Spring 5-8-2020

Assessment of Oral Motor Activity Variables During Consumption of Chocolate Using Surface Electromyography: Effects of Variation in Cocoa Solid Content

Kara Elizabeth Cook

Assessment of Oral Motor Activity Variables During Consumption of Chocolate Using Surface Electromyography: Effects of Variation in Cocoa Solid Content

By:

Kara Elizabeth Cook

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

Oxford

April 2020

Approved by

____

Advisor: Carol Britson, Ph.D.

____

Reader: Colin Jackson, Ph.D.

____

Reader: Sarah Liljegren, Ph.D.

ACKNOWLEDGMENTS

I must extend thanks to Dr. Carol Britson for allowing me the opportunity to complete my thesis under her guidance. This project would not have been possible without Dr. Britson's generosity with her time, ideas, and constructive criticism. I also would like to thank her for supporting my future career as a dentist. My experiences with Dr. Britson are integral in my academic career, and thanks to her, I will enter dental school with confidence in my critical thinking and research skills.

I would like to thank Dr. Sarah Lilljegren for the knowledge I obtained through her genetics course. In addition, I thank her for reading the following pages and for her constructive criticism. I also thank her for attending my thesis defense.

I would like to thank Dr. Colin Jackson for the knowledge he imparted as my former honors recitation leader. I appreciate his willingness to read my thesis and provide suggestions. I would also like to thank Dr. Jackson for attending my thesis defense.

ABSTRACT

KARA E. COOK: Assessment of Oral Motor Activity Variables During Consumption of Chocolate Using Surface Electromyography: Effects of Variation in Cocoa Solid Content
(Under the direction of Carol A. Britson)

The aim of this experiment is to assess how a wide range of cocoa content in different types of chocolate samples effects oral motor activity for mastication events, along with the time between consumption and swallowing. A prior study assessed individual oral motor activity using surface electromyography (sEMG) for mastication events while eating four different chocolate samples, and this information was used to determine which aspects of the masticatory process underlie differences in individual chewing behavior as well as whether subjects retain their general characteristic eating behavior across a variety of chocolate samples. The study found that with chocolate samples containing 0%, 30%, and 90% cocoa, most people preferred the sample with 30% cocoa; however, there is limited knowledge because a gradually increasing range of cocoa solids was not tested. In this experiment, the range of the cocoa content of the chocolates was increased to determine a clear relationship between cocoa content, oral motor activity and preference. By using more samples with cocoa contents between 30% and 94% and testing each type of chocolate with each subject, this experiment determines the transition points among subjects' positive and negative reactions to increasing cocoa content in chocolate. The results of this experiment reveal that while oral processing time and facial grimace status differed significantly across chocolate types, sEMG mastication did not. Regression analysis showed a significant correlation between oral processing time and some descriptive statistic rankings including hardness, bitterness, and satisfaction for certain chocolates. I hypothesize that (1) chocolate samples with higher cocoa composition will be preferred by subjects as assessed by subject evaluation of bitterness, hardness, mouth-coating, satiation, aftertaste, and overall ranking of four samples, (2) there will be a positive correlation between cocoa composition and preference

until reach a threshold is potentially reached, and (3) preference will be significantly correlated with oral motor events (e.g., sEMG activity of the masseter and suprahyoid muscles; total mastication time; facial grimace status). Earlier studies have found that faster chewing or a higher number of chews shows preference, meaning that the individual's eating behavior increases with increasing preference. However, this study did not support these findings, as it showed an overall trend of average oral processing time increasing as cocoa content increased and average satisfaction decreased. Through the use chocolates with an increased range of cocoa contents, this experiment ultimately expanded upon previous findings, supporting the hypothesis that there is a positive correlation between cocoa composition and preference until a threshold is reached.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Various attributes of chocolate affect the sensory experience of the consumer. Different cocoa types, varying ingredient proportions, and different processing methods help to determine the differences in sensory perceptions of chocolate (Afoakwa et al., 2007). The three main chocolate categories, dark, milk, and white, may be distinguished by differing amounts of cocoa solids, milk fat, and cocoa butter, which leads to variation in the amount of macronutrients such as carbohydrates, fats, and proteins (Afoakwa et al., 2007). Cocoa butter reduces chocolate viscosity, while milk fat adds a creaminess attribute (Gorty and Barringer, 2011). Novelty chocolate is characterized by the inclusion of plant-based fat sources in addition to cocoa butter (Afoakwa et al., 2007). These alternative fat sources, known as cocoa butter equivalents (CBEs), can be added to the chocolate without having a significant effect on texture, and are permitted up to 5% for the product to be sold as a chocolate (Afoakwa et al., 2007). Particle size affects the consumer's perception of flavor, viscosity, and texture. High quality chocolate is often characterized by a small particle size (Afoakwa et al., 2007). Large particles are important to mouth feel with respect to grittiness but can diminish sweetness (Afoakwa et al., 2007). The maximum particle size before reaching the point of grittiness is around 30 μm (Afoakwa et al., 2007). Beyond this point, chocolate contains a gritty taste with a high viscosity, which prolongs a pasty feeling in the mouth (Afoakwa et al., 2007). On the other hand, smaller particle sizes provide a creamier taste (Afoakwa et al., 2007). Optimizing the particle size distribution and reducing the fat content leads to a decrease in viscosity and reduction in hardness (Do et al., 2007). Chocolates with lower cocoa contents are associated with a melting, creamy sensation, while chocolates with higher cocoa content often produce a dry, mealy, and sticky mouth feel (Saltini et al., 2013).

Patterson (2017) assessed individual oral motor activity using surface electromyography (sEMG) for chewing events while eating four different chocolate samples, and this information was used to determine which aspects of the masticatory process [e.g. sEMG activity of master and suprahyoid muscles, total number of chewing actions, and time to last swallow (EGG)] underlie differences in individual chewing behavior as well as whether subjects retain their general characteristic eating behavior across a variety of chocolate samples. Patterson's (2017) experiment utilized white chocolate and novelty chocolate (which does not contain cocoa solids but does contain cocoa replacements), milk chocolate with 30% cocoa, and dark chocolate with 90% cocoa. However, the experiment lacked additional chocolate samples that fell between 30% and 90% cocoa content. The results of some of the statistical tests, such as the regression relationship between sEMG parameters and chocolate qualities such as bitterness, brought about limited conclusions because of this limited range along with the complete lack of cocoa in white chocolate, which made it difficult to draw conclusions regarding the significance of cocoa content. The aim of this experiment was to assess how a wide range of cocoa content in different types of chocolate samples affects oral motor activity for mastication events, along with the time between consumption and swallowing.

The physiological parameters of mastication may be measured using surface electromyography (sEMG),), a non-intrusive method of measuring muscle movements that does not interfere with the eating process. Carvalho-da-Silva et al. (2011) investigated variation in the individual eating behaviors of participants consuming chocolate using sEMG and determined that changes in eating behavior relate to textural differences between chocolate samples. Their study looked at five textural attributes, hardness, melting speed, smoothness, thickness, and mouth-coating as well as 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, first and last time of swallow, and swallow rate. Carvalho-da-Silva et al. (2011) described three types of eating behaviors: “fast chewers,” “thorough chewers,” and “suckers.” A similar study by Nasser et al. (2011) found that with chocolate specifically, sugar and cocoa content is positively correlated with the preference and desire for chocolate.

This research project assesses individual motor activity while eating different types of chocolate with a wide range of cocoa content using sEMG to measure mastication events. The goal was to determine which aspects of the masticatory process underlie differences in individual mastication behavior with a particular focus on the effect of cocoa content. I hypothesize that chocolate samples with higher cocoa composition will be preferred by subjects as assessed by subject evaluation of bitterness, hardness, mouth-coating, satiation, aftertaste, and overall ranking of four samples. I also hypothesize that there will be a positive correlation between cocoa composition and preference until a threshold is potentially reached. Finally, I hypothesize that preference will be significantly correlated with oral motor events (e.g., sEMG activity of the masseter and suprahyoid muscles; total mastication time; facial grimace status).

MATERIALS AND METHODS

Preparation for this experiment began with IRB approval in May 2019 (Protocol #19-102), followed by the recruitment of thirty-nine college students. All students were enrolled in Human Anatomy and Physiology I (BISC 206) at the University of Mississippi, and the majority of the students were between the ages of 18-22. Upon arrival to the research lab and prior to participating in the experiment, each participant read and signed an informed consent form.

