PERCEPTUAL INTERACTIONS BETWEEN COOLING AND FLAVORS

By

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ABSTRACT OF THE THESIS

Perceptual Interactions between Cooling and Flavors

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Thesis Director:

Doctor Beverly Tepper

There are many studies on the perceptual interactions between taste and smell, however, few studies have focused on interactions between flavor and cooling sensations.

To examine the interactions between these sensations, two studies were conducted. The objective of the first study was to determine whether perceptual interactions exist between cooling and flavors, whether these interactions are dependent on the congruency (appropriate pairing) of the flavor with cooling, and how this influences perceived intensity of cooling and flavor attributes. Based on studies of interactions between taste and smell, the expectation was that congruent pairings would enhance cooling and flavor intensities, while incongruent pairings would suppress or have no influence on attribute intensities. The objective of the second study was to understand how cooling compounds influence liking and emotions when added to congruent and incongruent flavored model beverages.

Concentrations corresponding to moderate and low cooling intensities were determined with a dose-response study of WS-3 (N-Ethyl-5-methyl-2-(1-methylethyl) cyclohexanecarboxamide). WS-3 concentrations were varied in flavored model beverages (apple, spearmint, caramel). Intensity scales were employed to study the effects of the mixtures on cooling and flavor intensities using ScentMove® and affective scales to study the hedonics and emotions related to these mixtures.

Results indicated that moderate intensity WS-3 significantly increased perceived intensity of spearmint flavor (a congruent pairing) over the flavor alone for a period of 2 minutes from tasting, while there was no influence of cooling intensity on apple or caramel intensities (incongruent pairings). For apple and caramel, increasing levels of WS-3 corresponded with decreased liking and positive emotions, as well as higher disgust. For spearmint, moderate intensity WS-3 slightly decreased positive emotions and slightly increased disgust.

Results suggest that congruency has an impact on perceptual interactions involving cooling. Cooling congruent flavor intensity can be boosted using a cooling agent. It is important to use caution in product design, as liking and emotional profiles are influenced by cooling intensities. Even with a congruent flavor, there is an optimal cooling intensity level.

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1. INTRODUCTION

1.1. Background:

While eating foods, the flavor experience can be a concert of sensations, including aroma, taste, temperature, texture and chemesthesis. These individual sensations can interact with each other to modify, enhance or suppress each other. There are many possible sensory interactions that can occur and this makes the perception of foods quite complex.

1.1.1. Chemesthesis:

Chemesthesis is defined by Green as “the chemical sensibility of the skin and mucous membranes” (2004). Chemesthetic sensations can be described in laymen’s terms as the bite from carbonation, the heat from chili peppers or the cooling from menthol (Lawless & Heymann, 1999). The somatosensory systems, responsible for thermoreception (temperature) and nociception (pain), contain neurons that are sensitive to certain chemicals and therefore will elicit sensations when activated (Carlson, 2004). This chemical activation of temperature or pain sensations can occur on all skin, although the sensations in the oral cavity are of interest for this research. Sensations produced by chemesthetic stimuli are mediated by the trigeminal, glossopharyngeal and vagus nerves in the oral cavity (Green, 1996). Although chemesthesis is sometimes referred to as ‘trigeminal sensitivity’, this is not wholly representative of chemesthetic sensations during food consumption since the glossopharyngeal and vagus nerves are also responsible for sensations in the posterior tongue and throat (Green, 1996, 2004).

Thermo transient receptor potential (TRP) channels are sensitive to changes in temperature and chemical cooling and warming agents, both in pleasant (innocuous - warm and cool) and irritating (noxious - hot and cold) ranges (Dhaka et al., 2006). Some of the most well studied and cited TRP channels known for sensing chemical cooling and warming agents are TRPA1, TRPV1 and TRPM8. TRPA1 is responsible for sensing pungent compounds, such as those found in mustard, wasabi and garlic. TRPV1 is activated by capsaicin from chili peppers (Patapoutian et al., 2003; Dhaka et al., 2006). TRPM8 is a transient receptor potential ion channel responsible for sensitivity to cool temperatures between 10°C and 25°C and is also sensitive to chemical cooling agents such as menthol, icilin and WS-3 (Behrendt, 2004; Green, 2004; McKemy et al., 2002).

Table 1: TRP Channels involved in sensing temperatures and non-thermal agonists

Receptor	Temperature Range	Non-thermal agonists
TRPA1	≤ 17°C	Cinnamaldehyde, mustard oil, allicin, icilin
TRPV1	≥ 42°C	capsaicin, camphor, acidic pH, ethanol
TRPM8	≤ 25°C	menthol, icilin, eucalyptol

(Dhaka et al., 2006; Patapoutian et al., 2003)

1.1.2. Sensory Interactions:

When odorants and tastants are experienced together, as is the case when eating, their perception can be quite different than if they were experienced alone. In some cases, for example, certain tastes may enhance perception of flavors or certain flavors may mask perception of tastes.

There are many possible sensory interactions and many of these interactions have been well studied. There are sensory modality specific interactions, for example, taste-taste interactions. These could include interactions between two compounds eliciting the same taste quality (e.g., two sweeteners) or two compounds eliciting different taste qualities (e.g., a sweetener and a bitter compound) (Keast & Breslin, 2002). Studies have shown that certain same taste quality mixtures (e.g. sweet, umami, bitter) stimuli tend to enhance that quality (Keast et al., 2003). This enhancement phenomenon usually occurs at concentrations of weaker intensity and lessens, or even turns to suppression, at higher stimuli concentrations (Keast & Breslin, 2002). One example is a study of fourteen sweeteners, in which researchers systematically tested binary sweetener mixtures to determine if there was additivity, enhancement or suppression of sweet taste. Researchers found that in most cases, there was either an additivity or enhancement of sweet quality. Results differed according to sweetener and concentration, with enhancement being more pronounced at lower concentrations (Schiffman et al., 1995).

Mixtures of differing taste qualities tend to be more variable than same taste quality mixtures. Depending on concentration, taste quality and stimuli, there can be mutual enhancement, suppression or asymmetrical enhancement of one quality and suppression of the other (Keast & Breslin, 2002). In addition to studies involving perceptual interactions within a modality, there can also be multi-sensory interactions between two sensory modalities, for example, taste-odor interactions. Similar to mixtures of differing taste quality stimuli, a variety of combinations of enhancement and suppression can be observed, depending on concentration, stimuli and how familiar are the stimuli pairings (Delwiche, 2004).

As described in the review article by Keast and Breslin, perceptual sensory interactions can be the result of three things. There might be chemical reactions between stimuli prior to ingesting. One example noted by Keast and Breslin is that acid and base in mixture will react to form a salt. In this way, intensities of sourness may decrease, while new taste sensations may arise (2002). Another level of interaction could be the influence of one stimulus on the receptors or transduction mechanisms of another stimulus. In their review, Keast and Breslin cite examples illustrating that sodium salts suppress bitterness of certain stimuli (2002). Lastly, there can be elevated cognitive interactions from experiencing multiple sensations at once. For example, mixture suppression is experienced when sweet and bitter compounds are mixed. Through experiments in which sweet and bitter compounds were applied separately to different sections of the tongue or together as a mixture, Kroeze and Bartoshuk (1985) provided evidence for higher level cognitive interactions causing mixture suppression (Keast & Breslin, 2002).

In summary, there are a variety of outcomes for sensory interactions, as described for taste-taste interactions by Keast & Breslin, including enhancement of the intensities of both sensations, suppression of the intensities of both sensations or an asymmetrical enhancement of one sensation and not the other (Keast & Breslin, 2003). These effects may depend on concentration, quality, stimuli and how familiar or unfamiliar are the pairings (2003; Delwiche, 2004). Of the interaction possibilities, some of the most recently studied are interactions between chemesthesis and other sensory modalities, such as taste or olfaction.

1.1.3. Congruency:

Results of various taste-aroma sensory interaction studies suggest that congruency can play a large role in perception. When sensations are normally experienced together, they are thought to be more congruent. One example by Frank and Byram, examined congruent pairings of strawberry aroma with sweet taste which enhanced ratings of the sweet taste intensity (1988). In a different condition of the same study, the incongruent pairing of strawberry aroma with salty taste had no effect on salt taste intensity ratings (Frank & Byram, 1988). In another study by Schifferstein and Verlegh, researchers found that, not only was sweetness enhanced when paired with congruent aromas, the congruent samples were also found to be more pleasant (1996). In their study, combinations of sucrose with strawberry or lemon flavors were even more pleasant than expected if pleasantness was additive. Sucrose combined with incongruent ham aroma had a suppressive effect on pleasantness (Schifferstein & Verlegh, 1996).

1.1.4. Review of chemesthetic interactions in oral cavity:

Of the research that has been done on interactions of chemesthesis with other sensory modalities, most of the work has centered around capsaicin, which elicits a warming, burning sensation and activates a different transient receptor potential. The research done on the perception of cooling and cooling interactions has included both menthol and WS-3. Research involving cooling perception is more limited than that of capsaicin and research involving perceptual cooling interactions is scarcer.

There are a couple of features of chemesthetic stimuli that distinguish them from other types of stimuli and could make interactions studies quite different. Particularly important phenomena related to chemical cooling and warming agents are sensitization and desensitization. Sensitization refers to the progressively increasing chemesthetic sensation with repeated exposure to a chemesthetic stimulus. Desensitization refers to the decreased chemesthetic sensation to a chemesthetic stimulus after repeated exposure (Dessirier et al., 1997). Other characteristics that distinguish chemesthetic stimulants from tastants are that their intensity develops more slowly and their effects can be longer lasting (Green, 1996).

1.1.5. Capsaicin and capsaicin interactions:

Capsaicin is a well-studied chemesthetic agent and is responsible for the burning and heat associated with chili peppers. Studies have investigated interactions between taste and capsaicin and between aroma and capsaicin. In a review by Green (2004), the author states that, surprisingly, even though people often say that they are unable to taste foods that are high in capsaicin, the only consistent effect of chemesthetic stimuli on taste is a slight decrease in sweetness. The author also states that only small influences on retronasal odor have been observed (Green, 2004).

Prescott and colleagues (1993) investigated the interactions between capsaicin and sucrose in solutions. They found no influence of sucrose on capsaicin irritation, but some suppression of sweetness dependent on sucrose and capsaicin concentration. Prescott also found a reduction of sweet intensity with increased capsaicin concentration in a soup base (1993). In a separate experiment, Prescott and colleagues investigated interactions between capsaicin and NaCl. They found little effect of capsaicin on saltiness, but an increase in burn intensity with added NaCl. The authors attribute this to NaCl having an irritation of its own, although there was no irritation produced by NaCl alone at the levels used (Prescott et al., 1993).

1.1.6. Cooling agent interactions:

Most of the cooling perception research has been done using menthol, which has a chemesthetic cooling quality, but also a strong ‘minty’ aroma. Since menthol has an accompanying aroma, it makes it difficult to use menthol to study sensory interactions between aroma and cooling. As menthol concentration increases, cooling intensity increases, but so does the accompanying menthol aroma. Like capsaicin, some cooling agents, do not have an accompanying aroma, making it possible to study sensory interactions between chemesthetic sensation and flavor. WS-3 is one of the cooling agents without an accompanying aroma. Two studies have used WS-3 to study sensory interactions and will be described in detail later.

Studies were done to understand sensitization and desensitization of menthol (Cliff & Green, 2004), as well as cross sensitization and desensitization to capsaicin and menthol (Cliff & Green, 2006). Researchers found that after menthol exposure there is desensitization to the irritation of menthol, but not the cooling of menthol (Cliff & Green, 2004), that menthol is cross-desensitized by capsaicin and that capsaicin is cross sensitized by menthol (Cliff & Green, 2006). There has also been research to suggest that menthol cross-desensitizes the irritation caused by nicotine (Dessirier et al., 2001).

Of the cooling perception research available, only a few studies have explored interactions between cooling sensation and other sensory modalities. In one study, researchers investigated the interaction between fat texture and menthol in a lozenge. They found that increased fat concentration decreased the oral and nasal cooling of menthol, and they suggest that more menthol may need to be used in products containing fat to achieve the same cooling as products that do not contain fat. While fat coating the receptors on the tongue could be a plausible explanation for decreased cooling sensation on the tongue, fat coating the receptors in the nasal passages is not a possible explanation for decreased cooling perception in the nose. The authors also found decreased intensity of other attributes related to menthol and they suggest that decreased oral and nasal cooling in their system is due to the hydrophobic fat in the lozenge solubilizing menthol and making it difficult to release into the headspace (Allison et al., 2001). This makes the study even less about sensory interactions at the peripheral or cognitive level and more about a chemical reaction occurring within the product and in the mouth.

Two studies have used WS-3 to investigate sensory interactions with cooling chemesthesis. The first studied interactions between color, coolant and aroma (Petit et al., 2007). In this study, the trained panel evaluated two types of samples: a congruent (green-coolant-melon) sample and an incongruent (purple-coolant-pineapple) sample. For their congruent samples, they found no influence of color, but an influence of melon aroma on cooling intensity and an influence of coolant on melon intensity. For the incongruent sample, they found that cooling intensity was only influenced by cooling agent concentration. Pineapple flavor intensity was dependent on pineapple concentration, and for one sample, purple color. After being exposed to either sample over a period of 5 weeks, the panelists in this study were asked to reassess the flavor and cooling intensity of the incongruent pairing. The authors found that panelists learned to associate the incongruent combination and this enhanced their ratings of cooling intensity and pineapple intensity (Petit et al., 2007). These findings imply that the novelty of an unfamiliar pairing can wear off. Congruency is important to sensory interactions and congruency can be learned after multiple exposures.

The other study using WS-3 to study perceptual interactions was an investigation between olfaction, taste, trigeminal, and texture perceptions varying the concentrations of odorant, acid and coolant in a viscous system, hence hitting on interactions between smell, taste, chemesthesis and texture within one study (Labbe et al., 2008). These authors studied peach and mint odorants. They found that olfaction not only influenced taste and chemesthetic perceptions, but also influenced taste-taste interactions and taste-trigeminal interactions. More specifically, they found that while aroma intensity was rated higher for peach samples than for mint samples, mint samples were perceived as more cooling, sour, sweet and bitter than peach samples. With increasing mint odorant concentration, authors found an increase in mint aroma, perceived cooling intensity, as well as sweetness and thickness. With increasing peach odorant, there was only an increase in perceived peach aroma. Increasing coolant concentration decreased perceived peach aroma and had no effect on perceived mint aroma. The coolant concentration had differing effects on taste and texture perception depending on the aroma. Coolant concentration reduced sweetness of peach samples and enhanced sweetness of mint samples. Increased thickness perception of mint samples was observed with increased coolant. Sourness of both peach and mint odorants was enhanced with coolant concentration (Labbe et al., 2008).

Our study was different from the two WS-3 studies described in several aspects. First, our range of WS-3 concentrations was quite different. Labbe and colleagues studied WS-3 concentrations of 1000 ppm and 2000 ppm (2008). Petit and colleagues studied solutions from 0-20 ppm WS-3 (2007). Our samples ranged from 0 to 100 ppm WS-3. The goal of our study was to have a range of cooling intensity from barely detectable to moderately strong cooling in a model beverage solution. While Petit and colleagues also prepared samples in a model beverage solution, their cooling intensity maximum was lower (2007). Labbe and colleagues were studying interactions in a model system that was less ecologically valid. With and without nose-clips, panelists tasted viscous solutions by the spoonful (2008).

To our knowledge, only one study has investigated how the combination of cooling and flavor may change the cooling perception or flavor perception over time. In the study by Su, Tepper and Green, cooling sensate concentration did not influence lemon-lime flavor intensity in a model beverage (2013). This study was only using one flavor and was not testing whether congruency of flavor had an influence. Also, we are unaware of any studies involving hedonics and emotions associated with cooling sensate mixtures.

1.2. Objectives and Hypotheses:

The objectives our study were to determine whether or not perceptual sensory interactions exist between chemesthetic cooling and flavor, whether these interactions were dependent on the congruency of the flavor with cooling and whether these interactions could influence the temporal perception of the beverage. Our study was concerned with understanding how cooling flavor mixtures influenced attribute intensities as well as hedonics and emotions. We were interested in exploring a cooling without accompanying aroma, such as is elicited by WS-3, in a somewhat realistic model beverage.

