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Examination of Electroglottography (EGG) and Surface Electromyography (sEMG) as Techniques to Assess Oral Motor Activity During Consumption of Four Forms of Chocolate

Taylor Patterson

University of Mississippi. Sally McDonnell Barksdale Honors College

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EXAMINATION OF ELECTROGLOTTOGRAPHY (EGG) AND SURFACE
ELECTROMYOGRAPHY (sEMG) AS TECHNIQUES TO ASSESS ORAL MOTOR
ACTIVITY DURING CONSUMPTION OF FOUR FORMS OF CHOCOLATE

By

Taylor E. Patterson

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of
the requirements of the Sally McDonnell Barksdale Honors College

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Approved by

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Advisor: Dr. Carol A. Britson

_____

Reader: Dr. Wayne L. Gray

_____

Reader: Dr. Colin R. Jackson

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ABSTRACT

Taylor Patterson: Examination of Electroglottography (EGG) and Surface
Electromyography (sEMG) as Techniques to Assess Oral Motor Activity during
Consumption of Four Forms of Chocolate
(Under the direction of Carol Britson)

The study assessed individual oral motor activity while eating different types of

chocolates through the use of electroglottography (EGG) and surface electromyography

(sEMG). I hypothesized that chocolate samples with higher milk solids and cocoa

composition would be preferred by subjects as assessed by subject evaluation of textural

attributes for the four chocolate samples. I predicted that preference will be significantly

and positively correlated with oral motor events [e.g. sEMG activity of master and

suprahyoid muscles, total number of chewing actions, and time to last swallow (EGG)].

The experiment used a subject group of 40 students enrolled at the University of

Mississippi between the ages of 18 and 24 with informed consent. Subjects were asked to

consume four chocolate samples as they normally would, but to consider and rank the

degree of bitterness, hardness, mouth coating, and satisfaction for each sample. Water

was consumed after each sample. The subject had sEMG electrodes positioned on each

side of the face at the masseter muscle and hyoid muscle, and one sEMG ground

electrode on the shoulder. EGG electrodes were positioned at each side of the thyroid

cartilage ('Adam's Apple), and one EGG ground electrode on the shoulder. The subject

consumed one randomly selected sample of chocolate to measure chewing and

swallowing events. Data collection began from the time the chocolate is placed in the

mouth, and conclude after the last swallowing event.

The overall preference was significantly different between chocolate types with gourmet milk chocolate being the most preferred, and gourmet dark chocolate the least. Significant differences were observed within each intake survey ranking. Significant relationships were observed between bitterness and mastication muscle forces and between hardness and chew time parameters. Mouth coating ranking showed a significant relationship to average masseter force and average chew time of chews 1-5. Finally, satisfaction showed a significant relationship with average chew time of chews 1-5 and chew rate. Each textural attribute significantly correlated with a mastication parameter from the first five chews indicating that textural attributes are decided in the beginning of mastication and flavor release. The experiment shows that a significant relationship exists among eating behavior, textural attributes, and preference.

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INTRODUCTION

Overview

In modern society, food texture has played an increasing role in consumer's food selection and preferences due to the wide variety of food available and a greater understanding of nutritional benefits (Brown, 1994). Chewing behavior is described as a combination of various physiological, anatomical, and psychological factors, such as coordination of jaw movement, size and strength of masticatory muscles, and learned patterns of chewing ranging from those who chew and swallow quickly to those who suck and allow the chocolate to melt in the mouth (Carvalho-da-Silva et al., 2011). However, the effectiveness with which the sample is manipulated in the mouth during mastication will contribute to the efficiency of food breakdown, which increases the surface area of the food, stimulates saliva secretion, and accelerates the rate of flavor release in the mouth (Brown et al., 1996).

Differences of sensory attributions of chocolate are attributed to different cocoa types, varying ingredient proportions, and processing methods (Afoakwa et al., 2007). The primary chocolate categories include dark, milk, and white chocolate that vary in ingredient composition of cocoa solids, milk fat, and cocoa butter, which lead to different proportions of carbohydrates, fat, and protein. Multiple versions of cocoa are used by different manufactures for different chocolates including cocoa butter, cocoa powder, cocoa liquor, cocoa butter equivalents, and cocoa butter replacements, such as lauric fats, palm kernel, and coconut oil (Afoakwa et al., 2007). Cocoa butter replacements are used completely in place of cocoa butter in novelty chocolates, but are poorly absorbed by the gut. The United States requires cocoa butter as the exclusive fat source for a product to be

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called a chocolate (U.S. Food and Drug Administration, 1988). However, cocoa butter and lecithin, a surfactant, reduce the chocolate viscosity, while milk fat softens the texture and adds to the creaminess attribute (Afoakwa et al., 2007). A high-quality chocolate contains a high fat content, small mean particle size, and a low viscosity. This is due to the fact that particle size distribution determines the perception of flavor, viscosity, and texture, and is a distinguishing factor of chocolate types (Afoakwa et al., 2007). Large particle sizes determine mouth feel, but diminish sweetness, while smaller particle sizes provide a creamier taste. The maximum particle size of a chocolate is 30 μm or the chocolate contains a gritty taste with a high viscosity that causes a pasty mouth feel to prolong in the mouth (Afoakwa et al., 2007). Dark chocolate has an optimum particle size of less than 35 μm, and milk chocolate has a bimodal distribution of particle size. Ziegler et al. (2001) demonstrated an inverse relationship between particle size and the effort to melt, manipulate, and swallow the chocolate sample. Fine chocolate with small particle sizes had a significantly high mastication effort.

Techniques to measure physiological parameters of chewing include surface electromyography (sEMG) to evaluate the activity of masticatory muscles, and electroglottography (EGG) to identify swallowing events (Carvalho-da-Silva et al., 2011). Both of these techniques are non-invasive, inexpensive, and do not interfere with the eating process. Carvalho-da-Silva et al. (2011) investigated variation in the individual eating behavior chocolate with the use of sEMG and EGG procedures, and determined that changes in eating behavior relate to textural differences between two chocolate samples. Forty chocolate consumers evaluated two different chocolate samples for five textural attributes (hardness, melting speed, smoothness, thickness, and mouth coating)

and sEMG data including total number of chews, time of last chew, total chewing time, total chew rate, total muscle work, total muscle work rate, total number of swallows, the first and last time of swallow, and swallow rate (Carvalho-da-Silva et al., 2011). The findings allowed separation of the subjects into three eating behavior groups including "fast chewers", "thorough chewers", and "suckers." A two-fold increase existed for the time the chocolate was kept in the mouth between the fast chewers (17s) and suckers (46s) while the thorough chewers (30s) had the median value. The suckers displayed the lowest number of chews (15), chew rate (0.36 s^{-1}), and muscle work (10.17 mV). The thorough chewers had the highest number of chews (26) and muscle work (28.02 mV), while the fast chewers had the highest chew rate (1.08 s^{-1}) (Carvalho-da-Silva et al., 2011). Similarly, Brown et al. (1994a) used sEMG to investigate the chewing patterns for a range of foods, and divided the population of fifty-two subjects into eating behavior groups of fast eaters (11.5%), slow eaters (21.2%), and energetic/inefficient eaters (9.6%) due to the discriminating factors of chew time, chew rate, and muscle work that showed significance across all foods tested. Another aspect of eating behavior investigated by de Lavergne et al. (2015) includes the analysis of different eating durations due to chewing behavior on texture perception and bolus properties. He divided subjects into two groups (fast and slow eaters), in which both groups had similar textural perceptions of the product in the beginning of mastication, but different towards the end. Short eaters had a higher first chew force, and did not break down the bolus as much resulting in larger, fewer bolus fragments, less adhesive bolus properties, and less saliva incorporation. Together these three studies suggest that eating behavior variation among consumers is

due to mastication parameters of chew time and muscle work and cause a significant effect in texture perception, but lack investigation of the influence of preference.