Seven different chocolate samples with varying ingredients were given to each participant. The samples included R.M. Palmer Company novelty chocolate (0% cocoa) [no expiration date], Lindt milk chocolate (35% cocoa) [expiration date 6/30/20], Ghirardelli dark chocolate squares (60% cocoa) [expiration date 9/30/20], Lindt dark chocolate (78% cocoa) [expiration date 6/30/20], Ghirardelli intense dark chocolate (86% cocoa) [expiration date 7/31/20], Lindt dark chocolate (90% cocoa) [expiration date 6/30/20], Lindt dark chocolate (95% cocoa) [expiration date 11/30/20]. The novelty chocolate contained soy lecithin and PGPR (emulsifiers), while the other chocolates contained cocoa butter.

The technique used in this experiment included surface electromyography (sEMG) in order to measure the movement of the masseter muscle, the anterior belly of the digastric of the suprahyoid muscle (chin), and the posterior belly of the digastric of the suprahyoid muscle. sEMG is a non-intrusive method of measuring muscle movements that does not interfere with the eating process. Two, 26T PowerLabs were connected in tandem to a computer running LabChart Version 8.1.8 software, licensed to the University of Mississippi. Two electrodes were placed on either side of the belly of each muscle and were connected to the PowerLab unit via a BioAmp cable. A ground electrode was placed on each shoulder. Muscular activity data were collected at a rate of 200 readings/second within a range of 2mV.

A push button switch was used to record swallowing events. Facial grimace events were recorded with event keys during recording.

Before electrodes were placed on the subject, they were shown where each electrode would be located and instructed to clean those specific areas of the face and neck using an alcohol wipe. Next, sEMG electrodes were placed on each masseter muscle, two sEMG electrodes on the anterior belly of the digastric of the suprahyoid muscle (under the chin), two sEMG electrodes on the posterior belly of the digastric of the suprahyoid muscle, and one sEMG ground electrode on each shoulder (Figure 1).

The testing protocol for this experiment began with calibration of the equipment. Participants were then asked to clench their teeth in order to measure maximum voluntary contraction (MVC) of the masseter muscle and to lower their mandible (open their mouth) to measure the voluntary action of the hyoid muscle. The first of the seven chocolate samples, which were scrambled and distributed in a random order each time, was then given to the participant. All chocolate samples were the same size and shape, weighing 5 g each. The participant was asked to consume it as they normally would, depressing the push button upon taking the last swallow. The participants were then verbally asked five post-consumption questions relating to bitterness, hardness, mouth-coating, degree of satisfaction, strength of aftertaste, and likelihood to consume the sample as a stand alone treat or as an ingredient within a recipe (Appendix A). After answering all of the questions, the participant consumed water to cleanse the palate, and the subject was then presented with the next sample. This procedure was repeated until all seven chocolate samples had been consumed. Data collection began from the time the chocolate was placed in the mouth and concluded after the last swallowing event.. The variables for which data was collected included oral processing time, time between when chocolate was first placed in mouth and last swallow, the absence or presence of a facial grimace, and the percent of maximum voluntary contraction of the masseter muscle, the anterior belly of the digastric of the suprahyoid muscle, and the posterior belly of digastric of the suprahyoid muscle. Once sampling was completed, the electrodes were removed from the subject's skin, and lotion was provided as needed for skin irritation. Finally, the subjects answered a two-question written survey regarding general preferences for chocolate and if/how those preferences had changed over time (Appendix B).

An alternate, online survey was available to BISC 206 students via Blackboard, and contained Likert style questions (Appendix C). The survey included questions about general chocolate preferences, oral processing habits involving chocolate, general sense of bitterness, hardness, satisfaction, aftertaste and mouth-coating when eating chocolate, and whether participants would be more likely to consume chocolate as a stand-alone treat or as an ingredient within a recipe.

Oral processing time, muscle activity, and facial grimace events were analyzed with a one-factor (e.g., chocolate type) analysis of variance (ANOVA). Except for facial grimace data which were recorded as yes/no, mastication variables that significantly differed across chocolate types were compared against the qualitative post consumption survey responses with a regression analysis. The level of significance was set at alpha = 0.05 for all analyses. Descriptive statistics including mean and standard deviation were calculated for the alternate survey data to develop a background profile of typical chocolate preferences in college students.

RESULTS

sEMG data

The average oral processing time differed significantly across all chocolate types ($F=7.059$; $df=6,266$; $p<0.0001$). The mean OPT for novelty chocolate was 26.929 seconds, mean OPT for 35% cocoa chocolate was 28.203 seconds, mean OPT for 60% cocoa chocolate was 29.664 seconds, mean OPT for 78% cocoa chocolate was 31.663 seconds, mean OPT for 86% cocoa chocolate was 38.444 seconds, mean OPT for 90% chocolate was 38.585 seconds, and mean OPT for 95% chocolate was 40.165 seconds (Figure 2).

The occurrence of facial grimacing for each chocolate type was found to differ significantly across chocolate types ($F=7.059$; $df=6,266$; $p<0.0001$). The mean facial grimace status for novelty chocolate was 0.026, 35% chocolate was 0.026, 60% chocolate was 0, 78% chocolate was 0.103, 86% chocolate was 2.56, 90% chocolate was .513, and 95% chocolate was .436 (Figure 3).

Analysis of muscle activity revealed that average percent of maximum voluntary contraction of the masseter did not differ significantly across chocolate types ($F=.481$; $df=6,266$; $P=.822$), nor did the average percent of maximum voluntary contraction of the suprahyoid posterior belly of the digastric ($F=.636$; $df=6,266$; $P=.702$) or the suprahyoid anterior belly of the digastric ($F=.393$; $df=6,266$; $P=.883$). For chocolates with lower cocoa contents, the average percentage of maximum voluntary contraction of the masseter muscle decreased as cocoa content increased. This trend was no longer evident, however, among 86%, 90%, and 95% chocolates (Figure 5). The average percentage of maximum voluntary contraction of the suprahyoid posterior belly and the suprahyoid anterior belly for each chocolate type did not exhibit any particular trend in conjunction with increasing cocoa content (Figure 5).

Post-Consumption Qualitative Rankings

The average bitterness ranking differed significantly across all chocolate types (F=108.384; df=6,266; P<0.0001), as did the average hardness ranking for all chocolate types (F=26.031; df=6,266; P<0.0001). The average bitterness ranking for each chocolate increased as cocoa content increased. Average hardness ranking also increased as cocoa content increased, with the exception of 95% chocolate, which received a slightly lower hardness ranking than 90% chocolate (Figure 5). Average mouth-coating ranking differed significantly across all chocolate types (F=7.651; df=6,266; P<0.0001). Overall, chocolates with higher cocoa contents generally received higher mouth-coating rankings, but average mouth-coating rankings did not consistently increase as cocoa content increased (Figure 5). Average satisfaction ranking differed significantly across all chocolate types (F=27.173; df=6,266; P<0.0001) . Generally, as cocoa content increased, average satisfaction rankings decreased, with the exception of novelty chocolate, which received a lower satisfaction ranking than 35% cocoa chocolate (Figure 5). The average strength of aftertaste ranking for all chocolate types (F=17.171; df=6,266; P<0.0001) differed significantly across all chocolate types. Generally, as cocoa content increased, average strength of aftertaste ranking increased as well, with the exception of novelty chocolate, which received a slightly higher strength of aftertaste ranking than 35% chocolate (Figure 5). The average mode of preferred consumption ranking for all chocolates also differed significantly across all chocolate types (F=18.813; df=6,266; P<0.0001). As cocoa content increased, subjects indicated a higher likelihood of consuming the chocolate as an ingredient within a recipe rather than as a stand alone treat. The only exception to this trend was for novelty chocolate, as average rankings indicated that subjects would be more likely to consume 35% chocolate as stand alone treat over novelty chocolate (Figure 6).

Regression analysis showed a significant correlation between oral processing time and at least one average qualitative characteristic for each chocolate type. For 60% chocolate, there was a significant negative correlation between oral processing time and hardness (Table 5). In 86% chocolate, there was a significant positive correlation between oral processing time and bitterness as well as a significant negative correlation between oral processing time and satisfaction (Table 7). For 90% chocolate, there was a significant negative correlation oral processing time vs. hardness rankings showed (Table 8). Finally, for 95% chocolate, there was a significant negative correlation between oral processing time and satisfaction rankings (Table 9).