Based on work done on congruency and pleasantness in odor-induced taste enhancement (Schifferstein & Verlegh, 1996), work done on congruency in colour-coolant-aroma interactions in model beverages (Petit et al., 2007) and work done on the impact of olfaction on taste, trigeminal and texture perceptions in viscous model systems (Labbe et al., 2008), it was hypothesized that the more congruent pairings of flavor and cooling (spearmint/cooling) would enhance flavor intensity and be more pleasant, while less congruent pairs (apple/cooling) would have no effect (or decrease) flavor intensity and be more unpleasant. It was also hypothesized that, as the temporal profile of the cooling agent is longer lasting than that of the flavor, and since the quality of cooling changes over time (possibly helping to avoid sensory adaptation), the flavor would last longer as in the case of longer lasting sweetener elongating flavors in gum (Davidson et al., 1999).

2. METHODS

This study was conducted in several parts. In Part 1, dose-response data was collected to determine concentrations for the mixtures used in Parts 2 & 3. Part 2 was concerned with measuring attribute intensities of the mixtures (cooling, warming, sweet, sour, bitter, flavor intensity) and Part 3 was concerned with measuring hedonics related to the mixtures (liking, familiarity, emotions, as well as overall intensity of the samples).

Table 2: Experimental Roadmap

Part 1 - Dose-response curves
Temporal dose-response relationship of WS-3
Dose-response curve of apple flavor
Dose-reponse curve of caramel flavor
Dose-response curve of spearmint flavor
Part 2 - Flavor-cooling mix intensities
Apple - WS-3 mixtures
Caramel - WS-3 mixtures
Spearmint - WS-3 mixtures
Part 3 - Measuring hedonics
Flavor - WS-3 mixtures from Part 2, randomized over 5 sessions, 2 samples per session

2.1. Samples:

All samples were a model beverage base solution which consisted of 5% sucrose and 0.01% citric acid in Poland Spring spring water. All samples were served in 10 mL portions in 1 oz soufflé cups, at room temperature.

2.1.1. Samples containing cooling agent:

Solutions of cooling agent WS-3 (((1R,3R,4S)-N-ETHYL-3-P-MENTHANECARBOXAMIDE) *Renessenz LLC*) were prepared by first creating a stock solution of 100,000 ppm WS-3 in solvent (1.25% Tween 20 in propylene glycol). An aliquot of the stock solution was then dosed into a model beverage solution. The concentrations prepared were: 20 ppm, 30 ppm, 44 ppm, 67 ppm and 100 ppm. An additional aliquot of solvent was added to each sample (if needed) to ensure that each sample contained 0.1% total solvent. There was also a 0 ppm solution, which consisted of 0.1% solvent in model beverage solution. Ranges chosen for panels were based on bench-top tasting with several people prior to testing, to ensure that samples ranged from barely detectable cooling to moderately strong cooling. The final sample set contained WS-3 at concentrations of : 0 ppm, 20 ppm, 30 ppm, 44 ppm, 67 ppm, 100 ppm.

2.1.2. Samples containing flavors:

Caramel, apple and spearmint flavors were all dilutions in propylene glycol and were obtained from Firmenich. Each sample was prepared by dosing an aliquot of flavor into model beverage base solution. Extra solvent was added to each sample to ensure that it was the correct ratio of Tween 20 and propylene glycol, and that total solvents in the mixtures were 0.1%. Ranges chosen for the panels were based on bench-top tasting with several people prior to testing, to ensure that samples ranged from barely detectable to strong flavor intensity.

Caramel flavor concentrations: 0%, 0.00313%, 0.00625%, 0.01250%, 0.02500%, 0.05000%

Apple flavor concentrations: 0%, 0.08000%, 0.02700%, 0.00900%, 0.00300%, and 0.00100%

Spearmint flavor concentrations: 0%, 0.00625%, 0.01250%, 0.02500%, 0.05000%, 0.10000%

2.2. Methodology:

The sensory tests were created on FIZZ software. All panelists were Firmenich employees who are not trained, but who have experience participating in sensory panels. Panelists were asked to rate samples on visual analog scales for all attributes listed from ‘Not at all intense’ to ‘Very intense’ translating from 0-10. Dumping occurs when panelists are given too few appropriate scales to rate for a product, so they ‘dump’ ratings from missing scales onto other scales. In order to avoid problems of dumping and to make sure no information was lost, we used six scales: flavor intensity (specific to whichever flavor was being tested), cooling intensity, warming intensity, bitterness, sweetness and sourness intensity. We decided to use a scale from 0 to 10 since our panelists were not trained, but had experience paneling and were familiar with using that scale for all other panels.

While designing the experiment, we tested a ten minute exposure interval with WS-3, by running a pilot panel with a few panelists. Panelists were exposed to the 100 ppm concentration, 3 times, once every 10 minutes. They were asked to rate the intensity of the solution (they were not informed that it was the same concentration). After finding no difference through repeated measures ANOVA (95% confidence level), we felt comfortable with the ten minute inter-stimulus interval.

Prior to the first session, a short introductory session was held. Panelists were invited to taste a sample without flavor or cooling and were told that it was not cooling. They were then given a mid-level cooling sample and were told that they would be tasting similar samples and rating various attribute intensities. They were given a brief introduction to the timing of the rating, the use of the Fizz software, and the tasting and rinsing regimens through a practice rating of the mid-level cooling sample level.

2.2.1. Part 1 - Dose-response curves:

Sessions were conducted with the same 33 panelists. All panelists were internal employees, had experience paneling, but were not trained and ranged in age. Nine panelists were males and twenty four panelists were females. Eight panelists were 18-35 years old, nine panelists were 36-45 years old, seven panelists were 46-55 and nine panelists were 56-65 years old. Panelists were asked to taste each sample and rate attribute intensities. For the tasting regimen, they were asked to taste each sample by taking the whole sample into their mouths, swishing for 10 seconds, expectorating, keeping their mouths closed for 5 seconds and then breathing gently through their mouths.

For samples containing cooling agent, panelists were asked to rate the sensations on all scales immediately, then again at 30 seconds, 1 minute, 2 minutes, 3 minutes, 4 minutes and 5 minutes. The panelists then entered a rest period where they were asked to eat a bite of cracker and rinse vigorously twice with spring water. The rest period lasted for 5 minutes, at the end of which, panelists received their next sample and the process was repeated. Attribute scales for cooling samples were cooling, warming, sweet, sour and bitter.

For samples containing flavor only, panelists were asked to taste in the same way described for cooling samples, rate all sensations on all scales immediately, then again at 30 seconds, 1 minute and 2 minutes. There was no rest period, panelists were able to taste the next sample after eating a bite of cracker and rinsing twice with water. Attribute scales for flavor samples were flavor intensity (specific to the flavor being tasted), sweet, sour and bitter.

In each session, panelists tasted a total of six samples, which ranged in concentration and were served in a randomized order (Latin-square determined by FIZZ). One session was dedicated to each flavor and one session was dedicated to cooling agent only.

2.2.2. Part 2 - Flavor-cooling mixture intensities:

The same 33 panelists who participated in all the dose-response curve panels participated in all the flavor-cooling mixture panels. From the dose-response panels, moderate iso-intense concentrations (equivalent to an average score of ‘5’ on the intensity scale), were chosen for each flavor. The moderate concentration flavor was paired with two of the WS-3 concentrations from the WS-3 dose-response curve, corresponding to a low (mean perceived intensity of ‘3’) and a moderate (mean perceived intensity of ‘5’) cooling intensity (30 ppm & 100 ppm WS-3 respectively). A sample of flavor without cooling was also evaluated, for a total of 3 samples per session. Panelists were asked to use the same tasting regimen and rating as during the dose-response collection for samples containing cooling agent. They were asked to rate cooling, warming, sweet, sour, bitter and flavor intensity.

2.2.3. Part 3 – Measuring hedonics:

The same mixtures as were prepared for Part 2 – Flavor-cooling mixtures were used for Part 3. Additionally, there was a model beverage base solution with 0.1% solvent, but no flavor or coolant. The 10 samples were randomized over 5 sessions – two samples were served in random order per session. There were 36 panelists who participated in all sessions. These panelists were not necessarily the same who participated in Parts 1 and 2, however, participants from Parts 1 and 2 were not excluded.

For tasting the samples, panelists were asked to sip as much as they needed to form an opinion, then rate their emotions on the ScentMove® scales ('No feelings' to 'Very intense feelings'), followed by familiarity ('Not at all familiar' to 'Very familiar'), intensity ('None' to 'Very Intense') and liking (bipolar: 'Dislike very much' to 'Like very much') scales. In between samples, panelists were asked to eat a cracker and drink water to cleanse their palates. There was a 10 minute rest in between samples.

The nine ScentMove® scales are the following:

1) Happy – Well-being – Pleasantly surprised
2) Romantic – Desire – Sensual
3) Disgusted – Irritated – Unpleasantly surprised
4) Relaxed – Comforted – Soothed
5) Energetic – Refreshed – Revitalized
6) Mouthwatering – Thirsty – Famished
7) Interested – Amusement – Impressed
8) Nostalgic – Melancholic – Sad
9) Spiritual feeling

2.3. Data analysis:

One-way ANOVA refers to one factor being varied and two-way ANOVA refers to two factors being varied.

2.3.1. Intensity Ratings for dose-response and flavor-cooling mixtures:

For all attributes, rating data were organized in Excel 2007. The means and confidence intervals were calculated for each point. Data were analyzed using Repeated Measures ANOVA in XLStat 2012. An alpha level of 0.05 was used for all statistical tests and all tests were two-tailed. All post-hoc tests were done using Duncan's Multiple Range Test criterion. In any Repeated Measures ANOVA tests in which the assumption of sphericity was violated (indicated by significance for Mauchly's test statistic), the degrees of freedom were adjusted for within-subject effects using the Greenhouse-Geisser correction. This correction makes the F-ratio more conservative.

For the WS-3 dose-response relationship, one-way Repeated Measures ANOVA was used and the fixed effect was WS-3 concentration. For flavor-cooling mixtures, two-way Repeated Measures ANOVA was used and the fixed effects were WS-3 concentration and flavor.

2.3.2. Affective ratings for measuring hedonics:

For all attributes, rating data was organized in Excel 2007. The means and confidence intervals were calculated for each point. Data were analyzed using two-way ANOVA in XLStat 2012. An alpha level of 0.05 was used for all statistical tests and all tests were two tailed. All post-hoc tests were done using the Duncan criterion.

3. RESULTS

3.1. Part 1 – Dose-response curves

3.1.1. ***WS-3 dose-response:***

3.1.1.1. Cooling data for WS-3 dose-response:

Between subject effects showed that, collapsed across time, WS-3 concentration had a significant effect on cooling intensity ($F(5,204) = 22.939$, $p < 0.0001$), where higher WS-3 concentration equaled more intense cooling. Mauchly's test indicated that the assumption of sphericity was violated, so degrees of freedom were adjusted for within-subject effects using Greenhouse-Geisser estimates of sphericity ($\varepsilon=0.367$). Time had a significant effect on cooling intensity ($F(2.2,449.21) = 111.755$, $p < 0.0001$), as well as the interaction between time and WS-3 concentration ($F\ (11.01,449.21) = 4.161$, $p < 0.0001$). The means were plotted together across time *(Figure 1)*.

Time 0 seconds was chosen as the I_{max} (maximum intensity) for all samples as it was the I_{max} for three of the four samples containing WS-3 (100, 67 and 30 ppm WS-3). The only other sample containing WS-3 that had a different I_{max}, was 20 ppm WS-3. Its I_{max} (at 30 seconds) was not significantly different from its intensity at 0 seconds.

A dose-response relationship was plotted for the I_{max} (time 0 seconds) (*Figure 2*). A one-way ANOVA showed that there was a significant effect of WS-3 concentration on cooling intensity ($F(5,204)=18.736$, $p <0.0001$).

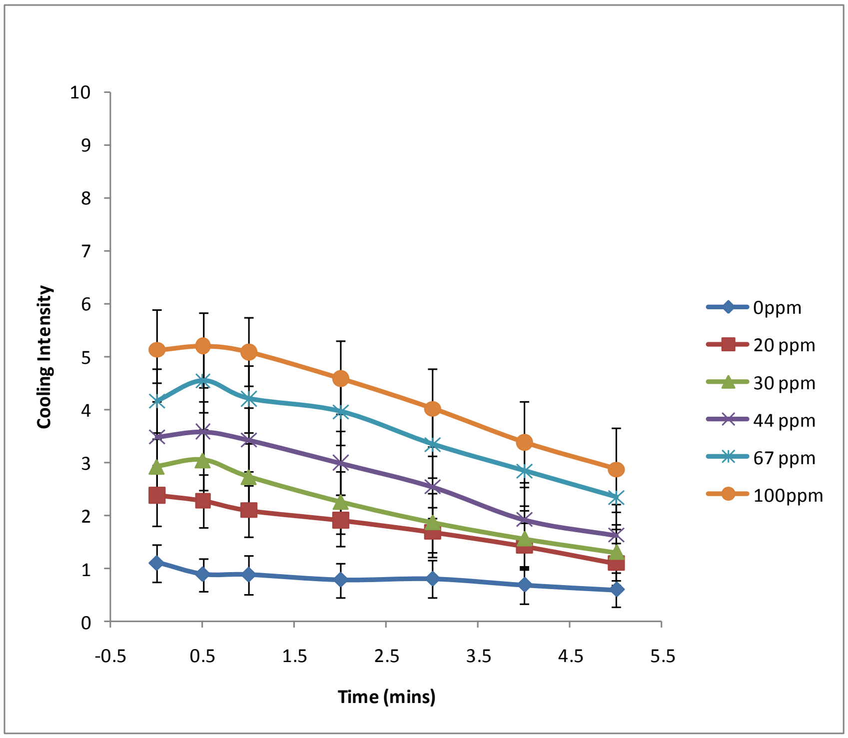


Figure 1.WS-3 Dose Response Cooling Intensities Over Time

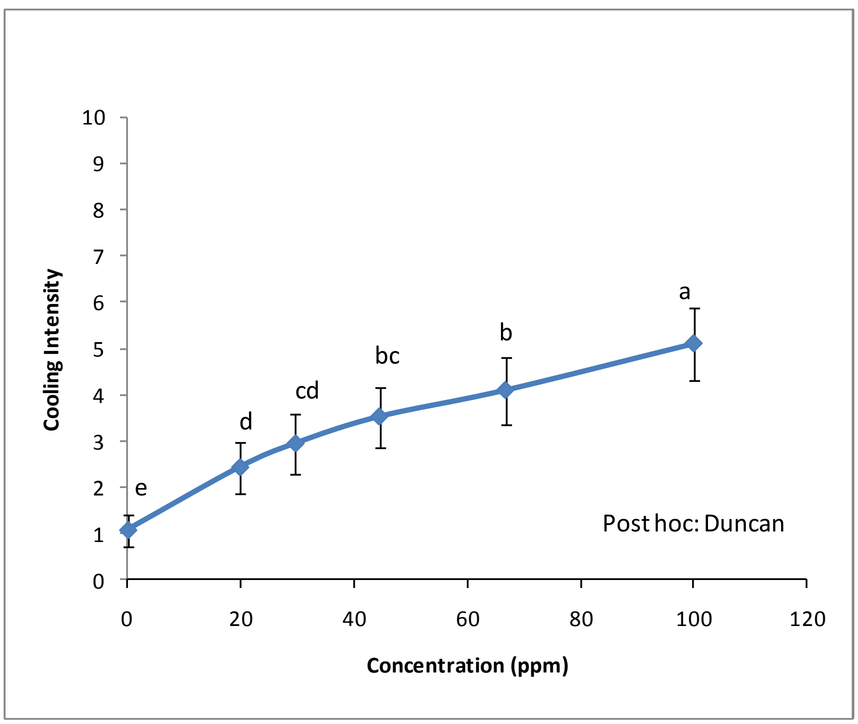


Figure 2. Mean Scores WS-3 Samples at I_{max}
(Duncan means comparison; values with different letters are significantly different at the 95% confidence level)

Based on the I_{max} one-way ANOVA results for cooling intensity, levels were chosen for pairing with flavors in Sessions 5-7. The moderate level concentration chosen was 100 ppm WS-3 and the low level was 30 ppm WS-3. These levels were chosen because they were spread along the dose-response curve and they were significantly different from each other, and from 0 ppm WS-3, at their $I_{max.}$

<u>3.1.1.2.</u> *Warming data for WS-3 dose-response:*

Between subject effects showed that WS-3 concentration had a significant effect on warming intensity when collapsed over time ($F(5,204) = 2.422$, $p < 0.05$), where higher WS-3 concentrations meant higher warming intensity. Mauchly's test indicated that the assumption of sphericity was violated, so degrees of freedom for within subject effects were adjusted using Greenhouse-Geisser estimates of sphericity (ε=0.329). Time had a significant impact on warming intensity ($F(1.974,402.696) = 72.362$, $p < 0.0001$), however, the interaction of time and WS-3 concentration was not significant(F (9.87,402.696) = 1.458, $p = 0.153$). The means were plotted together across time *(Figure 3)*. Even at the highest WS-3 concentration, warming intensity was quite low.