Differences in eating behaviors may contribute to preference because acceptability and liking of a food is based on the ease in which a product can be manipulated and managed in the mouth. Specifically, chew rate is positively correlated to preference; the faster a subject chews, the easier the product is manipulated in the mouth, and the higher the preference (Brown and Braxton, 2000). Nasser et al. (2011) found that with chocolate specifically, the sugar and cocoa content of chocolate is positively correlated with the preference and desire for chocolate. Chocolates are semi-solid suspensions of fine solid particles from sugar and cocoa in a continuous fat phase. The continuous lipid phase composition of chocolate influences mouth coating because chocolate melts during consumption to give a smooth suspension of solids in cocoa butter and milk fat (Afoakwa et al., 2007). As chocolate melts in the mouth, the continuous fat phase inverts into a continuous aqueous phase that mixes with saliva to dissolve sugar particles, while lipids and cocoa solids coat the mouth's epithelial surfaces. This final phase inversion of oral dissolution provides the final assessment of texture.

Not only do eating behaviors contribute to preference, but also perceived textural attributes. Chang and Chen (2013) found that chew force was at a maximum, meaning masseter muscles were most active, during the early chewing phases, and progressively decreased as mastication continued due to the progressively fragmented bolus. Food texture properties had the strongest relationship with muscle activity in the first bite; chew force positively correlated with hardness, while chew work positively correlated with breaking energy. Iguchi et al. (2015) concluded that chewing performance and the

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resultant muscle activity depends on food hardness; the harder the food, the more

chewing cycles, the longer the chew duration, and the greater the masseter and hyoid

force. Previous studies have also concluded that the number of chews, swallowing events,

and residence time in mouth for chocolate are positively correlated to mouth coating

(Carvalho-da-Silva et al., 2011). Overall, previous studies tested eating behavior in

relation to preference or textural attributes proving that significant relationships exist

among all three variables, but the relationship has not been tested to understand how the

oral breakdown of food gives rise to preference and perceived textural attributions.

Purpose

This research project assessed individual oral motor activity while eating different

types of chocolate samples using EGG for swallowing events and sEMG for chewing

events. With this information, I aimed to determine which aspects of the masticatory

process underlie differences in individual chewing behavior. I hypothesized that

chocolate samples with a higher milk solid and cocoa composition would be preferred by

subjects as assessed by subject evaluation of four chocolate samples for bitterness,

hardness, mouth coating, and satiation. I also hypothesized that preference will be

significantly correlated with oral motor events, such as total chews, muscle force, and

time to last swallow.

MATERIALS AND METHODS

This study began by recruiting by email forty, male or female, college students ages 18-24 enrolled at the University of Mississippi with no history of adverse reactions to chocolate, and no dental work that would interfere with mastication. The experiment was reviewed and approved by the Institutional Review Board (#16-066) at the University of Mississippi. If participants were interested in volunteering, informed consent was obtained and an intake survey was given to the participants (Appendix A).

Four different types of chocolate samples of the same size and shape with varying ingredients were used in my experiment (Table 1): gourmet milk chocolate (Lindt and Sprüngli, 30% cocoa), gourmet dark chocolate (Lindt and Sprüngli, 90% cocoa), gourmet white chocolate (Lindt and Sprüngli), and novelty milk chocolate (R.M. Palmer Company). All chocolates were purchased locally. All of the Lindt chocolate types had expiration dates within one month of each other, but the novelty milk chocolate had no expiration date. This novelty milk chocolate does not contain cocoa butter but instead contains vegetable oils. The intake survey evaluated the subject's perception of each chocolate sample's textural attributes, along with their overall preference. Participants were given an intake survey form in an envelope, and 10 mg of each of the four chocolate samples were randomly presented to the subject as A, B, C, and D. For each chocolate sample, the subjects were instructed to consume the chocolate as they normally would, but to consider the degree of bitterness, hardness at first bite (force to bite into the chocolate), mouth coating (extent to which residue coats the mouth after swallowing), and satisfaction. Water was consumed by the subjects after each chocolate sample to

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cleanse the mouth. After all chocolate samples were tested, the subjects ranked the chocolate samples in order of overall preference, and placed the intake survey back into the envelope. My advisor assigned a four-digit code to the envelope to ensure that I did not know which codes were matched with which subject until completion of the experimental data collection.

Surface electromyography was used to evaluate masticator muscle activity while chewing, and electroglottography was used to record swallowing events from the closing and opening of the vocal folds (Hort et al., 2011). Both methods are non-intrusive, and do not interfere with the eating process. A PowerLab 26T (LTS) electronic data acquisition system from ADInstruments Inc. recorded the EGG and sEMG data using LabChart software package version 8.0.2.

I used a ML317 electrooculography (EOG) recorder connected to the PowerLab system to record the EGG data. The EOG pod is designed to detect fine scale muscle activity and in addition to oculomotor activity can be used to produce electrogastrographs and electroglottographs. Three MLA2504 Shielded Lead Wires were connected to the EOG pod according to the color-coded, positive, negative and ground leads. The EOG pod was plugged into the Input 1 Pod Port on the front panel of the PowerLab system. Disposable adhesive electrodes were attached to each lead before being placed on the subject. The EOG pod was calibrated to 0 mV before the experiment began, and the sensitivity range for the pod is $\pm 4mV$. A push button switch was also connected to the front panel of the PowerLab system via the Input 2 Pod Port in order to ensure the recording of the last swallow.

For the sEMG equipment, a MLA2540 Five-lead Shielded Bio Amp Cable and MLA2505 snap-connected Lead Wires were used. The Bio Amp cable was connected to the PowerLab system via the Bio Amp socket. Five MLA1010 disposable adhesive electrodes were attached to the five snap-connected Lead Wires before placed on the subject. The sensitivity range for the MLA2540 Five-lead Shielded Bio Amp Cable is $\pm$ 5μV to ±100 mV.

Before beginning the experimental portion of the project, subjects were shown where the sEMG and EGG electrodes would be placed on their skin. They were instructed to cleanse those areas of their own skin with an alcohol wipe in order to guarantee secure attachment of the electrodes to the skin. Four sEMG electrodes were attached to each side of the subject's face at the masseter muscle and hyoid muscle. The other sEMG electrode was placed on the subject's shoulder to act as a ground. Two EGG electrodes were placed on each side of the thyroid cartilage ("Adam's Apple), and one EGG electrode was placed on the shoulder to act as a ground (Figure 1).

Following electrode attachment, subjects were asked to perform three voluntary actions for the purpose of calibrating the equipment. First, subjects clenched their teeth to measure the maximum voluntary contraction (MVC) of the masseter muscle. Second, subjects lowered their mandible (opened mouth) to measure the voluntary action of the hyoid muscle. Third, subjects took a sip of water and swallowed in order to calibrate a swallowing event. The subjects were then presented with one randomly selected chocolate sample (10 mg), asked to consume as they normally would, and depress the push button when taking their last swallow. Data collection began from the time the chocolate was placed in the mouth, and concluded after the last swallowing event. After

consumption, data files were stored on a computer using a randomly assigned four-character code. Upon completion of consumption and recordings, the electrodes were removed from the subject's skin, and lotion was provided as needed for skin irritation. At the end of the experimental data collection, the randomly assigned four-digit codes were matched with the four character codes for each subject to allow for analysis testing of the correlation between preference and oral motor activity from the single-blind experiment.

Analytical Methods

Chi-square analysis (Siegel and Castellan, 1988) was used to detect significant difference in preferred chocolate. Descriptive statistics, such as mean, standard deviation, and standard error, were calculated for the intake survey rankings of each chocolate for mouth coating, hardness, bitterness, satisfaction, and overall preference. One-Way Analyses of Variance (ANOVA) were performed to evaluate the rankings of each textural attribute from the intake survey data for each of the four chocolate samples. Descriptive statistics were also calculated for the sEMG and EGG variables including average chew force, average time per chew, % of max masseter force, % of max hyoid force, total number of chews, total chewing time, chew rate, and time of last swallow. Regression analyses were used to identify significant relationships between intake ranking and the sEMG variables for the specific chocolate sample that the subject consumed during the sEMG and EGG data recording. Analyses were conducted using Microsoft Excel and the level of significance was set at $\alpha=0.05$ for all tests.

RESULTS

Intake Survey

When asked to rank their overall preference of the four chocolate samples, 24 students preferred gourmet milk chocolate over the 0 students who preferred gourmet dark chocolate, 13 who preferred gourmet white chocolate, or 3 who preferred novelty milk chocolate. The overall preference ranking of the four chocolate samples was statistically significant ($\chi 2$= 35.4, df=3, p<0.05).