For novelty chocolate, regression analysis of oral processing time vs. rankings of bitterness, hardness, mouth-coating, satisfaction, and aftertaste showed that these values were not significantly correlated (Table 3). Similarly, for 35% chocolate (Table 4) and 78% chocolate (Table 6), regression analysis revealed that oral processing time and average rankings for all descriptive statistics were not significantly correlated.

Post-Testing Qualitative Questionnaire

When asked what their preferred type of chocolate was after participating in the experiment, 26 subjects said milk chocolate, 12 subjects said dark chocolate, and one subject said white chocolate. When asked if their preference has changed over time, nine subjects said it has changed from milk to dark, one subject said it has changed from milk to white, and one subject said it has changed from white to milk. In addition, 25 subjects said they have always preferred milk chocolate, and three subjects said they have always preferred dark. Of the students who said their preference has changed at some point, one subject said it changed around age 11, five subjects said it changed around age 14-15, one subject said it changed around 18-19, one subject said it changed when she became lactose intolerant, and three subjects did not specify when it changed.

Alternate Activity

The alternative activity data includes responses from 54 students. When asked to select their generally preferred type of chocolate, 29 students selected milk chocolate (35% cocoa), making it the most popular choice by far. The next most popular response, dark chocolate (60%) cocoa, was chosen by nine students (Figure 7). When asked to indicate their general preference for chocolate, "prefer it a moderate amount" was the most popular response, followed by "prefer a lot" and "prefer a great deal." The least popular response, "do not prefer," was chosen by only two students (Figure 8). When asked to estimate their average oral processing time when eating chocolate, the majority of students selected "5-7 seconds", followed by "3-5 seconds." "10 or more seconds" was the least popular response (Figure 9). When ranking the general degree of bitterness, hardness, mouth-coating, and strength of aftertaste of chocolate (particular chocolate type was not specified) on a scale from one to ten, most students chose five for all the qualities except for satisfaction. When ranking the general degree of satisfaction experienced while eating chocolate, however, most students chose eight to ten, indicating a very high degree of satisfaction (Figure 10). When asked to indicate their likelihood to consume chocolate as a stand-alone treat or as an ingredient within a recipe, most students indicated that they would be equally as likely to do either. (Figure 11).

DISCUSSION

Patterson (2017) found that faster chewing or a higher number of chews showed preference, meaning that these eating behaviors increased with increasing preference. Patterson's (2017) findings aligned with a study by de Lavergne et al. (2015) who found that the longer the oral exposure time to food, the higher the satiation. However, this study did not support these findings. Instead, an overall trend was shown of average oral processing time increasing as cocoa content increased and average satisfaction decreased. These findings align with those of Do et al. (2007), who stated that reducing the fat content leads to a decrease in viscosity and reduction in hardness, because as cocoa content and average hardness rankings increased, average oral processing time increased as well. The results of this experiment are also supported by Saltini et al. (2013)'s statement that chocolates with higher cocoa content often produce a dry, mealy, and sticky mouth feel, which leads to an increased oral processing time. In this experiment, the chocolate with the highest cocoa content had the highest oral processing time and the lowest average satisfaction ranking, while the chocolate with the lowest cocoa content had the lowest oral processing time and the third highest satisfaction ranking. Regression analysis showed a significant negative correlation between oral processing time and satisfaction rankings for several chocolates, including 86% cocoa chocolate and 95% cocoa chocolate. The regression relationship for 90% cocoa chocolate also exhibited a negative correlation between oral processing time and satisfaction rankings, and though it is not statistically significant ($p=.069$), it is representative of the overall trend of increasing oral processing time and decreasing satisfaction ranking in chocolates with high cocoa contents. This contrasts with the regression analysis of satisfaction rankings and oral processing time for all chocolates with cocoa contents less than 86%, which did not show a significant correlation. These results indicate that at lower levels, cocoa content does not directly affect preference/satisfaction and oral processing time, but as cocoa content reaches higher levels, such as 86% and beyond, it begins to have a significant positive effect on oral processing patterns, which is a reflection of a decrease in satisfaction/preference. Because this study implemented more chocolates samples covering a wider range of cocoa content, it is possible that these results are a better representation of the relationship between preference, satisfaction, and oral processing time than that of previous studies such as Patterson's (2017).

The average oral processing time of the subjects in this experiment differed significantly across chocolate types. As cocoa content of each sample increased, oral processing time increased as well (with the exception of very slight difference in the oral processing times of 86% chocolate and 90% chocolate). Harwood et al. (2012) found that as the bitterness of chocolate samples increased, preference decreased. The results of this experiment align with the findings of Harwood et al. (2012); the verbal rankings from taste tests show that as cocoa content increased, average bitterness rankings also increased and average satisfaction rankings consistently decreased (with the exception of the satisfaction ranking of novelty chocolate). In addition, regression analysis showed a significant correlation existed between bitterness and oral processing time for 86% chocolate. In the regression analysis for bitterness and oral processing time for novelty chocolate, the p-value for the r^2 value is not significant, but there appears to be a relationship between the average bitterness ranking of novelty chocolate, which is by far the lowest average bitterness rankings among all chocolates, and the average oral processing time for novelty chocolate, which is also the lowest average oral processing time among all chocolates.

Along with OPT, the average facial grimace status differed significantly across all chocolate types as well. Facial grimace status was recorded because it is a clear visible representation of a subject's displeasure when eating a chocolate sample. For the 78%, 86%, and 90% dark chocolate samples, facial grimace status increased as cocoa content increased.

However, facial grimace status of the 95% cocoa chocolate was actually slightly lower than for 90% cocoa chocolate. Similarly, the average mouth coating ranking for 95% cocoa chocolate was slightly lower than that of 90% chocolate. It is possible that the lower facial grimace status for 95% cocoa may be attributed to the lower prevalence of mouth coating while eating this chocolate. It is also interesting to note that a small amount of subjects exhibited facial grimaces for novelty chocolate and 35% cocoa chocolate, which have very low cocoa contents, but the only chocolate for which no facial grimaces were recorded was the 60% cocoa. Novelty chocolate and 35% cocoa chocolate are the only two chocolates with sugar as their primary ingredient, and several subjects who preferred dark chocolate expressed their dislike for chocolates that were “too sweet” in the post-testing qualitative questionnaire. This data suggests that perhaps 60% cocoa chocolate was the only chocolate not linked to any facial grimaces because it is least “extreme” in any category, as evidenced by its medium average ranking in all of the descriptive statistics as well as its sugar and cocoa content.

While oral processing time and facial grimace status differed significantly across all chocolate types, mastication parameters from sEMG data, including %MVC of the masseter, suprahyoid (posterior belly of digastric), and suprahyoid ("chin", anterior belly of digastric), did not differ significantly across all chocolate types. This data does not support the hypothesis that preference would be significantly and positively correlated with oral motor events. It is possible that the force with which subjects chew and process their food simply is not strongly related to one’s preference. It is also possible that subjects modified their normal mastication patterns, whether consciously or subconsciously, because they knew their muscle movement was being monitored. Finally, it is possible that the lack of a significant variation is due to preexisting differences in mastication patterns such as the strength of the masticatory muscles and the implementation, or lack thereof, of table manners regarding chewing. While many factors could have affected the %MVC each subject exhibited, it is impossible to conclude with certainty the reasons for mastication force did not differ significantly across all chocolate types.