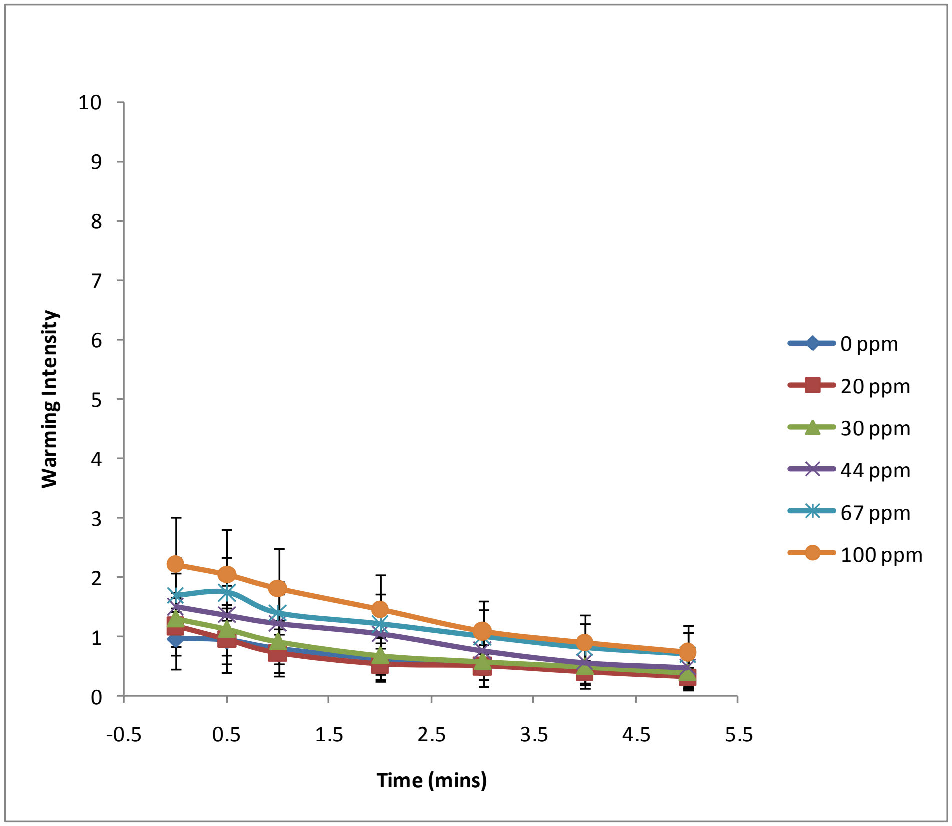


Figure 3. WS-3 Dose Response Warming Intensities Over Time

3.1.1.3. *Sweet data for WS-3 dose-response:*

Between subject effects indicated that, collapsed over time, WS-3 concentration did not have a significant effect on sweet intensity ($F(5,204) = 0.813$, $p = 0.541$). Mauchly's test indicated that the assumption of sphericity was violated, so degrees of freedom for within subject effects were adjusted using Greenhouse-Geisser estimates of sphericity (ε=0.302). Time had a significant impact on sweet intensity ($F(1.812,369.648)=213.210$, $p < 0.0001$), however, the interaction between time and sweet intensity was not significant ($F(9.06,369.648) = 1.317$, $p = 0.225$). The means were plotted together across time *(Figure 4)*.

<u>3.1.1.4.</u> *Sour data for WS-3 dose-response:*

Between subject effects indicated that, collapsed across time, WS-3 concentration did not have a significant effect on sour intensity (F(5,204) = 1.407, p = 0.223). Mauchly's test indicated that the assumption of sphericity was violated, so degrees of freedom were adjusted for within subject effects using Greenhouse-Geisser estimates of sphericity (ε=0.369). Time had a significant impact on sourness intensity (F(2.214,451.656) = 5.202, p < 0.01), however the interaction between time and sourness intensity was not significant (F(11.07,451.656) = 1.512, p = 0.123). The means were plotted together across time *(Figure 4)*.

<u>3.1.1.5.</u> *Bitter data for WS-3 dose-response:*

Between subject effects indicated that WS-3 concentration had a significant effect on bitter intensity, collapsed across time (F (5,204) = 2.379, p < 0.05). Mauchly's test indicated that the assumption of sphericity was violated, so degrees of freedom were adjusted for within subject effects using Greenhouse-Geisser estimates of sphericity (ε=0.312). Time had a significant effect on bitter intensity (F(1.872,381.189)=6.485, p < 0.01) as well as the interaction between WS-3 concentration and time (F(9.36,381.888)=2.507, p < 0.01). The means were plotted together across time *(Figure 4)*.

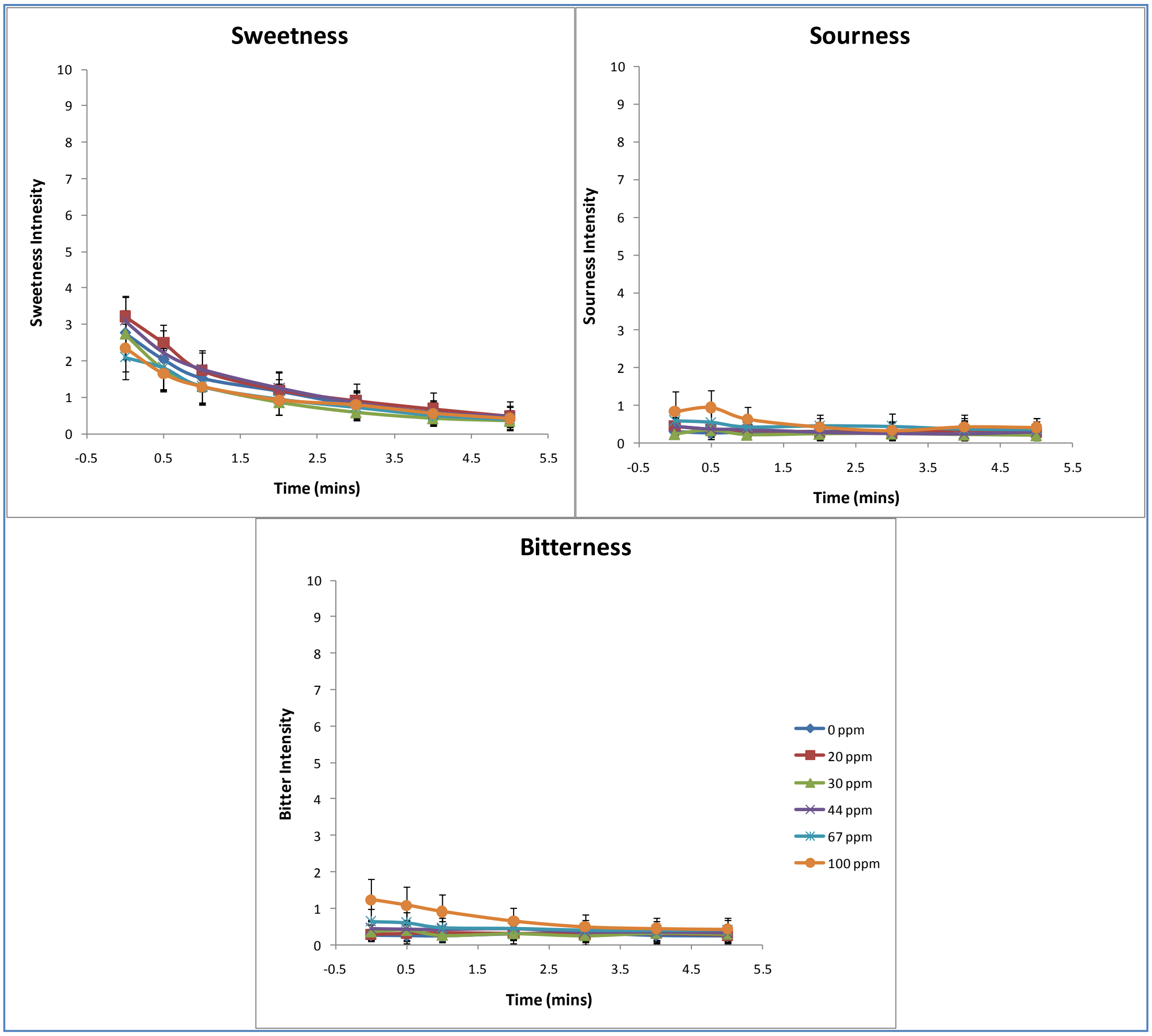


Figure 4. WS-3 dose response sweet, sour and bitter intensities over time

3.1.2. Flavor dose-response:

As we wanted to use one concentration for each flavor, and we wanted the flavors to be matched at moderate intensity, we collected dose-response curves over time for each of the three flavors (spearmint, caramel and apple). The means were plotted together across time for each flavor *(Figure 5)*.

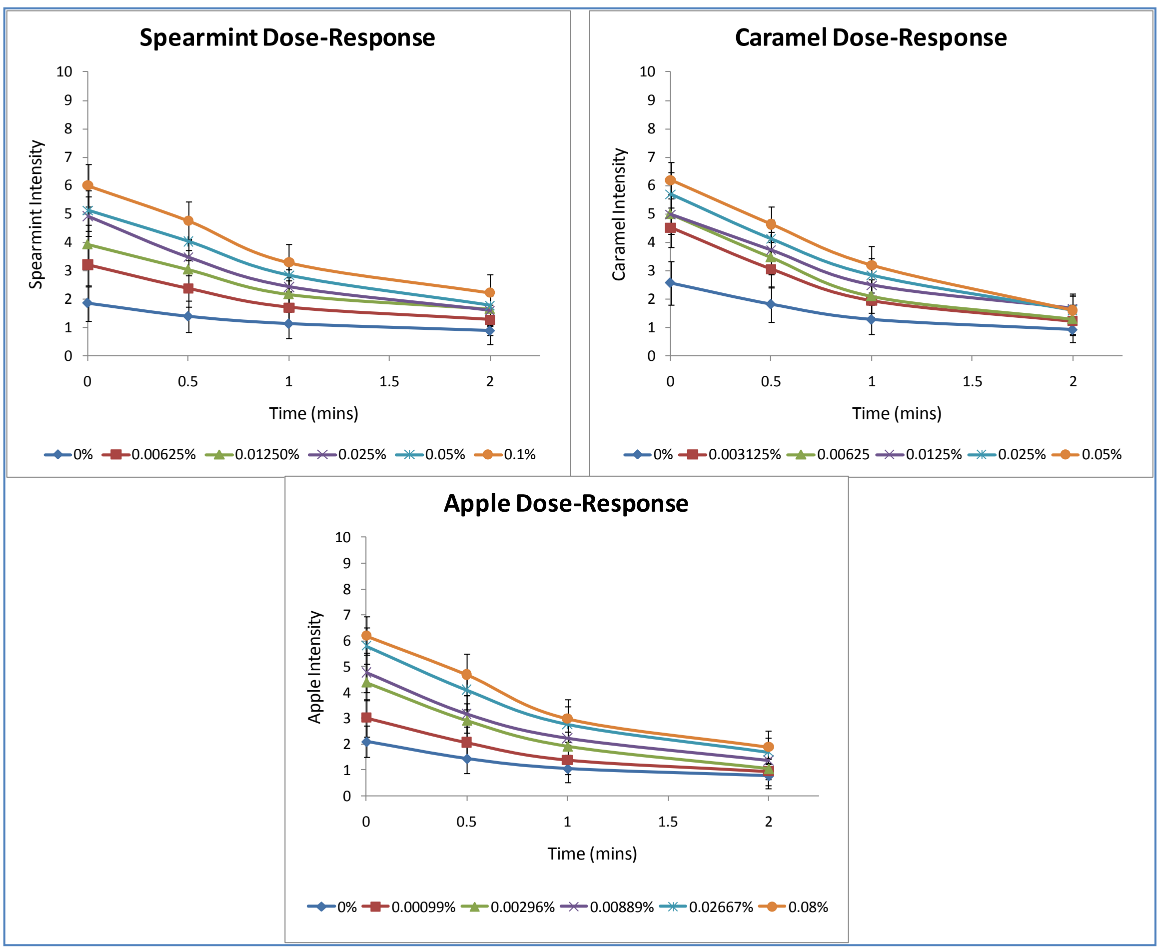


Figure 5. Flavor Dose-Response Intensities Over Time

From the flavor dose-response curves, concentrations that were moderate and iso-intense (at a flavor intensity of '5') were chosen. The chosen concentrations are plotted together in *Figure 6*.

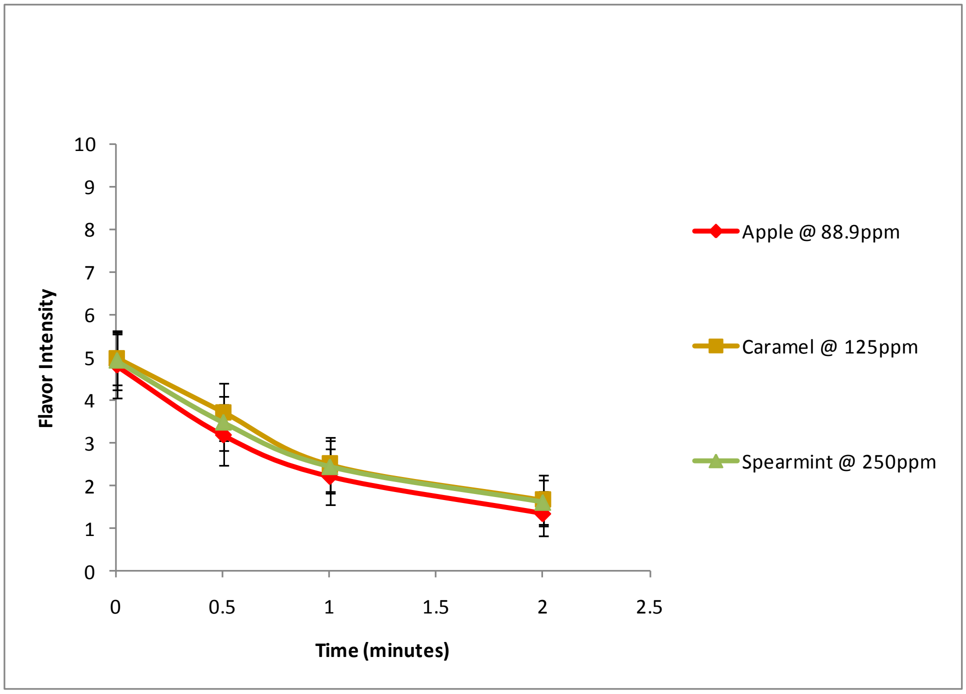


Figure 6. Moderate, iso-intense flavor concentration choices

3.2. Part 2 - Cooling-flavor mixture intensities:

The objective of this experiment was to understand if there are perceptual interactions between cooling and flavors, if these interactions were dependent of the congruency of the flavor pairing and if there was an influence of the interaction on the temporal profiles of the attributes.

3.2.1. Cooling data for Mixtures:

According to the between subjects effects, WS-3 concentration had a significant effect on cooling intensity, collapsed across time and flavor ($F(2,384) = 165.147$, $p < 0.0001$). Flavor did not have a significant effect on cooling intensity when collapsed over time and WS-3 concentration ($F(3,384) = 1.192$, $p = 0.312$). There was not a significant interaction between flavor and cooling ($F(6,384) = 0.469$, $p = 0.831$), however, trends showed that cooling was more intense when apple flavor was paired with WS-3 at 30 ppm and 100 ppm. Mauchly's test indicated that the assumption of sphericity was violated, so degrees of freedom were adjusted for within subject effects using Greenhouse-Geisser estimates of sphericity (ε=0.326). Time had a significant effect on cooling intensity ($F(1.956,751.1) = 233.290$, $p < 0.0001$). The interaction between WS-3 concentration and time was significant ($F(3.912,751.1) = 26.880$, $p < 0.0001$). Neither the interaction between flavor and time ($F(5.868,751.1) = 1.751$, $p = 0.108$), nor the interaction between flavor, concentration and time ($F(11.736,751.1) = 0.846$, $p = 0.600$) was significant. The means of the different flavor mixtures are plotted over time, according to the WS-3 concentration in the mixture *(Figure 7)*.