Gourmet dark chocolate was ranked as having the highest bitterness, highest hardness, and lowest satisfaction among all chocolate samples, while gourmet milk chocolate was overall perceived as having the highest mouth coating and highest satisfaction. Gourmet white chocolate was ranked as the lowest perception of bitterness while the novelty milk chocolate had the lowest hardness and the lowest mouth coating properties. Statistical differences were observed within the ranked bitterness rankings of the four chocolates ($F_{(3,156)}$=126.97, p<0.001; Fig. 2), as well as within hardness perception rankings ($F_{(3,156)}$=38.53, p<0.001; Fig. 2), mouth coating rankings ($F_{(3,156)}$=8.07, p<0.001; Fig. 2), and satisfaction rankings ($F_{(3,156)}$=44.84, p<0.001; Fig. 2).

sEMG Data

Each chocolate type was consumed by 10 volunteers randomly selected from the subjects during sEMG analysis of mastication activity. Mastication force variables (Fig. 3) and chewing time, count, and rate variables (Fig. 4) were statistically analyzed using a regression analysis. The EGG equipment was not consistently reliable across all trials, so

only the time of last swallow parameter was examined from the use of the red push button.

For testing the regression relationship of mastication parameters vs. ranked bitterness, only intake rankings for bitterness of novelty milk chocolate (for example) were analyzed against mastication parameters of subjects consuming novelty milk chocolate during the sEMG test. Thus, n=10 for all regression tests of each textural attribute ranking. Every chocolate had at least one significant relationship in each ranking regression, but it was not always significant or not across all regressions.

Regression analysis of sEMG mastication data and bitterness rankings (Table 2) indicated that the ranked bitterness for all chocolate samples is significantly correlated with percent maximum masseter force of chews 1-5 and chew rate. The bitterness ranking of gourmet milk chocolate significantly correlated with all mastication parameters except average masseter force for chews 1-5 and the percentage of maximum hyoid force of the entire trial. Gourmet white chocolate's bitterness ranking significantly correlated with all sEMG parameters except the percentage of maximum masseter of the entire trial. The highest significant r^2 value existed for the percent maximum hyoid chew force of the entire trial for gourmet white chocolate's ranked bitterness. However, every subject ranked bitterness as 1, so it distorted the results of the statistical test. Significant relationships with less variance were also observed between ranked bitterness of novelty milk chocolate and both percent maximum masseter force for chews 1-5 (Figure 5) and percent maximum masseter force of the entire trial.

The regression of ranked hardness and its collective sEMG data (Table 3) showed that hardness ranking for all chocolate samples significantly correlated with both the

average chew time for chews 1-5 and the maximum hyoid chew time. However, only gourmet white chocolate showed significant correlation of hardness ranking and time at last swallow, total chews, and total chewing time. Novelty milk chocolate showed significant correlation between hardness and all mastication variables except time at last swallow, total chews, and total chewing time. Maximum masseter chew time showed a significant relationship with less variance for gourmet milk chocolate. The highest significant r^2 values existed for gourmet dark chocolate's average masseter force for chews 1-5 (Fig. 6) and maximum hyoid chew time. Hardness also displayed a significant relationship with high r^2 values meaning less variance around the best fit line for average chew time of chews 1-5 for novelty milk chocolate.

The regression of mouth coating ranking and sEMG mastication parameters (Table 4) indicated that mouth coating ranking significantly correlated with chew rate for all chocolate samples. Within this regression, gourmet dark chocolate showed significant correlation of mouth coating ranking and all mastication parameters except average masseter force for chews 1-5, while gourmet milk chocolate's mouth coating ranking was only significantly correlated with chew rate. The only strong significant correlations with the least variance existed for gourmet white chocolate's mouth coating ranking, in which the average masseter force for chews 1-5 (Fig. 7) displayed a three-fold difference from average chew time for chews 1-5.

The final regression of chocolate sample satisfaction and sEMG data (Table 5) shows significant correlations for all of the chocolates satisfaction rankings and the average chew time of chews 1-5, percent maximum hyoid force of the entire trial, and time at last swallow. These were the only parameters that gourmet milk chocolate showed

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significance correlations with satisfaction. Significant relationships with less variance existed between satisfaction raking and both average chew time of chews 1-5 and percent maximum hyoid chew force for gourmet dark chocolate. Gourmet white chocolate's satisfaction ranking showed significant correlation with less variance for percent maximum hyoid force as well. Novelty milk chocolate's satisfaction ranking of less variance significantly correlated with chew rate, total chewing time, total chews, time at last swallow, and percent maximum masseter chew force of the entire trial. The highest r^2 value existed for novelty milk chocolate's time at last swallow (Fig. 8).

DISCUSSION

This experiment provides statistically significant evidence that preference and textural attributes of chocolate have an impact upon individual eating behavior. From examining the literature of Nasser (2011), it was expected that the chocolate with the highest sugar and cocoa content would be highly preferred. This holds true for the gourmet milk chocolate, which contains the two largest proportions of both sugar and cocoa content (30%), as well as obtained the overall most preferred ranking, and highest satisfaction ranking. Gourmet dark chocolate was the least preferred chocolate and had the lowest satisfaction ranking; this is because it contains the largest proportion of cocoa (90%), but minimum sugar causing its high bitterness evaluation. Dark chocolate is also the only chocolate sample that does not contain any milk products that add to the creaminess and therefore sweetness attribute. Because of its high level of cocoa, gourmet milk chocolate was also perceived to have the highest mouth coating perception. Afoaka (2007) explains that this is because lipids and cocoa solids coat the oral epithelial surface providing the perception of mouth coating and melting properties. As chocolate melts in the mouth, the continuous fat phase inverts into an aqueous phase mixing with saliva to dissolve sugar. Because gourmet dark chocolate has a minimum level of sugar dissolved, all that is tasted is the inherently bitter cocoa coated on the mouth. Similarly, Harwood et al. (2012) found that as bitterness of chocolate increased, preference decreased; also, subjects that preferred dark chocolate displayed significantly higher rejection thresholds of bitterness than those who preferred milk chocolate. Another aspect that could influence preference of dark chocolate is the associated cardiovascular health benefits such as decreased blood pressure and overall cardiovascular mortality. Dark chocolate's

manufacturing process retains the compound epicatechin from cacoa, which is thought to be responsible for the associated health benefits. Current data suggests that chocolate containing at least 60% cacoa and consumed at a maximum of 2 g per day is optimum for preventing cardiovascular disease (Higginbotham and Taub, 2015).

Both gourmet white chocolate and novelty milk chocolate have sugar as their primary ingredient, but a lesser cocoa content. Gourmet white chocolate has only cocoa butter as its cocoa content causing the significantly lowest perceived bitterness ranking. The novelty milk chocolate lacks cocoa butter, and instead gets its fat from vegetable oil. For a chocolate to legally be considered a chocolate, it must have a minimum percentage of cocoa including cocoa butter, or it must be called "chocolate flavored," as is the case with the novelty milk chocolate (U.S. Food and Drug Administration, 1988). Without cocoa butter a chocolate does not have to go through the tempering process, in which a series of melting and agitation forces the proper crystallization of cocoa butter crystals in order to further provide creaminess and hardness to the chocolate (Afoaka, 2007). This makes the chocolate cheaper to manufacture, and easier to mold. The novelty milk chocolate has the lowest amount of cocoa resulting in its significant low mouth coating ranking, and also has the significantly lowest hardness ranking because of the lack of the tempering process. The novelty milk chocolate also was the only chocolate with no expiration date. These relationships signify that both high, close proportions of sugar and cocoa positively correlate with preference of chocolate.

Chewing parameters were also indicators of preference among subjects. Average chew time of chews 1-5, percent maximum masseter chew force across the entire trial, percent maximum hyoid chew force of the entire trial, time at last swallow, total chews,

total chewing time, and chew rate were all variables significantly correlated with the preference of a chocolate sample type. Chew rate's correlation to preference is due to the ease of manipulating the chocolate in the mouth (Brown 2000). However, chew rate and satisfaction ranking significantly correlated for all of the chocolates except gourmet milk chocolate possibly due to its significantly high mouth coating ranking. de Lavergne (2015) concluded that the longer the oral exposure time to the food and the higher the eating effort, defined as total muscle work, total number of chews, and chew frequency, the higher the satisfaction. Satisfaction and longer oral exposure time could be related to the adaptation of a taste because instinctively people desire to rid of a bad stimulus from the oral cavity with quick eating behaviors or expelling it from the mouth as seen in the gourmet dark chocolate's significantly low number of total chews and total chewing time. Facial grimaces were also recorded for subjects eating the gourmet dark chocolate during the sEMG recording. I can also conclude that satisfaction and preference of chocolate significantly correlate with average chew time for chews 1-5, percent maximum hyoid force of the entire trial, and time at last swallow across all chocolate samples. Overall, the faster the chewing or the higher number of chews showed preference meaning that individual's eating behavior increased with increasing preference.