According to a study by Nasser et al. (2011), participants consuming chocolate samples with cocoa content ranging from 0-85% were more likely to want more of the sample if it had high sugar and cocoa content. The results of this experiment mostly aligned with Nasser et al.'s (2011) ideas about the effect of sugar content, but with the exception of novelty chocolate, increasing cocoa content was correlated with decreasing satisfaction ranking. When participants in this study were asked to rank their degree of satisfaction after consuming each sample, the three chocolate samples with the lowest amount of cocoa content received the highest satisfaction rankings. 35% cocoa milk chocolate received the highest average satisfaction ranking, followed by 60% cocoa milk chocolate and novelty chocolate. For the remaining 78%, 86%, 90%, and 95% cocoa chocolate samples, average satisfaction ranking decreased as cocoa content increased and sugar cocoa content decreased. While the Nasser et al.'s (2011) findings on the effects of sugar content in the sense that the three chocolate samples which received the highest satisfaction rankings were those with the highest sugar content, it is interesting to note that the chocolate which received the highest satisfaction ranking overall, 35% cocoa, did not have the highest sugar content. The 35% cocoa sample contains 2.6 grams of sugar, while novelty chocolate contains 2.9 grams of sugar. This data suggests that novelty chocolate is likely less satisfactory than 35% chocolate because it has 0% cocoa solids, and the ideal amount of cocoa solids for satisfaction is around 35%.

In the alternate activity survey, participants reported preferences similar to those seen in the taste test satisfaction rankings, as 53.7% of the participants chose milk chocolate (35% cocoa) as their preferred type of chocolate, which was significantly higher than any other type. However, the next most popular answer was dark chocolate (60%), followed by dark chocolate (86%), white chocolate (0% cocoa), dark chocolate (95% cocoa), and dark chocolate (78% cocoa), and dark chocolate (90% cocoa). Based upon these answers, it would be expected that 35% milk chocolate would receive significantly higher satisfaction rankings during a taste test, but this was not the case--the average satisfaction ranking for 35% milk chocolate, was closely followed by the satisfaction ranking for 60% dark chocolate. These data suggest that many people may be unaware that they actually like dark chocolate that is on the lower end of the cocoa content scale (such as 60% dark chocolate) almost as much as they like milk chocolate with low cocoa content (such as 35% milk chocolate).

Brown et al. (1996) found that mastication rate was significantly correlated with preference, and that individuals chew faster and prefer the samples in which they find easiest to manipulate and manage in the mouth. Iguchi et al. (2015) found that chewing performance parameters, such as number of chews, chew time, and chew force, are correlated with food hardness. With the exception of the 95% chocolate sample, the average hardness ranking for each chocolate increased as cocoa content increased, as did average oral processing time. This suggests that chocolates with higher cocoa content are typically harder and thus take longer to chew and process. The regression analysis of average mouth-coating ranking and oral processing time in this experiment did not directly support the findings of Brown et al. (1996), study, as these factors did not show a significant correlation for any chocolates. The results of this experiment support the findings of Brown et al. (1996) and Iguchi et al. (2015), however, in regards to the relationship between average hardness rankings and preference/satisfaction rankings. As an example, 90% cocoa chocolate received the highest average hardness ranking, was reported as the least preferred chocolate in the alternate survey, and received the second lowest satisfaction ranking. In addition, regression analysis showed a significant relationship between average hardness rankings and oral processing times for both 60% cocoa chocolate and 90% cocoa chocolate. These findings indicate that while the hardness of a chocolate sample has a predictable effect on subjects' satisfaction and preference, the effect mouth-coating on satisfaction and preference is not as clear. Hardness likely displays a predictable effect on satisfaction on and preference primarily because it is closely linked with cocoa content, which, according to the results of this experiment, has a strong effect on satisfaction and preference. According to Afoakwa et al. (2007), mouth-coating involves cocoa and lipids coating the epithelial surface, so it is possible that the subject's perception of mouth-coating is influenced more by the prevalence of lipids than cocoa content, meaning that mouth-coating does not necessarily increase as cocoa content increases. In this way, the influence of lipids over cocoa content on mouth-coating serves as a possible explanation for why hardness has a predictable effect on satisfaction and preference while mouth-coating does not.

LIST OF REFERENCES

Afoakwa, E.O., Paterson, A., Fowler, M. (2007). Factors influencing rheological and textural qualities in chocolate—a review. Trends in Food Science & Technology 18, 290-298.

Brown, W. E., Dauchel, C., Wakeling, I. (1996). Influence of chewing efficiency on texture and flavour perceptions of food. Journal of Texture Studies 27, 433-450.

Carvalho-Da-Silva, A.M., Damme, I.V., Wolf, B., Hort, J. (2011). Characterisation of chocolate eating behaviour. Physiology & Behavior 104.5, 929-933.

De Lavergne, D., Derks, J.A.M., Ketel, E.C., de Wijk, R.A., Stieger, M. (2015). Eating behavior explains differences between individuals in dynamic texture perception of sausages. Food Quality and Preference 41, 189-200.

Do, T.-A., Hargreaves, J., Wolf, B., Hort, J. and Mitchell, J. (2007), Impact of particle size distribution on rheological and rextural properties of chocolate models with reduced fat content. Journal of Food Science, 72: E541-E552. doi:10.1111/j.1750-3841.2007.00572.x

Gorty, A.V., Barringer, S.A. (2011). Electrohydrodynamic spraying of chocolate. Journal of Food Processing & Preservation, 35(4), 542–549. https://doi-org.umiss.idm.oclc.org/10.1111/j.1745-4549.2010.00500.x

Harwood, M., Ziegler, G.R., Hayes, J.E. (2012). Rejection threshold in solid chocolate-flavored compound coating. Journal of Food Science 77, 390-393.

Iguchi, H., Magara, J., Nakamura, Y., Tsujimura, T., Ito, K., Inoue, M. (2015). Changes in jaw muscle activity and the physical properties of foods with different textures during chewing behaviors. Physiology & Behavior 152, 217-224.

Nasser, J.A., Bradley, L.E., Leitzsch, J., Chohan, O., Fasulo, K., Haller, J., Jaeger, K., Szulanczyk, B., Del Parigi, A. (2011). Psychoactive effects of tasting chocolate and desire for more Chocolate. Physiology & Behavior 104.1, 117-121.

Patterson, T. (2017). Examination of electroglottography (EGG) and surface electromyography (sEMG) as techniques to assess oral motor activity during consumption of four forms of chocolate. Undergraduate thesis, under the direction of Carol Britson from Biology, University of Mississippi.

Saltini, R., Akkerman, R., Frosch, S. (2013). Optimizing chocolate production through traceability: a review of the influence of farming practices on cocoa bean quality. Food Control 29:167–187.

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

Novelty chocolate (0% cocoa) (R.M. Palmer Company)	Milk chocolate (35% cocoa) (Lindt)	Dark chocolate (60% cocoa) (Ghirardelli)	Dark chocolate (78% cocoa) (Lindt)	Dark chocolate (86% cocoa) (Ghirardelli)	Dark chocolate (90% cocoa) (Lindt)	Dark chocolate (95% cocoa) (Lindt)
-Sugar -Cocoa butter -Whole milk -Chocolate -Soy lecithin & PGPR (emulsifiers) -Vanilla	-Sugar -Cocoa butter -Milk -Chocolate -Skim milk -Soy lecithin (emulsifier) -Barley malt powder -Artificial flavor	-Unsweetened chocolate -Sugar -Cocoa butter -Milk fat -Soy lecithin (emulsifier) -Vanilla	-Chocolate -Cocoa butter -Sugar -Cocoa powder (processed with alkali) -Milk fat	-Unsweetened chocolate -Cocoa butter -Sugar -Milk fat -Soy lecithin (emulsifier) -Vanilla -Natural flavor	-Chocolate -Cocoa butter -Cocoa powder (processed with alkali) -Sugar -Bourbon vanilla beans	-Chocolate -Cocoa butter -Cocoa powder (processed with alkali) -Sugar

Table 2: Chocolate nutrient content for each 5 g chocolate sample.

	Novelty chocolate (35% cocoa) (R.M. Palmer Company)	Milk chocolate (35% cocoa) (Lindt)	Dark chocolate (60% cocoa) (Ghirardelli)	Dark chocolate (78% cocoa) (Lindt)	Dark chocolate (86% cocoa) (Ghirardelli)	Dark chocolate (90% cocoa) (Lindt)	Dark chocolate (95% cocoa) (Lindt)
Protein (g)	0.25	0.5	0.5	0.5	0.5	.67	.65
Fat (g)	1.65	1.6	1.8	2.4	2.7	2.7	2.8
Sugar (g)	2.9	2.6	1.8	0.9	0.5	0.33	0.2

Table 3: Regression analysis of oral processing time vs. ranked descriptive statistics for novelty chocolate. Significant r^2 values ($p \leq 0.05$) are shown in bold.