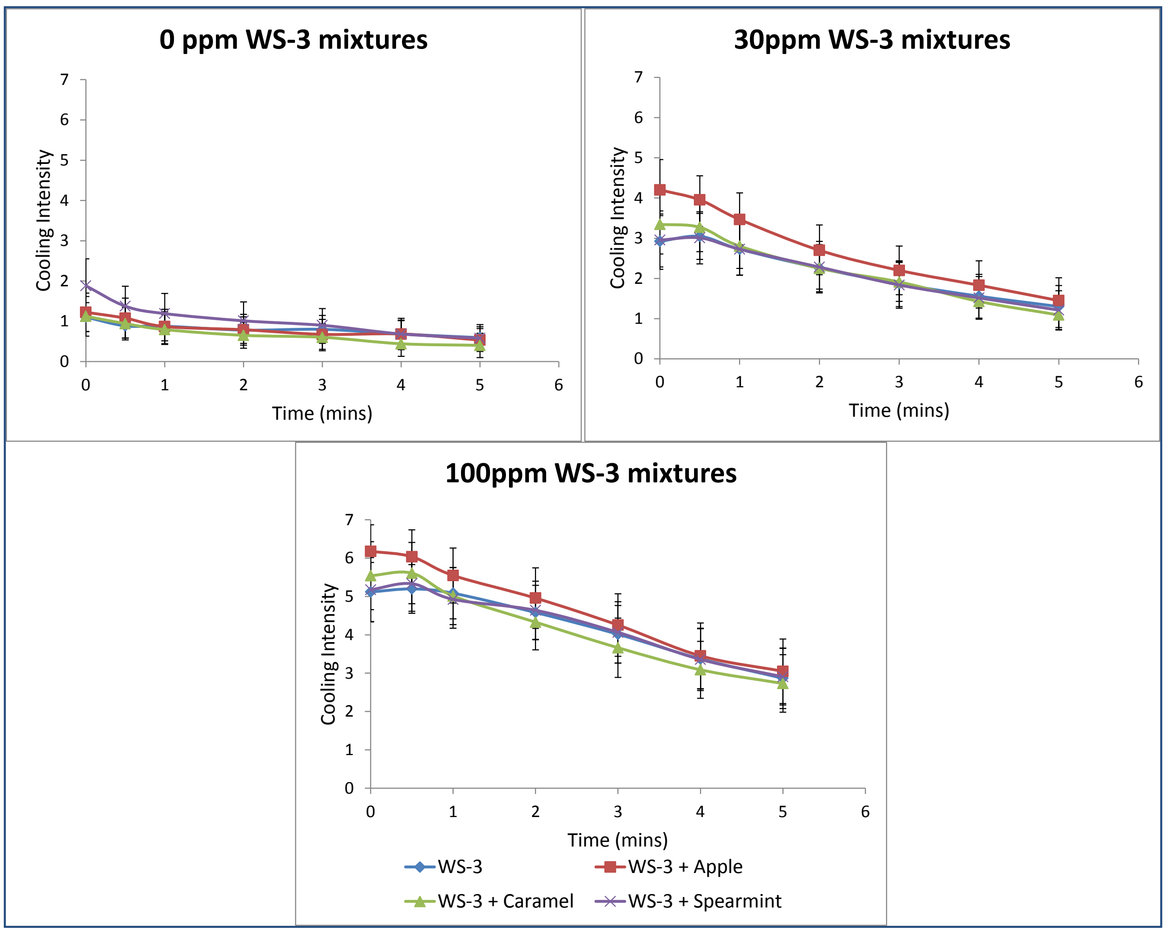


Figure 7. Cooling Over Time: Flavor Only, Flavors with 30 ppm WS-3 and Flavors with 100 ppm WS-3

Since there were significant effects from the two-way Repeated Measures ANOVA and trends in the means, further analysis was done looking only at the I_{max}. The I_{max} was chosen as time 0 seconds because, in most samples in which WS-3 was added, the cooling I_{max} was at either 0 or 30 seconds. For the other attributes, the I_{max} was mostly at 0 seconds.

Bar charts were created for I_{max} with samples compared by flavor or by WS-3 concentration *(Figures 8 & 9, respectively)*. In the case which they are compared across flavor, there are no significant differences between flavors. We expected to see spearmint samples be the most cooling, if any. In the case which there is comparison across WS-3 concentrations per flavor, each concentration is significantly different from one another, except with spearmint. With spearmint flavor, 0 ppm and 30 ppm WS-3 are not significantly different from each other. This reinforces the idea of congruency – even though no cooling was present in 0 ppm samples, panelists expected cooling with mint flavor.

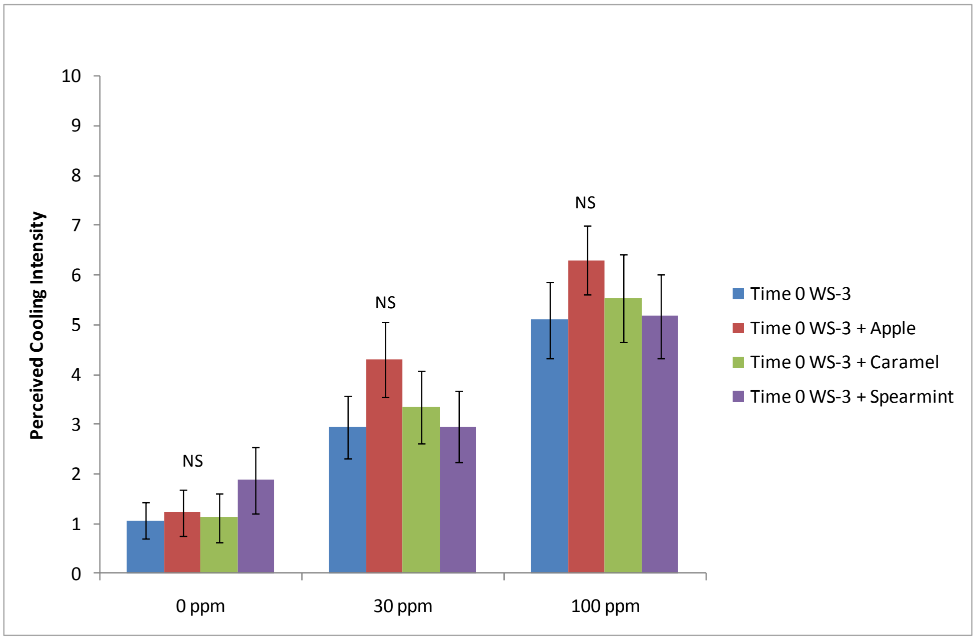


Figure 8. Mean Cooling Intensity of Mixes, at I_{max}, by Concentration
(Duncan means comparison, NS = no significant difference and values with different letters are significantly different at the 95% confidence level)

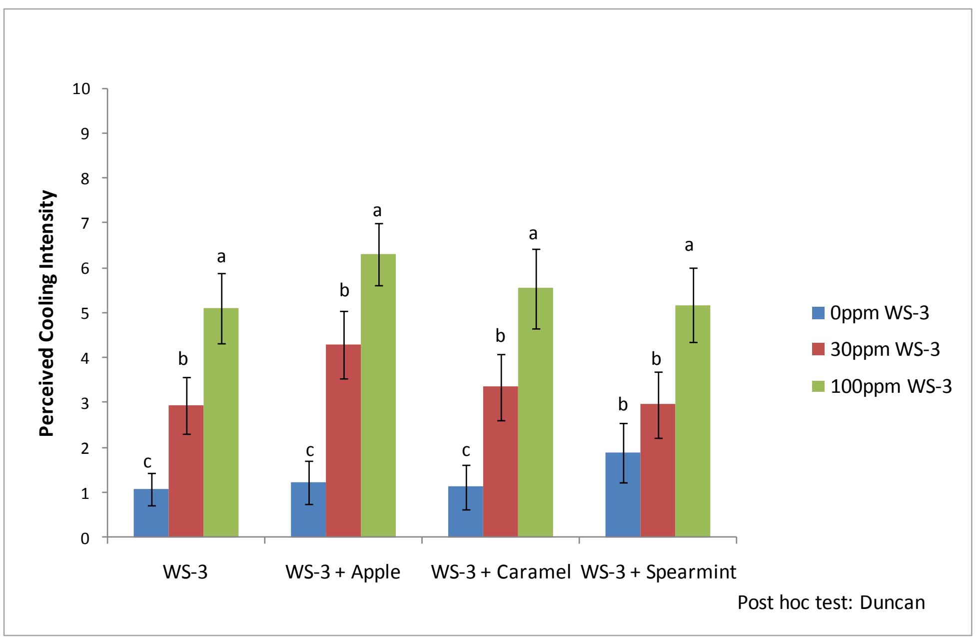


Figure 9. Mean Cooling Intensity of Mixes, at I_{max}, by Flavor
(Duncan means comparison, NS = no significant difference and values with different letters are significantly different at the 95% confidence level)

3.2.2. Warming data for Mixtures:

According to between subject effects, WS-3 concentration had a significant effect on warming intensity, collapsed across time and flavor ($F(2,384) = 11.659$, $p < 0.0001$). Flavor did not have a significant effect on warming intensity when collapsed over time and WS-3 concentration ($F(3,384) = 1.414$, $p = 0.238$), nor did the interaction between WS-3 concentration and flavor ($F(6,384) = 0.126$, $p = 0.993$). Mauchly's test indicated that the assumption of sphericity was violated, so degrees of freedom were adjusted for within subject effects using Greenhouse-Geisser estimates of sphericity ($\varepsilon=0.269$). Time had a significant effect on warming intensity ($F(1.614,619.776) = 135.929$, $p < 0.0001$). The interaction between WS-3 concentration and time was significant ($F(3.228,619.776) = 9.383$, $p < 0.0001$). Neither the interaction between flavor and time ($F(3.228,619.776) = 1.229$, $p = 0.294$) nor the interaction between time, flavor and concentration ($F(11.298,619.776) = 0.804$, $p = 0.639$) was significant. These results suggest that warming intensity ratings, and their evolution over time, were only influenced by WS-3 concentration, not flavor.

Since the two-way Repeated Measures ANOVA showed significant effects, the I_{max} was investigated further. When concentrations were compared within a flavor, at I_{max}, it was found that all 0 ppm samples were significantly weaker than 100 ppm WS-3 samples, except for spearmint flavor. Within spearmint flavor, there are no significant differences between WS-3 concentrations (although the pattern is the same as for the other flavors *(Figure 10)*. These results suggest that panelists may associate spearmint flavor with a warming sensation, making even their ratings for samples without WS-3 insignificantly different from samples containing the highest level of WS-3.

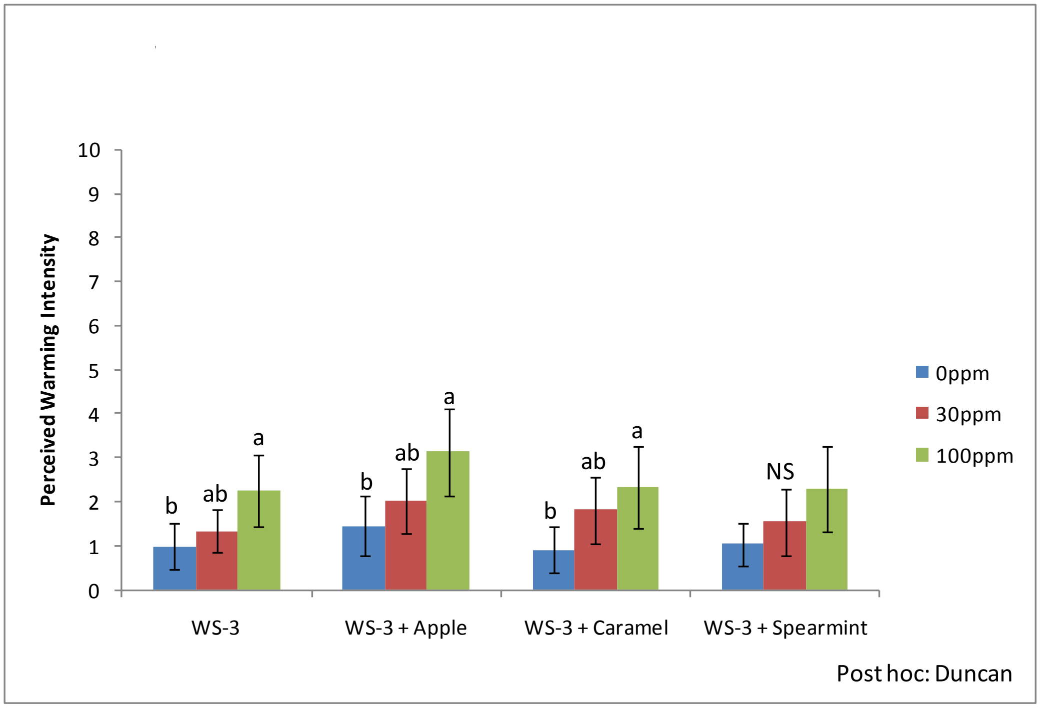


Figure 10. Mean Warming Intensity of Mixes, at I_{max}, by Flavor (Duncan means comparison, NS = no significant difference and values with different letters are significantly different at the 95% confidence level)

<u>*3.2.3.*</u> Sweet data *for Mixtures:*

Between subject effects indicated that flavor did not have a significant effect on sweet intensity ($F(3,384) = 2.359$, $p = 0.071$). WS-3 concentration ($F(2,384) = 2.193$, $p = 0.113$) had no significant effect on sweet intensity when ignoring time and each other. Mauchly's test indicated that the assumption of sphericity was violated, so degrees of freedom for within subject effects were adjusted using Greenhouse-Geisser estimates of sphericity ($\varepsilon=0.345$). Time had a significant effect on sweet intensity ($F(2.07,794.88) = 552.535$, $p < 0.0001$). The interaction between WS-3 concentration and time was significant ($F(4.14,794.88) = 3.792$, $p < 0.01$) as was the interaction between flavor and time ($F(6.21,794.88) = 3.221$, $p < 0.01$). The interaction between time, WS-3 concentration and flavor did not have a significant effect on sweet intensity

($F(14.49,794.88) = 1.112$, $p = 0.341$). The means of the different flavor mixtures are plotted over time, according to the WS-3 concentration in the mixture *(Figures 11)*.