Chang and Chen (2013) established that food textural properties have the strongest relationship with muscle activity at first bite causing a correlation between chew force and hardness of food. Similarly, Iguchi et al. (2015) added that chewing performance parameters, such as number of chews, chew time, and chew force, are correlated with food hardness. Supporting these previous findings, average masseter force for chews 1-5, average chew time for chews 1-5, and maximum masseter and hyoid chew

time all displayed significant, positive relationships to hardness. High force for the first chews is needed to progressively break apart the chocolate into smaller fragments, which subsequently increases the time of these chews because of the still large bolus in the mouth. Gourmet dark chocolate had the significantly highest hardness ranking, which was ranked from the perception of hardness from the first bite of chocolate. However, gourmet dark chocolate had the lowest mean number of chews, chew time, and chew rate, which contradicts Iguchi et al.'s (2015) conclusion. This is because, unlike this experiment, Iguchi et al.'s (2015) experiment did not relate hardness, and the chewing parameters to preference.

Similar to hardness, the mouth coating ranking also showed a significant positive relationship with average masseter force for chews 1-5 and average chew time for chews 1-5. With an increasing mouth coating attribute, chewing time is increased because of the added difficulty due to an oral epithelial coat of milk solids and cocoa particles. Chew rate is found to be significant across all chocolate samples for mouth coating rankings. The gourmet milk chocolate had the highest mouth coating ranking, which was significantly correlated to only chew rate. However, gourmet dark chocolate had the second highest mouth coating ranking that significantly correlated with all mastication parameters except average masseter force of chews 1-5. The mastication parameters involving muscle force were all correlated with different attributes of the chocolate samples providing significant evidence of a relationship between eating behavior and textural attributes. In the first five chews, all textural attributes were found to significantly correlate with a mastication parameter. This indicates that perception of textural attributes occurs at the beginning of mastication.

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In previous experiments, such as Carvalho-da-Silva et al. (2011) and Brown (1994a), subjects were grouped by significant differences in their eating behavior. Carvalho-da-Silva et al. (2011) grouped subjects into "fast chewers, thorough chewers, and suckers" based on differences in chew rate, number of chews, time chocolate was kept in mouth, and muscle work. Brown (1994a) grouped subjects into "fast, slow, energetic/inefficient, and normal eaters" based on differences in chew rate, total chew time and muscle work, and concluded that chewing behavior is responsible for variability in texture and flavor perception. Similarly, my experiment suggests that the underlying differences in chocolate eating behavior is the chew rate and average chew time of chews 1-5. In the regressions for both bitterness and mouth coating versus the sEMG parameters, chew rate was significant across all chocolate samples. In the regressions for both hardness and satisfaction versus the EMG parameters, average chew time of chews 1-5 was significant across all chocolate samples. This data adds to the conclusion that different chewing behavior causes different flavor and texture perception, and warrants future experimental investigation to find the causal relationships between food texture, preference, and eating behavior.

de Lavergne (2015) grouped subjects into fast and slow eaters, and found that both groups had similar textural perceptions in the beginning, but different towards the end of mastication. Future experiments may provide a taste evaluation before and after consuming the chocolate sample in order to better understand this relationship between chewing behavior, flavor perception, and preference. Also, the blind tasting of chocolate could be conducted in order to reduce prior preferences. The impact of eating behavior on sensory perception and preference warrants further study on other food products with

different textural attributes. Different methods to examine swallowing parameters might be explored as well. One objective of this experiment was to test both chewing and swallowing parameters in order to determine the underlying differences in individual mastication. However, the EGG equipment did not consistently provide the needed information, so the red push button determined the time at last swallow only. Furthermore, the impact of satisfaction on eating behavior should be further studied in order to determine its benefits and possible effects on health and food intake levels.

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Table 1: Chocolate ingredient composition for each chocolate sample in descending order of quantity.

Milk Chocolate (Lindt and Sprüngli)	Dark Chocolate (Lindt and Sprüngli, 90% cocoa)	White Chocolate (Lindt and Sprüngli)	Milk Chocolate (R.M. Palmer Company)
Sugar Cocoa butter Milk Chocolate Skim milk Soya lecithin Barley malt powder Artificial flavor	Chocolate Cocoa powder Cocoa butter Sugar Bourbon vanilla beans	Sugar Cocoa butter Milk Skim milk Soya lecithin Artificial flavor	Sugar Partially hydrogenated vegetable oil Whey Cocoa Lactose Skim milk Soy lecithin Vanillin

Table 2: Regression analyses of ranked bitterness vs. mastication parameter by chocolate type. Significant r^2 values (p≤0.05) are shown in bold. Asterisks indicate less variance about the best fit line ($r^2 > 0.3$).

	Gourmet Milk Chocolate (n=10)	Gourmet Dark Chocolate (n=10)	Gourmet White Chocolate (n=10)	Novelty Milk Chocolate (n=10)
Average masseter force, chews 1-5 (mV)	0.2211	0.0380	**0.0086**	**0.2191**
Average chew time, chews 1-5 (s/chew)	**0.0375**	0.0469	**0.00098**	**0.0313**
% max masseter force, chews 1-5 (mV)	**0.0813**	**0.0523**	0.0017	**0.3397****
% max masseter chew force, entire trial (mV)	**0.1165**	**0.0010**	0.0032	**0.2808****
Max masseter chew time (s)	**0.0296**	6.5921E-07	**0.0010**	**0.0495**
% max hyoid, entire trial (mV)	0.5755	**0.0113**	**0.3948****	0.2792
Max hyoid chew time (s)	**0.0058**	**0.0413**	**0.0267**	0.0004
Time at last swallow (s)	**0.0525**	0.0258	**0.0069**	0.0799
Total chews	**0.0077**	**0.1293**	**0.0007**	0.0793
Total chewing time (s)	**0.0621**	0.0222	**0.0082**	0.0850
Chew rate/s	**0.0047**	**0.0755**	**0.0348**	**0.0095**

Table 3: Regression analyses of ranked hardness vs. mastication parameter by chocolate type. Significant r^2 values (p≤0.05) are shown in bold. Asterisks indicate less variance about the best fit line (r^2>0.3).

|	Gourmet Milk Chocolate (n=10)	Gourmet Dark Chocolate (n=10)	Gourmet White Chocolate (n=10)	Novelty Milk Chocolate (n=10)
Average masseter force, chews 1-5 (mV)	0.0012	**0.5819****	**0.0139**	**0.1329**
Average chew time, chews 1-5 (s/chew)	**0.0775**	**0.1976**	**0.1150**	**0.2788****
% max masseter force, chews 1-5 (mV)	0.0078	0.3205	**0.2233**	**0.1744**
% max masseter chew force, entire trial	**1.8057E-06**	**0.1476**	0.1711	**0.0061**
Max masseter chew time (s)	**0.2630****	0.1617	0.2872	**0.0630**
% max hyoid, entire trial (mV)	0.2477	0.0152	**0.0926**	**0.2183**
Max hyoid chew time (s)	**0.0357**	**0.4821****	**0.2257**	**0.0854**
Time at last swallow (s)	0.0201	0.0050	**0.0327**	0.1929
Total chews	0.0312	0.1098	**0**	0.1073
Total chewing time (s)	0.0108	0.0284	**0.0402**	0.1780
Chew rate/s	**0.2270**	0.6162	**0.1357**	**0.0325**

Table 4: Regression analyses of ranked mouth coating vs. mastication parameter by chocolate type. Significant r^2 values ($p \leq 0.05$) are shown in bold. Asterisks indicate less variance about the best fit line ($r^2 > 0.3$).