	R square	F	Sig	df
Bitterness	0.083	3.371	0.074	6,266
Hardness	0.034	1.309	0.26	6,266
Mouth-coating	0.011	0.413	0.524	6,266
Satisfaction	0.005	0.18	0.674	6,266
Stand-alone vs. ingredient in recipe	0.024	0.917	0.344	6,266
Aftertaste	0.011	0.412	0.525	6,266

Table 4: Regression analysis of oral processing time vs. ranked descriptive statistics for 35% cocoa chocolate. Significant r^2 values ($p \leq 0.05$) are shown in bold.

	R square	F	Sig	df
Bitterness	0.039	1.516	0.226	6,266
Hardness	0.002	0.089	0.767	6,266
Mouth-coating	0.007	0.251	0.619	6,266
Satisfaction	0	0.008	0.93	6,266
Stand-alone vs. ingredient in recipe	0	0	0.983	6,266
Aftertaste	0	0.016	0.9	6,266

Table 5: Regression analysis of oral processing time vs. ranked descriptive statistics for 60% cocoa chocolate. Significant r^2 values ($p \leq 0.05$) are shown in bold.

	R square	F	Sig	df
Bitterness	0.018	0.663	0.421	6,266
Hardness	**0.125**	**5.293**	**0.027**	6,266
Mouth-coating	0.001	0.034	0.855	6,266
Satisfaction	0.04	1.559	0.22	6,266
Stand-alone vs. ingredient in recipe	0.019	0.734	0.397	6,266
Aftertaste	0.002	0.065	0.8	6,266

Table 6: Regression analysis of oral processing time vs. ranked descriptive statistics for 78% cocoa chocolate. Significant r^2 values ($p \leq 0.05$) are shown in bold.

	R square	F	Sig	df
Bitterness	0	0.005	0.943	6,266
Hardness	0.014	0.531	0.471	6,266
Mouth-coating	0.01	0.374	0.545	6,266
Satisfaction	0.061	2.406	0.129	6,266
Stand-alone vs. ingredient in recipe	0.021	0.774	0.385	6,266
Aftertaste	0.01	0.357	0.554	6,266

Table 7: Regression analysis of oral processing time vs. ranked descriptive statistics for 86% cocoa chocolate. Significant r^2 values ($p \leq 0.05$) are shown in bold.

	R square	F	Sig	df
Bitterness	**0.207**	**9.649**	**0.004**	**6,266**
Hardness	0.035	1.352	0.252	6,266
Mouth-coating	0.001	0.022	0.882	6,266
Satisfaction	**0.137**	**5.868**	**0.02**	**6,266**
Stand-alone vs. ingredient in recipe	0.001	0.047	0.829	6,266
Aftertaste	0.009	0.329	0.57	6,266

Table 8: Regression analysis of oral processing time vs. ranked descriptive statistics for 90% cocoa chocolate. Significant r^2 values ($p \leq 0.05$) are shown in bold.

	R square	F	Sig	df
Bitterness	0.02	0.748	0.393	6,266
Hardness	**0.105**	**4.354**	**0.044**	**6,266**
Mouth-coating	0.013	0.493	0.487	6,266
Satisfaction	0.087	3.504	0.069	6,266
Stand-alone vs. ingredient in recipe	0.02	0.758	0.39	6,266
Aftertaste	0	0	0.984	6,266

Table 9: Regression analysis of oral processing time vs. ranked descriptive statistics for 95% cocoa chocolate. Significant r^2 values ($p \leq 0.05$) are shown in bold.

	R square	F	Sig	df
Bitterness	0.066	2.597	0.116	6,266
Hardness	0.063	2.481	0.124	6,266
Mouth-coating	0	0.001	0.981	6,266
Satisfaction	**0.103**	**4.255**	**0.046**	**6,266**
Stand-alone vs. ingredient in recipe	0.014	0.524	0.474	6,266
Aftertaste	0.003	0.124	0.726	6,266

Figure 1: Electrode placement on participant includes four electrodes under the chin for measuring the contraction of the suprahyoid muscle (two on the anterior belly of digastric and two on the posterior belly of digastric), single electrodes on both masseter muscles, and single ground electrodes on both shoulders.

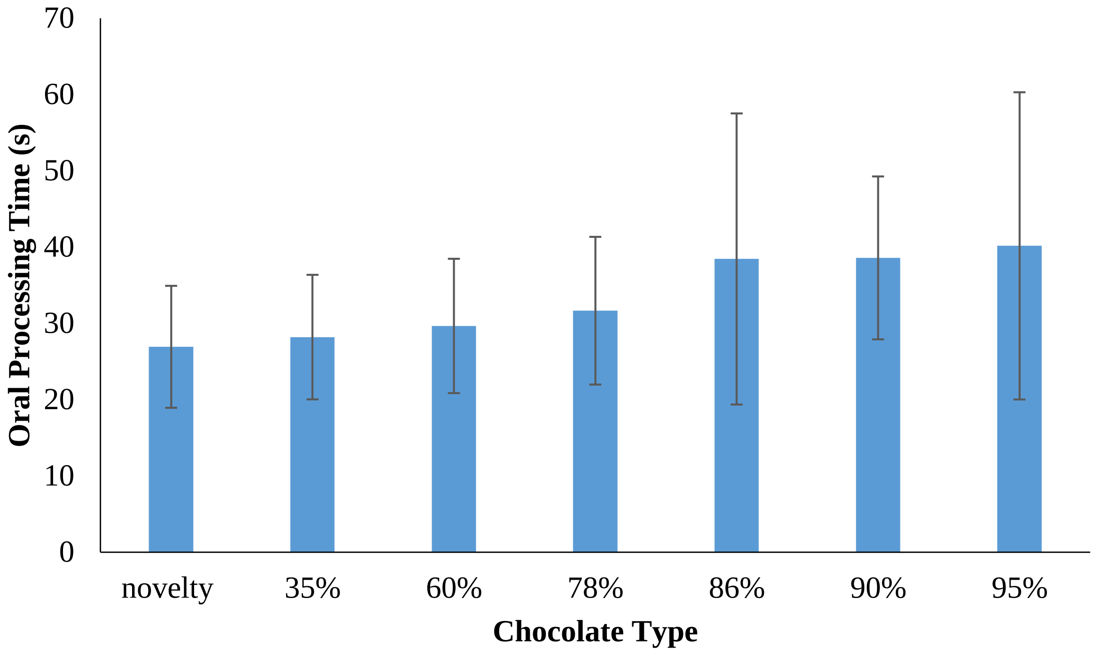

Figure 2: Average oral processing time for all chocolate samples. Error bars represent one standard deviation around the mean.

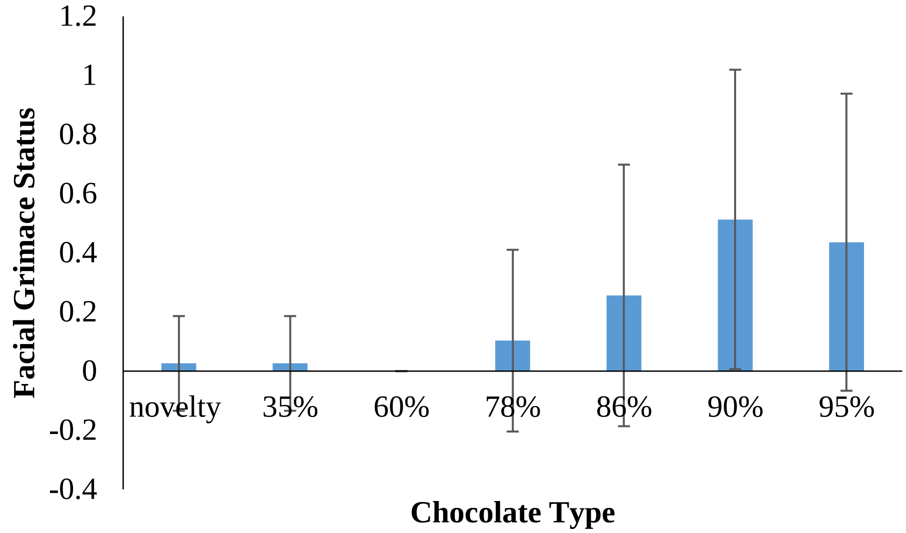

Figure 3: Average facial grimace status for each chocolate type. The absence of a facial grimace is represented as y=0, and the presence of a facial grimace is represented as y=1. Error bars represent one standard deviation around the mean.