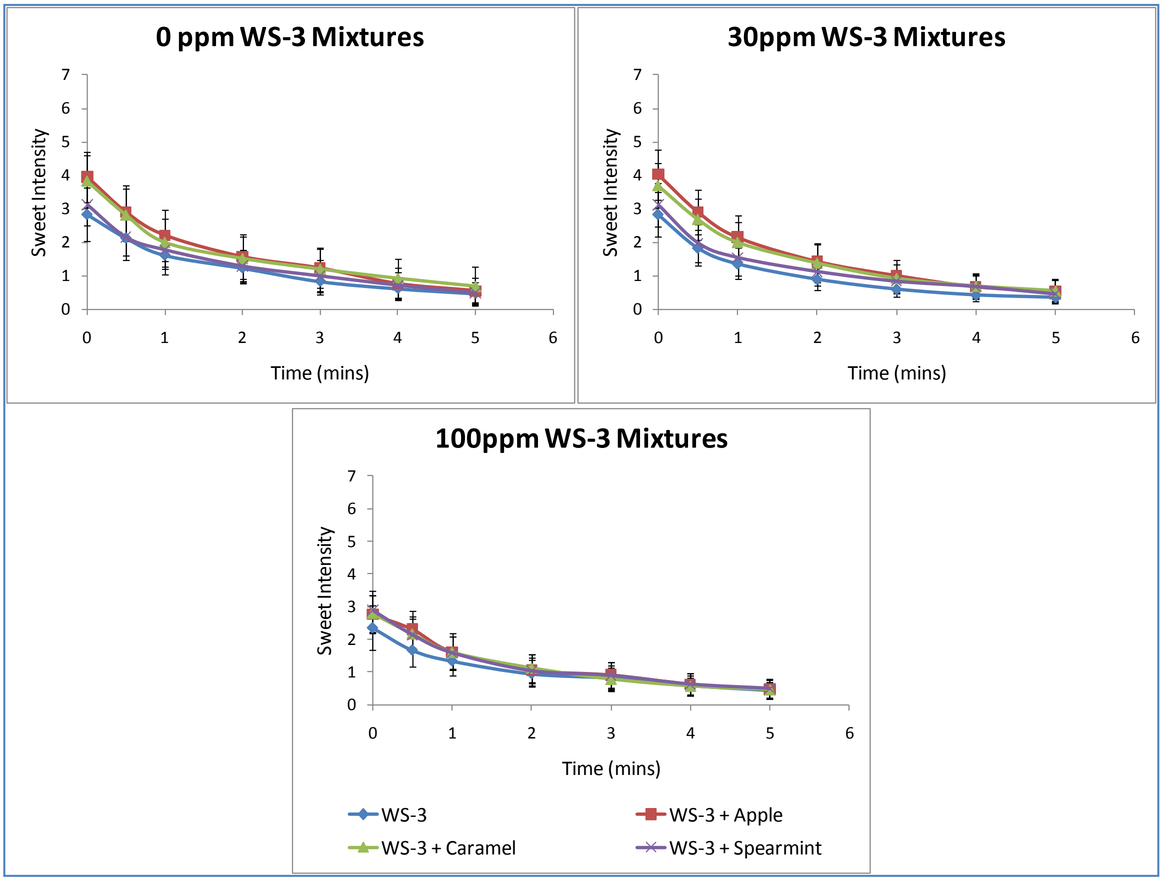


Figure 11. Sweetness Over Time: Flavor Only, with 30 ppm WS-3 and with 100 ppm WS-3

Since the repeated measure ANOVA showed significant effects, the I_{max} was investigated further. When concentrations were compared within a flavor, the trend showed that 100 ppm samples were less sweet than other WS-3 concentrations, however, this was only significant when apple flavor was paired with WS-3 *(Figure 12)*. When compared within a concentration, there are no significant differences in sweetness I_{max} between flavors *(Figure 13)*.

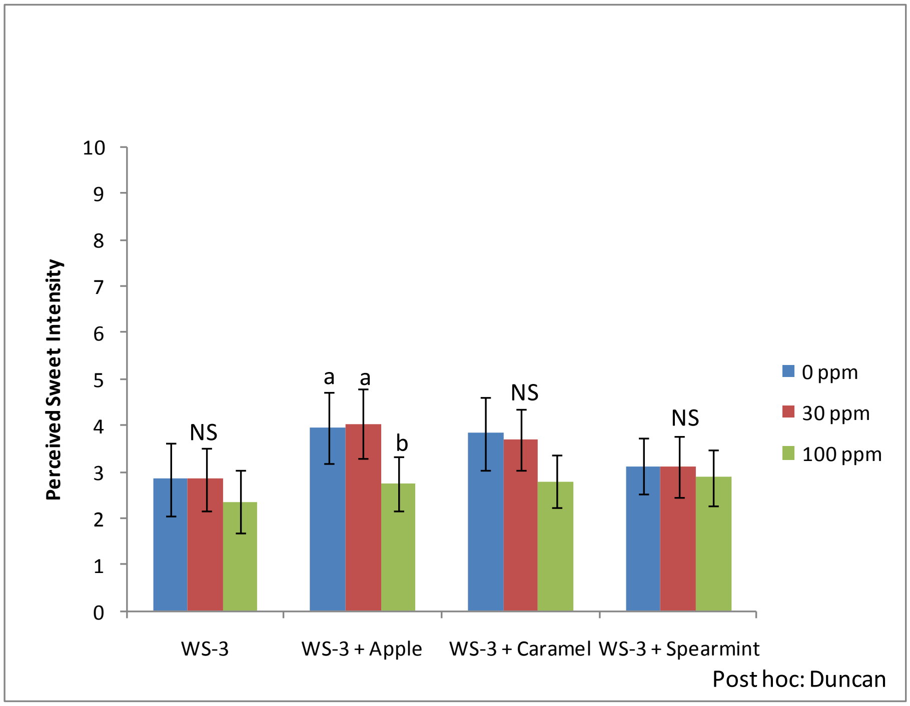


Figure 12. Mean Sweetness Intensity of Mixes, at I_{max}, by Flavor (Duncan means comparison, NS = no significant difference and values with different letters are significantly different at the 95% confidence level)

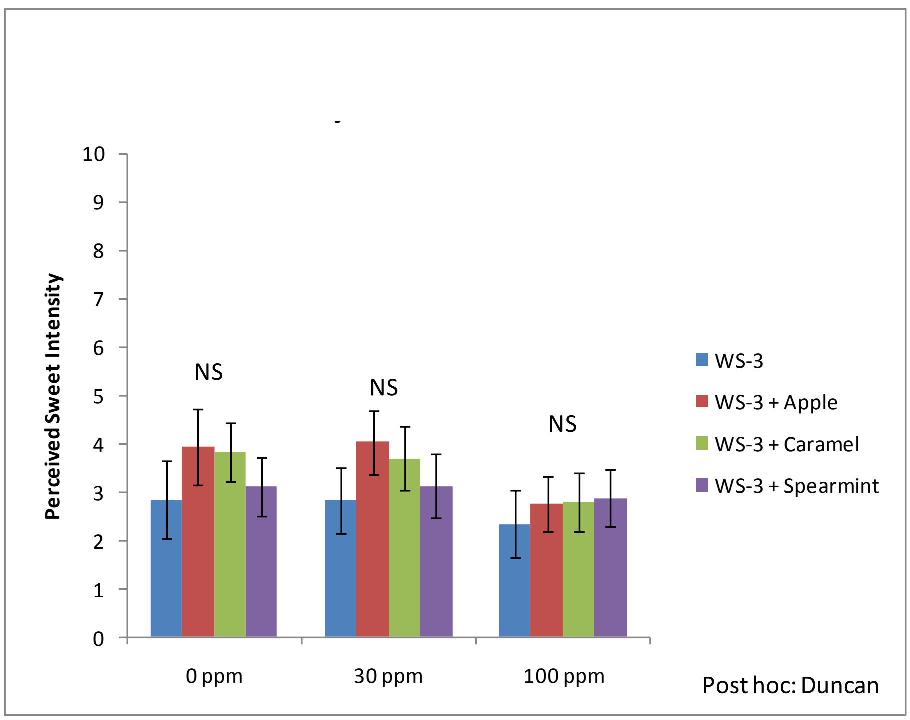


Figure 13. Mean Sweetness Intensity of Mixes, at I_{max}, by Concentration (Duncan means comparison, NS = no significant difference and values with different letters are significantly different at the 95% confidence level)

3.2.4. Sour data for Mixtures:

Between subject effects indicated that both flavor ($F(3,390) = 3.717$, $p < 0.05$) and WS-3 concentration ($F(2,390) = 3.960$, $p < 0.05$) had a significant effect on sour intensity when collapsed across time and each other. The interaction between WS-3 concentration and flavor did not have a significant effect on sour intensity ($F(3,384) = 0.443$, $p = 0.850$). Mauchly's test indicated that the assumption of sphericity was violated, so degrees of freedom for within subject effects were adjusted using Greenhouse-Geisser estimates of sphericity ($\varepsilon=0.280$). Time had a significant effect on sour intensity ($F(1.68,645.12) = 7.572$, $p < 0.01$). The interaction between WS-3 concentration and time was significant ($F(3.36,645.12) = 3.591$, $p < 0.05$) as was the interaction between flavor and time ($F(5.04,645.12) = 4.043$, $p < 0.05$). The interaction between time, WS-3 concentration and flavor did not have a significant effect on the sour intensity ($F(11.76,645.12) = 1.405$, $p = 0.160$). The means of the different flavor mixtures are plotted over time, according to the WS-3 concentration in the mixture *(Figure 14)*.

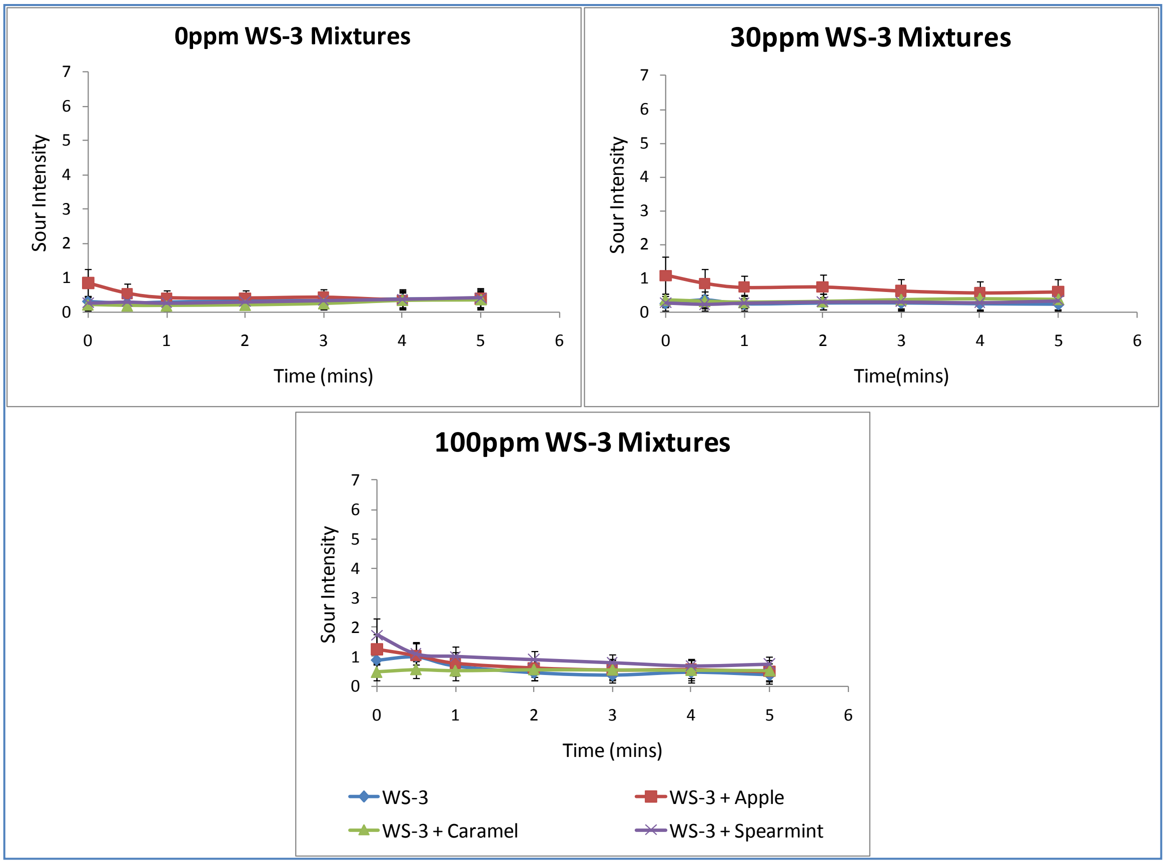


Figure 14. Sourness Over Time: Flavor Only, with 30 ppm WS-3 and with 100 ppm WS-3

Since the repeated measure ANOVA showed significant effects, the I_{max} was investigated further. When concentrations were compared within a flavor, at I_{max}, the trend showed that 100 ppm samples were more sour than other WS-3 concentrations, however, this was only significant in the WS-3 only condition *(Figure 15)*. When compared within a concentration, samples with apple were significantly more sour with 0 ppm and 30 ppm WS-3 *(Figure 16)*.

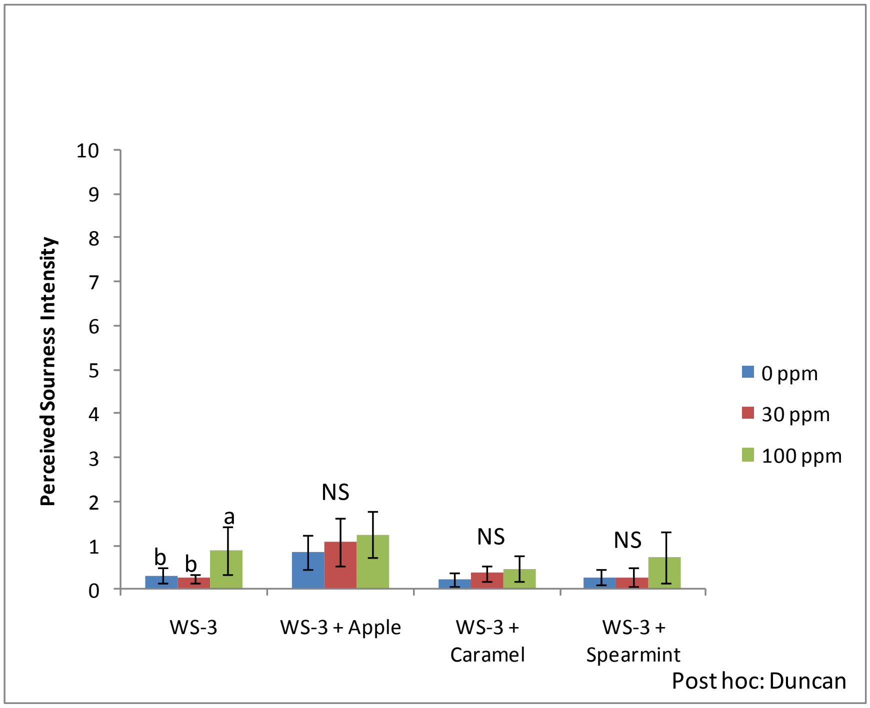


Figure 15. Mean Sourness Intensity of Mixes, at I_{max}, by Flavor (Duncan means comparison, NS = no significant difference and values with different letters are significantly different at the 95% confidence level)

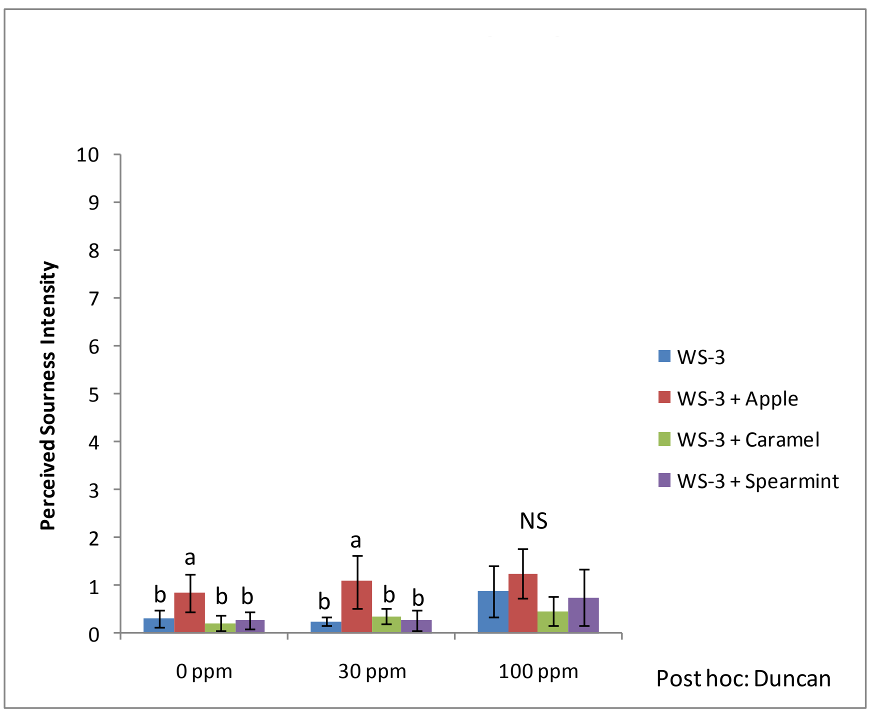


Figure 16. Mean Sourness Intensity of Mixes, at I_{max}, by Concentration (Duncan means comparison, NS = no significant difference and values with different letters are significantly different at the 95% confidence level)

3.2.5. Bitter data for Mixtures:

Between subject effects indicated that both flavor ($F(3,384) = 4.010$, $p < 0.05$) and WS-3 concentration ($F(2,384) = 3.056$, $p < 0.05$) had a significant effect on bitter intensity when collapsed across time and each other. The interaction between flavor and WS-3 concentration did not have a significant effect on bitter intensity ($F(6,384) = 0.431$, $p = 0.858$) Mauchly's test indicated that the assumption of sphericity was violated, so degrees of freedom for within subject effects were adjusted using Greenhouse-Geisser estimates of sphericity ($\varepsilon=0.366$). Time had a significant effect on bitter intensity ($F(2.196,843.264) = 8.450$, $p < 0.01$). The interaction between WS-3 concentration and time was not significant ($F(4.392,843.264) = 1.232$, $p = 0.294$), nor was the interaction between flavor and time ($F(6.588,843.264) = 1.905$, $p = 0.070$). The interaction between WS-3 concentration, flavor and time did not have a significant effect on bitter intensity either ($F(15.372,843.264) = 1.314$, $p = 0.184$).