|	Gourmet Milk Chocolate (n=10)	Gourmet Dark Chocolate (n=10)	Gourmet White Chocolate (n=10)	Novelty Milk Chocolate (n=10)
Average masseter force, chews 1-5 (mV)	0.0592	0.0355	**0.6200****	0.0480
Average chew time, chews 1-5 (s/chew)	0.0893	**0.0069**	**0.2741****	**0.1189**
% max masseter force, chews 1-5 (mV)	0.1310	**0.0901**	0.0827	**0.1195**
% max masseter chew force, entire trial (mV)	0.0102	**0.0006**	0.0252	**0.1338**
Max masseter chew time (s)	0.0670	**0.0388**	0.0004	**0.0442**
% max hyoid, entire trial (mV)	0.1463	**0.1073**	**0.1139**	**0.0614**
Max hyoid chew time (s)	0.0304	**0.0207**	0.0001	0.0410
Time at last swallow (s)	0.2872	**0.1062**	**0.0060**	0.0231
Total chews	0.0503	**0.0095**	**0.0128**	0.0235
Total chewing time (s)	0.2812	**0.0176**	**0.0001**	0.0321
Chew rate/s	**0.1883**	0.0046	0.0328	**0.0107**

Table 5: Regression analyses of ranked satisfaction vs. mastication parameter by chocolate type. Significant r^2 values (p≤0.05) are shown in bold. Asterisks indicate less variance about the best fit line ($r^2 > 0.3$).

|	Gourmet Milk Chocolate (n=10)	Gourmet Dark Chocolate (n=10)	Gourmet White Chocolate (n=10)	Novelty Milk Chocolate (n=10)
Average masseter force, chews 1-5 (mV)	0.1882	0.0390	**0.0545**	0.4441
Average chew time, chews 1-5 (s/chew)	**0.0003**	**0.3428****	**0.0106**	**0.0096**
% max masseter force, chews 1-5 (mV)	0.0097	**0.0003**	**0.1691**	0.2748
% max masseter chew force, entire trial (mV)	0.0041	**0.0550**	0.0721	**0.2596****
Max masseter chew time (s)	0.0503	0.4798	0.0711	**0.1925**
% max hyoid force, entire trial (mV)	**0.0884**	**0.2555****	**0.2880****	**0.1141**
Max hyoid chew time (s)	0.2246	**0.2030**	**0.0805**	0.0011
Time at last swallow (s)	**0.1314**	0.0729	0.0576	**0.3476****
Total chews	0.0698	**0.0084**	0.1319	**0.3436****
Total chewing time (s)	**0.1222**	0.1254	**0.0243**	**0.3210****
Chew rate/s	0.0252	**0.2150**	**0.0430**	**0.2505****
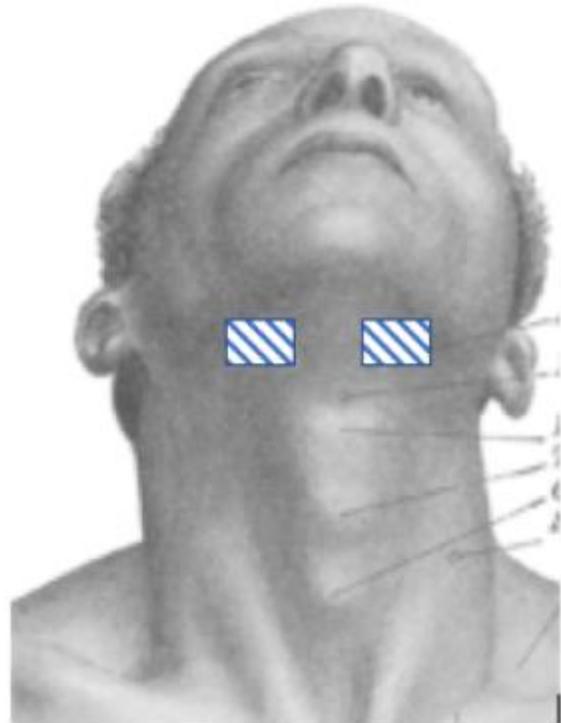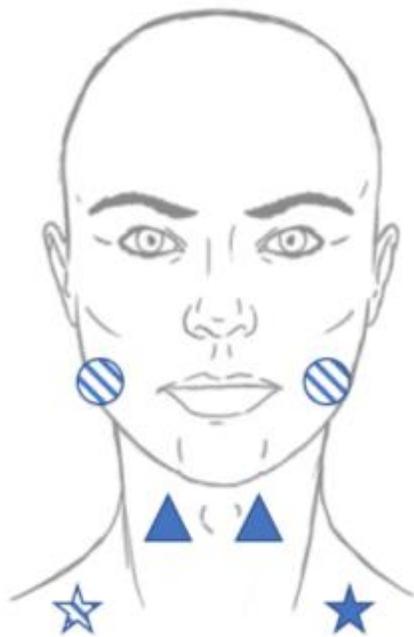
⊘ sEMG masseter muscle electrodes