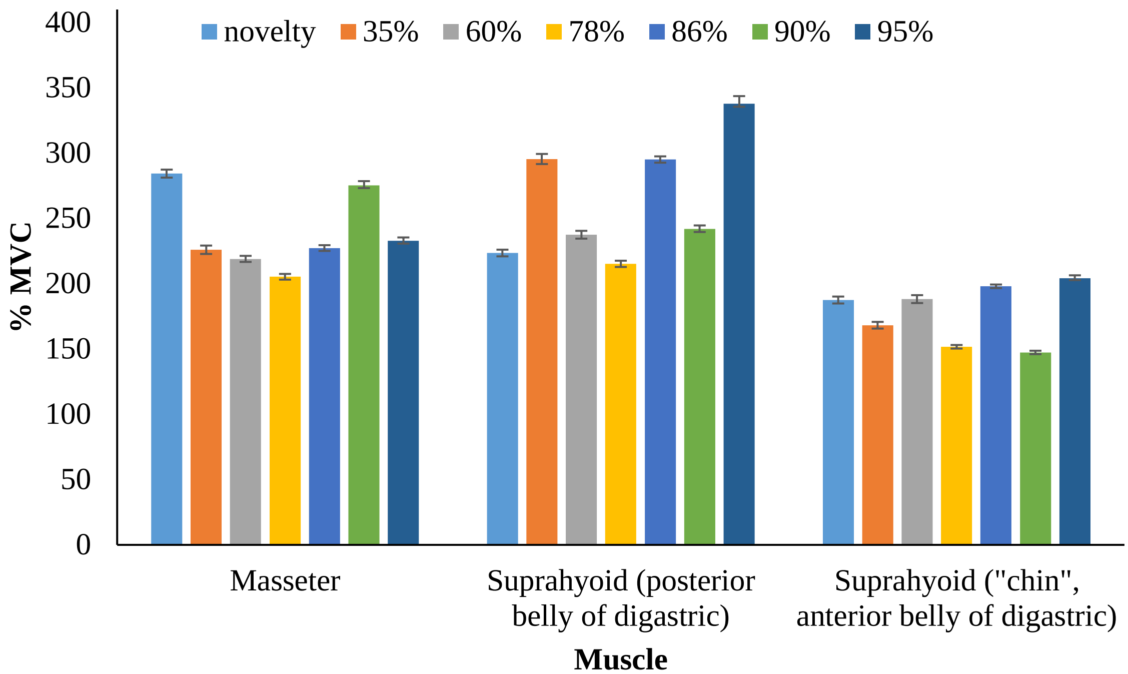

Figure 4: Average percent of maximum voluntary contraction (MVC) of the masseter, suprahyoid (posterior belly of digastric), and suprahyoid (“chin”, anterior belly of digastric) for each chocolate type. Error bars represent one standard deviation around the mean.

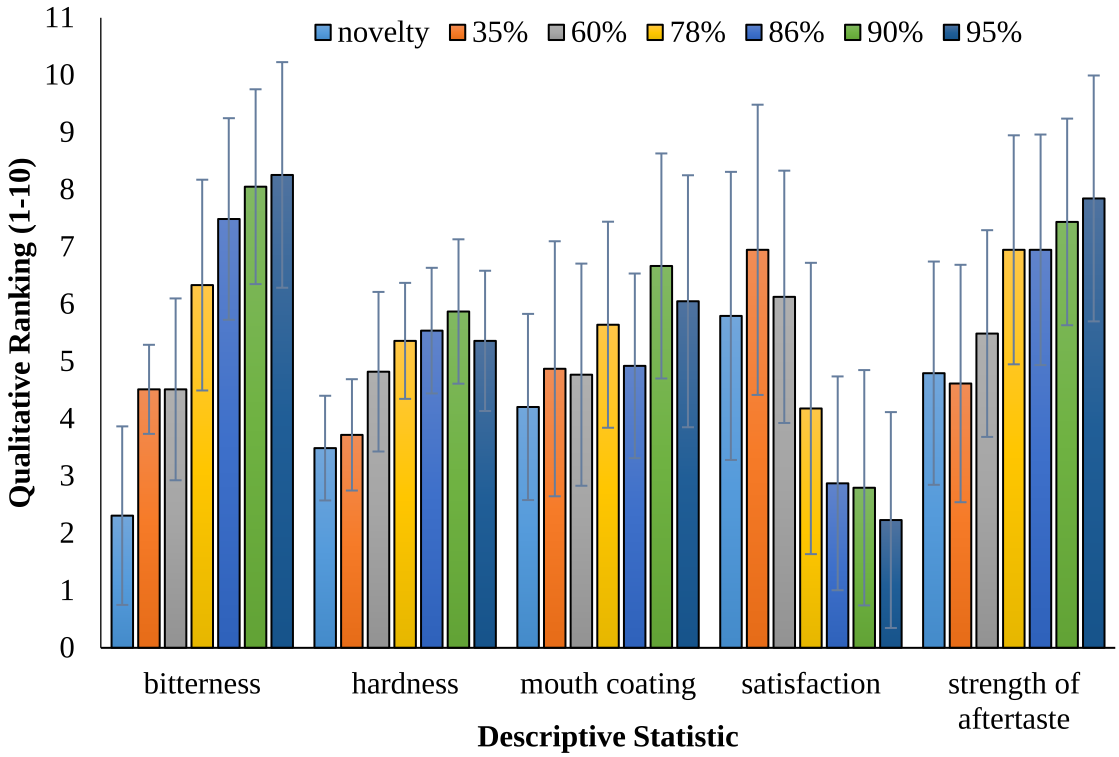

Figure 5: Average qualitative rankings of descriptive statistics for each chocolate including degree of bitterness, hardness, mouth-coating, satisfaction, and strength of aftertaste on a scale of 1-10, with 1 being the lowest degree of each quality and 10 being the highest degree. Error bars represent one standard deviation around the mean.

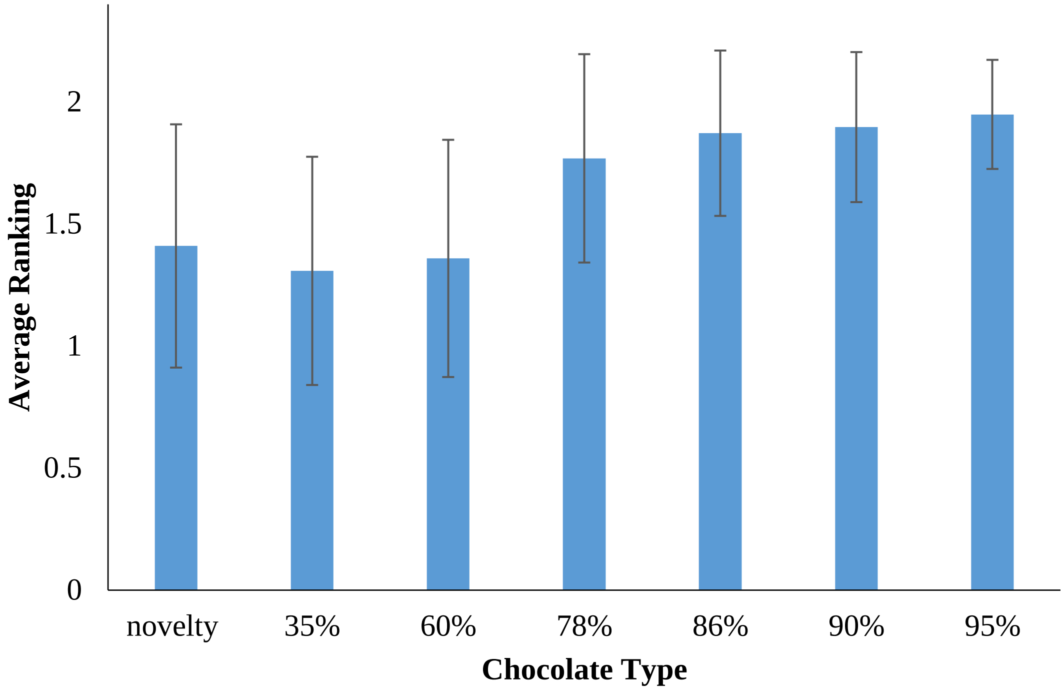

Figure 6: Average rankings for likelihood to consume each type of chocolate as a stand alone treat (1) or as an ingredient within a recipe (2). Error bars represent one standard deviation around the mean.