Since the between subject effects indicated differences based on flavor and WS-3 concentration, further one-way ANOVA of the I_{max} was done. For all, when grouped by flavor, 100 ppm WS-3 samples were more bitter than 0 ppm and more than 30 ppm samples in all cases except apple *(Figure 17)*. When grouped by WS-3 concentration, the trend shows that apple samples are slightly more bitter than other flavors, however, there were no significant differences *(Figure 18)*.

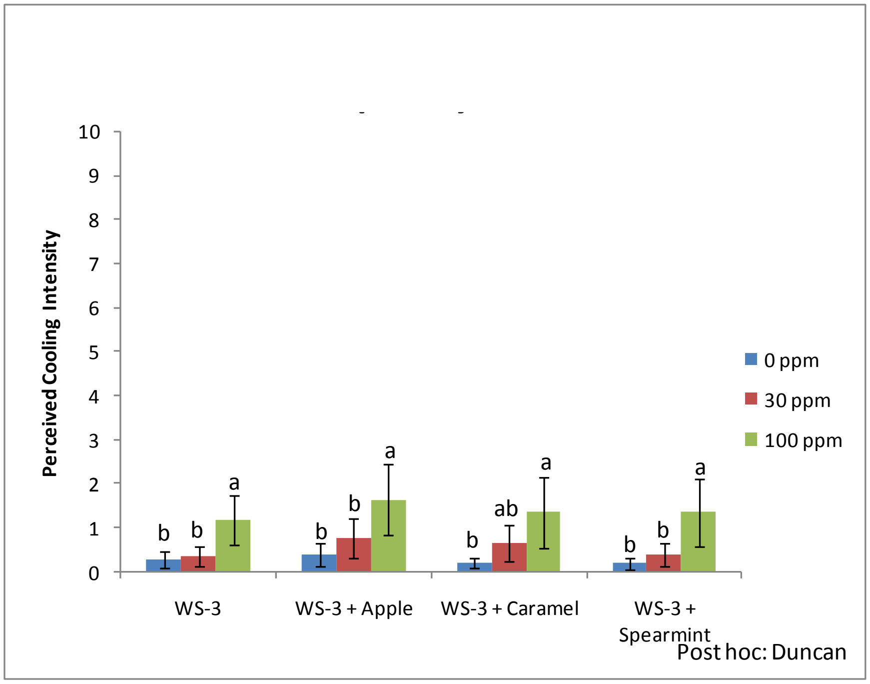


Figure 17. Mean Bitterness Intensity of Mixes, at I_{max}, by Flavor (Duncan means comparison, NS = no significant difference and values with different letters are significantly different at the 95% confidence level)

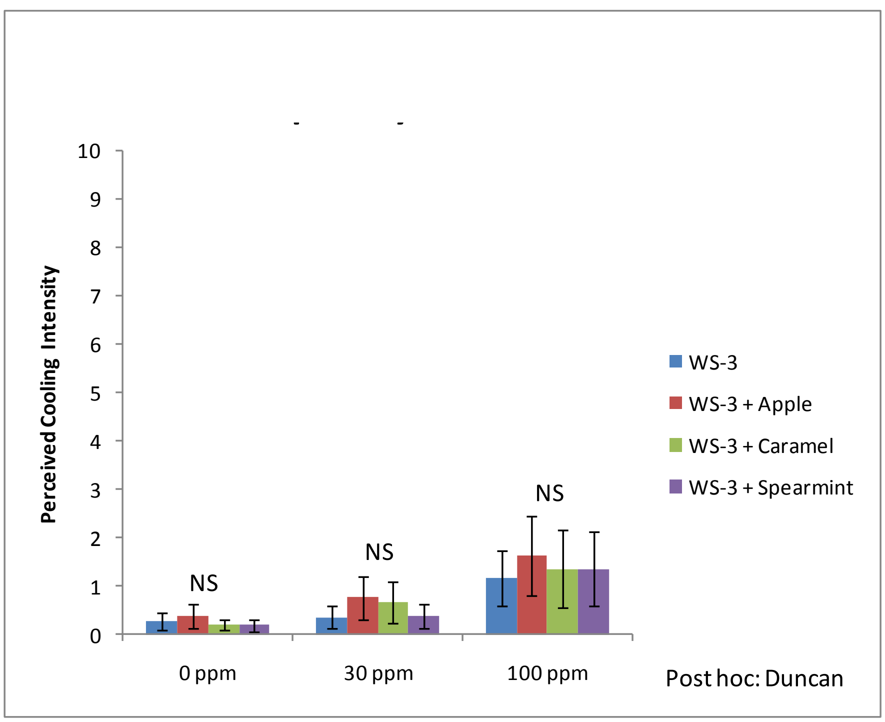


Figure 18. Mean Bitterness Intensity of Mixes, at I_{max}, by Concentration (Duncan means comparison, NS = no significant difference and values with different letters are significantly different at the 95% confidence level)

3.2.6. Apple data for Mixtures:

Between subject effects indicated that WS-3 concentration ($F(2,102) = 0.095$, $p = 0.909$) did not have a significant effect on apple flavor intensity when collapsed across time. Mauchly's test indicated that the assumption of sphericity was violated, so degrees of freedom for within subject effects were adjusted using Greenhouse-Geisser estimates of sphericity ($\varepsilon=0.396$). Time had a significant effect on apple intensity ($F(2.376,242.352) = 290.789$, $p < 0.0001$). The interaction between WS-3 concentration and time was not significant ($F(4.752,242.352) = 0.772$, $p = 0.564$). The means for apple intensity for the WS-3 concentrations were plotted together over the 5 minutes *(Figure 19)*.

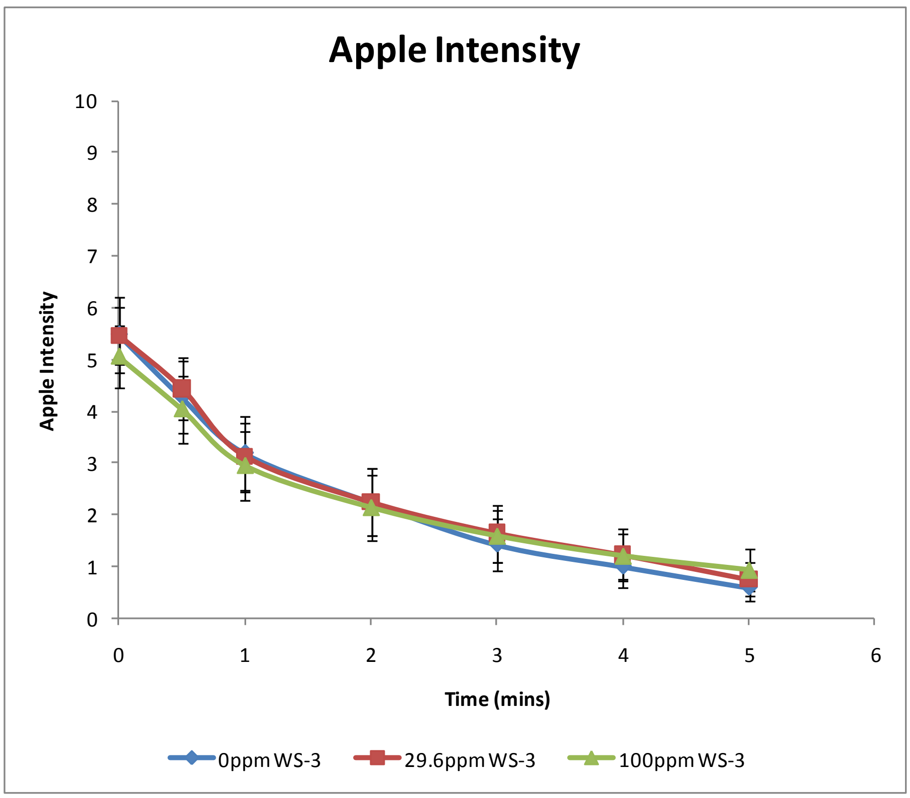


Figure 19. Mean Apple Flavor Intensities Over Time

3.2.7. Caramel data for WS-3/Flavor Mixtures:

Between subject effects indicated that WS-3 concentration ($F(2,96) = 0.351$, $p = 0.705$) did not have a significant effect on caramel flavor intensity when collapsed across time. Mauchly's test indicated that the assumption of sphericity was violated, so degrees of freedom for within subject effects were adjusted using Greenhouse-Geisser estimates of sphericity ($\varepsilon=0.369$). Time had a significant effect on apple intensity ($F(2.214,212.544) = 244.782$, $p < 0.0001$). The interaction between WS-3 concentration and time was not significant ($F(4.428,185.679) = 0.916$, $p = 0.462$). The means for caramel intensity for the WS-3 concentrations were plotted together over 5 minutes *(Figure 20)*.

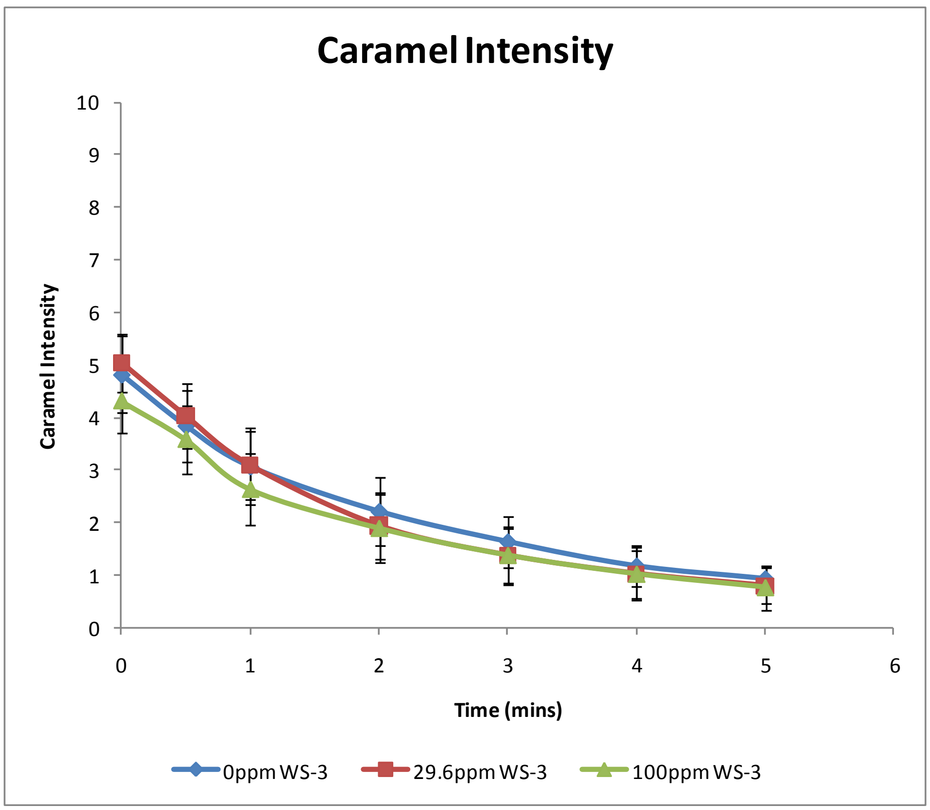


Figure 20. Mean Caramel Flavor Intensities Over Time

3.2.8. Spearmint data for Mixtures:

In contrast to the other flavors, between subject effects indicated that WS-3 concentration ($F(2,96) = 3.698$, $p < 0.05$) had a significant effect on spearmint flavor intensity when collapsed across time. Mauchly's test indicated that the assumption of sphericity was violated, so degrees of freedom for within subject effects were adjusted using Greenhouse-Geisser estimates of sphericity ($\varepsilon=0.311$). Time had a significant effect on spearmint intensity ($F(1.866,179.136) = 252.277$, $p < 0.0001$). The interaction between WS-3 concentration and time was not significant ($F(3.732,179.136) = 1.462$, $p = 0.218$). The means for spearmint intensity for the WS-3 concentrations were plotted together over 5 minutes *(Figure 21)*. ANOVA was calculated to compare difference between concentrations within a time point. Samples with 100 ppm WS-3 were significantly stronger in spearmint flavor intensity than those without WS-3 over the period of 0 seconds to 2 minutes.

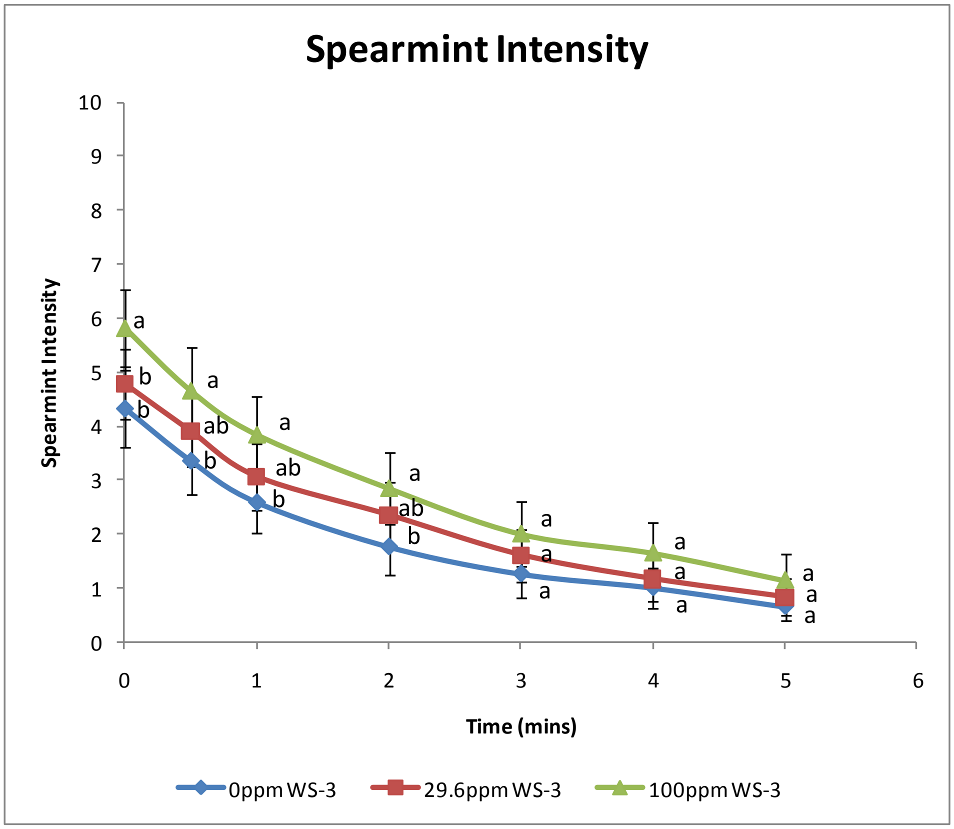


Figure 21: Mean Spearmint Flavor Intensities, Over Time
(One-way ANOVA per time point done with Duncan mean comparison at the 0.05 confidence level)

3.3. Part 3 - Emotions and Liking:

The means and confidence intervals were calculated for each point. Data was analyzed using two-way ANOVA in XLStat 2012. An alpha level of 0.05 was used for all statistical tests. All post-hoc tests were done using the Duncan criterion.

An overall two-way ANOVA was done to investigate the effects of flavor and WS-3 concentration on each of the attributes measured. Each marked box in *Table 1* indicates a statistically significant effect.

Table 3. ANOVA Significance for Hedonic Measures

	Happy	Sensual	Disgust	Relax	Energy	Hunger	Interest	Nostalgia	Spiritual	Intensity	Familiar	Liking
Flavor					•						•	
Conc	•	•	•	•			•			•	•	•
Flav x Conc												•

Further comparisons were done in within a flavor, across WS-3concentrations (*Figure 22, 23 & 24*). One-way ANOVAs were done to compare the attributes within each flavor.