▨ sEMG hyoid muscle electrodes

☆ sEMG shoulder ground electrode

▲ EGG 'Adam's Apple' electrodes

★ EGG shoulder ground electrode

Figure 1: Electroglottography and surface electromyography electrode placement on the subject for chewing and swallowing parameter recordings.
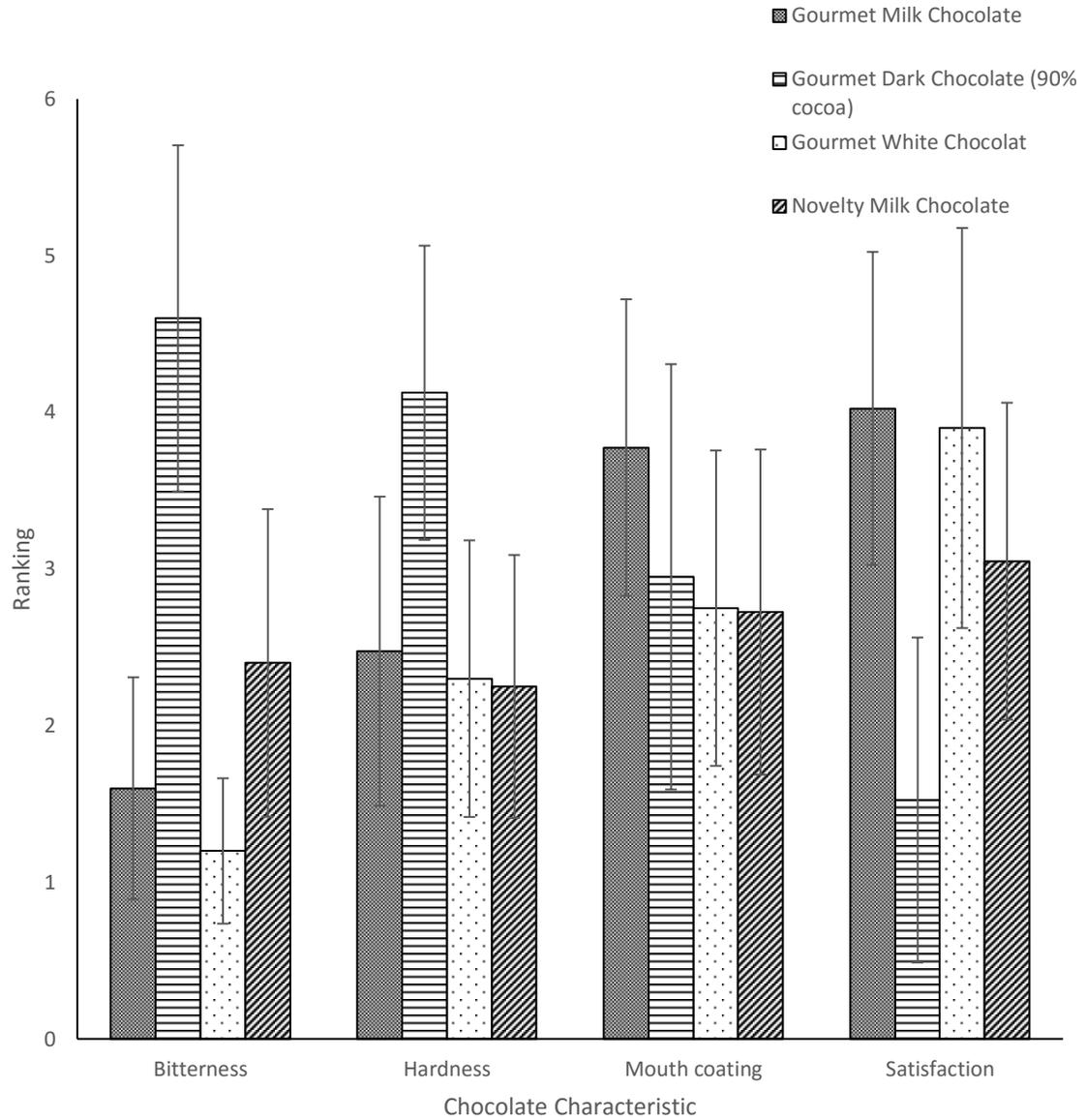
Figure 2: Average of student rankings of chocolate characteristics rated on a scale of 1-5 (1=low characteristic perception, 5= high characteristic perception) for each chocolate sample tested. Error bars represent one standard deviation from the mean.
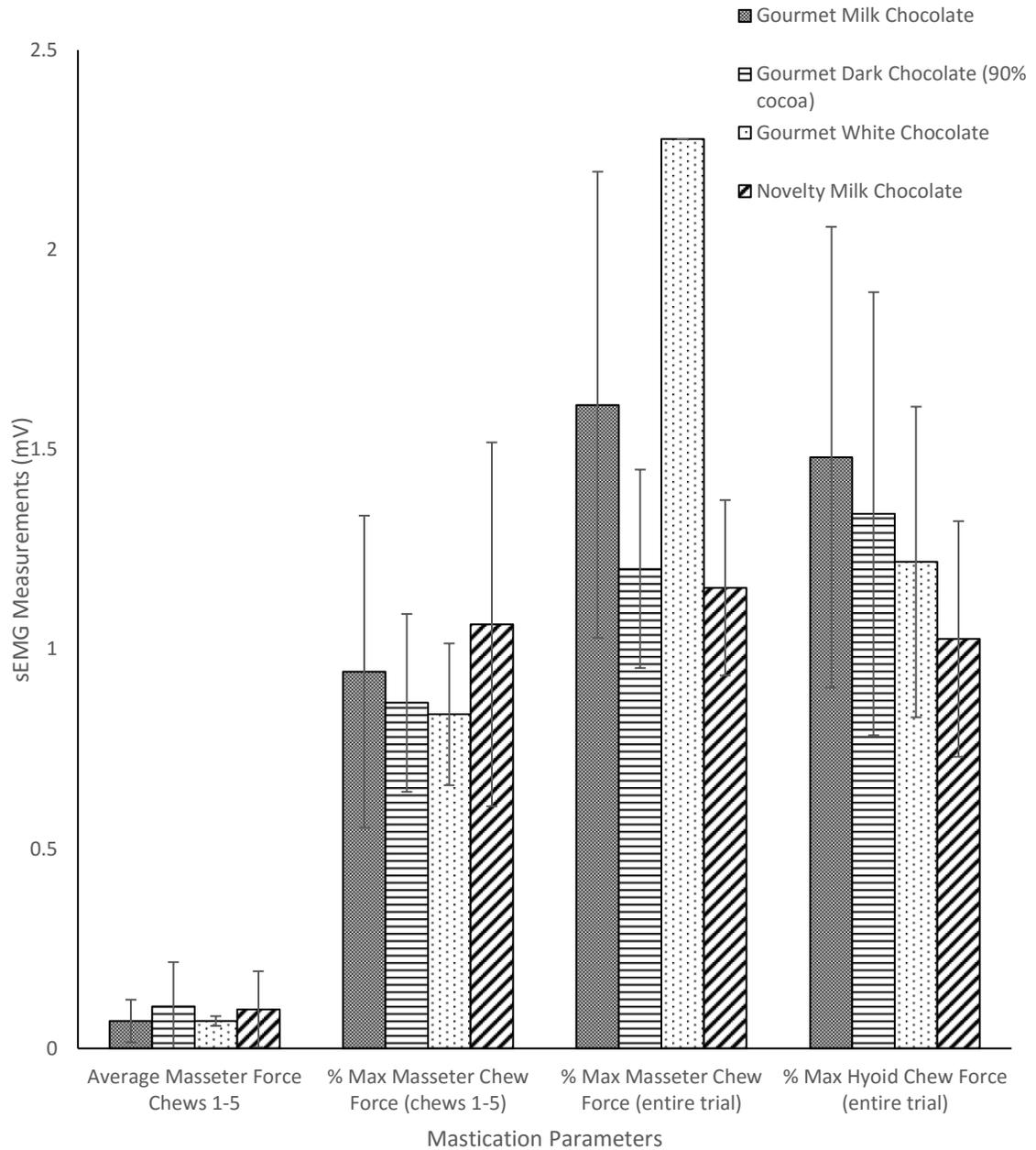
Figure 3: Average sEMG recordings for mastication force parameters for all chocolate samples. The gourmet white chocolate error bar (SD=4.12) was removed because it was obscuring visual relationships among groups.
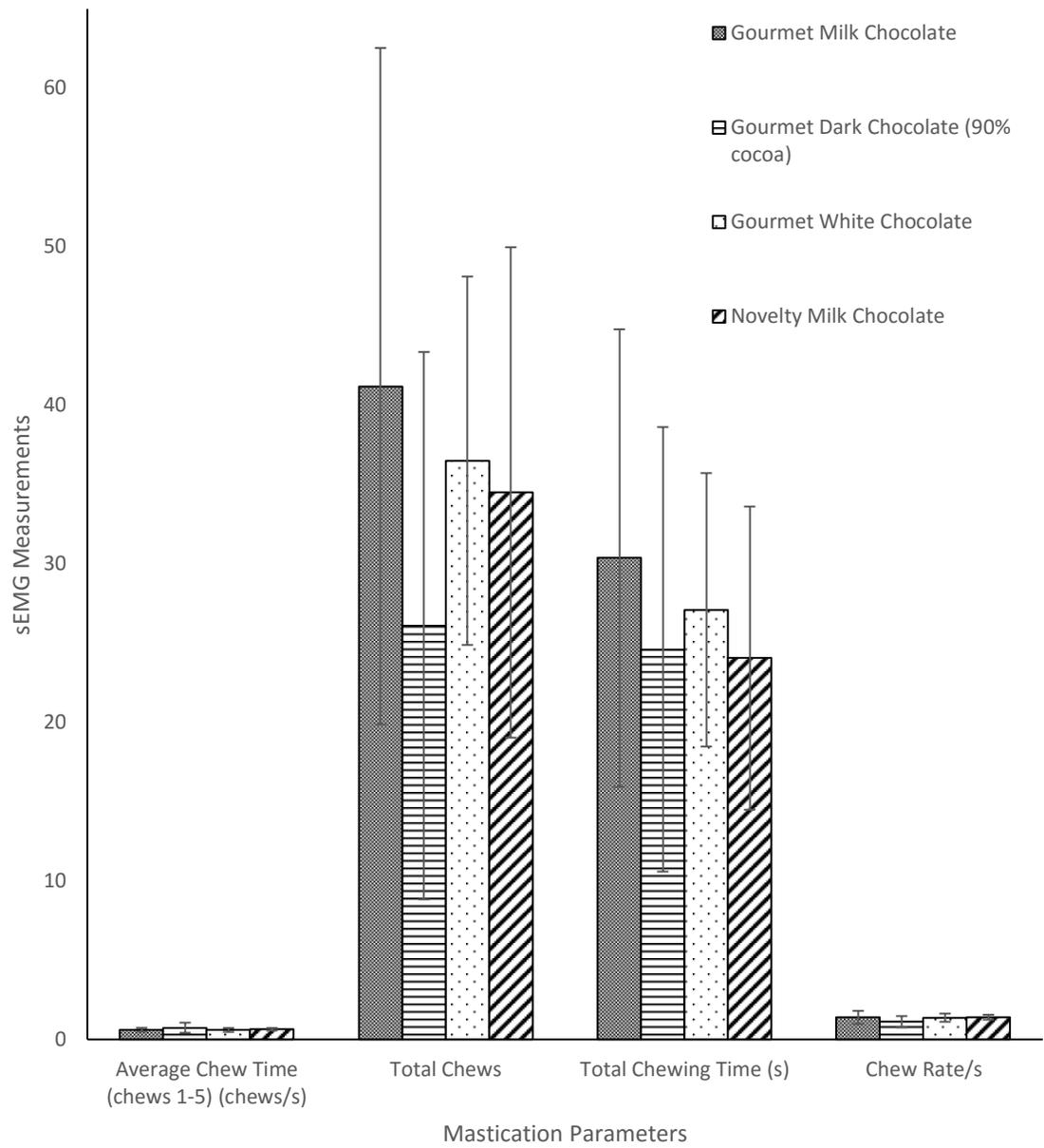
**Figure 4:** Average sEMG recordings for chewing time, count, and rate for all chocolate samples.
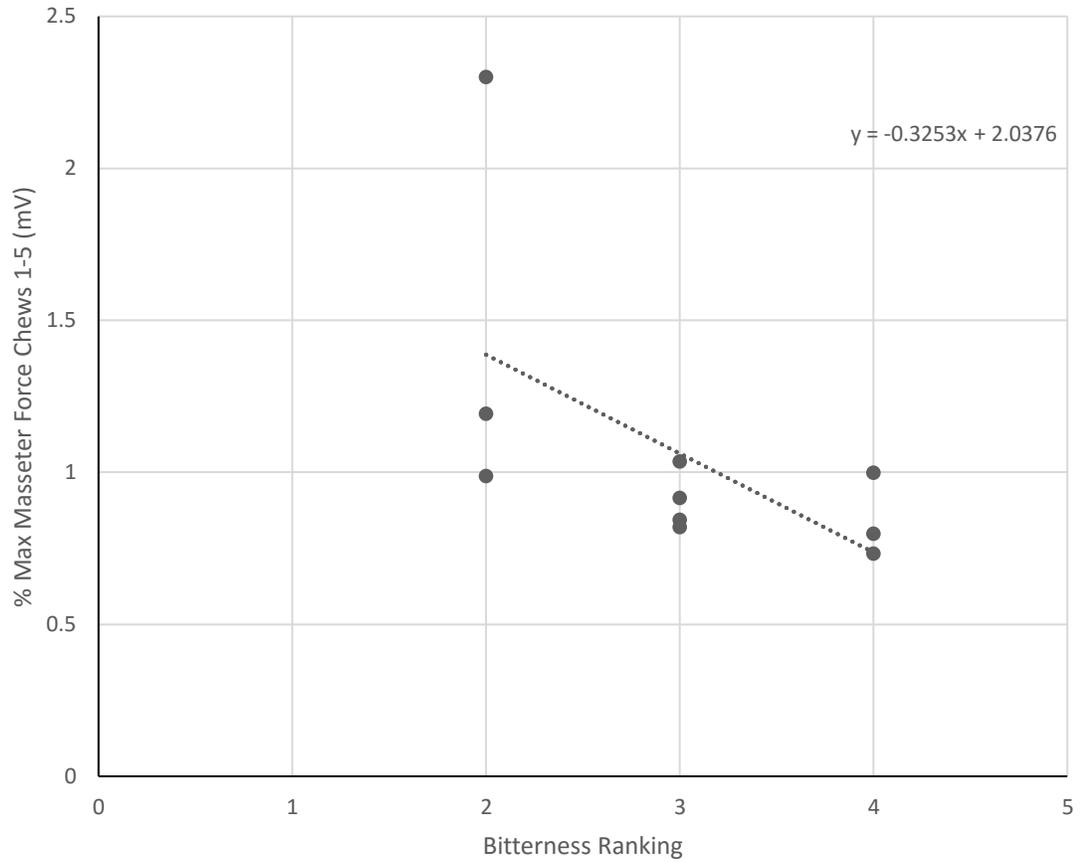
Figure 5: The regression analysis of % max masseter force chews 1-5 vs. bitterness ranking for novelty milk chocolate with a significant, high r^2 value.
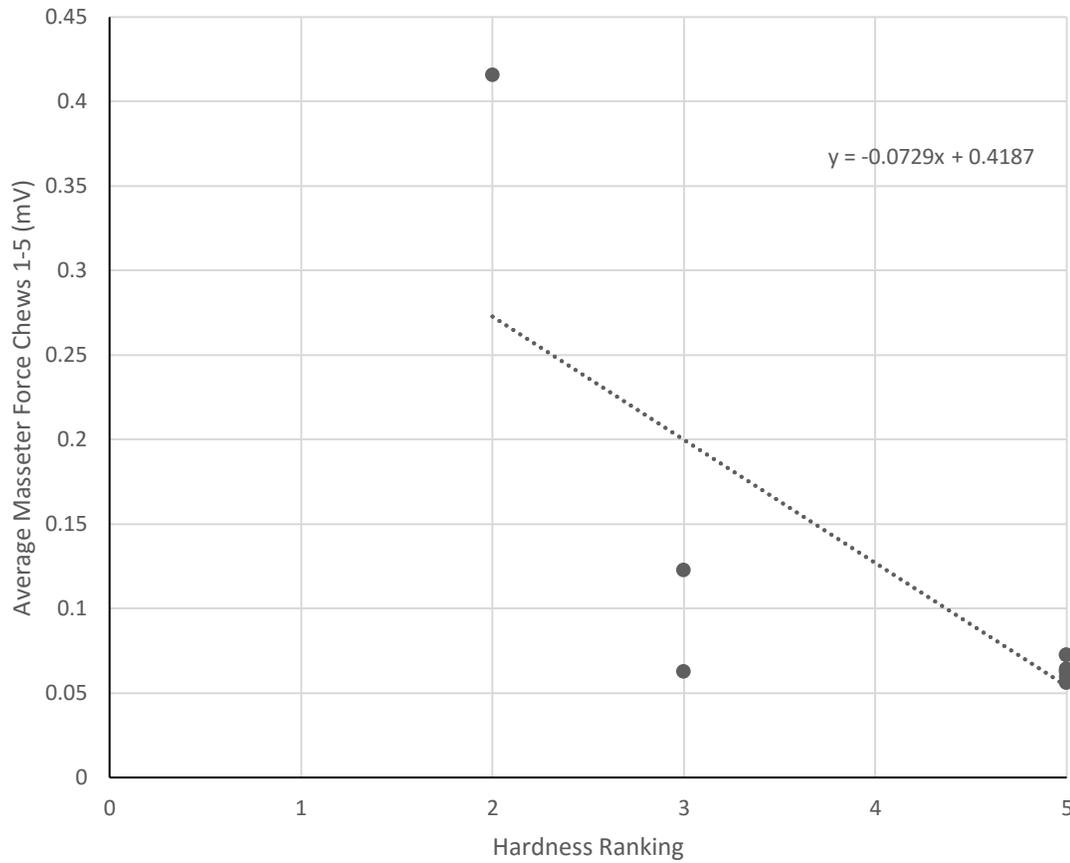
Figure 6: The regression analysis of the average masseter force chews 1-5 vs. hardness ranking of gourmet dark chocolate with the highest, significant r^2 value.

