preference."

___

Please check the answer that best corresponds to your usage of mouthwash or other rinse.

Are you a regular user of mouthwash or other rinse?

☐ Yes ☐ No

If yes, how often do you use mouthwash?

☐ Daily
☐ Weekly
☐ Monthly
☐
N/A

What brand and flavor mouthwash do you usually use? (Example: Scope Cinnamon Ice). If you do not know the exact brand and type, write as much as you can remember. If you have no preference, please write "no preference."

Please check the answer that best corresponds to your usage of toothpaste.

Are you a regular user of toothpaste?

☐ Yes ☐ No

If yes, how often do you use toothpaste?

☐ Daily
☐ Weekly
☐ Monthly
☐ N/A

What brand and flavor toothpaste do you usually use? (Example: Crest Whitening Expressions Vanilla Mint). If you do not know the exact brand and type, write as much as you can remember. If you have no preference, please write "no preference."

____

Have you ever tried beverages that deliver cooling sensation?

☐ **Yes** ☐ **No**

If "yes", how often do you consume those beverages:

☐ Never
☐ Rarely
☐ Some of the time
☐ Most of the time

APPENDIX III

FIZZ Network Ballot for Cooling Ingredient Taste Study

Welcome to the cooling ingredient taste study. Thank you for your participation!

During this session, you will be asked to rate it four times for the INTENSITY and LIKING of each of the following attributes: cooling, heat/burning, tingling, sweetness, bitterness and overall flavor of four samples over a 55 minute time span. You will also be given a 5-minute break between samples, during which you may be asked to answer a few brief questionnaires.

You will now be receiving the first sample. You will be asked to taste the sample only once, and then rate the attributes immediately after tasting, after 2.5 minutes, after 5 minutes, and after 10 minutes. Once you have rated the sample four times, you will be given a second sample to rate in the same manner.

There will be a timer in the lower right- hand corner of the screen and you will be asked to wait until the timer reaches a specific time before you click “Next Page” and continue the test.

Please pay attention to the timer and the instructions carefully.

Please rinse your mouth with water at this time.

As soon as you click “Next Page”, you MUST check to make sure the sample code matches the code on top right corner of the screen, then taste the sample.

Please click “ Next Page” when you have received the first sample.

Please turn on your signal light to indicate to the server you are ready for the next sample.

Rinse your mouth with water before next sample.

Did you taste any other flavor or any other attributes in the previous sample? Use the space below to comment if desired.

K