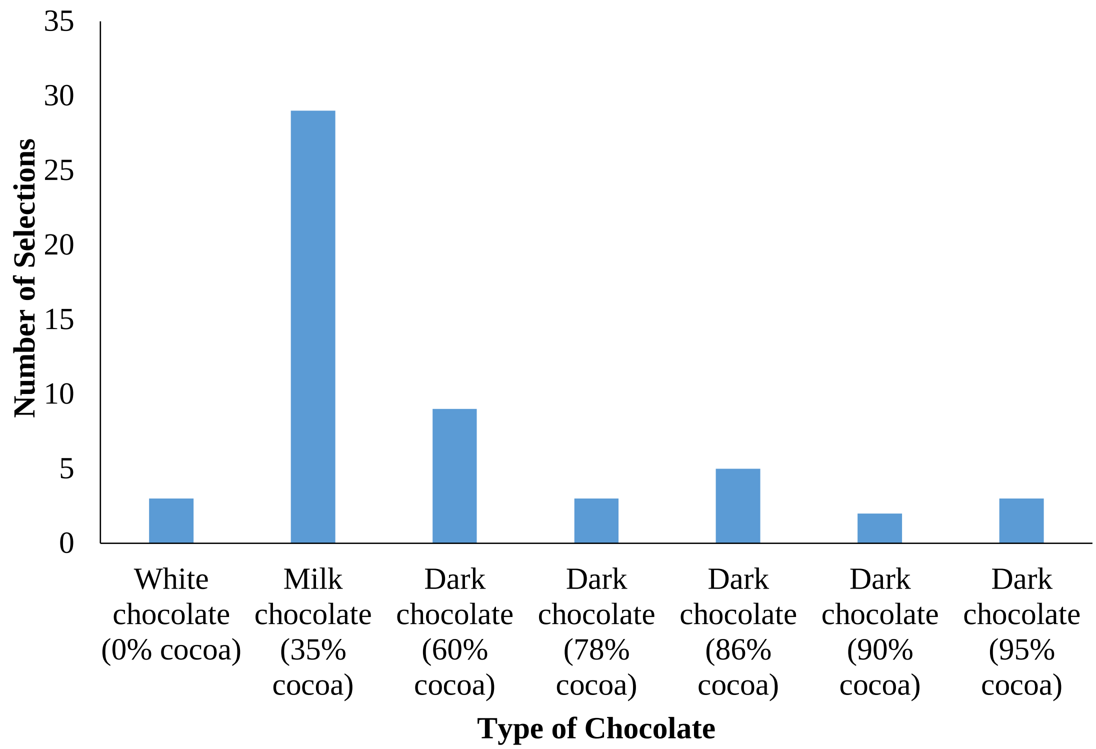

Figure 7: Alternate survey results for generally preferred type of chocolate.

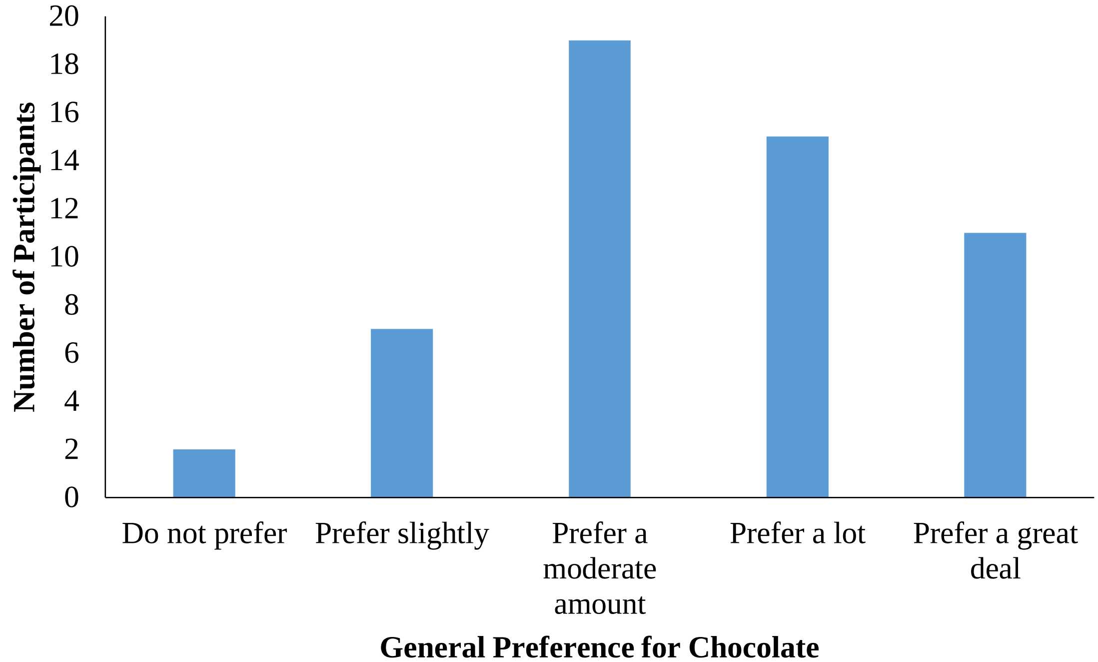

Figure 8: Alternate survey results for general preference for chocolate.

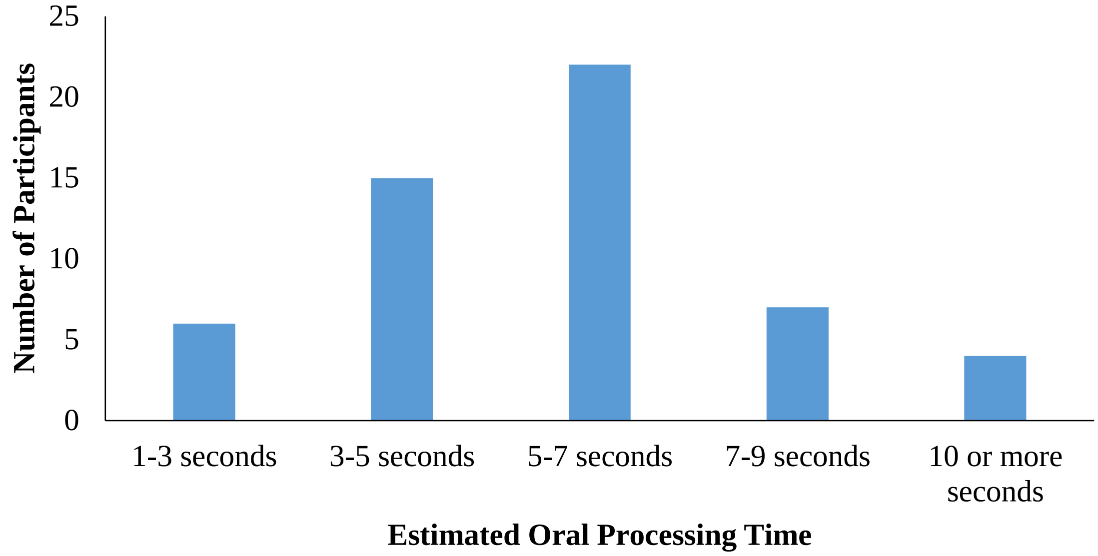

Fig 9: Alternate survey results for estimated time between when a piece of chocolate is put in the mouth and when it is swallowed.