$y = -0.0114x + 0.0959$
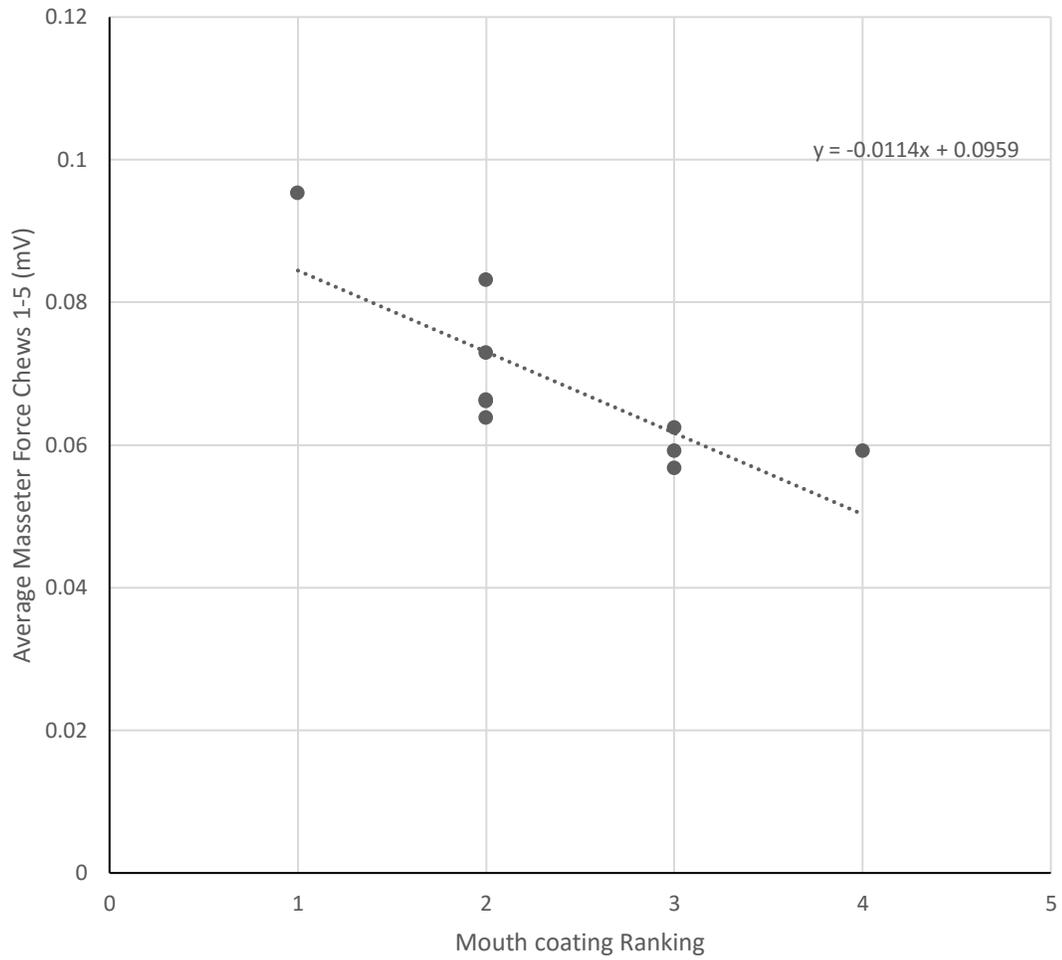
Figure 7: The regression analysis of average masseter chew force chews 1-5 vs. mouth coating ranking of gourmet white chocolate with the highest, significant r^2 value.
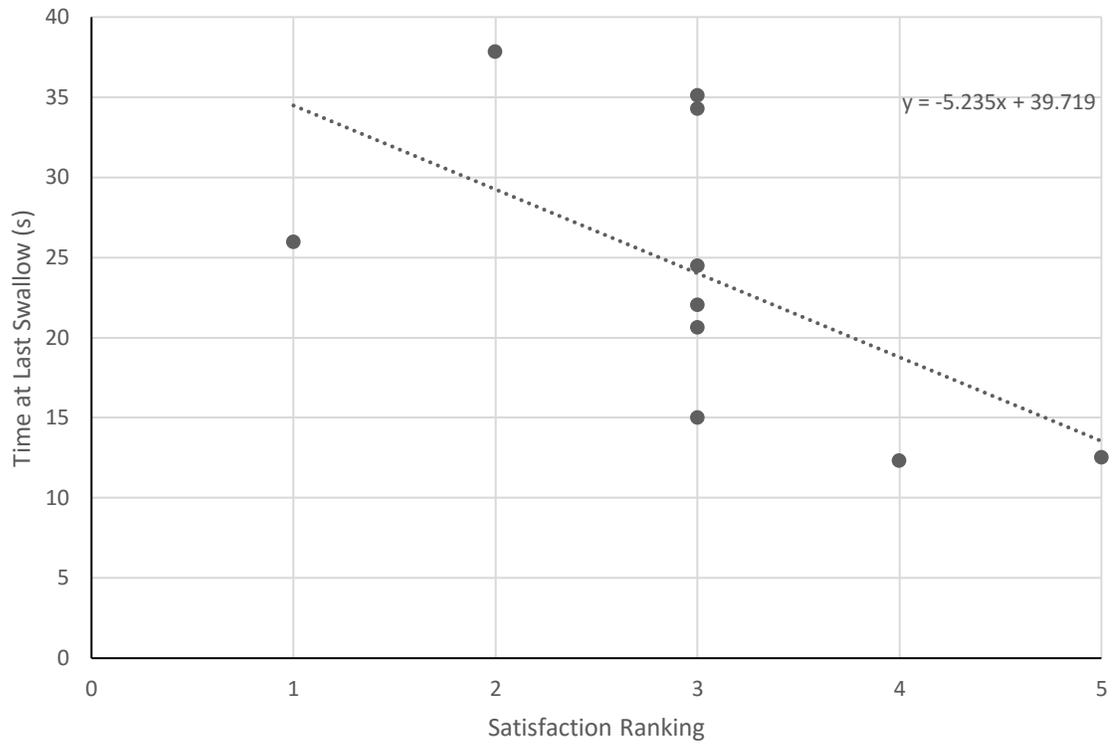
Figure 8: The regression analysis of time at last swallow vs. satisfaction ranking of novelty milk chocolate with the highest, significant r^2 value.