3.3.1. ***Caramel Samples***

A one-way ANOVA was conducted with WS-3 concentration as the factor. The means are shown for each caramel sample with each concentration in *Table 4.* When caramel flavor was mixed with varying levels of WS-3, there were differences between the concentrations with many attributes, as pictured in *Figure 22.*

Table 4: Mean Hedonic Scores for Caramel Samples

Sample	Liking	Familiarity	Intensity	Happy	Sensual	Disgust	Relax	Energy	Hunger	Interest	Nostalgia	Spiritual
0 ppm	7.4^{a}	7.0^{a}	5.9^{b}	6.3^{a}	3.3^{a}	0.5^{b}	5.2^{a}	4.1	3.1	5.1^{a}	2.7^{a}	1.4
30 ppm	6.2^{b}	6.1^{ab}	6.2^{b}	5.6^{a}	2.5^{ab}	0.8^{b}	3.2^{b}	4.4	2.3	4.5^{a}	1.9^{ab}	1.0
100 ppm	3.3^{c}	5.5^{b}	8.1^{a}	3.0^{b}	1.6^{b}	3.2^{a}	1.9^{c}	3.5	2.1	3.0^{b}	0.9^{b}	0.8

(Duncan means comparison; values with different letters are significantly different at the 95% confidence level; values with no letters indicates no significant differences between concentrations)

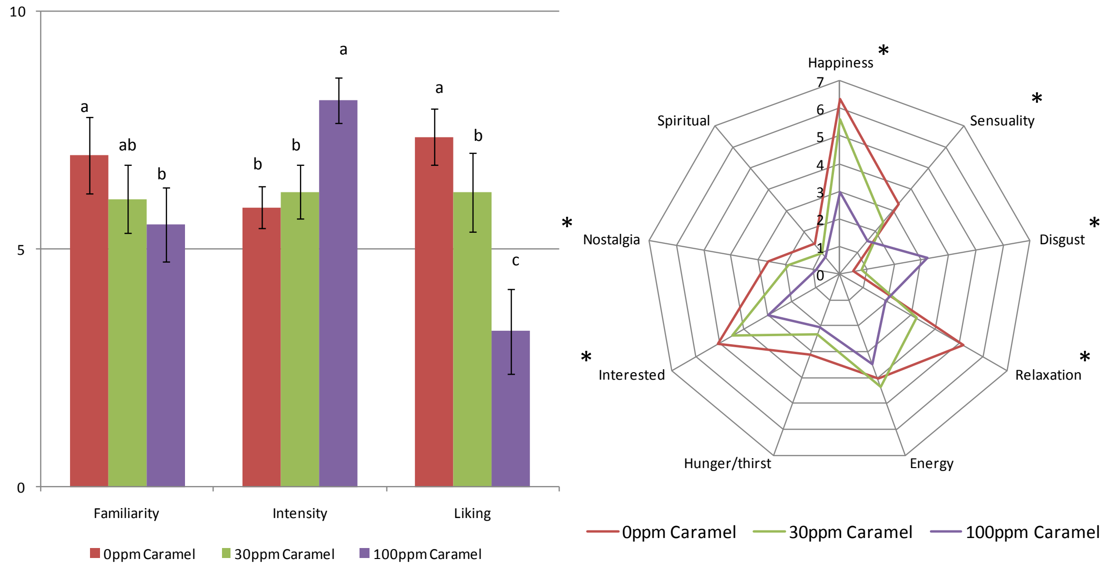


Figure 22.Hedonic Results of Samples with Caramel Flavor
*NS indicates no significant difference, * indicates a significant difference at 0.05 confidence level*

Happiness and interest were significantly higher when paired with 30 ppm or 0 ppm WS-3 than with 100 ppm. Samples paired with 100 ppm were significantly higher in both disgust and intensity than those paired with 30 ppm or 0 ppm WS-3. Relaxation and liking were significantly less intense with increased WS-3 concentration. 100 ppm WS-3 with caramel flavor was disliked, while the other samples were not. Sensuality, familiarity and nostalgia were significantly higher when paired with 0 ppm WS-3 than with 100 ppm WS-3.

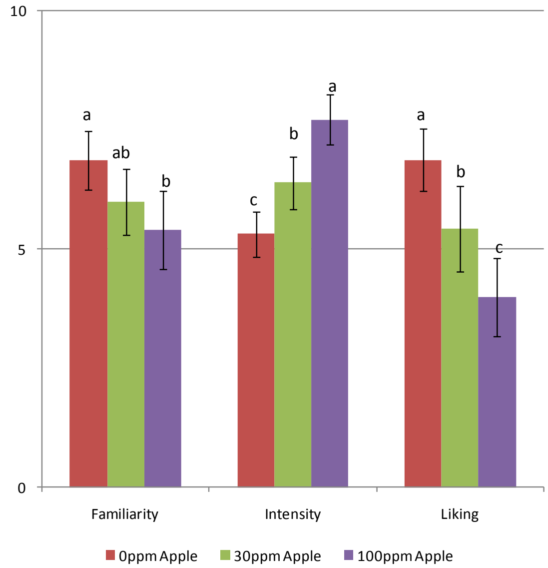
3.3.2. ***Apple Samples***

A one-way ANOVA was conducted with WS-3 concentration as the factor. The means are shown for each apple sample with each concentration in *Table 5.* Like caramel, when apple flavor is mixed with varying levels of WS-3, there are many differences within an attribute, as pictured in *Figure 23.*

Table 5: Mean Hedonic Scores for Apple Samples

Sample	Liking	Familiarity	Intensity	Happy	Sensual	Disgust	Relax	Energy	Hunger	Interest	Nostalgia	Spiritual
0 ppm	6.9[a]	6.9[a]	5.3[c]	5.9[a]	2.9[a]	0.2[b]	4.5[a]	3.8	2.9	4.9[a]	2.4	1.3
30 ppm	5.4[b]	6[ab]	6.4[b]	5.2[a]	2.3[ab]	1.2[b]	3.6[ab]	4.2	2.6	4.0[ab]	2.1	1.4
100 ppm	4.0[c]	5.4[b]	7.7[a]	3.4[b]	1.6[b]	3.2[a]	2.5[b]	3.5	2.0	3.1[b]	1.7	1.1

(Duncan means comparison, values with different letters are significantly different at the 95% confidence level; values with no letters indicates no significant differences between concentrations)



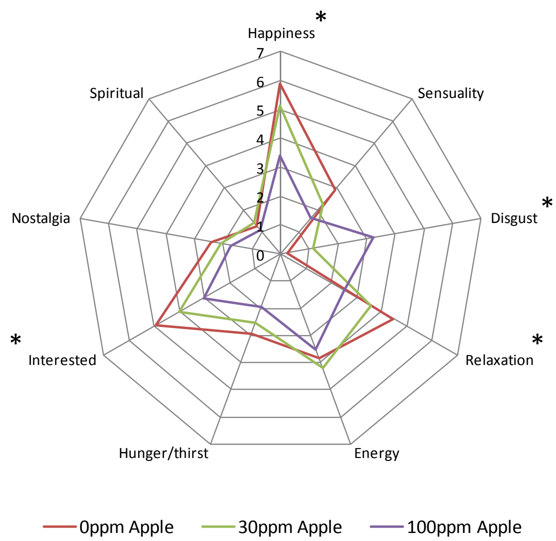


*Figure 23. NS indicates no significant difference, * indicates a significant difference at 0.05 confidence level*

Happiness and liking decrease significantly with each increase in WS-3 concentration. Apple flavor paired with 100 ppm WS-3 was disliked, while 30 ppm WS-3 was close to neutral and 0 ppm WS-3 was liked. In the cases of sensuality, relaxation, interest and familiarity, 0 ppm WS-3 was significantly higher rated than 100 ppm WS-3. With increasing WS-3 concentration, disgust and intensity increase significantly.

3.3.3. ***Spearmint Samples***

A one-way ANOVA was conducted with WS-3 concentration as the factor. The means are shown for each spearmint sample with each concentration in *Table 6.* When changing the WS-3 concentration of spearmint samples there are changes in some of the attributes, as pictured in *Figure 24*.

Table 6: Mean hedonic scores for Spearmint Samples

Sample	Liking	Familiarity	Intensity	Happy	Sensual	Disgust	Relax	Energy	Hunger	Interest	Nostalgia	Spiritual
0 ppm	6.8^{a}	7.4	5.4^{c}	5.7^{a}	2.7^{a}	0.5^{b}	4.6^{a}	4.7	2.7	4.6^{a}	1.8	1.5
30 ppm	6.3^{a}	7.9	6.8^{b}	5.2^{a}	2.1^{ab}	0.6^{b}	4.3^{a}	5.4	3.0	3.7^{ab}	1.7	1.9
100 ppm	5.1^{b}	7.7	8.4^{a}	3.3^{b}	1.5^{b}	2.4^{a}	2.4^{b}	4.9	2.5	3.1^{b}	1.7	1.3

(Duncan means comparison, values with different letters are significantly different at the 95% confidence level; values with no letters indicates no significant differences between concentrations)

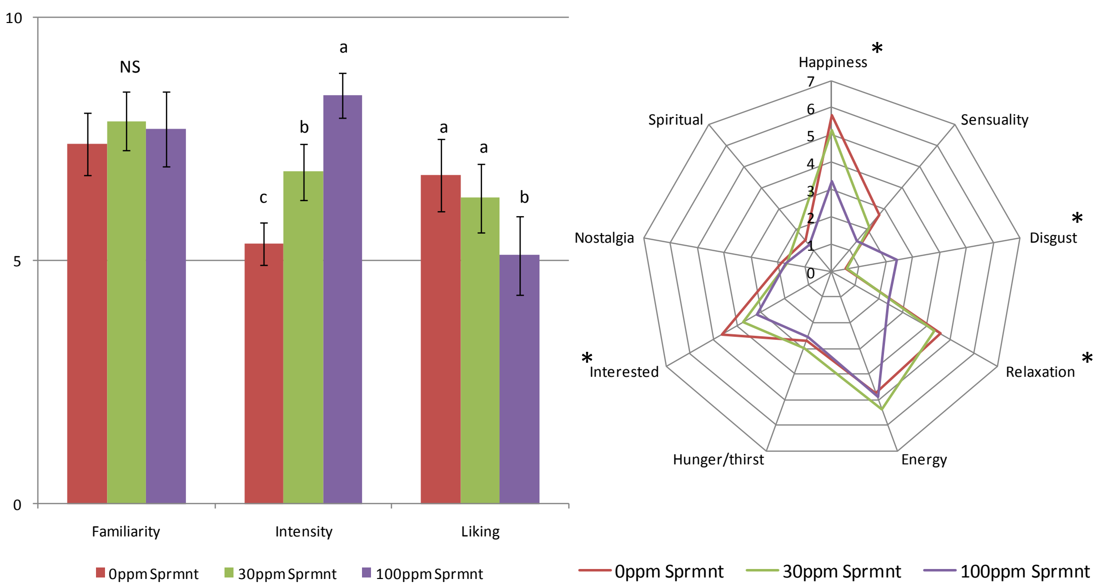


*Figure 24. NS indicates no significant difference, * indicates a significant difference at 0.05 confidence level*

Intensity increases with increasing WS-3 concentration, while disgust also increases. Happiness, relaxation and liking were significantly less at 100 ppm than 0 ppm and 30 ppm WS-3. Interest was significantly higher at 0 ppm than at 100 ppm WS-3.

4. DISCUSSION

For discussion and conclusion sections, 0 ppm WS-3 will be referred to as 'no cooling', 30 ppm WS-3 will be referred to as 'low cooling' and 100 ppm WS-3 will be referred to as 'moderate cooling'.

4.1. Part 1 – Intensity Ratings

Through this work, we found that cross-modal sensory interactions exist between chemesthetic cooling and flavor (from retro-nasal aroma– the perception of volatiles from the mouth through the back of the nasopharynx (Lawless & Heymann, 1999)). This helps to expand knowledge on sensory interactions into areas other than taste-taste and taste-aroma. This interaction, as has been found in other cross-modal interaction studies, was dependent on the congruency of the mixture (Petit et al., 2007, Labbe et al., 2008, Schifferstein & Verlegh, 1996). While we hypothesized that there would be both an effect of cooling intensity on flavor and an effect of flavor on cooling intensity, we found that the interaction only worked one way. Cooling concentration increased perceived flavor intensity of a congruent flavor but flavor, congruent or not, did not influence cooling intensity.

In the flavor cooling mixtures, we found that WS-3 concentration, as hypothesized, influenced the perceived cooling intensity. Concentration also influenced the way that the cooling intensity changed over time. This is what we would expect – low cooling dissipates rapidly, while stronger cooling concentrations linger longer. We hypothesized that, with a congruent flavor (spearmint), we would find an increase in cooling intensity, as researchers Petit (2007) and Labbe (2008) had found with their congruent cooling-flavor mixtures. The data showed no significant differences between the flavors for cooling intensity.

Our results for the influence of flavors on cooling intensity were not in line with what we had expected. We hypothesized that congruent flavors would influence cooling intensity and incongruent flavors would not, similar to what Petit and colleagues had found with their model beverages (2007). Instead, we found no significant influence of flavors on the cooling perception. Some explanations for the differences between our findings could be that we used ANOVA to assess whether or not the flavors significantly influence cooling, while Petit used the significance of polynomial terms from a response surface analysis. Another difference between our studies is that we asked our panelists to rate warming, sweet, sour and bitter, in addition to cooling and flavor intensity. Petit and colleagues only asked cooling and flavor intensity, so a “dumping effect” may have caused different results. A “dumping effect” is a phenomena that occurs when panelists are given too few rating scales, so they ‘dump’ ratings onto inappropriate scales for lack of an appropriate place to rate them (Lawless & Heymann, 1999).

It is possible that odor-induced chemesthetic enhancement works similarly to odor-induced taste enhancement, as described by many and summarized by Schifferstein and Verlegh (1996). This is an explanation for why some sensory interactions studies find taste enhancement by odor and other studies do not. They describe the ‘appropriate descriptor hypothesis’, in which subjects think of their target sensation rather broadly. When they are given many suitable other descriptors to rate, their focus narrows and usually taste enhancement disappears (Schifferstein & Verlegh, 1996; Frank et al., 1993; Prescott, 1999). In a couple of studies investigating odor enhancement by taste, researchers have found repeatedly that sucrose enhances odor intensity, but that odor intensity had little or variable effects on sweet intensity (Green et al., 2012; Fujimaru and Lim, 2013). In both instances, the researchers hypothesize that the enhancement by sucrose has something to do with nutrition related to macronutrients in foods. In both cases, sucrose enhances the low intensity fruit odor in order to aid in identification of a nutritive food. They are doing further research to explore this hypothesis.Our findings of chemesthetic cooling induced odor enhancement do not seem to fit in-line with this, although there is quite a difference between chemesthetic-odor interactions and taste-odor interactions.

For sweet perception, we found that both WS-3 concentration and flavor influenced the way sweet perception changed over time. With moderate cooling, sweet intensity seemed to be suppressed and to decrease more quickly than with low or no cooling. This was found to be significant at the I_{max} in the case of apple flavor. There were some significant differences for sourness and bitter intensities, but the levels of both were low for all samples. At their I_{max}, we saw that moderate cooling samples were significantly more bitter than other low or no cooling samples. Apple flavored samples were significantly more sour than other flavors.

While flavors did not have a significant effect on cooling intensity, cooling agent concentration did have a significant effect on perceived flavor intensity. As we hypothesized, there was no effect of cooling concentration on apple or caramel flavor perception. For spearmint flavor, which is the flavor congruent with cooling, we saw an increase in spearmint flavor intensity when the solution contained moderate cooling versus low or no cooling.

Enhancement of odors by taste has been highlighted by various studies, and in all cases, the odors and tastants were congruent. In a study of taste, trigeminal and aroma interactions, the authors found that chemesthesis from carbonation decreased sweet perception and enhanced both sourness and aroma perception (Saint-Eve et al., 2010). Even though it is a different kind of chemesthesis, we found these same effects with our moderately cooling samples. The trends showed our moderately cooling samples were more sour, less sweet, and we saw a significant increase in perceived odor intensity with congruent spearmint odor.

4.2. Part 2 – Emotions and Liking

An overall two-way ANOVA of the hedonic data (Table 3) showed that flavor influenced energy and familiarization. WS-3 concentration had an influence on many more attributes: happiness, sensuality, disgust, relaxation, interest, intensity, familiarity and liking. The interaction of flavors and WS-3 concentration only influenced liking. To investigate these effects further, we compared WS-3 concentrations within a flavor.