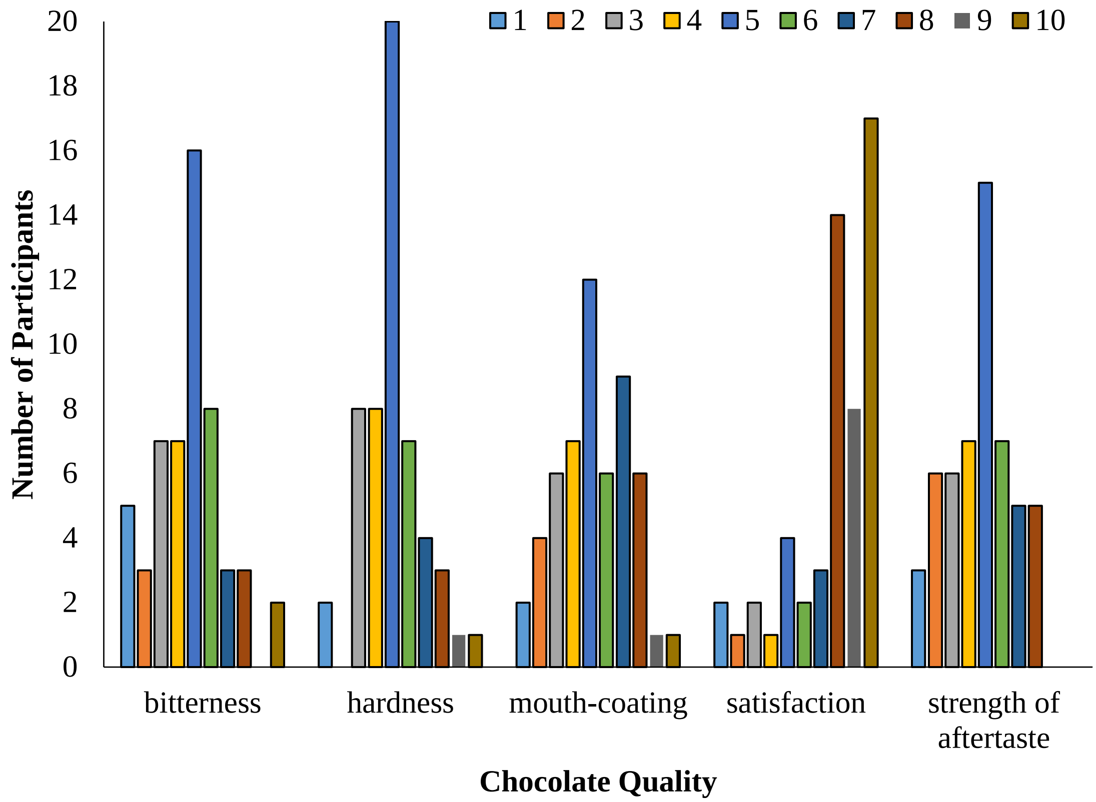

Fig 10: Alternate survey results for ratings (on a scale of 1-10) of generally preferred degree of bitterness, hardness used to bite, mouth-coating experienced, degree of satisfaction experienced, and strength of aftertaste when eating chocolate. For each quality, 1 represents the lowest degree and 10 represents the highest degree.

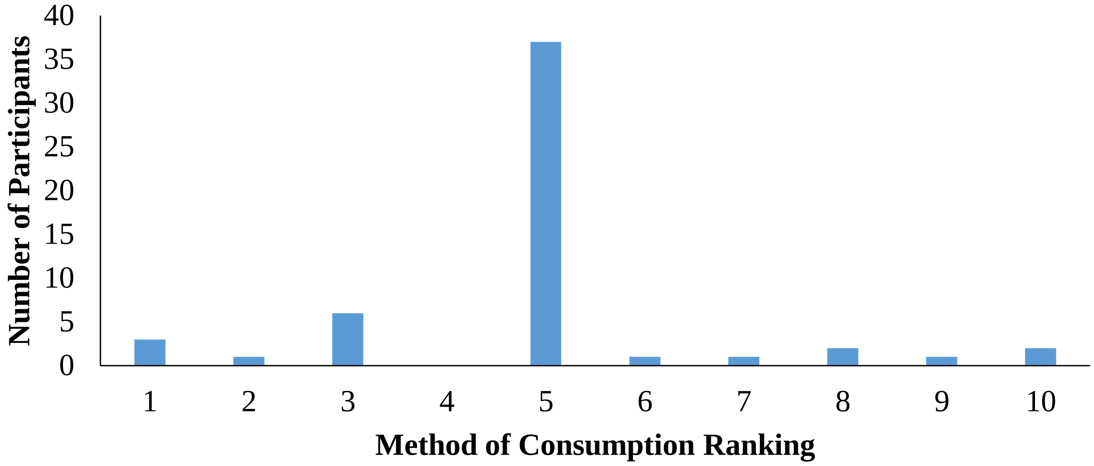

Fig 11: Alternate survey results for likelihood to consume chocolate as a stand-alone treat or as an ingredient within a recipe, with 1=as a stand-alone treat, 5=equally as likely to do either, 10=only within a recipe.

APPENDIX A

Fall 2019 Taste Test Evaluation of Chocolate Samples

Rate the degree of bitterness experienced during consumption on a scale from 1 to 10, with1 being equivalent to the least bitter taste you have ever experienced, and 10 being equivalent to the most bitter taste you have ever experienced. (1=lowest degree of bitterness, 10=highest degree of bitterness).

1 2 3 4 5 6 7 8 9 10

Rate the degree of hardness at first bite on a scale from 1 to 10 (1=lowest degree of hardness (low force used), 10=highest degree of hardness (high force used)). Imagine that 1 is equivalent to water, while 10 is equivalent to a jaw breaker candy.

1 2 3 4 5 6 7 8 9 10

Rate the degree of mouth-coating experienced after swallowing on a scale from 1 to 10 (1= low degree of mouth-coating, 10= high degree of mouth-coating).

1 2 3 4 5 6 7 8 9 10

Rate the degree of satisfaction experienced during consumption on a scale from 1 to 10 (1=low satisfaction, 10=high satisfaction).

1 2 3 4 5 6 7 8 9 10

Would you be more likely to consume this type of chocolate as a stand-alone treat or as an ingredient within a recipe?

Stand-alone treatIngredient

Rate the strength of the aftertaste of this chocolate on a scale from 1 to 10 (1=no aftertaste, 10=very strong aftertaste).

1 2 3 4 5 6 7 8 9 10

APPENDIX B

Fall 2019 Post-Testing Qualitative Questionnaire

Questionnaire to Evaluate Chocolate Preferences of Testing Subjects.

- Generally, what is your preferred type of chocolate?

- Have you always had this preference, or has it changed at some point? If so, what was it before, and when did it change?

APPENDIX C

Fall 2019 Alternate Activity Survey

- Generally, what is your preferred type of chocolate?
  - White chocolate (0% cocoa)
  - Milk chocolate (35% cocoa)
  - Dark chocolate (60% cocoa)
  - Dark chocolate (78% cocoa)
  - Dark chocolate (86% cocoa)
  - Dark chocolate (90% cocoa)
  - Dark chocolate (95% cocoa)
- Generally, how much do you like chocolate?
  - Do not prefer
  - Prefer slightly
  - Prefer a moderate amount
  - Prefer a lot
  - Prefer a great deal
- Between the time you put a piece of chocolate in your mouth and the time you swallow it, about how long do you think you usually have it in your mouth?
  - 1-3 seconds
  - 3-5 seconds
  - 5-7 seconds
  - 7-9 seconds
  - 10 or more seconds
- In general, when you eat a piece of chocolate, rate the degree of bitterness that you prefer on a scale from 1 to 10, with 1 being the most bland taste you have ever experienced, and 10 being equivalent to the most bitter.
  - 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9
  - 10
- In general, when you eat a piece of chocolate, rate the degree of hardness that you think you use at first bite on a scale from 1 to 10 (1=low degree of hardness (low force used), 10=high degree of hardness (high force used)). Imagine that 1 is equivalent to water, while 10 is equivalent to a jaw breaker candy.
  - 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7

- 8
- 9
- 10

- In general, when you eat a piece of chocolate, rate the degree of mouth-coating experienced after swallowing on a scale from 1 to 10 (1= low degree of mouth-coating, 10= high degree of mouth-coating).
  - 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9
  - 10

- In general, when you eat a piece of chocolate, rate the degree of satisfaction experienced during consumption on a scale from 1 to 10 (1=low satisfaction, 10=high satisfaction).
  - 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9
  - 10
- Would you be more likely to consume chocolate as a stand-alone treat or as an ingredient within a recipe? 1=as a stand-alone treat, 5=equally as likely to do either, 10=only within a recipe.
  - 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9
  - 10
- In general, when you eat a piece of chocolate, rate the strength of the aftertaste of the chocolate on a scale from 1 to 10 (1=no aftertaste, 10=very strong, unpleasant aftertaste).
  - 1

- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10