Taste Test Evaluation of Chocolate Samples

Chocolate Sample: A

Rate the degree of bitterness experienced during consumption on a scale from 1 to 5 (1=low degree of bitterness, 5=high degree of bitterness).

1 2 3 4 5

Rate the degree of hardness at first bite on a scale from 1 to 5 (1=low degree of hardness (low force used), 5=high degree of hardness (high force used)).

1 2 3 4 5

Rate the degree of mouth coating experienced after swallowing on a scale from 1 to 5 (1= low degree of mouth coating, 5= high degree of mouth coating).

1 2 3 4 5

Rate the degree of satisfaction experienced during consumption on a scale from 1 to 5 (1=low satisfaction, 5=high satisfaction).

1 2 3 4 5

Taste Test Evaluation of Chocolate Samples

Chocolate Sample: B

Rate the degree of bitterness experienced during consumption on a scale from 1 to 5 (1=low degree of bitterness, 5=high degree of bitterness).

1 2 3 4 5

Rate the degree of hardness at first bite on a scale from 1 to 5 (1=low degree of hardness (low force used), 5=high degree of hardness (high force used)).

1 2 3 4 5

Rate the degree of mouth coating experienced after swallowing on a scale from 1 to 5 (1= low degree of mouth coating, 5= high degree of mouth coating).

1 2 3 4 5

Rate the degree of satisfaction experienced during consumption on a scale from 1 to 5 (1=low satisfaction, 5=high satisfaction).

1 2 3 4 5

Taste Test Evaluation of Chocolate Samples

Chocolate Sample: C

Rate the degree of bitterness experienced during consumption on a scale from 1 to 5 (1=low degree of bitterness, 5=high degree of bitterness).

1 2 3 4 5

Rate the degree of hardness at first bite on a scale from 1 to 5 (1=low degree of hardness (low force used), 5=high degree of hardness (high force used)).

1 2 3 4 5

Rate the degree of mouth coating experienced after swallowing on a scale from 1 to 5 (1= low degree of mouth coating, 5= high degree of mouth coating).

1 2 3 4 5

Rate the degree of satisfaction experienced during consumption on a scale from 1 to 5 (1=low satisfaction, 5=high satisfaction).

1 2 3 4 5

APPENDIX A continued

Taste Test Evaluation of Chocolate Samples

Chocolate Sample: D

Rate the degree of bitterness experienced during consumption on a scale from 1 to 5 (1=low degree of bitterness, 5=high degree of bitterness).

1 2 3 4 5

Rate the degree of hardness at first bite on a scale from 1 to 5 (1=low degree of hardness (low force used), 5=high degree of hardness (high force used)).

1 2 3 4 5

Rate the degree of mouth coating experienced after swallowing on a scale from 1 to 5 (1= low degree of mouth coating, 5= high degree of mouth coating).

1 2 3 4 5

Rate the degree of satisfaction experienced during consumption on a scale from 1 to 5 (1=low satisfaction, 5=high satisfaction).

1 2 3 4 5

APPENDIX A continued

Taste Test Evaluation of Chocolate Samples

Rank the chocolate samples in order of preference (1=highly preferred, 4=least preferred).

A   _____

B   _____

C   _____

D   _____