Across caramel samples (Figure 22), increasing cooling intensity decreased all positive emotions and increased the disgust ratings. While a moderate cooling level was significantly more intense than the others, it was significantly less liked than the others also. It differed significantly in familiarity from the sample without cooling. This was as expected; less congruent flavor is less liked and less familiar with added cooling. It is not unreasonable to think that all emotions would decrease except for disgust.

Apple samples behaved much in the same way as caramel with one subtle difference. As shown in Figure 23, with low cooling, the apple sample was significantly more intense than the no cooling. While it was less liked than the no cooling sample, it was not disliked. This suggests that there may be an optimal level of cooling that can enhance intensity without antagonizing the emotions of the consumer. The emotional profiles were not very different from each other, although the samples with low cooling elicited less intense emotions than samples without cooling. Although the trends for caramel were similar to those for apple, the no cooling caramel and low cooling caramel samples did not show a significant difference in overall intensity. This suggests that the interaction is flavor dependent. It is possible that apple is considered congruent to cooling by some people. It is combination has been put together in some gums, candies and soft drinks, so there may be a learned congruence between the two. One example of this combination on the market is a discontinued gum called Cool Green Apple, which was a part of the Wrigley Extra® Gum line ("Wrigley Extra® Gum Facts and History", n.d.). The most relevant example of learned congruency is that between pineapple aroma and cooling in the work done by Petit and colleagues, in which there were enhanced ratings of this previously incongruent pairing after repeated exposure to that pairing (2007).

For spearmint flavored samples, moderate cooling was significantly less liked and less happy than no cooling or low cooling samples, as pictured in Figure 24. The intensity of spearmint samples was significantly higher with each increase in WS-3 concentration. What is striking about the emotions portion is that the moderate cooling sample is significantly less intense in sensuality and relaxation dimensions, however, energy stays the same across concentrations. This result suggests that varying levels of cooling can change your product from relaxing and energizing to energizing only. While liking was significantly less when paired with moderate cooling, none of the samples were disliked. The optimal level of WS-3 with spearmint seemed to be 30 ppm, because it was intense, well-liked and maintained a majority of positive emotions.

One interesting note about liking between the flavors is that at low cooling levels, there was no significant difference in liking between the flavors. This was somewhat unexpected. In odor-induced taste enhancement, combinations that are highly congruent are more pleasant than expected and combinations that are not congruent are less pleasant (Schifferstein & Verlegh, 1996) and we expected to see similar results with low and moderate cooling samples. At a moderate cooling intensity, we observe that spearmint is liked more than caramel, which is what we expected. These results strongly suggest that, an interaction between chemesthetic cooling and flavor is not only dependent on the congruency of the flavor, but also the intensity of the cooling. This is also illustrated by the significance of the interaction term for flavor and cooling intensity for liking in our global two-way ANOVA (Table 1).

There are some limits to the work that was done. One limitation is that we did not test varying concentrations of flavors to see how that influenced intensity perceptions or hedonics. A moderate level was chosen because it would be what would be most likely used in a flavor application. It is possible that significant interactions were missed. There has been a recent study, for example, that investigated odor enhancement by taste, using sucrose and citral. The researchers found that enhancement of odor by sucrose was greater when odors were weak (Fujimaru & Lim, 2013). It's possible that there is a similar phenomenon with odor enhanced by cooling. It is also possible that low flavor levels could have influenced cooling intensity in a way that moderate levels had not. It has been shown, however, that odor concentration has little influence on taste enhancement (Stevenson et al., 1999). Another limitation is that we cannot be entirely sure whether or not the enhancement of spearmint intensity is a central or peripheral event. Being as the enhancement occurred specifically with the congruent spearmint flavor at the highest WS-3 concentration, it is surmised that it is more of a perceptual interaction. Lastly, we determined the interactions and their effects on attribute intensity. We do not know, however, how the interactions may have changed the quality of the cooling or quality of the flavor. To address this, further experimentation could be done, such as similarity testing or profiling.

This study was done to understand the basic perceptual interactions of cooling sensates with differing flavors. Our results showed decreased liking and positive emotions anytime cooling was added to a flavored beverage. This indicates that cooling in beverages might not be a welcome addition. This could be dependent on other factors, such as culture and age. It is also likely that the applications in which the mixtures are present could have a profound effect on panelists' responses. For example, for mouthwash, toothpaste or gum, people may like a combination of a minty flavor with strong cooling. The strong cooling sensation may make them feel clean and refreshed. This same intensity and combination may not be as desirable in soft drink, however. Another possibility for further studies could be a more in depth hedonic study including more flavors, especially the most pertinent flavors used in applications in which cooling sensates are added. It could also be interesting to investigate different types of apple flavors or mint flavors. Maybe some are more congruent with cooling than others and have different emotional profiles.

5. CONCLUSIONS

Chemesthetic cooling can influence the perceived intensity of a congruent flavor over a period of time. In our study, we observed that a moderate level of cooling increased the intensity of spearmint flavor over the course of two minutes, when compared to the same sample without added cooling agent. We did not observe that the flavor influenced the cooling intensity. One caveat, however, is that we did not look into how varying the flavor concentration might have had an influence on cooling intensity; we only investigated a moderate flavor level. Our results imply that, congruent cooling-flavor mixtures can be used to extend the lasting capacity of the flavor intensity.

Cooling agent intensity can influence and differentiate otherwise identical samples along the hedonic and emotional profiles. The outcomes are dependent on the flavor. While increasing cooling intensity increased overall intensity for all flavor samples, how this correlated with liking and emotions depending on the flavor. The more congruent the flavor, the more positive ratings the samples received. With this model beverage system, low level cooling seemed to be optimal for spearmint flavor and apple flavor, in that it increased overall intensity while maintaining liking and emotions. This most likely depends on the application in which the combination is present.

6. APPENDICES

6.1. WS-3 Dose-Response Example Ballot

Instruction Screen:

On the next screen, you will rate cooling, warming, sweet, sour and bitter sensations.

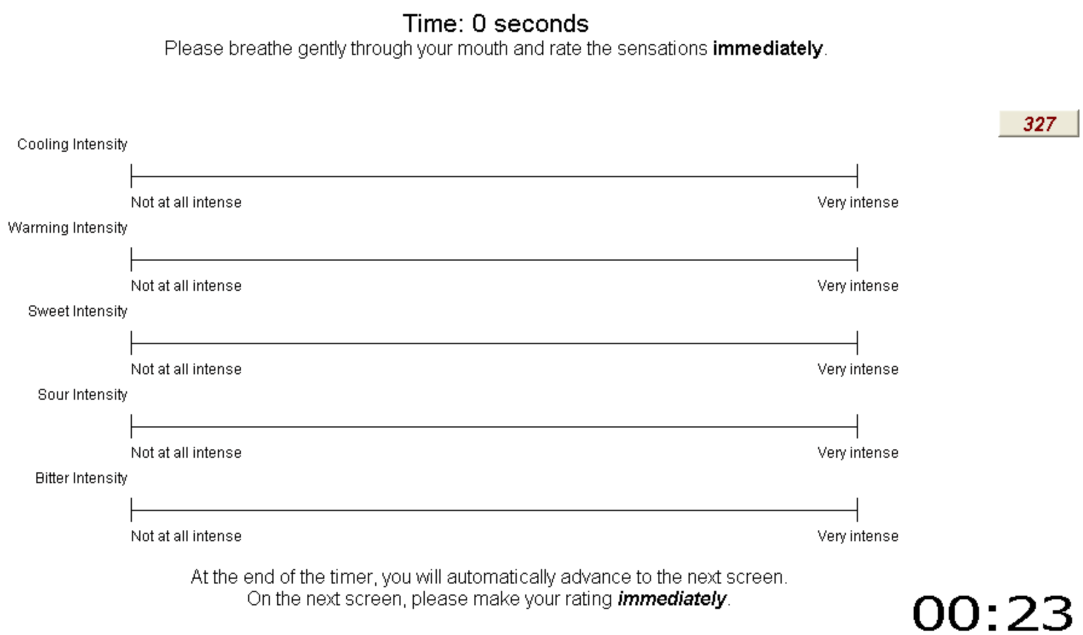
For each sample, you will sip the entire sample, swish for 10 seconds, expectorate, wait 5 seconds, then breathe gently through your mouth and immediately rate the sensations.

Now, please taste the sample, as described above, and immediately advance to the next page.

327

Next screen

Attribute Rating Page (duplicate pages for 0 sec, 30 sec, 1 min, 2 min, 3 min, 4 min and 5 min):



Rest Period Page (one page in between each sample):

You have begun your 5 minute rest period.

As soon as you begin, to cleanse your palate, please take at least one bite of cracker. Then swish vigorously with water, at least twice.

Please push back your tray when you are ready for your next sample.

During the rest of the period, relax!

04:57

Next screen

6.2. Flavor Dose-Response Example Ballot

Instruction Screen:

On the next screen, you will rate caramel flavor intensity, as well as sweet, sour and bitter sensations.

For each sample, you will sip the entire sample, swish for 10 seconds, expectorate, wait 5 seconds, then breathe gently through your mouth and immediately rate the sensations.

Now, please taste the sample, as described above, and immediately advance to the next page.

243

Next screen

Attribute Rating Page (duplicate pages for 0 sec, 30 sec, 1 min and 2 min):

Time: 0 seconds

Please breathe gently through your mouth and rate the sensations **immediately**.

243

Caramel Intensity

Not at all intense — Very intense

Sweet Intensity

Not at all intense — Very intense

Sour Intensity

Not at all intense — Very intense

Bitter Intensity

Not at all intense — Very intense

At the end of the timer, you will automatically advance to the next screen.
On the next screen, please make your rating ***immediately***.

00:27

Rest Period Page (one page in between each sample):

Please take at least one bite of cracker and rinse with water.

As soon as you are ready, advance to the next screen.

6.3. WS-3 Flavor-Mixture Example Ballot

Instruction Screen:

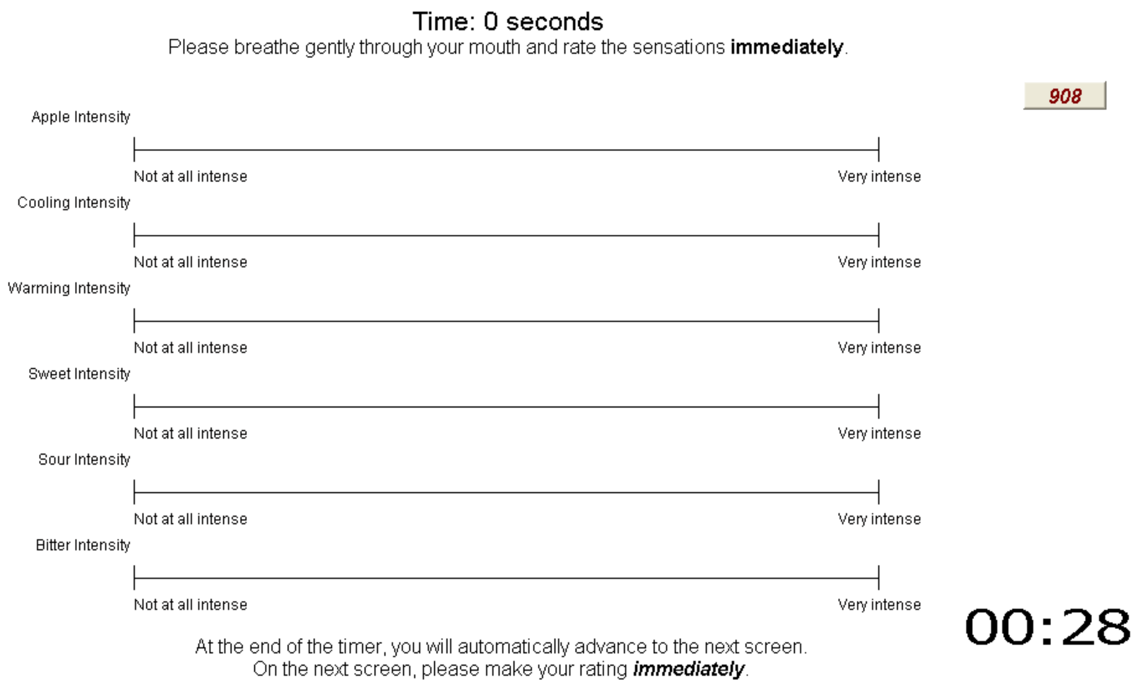
On the next screen, you will rate apple flavor, cooling, warming, sweet, sour and bitter sensations.

For each sample, you will sip the entire sample, swish for 10 seconds, expectorate, wait 5 seconds, then breathe gently through your mouth and immediately rate the sensations.

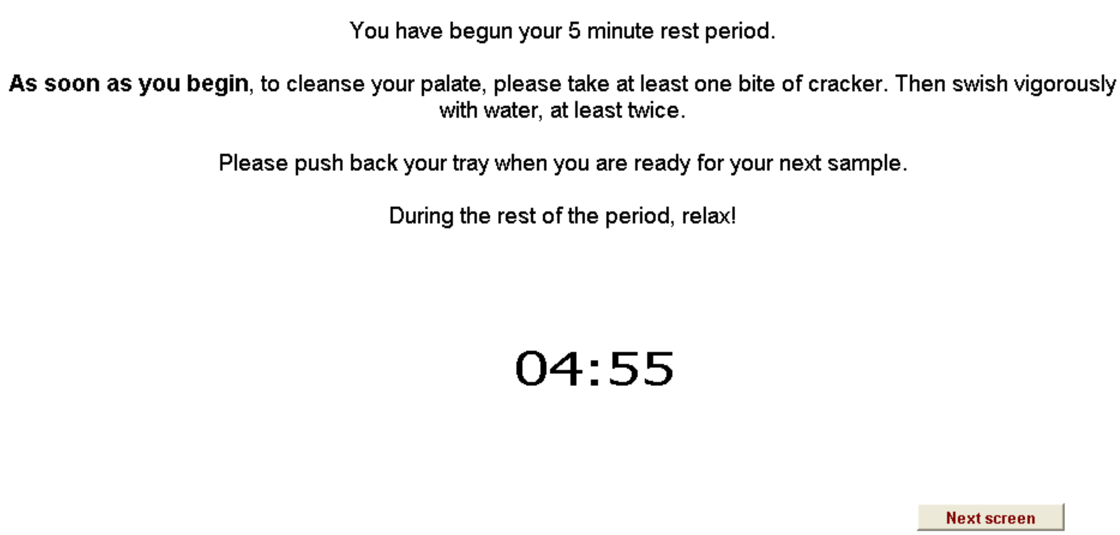
Now, please taste the sample, as described above, and immediately advance to the next page.

908

Attribute Rating Page (duplicate pages for 0 sec, 30 sec, 1 min, 2 min, 3 min, 4 min and 5 min):



Rest Period Page (one page in between each sample):



6.4. Hedonic Example Ballot

Instruction Screen:

Please taste the samples as prompted and rate your emotions, liking, familiarity and intensity on the scales provided. Please feel free to re-sip if needed.

In between samples, there is a 10 minute rest. Please sip water and have crackers to cleanse your palate.

Next screen

Attribute Rating Pages:

Please take a few sips of the sample and then use the cursor to indicate the intensity of your feelings on each of the following scales.

618

Happy - Well-being - Pleasantly surprised

No feelings | Very intense feelings

Romantic - Desire - Sensual

No feelings | Very intense feelings

Disgusted - Irritated - Unpleasantly surprised

No feelings | Very intense feelings

Relaxed - Comforted - Soothed

No feelings | Very intense feelings

Energetic - Refreshed - Revitalized

No feelings | Very intense feelings

Mouthwatering - Thirsty - Famished

No feelings | Very intense feelings

Next screen

618

Interested - Amusement - Impressed

No feelings — Very intense feelings

Nostalgic - Melancholic - Sad

No feelings — Very intense feelings

Spiritual Feeling

No feelings — Very intense feelings

Next screen

Using the cursor, please indicate the intensity, familiarity and liking of the sample on the scales below.

840

Intensity

None — Very Intense

Familiarity

Not at all familiar — Very familiar

Liking

Dislike very much — Neutral — Like very much

Next screen

Rest Period Page (one page in between each sample):

You have entered the 10 minute rest period.

Please sip water and eat a cracker to cleanse your palate.

09:57

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